Opportunities to reduce early antiretroviral therapy mortality in sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care

Francis Andrew Auld

Clinical Research Department

Faculty of Infectious & Tropical Diseases

London School of Hygiene & Tropical Medicine

July 2020

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

The research studies included in the thesis were supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the U.S. Centers for Disease Control and Prevention (CDC).

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

DECLARATION OF OWN WORK

All students are required to complete the following declaration when submitting their thesis.

Please note: Assessment misconduct includes any activity that compromises the integrity of your research or assessment of is will be considered under the Assessment Irregularity Policy. This includes plagiarism, cheating and failure to follow correct progression and examination procedures.

Please see the following documents for further guidance:

- Research Degrees Handbook
- Assessment Irregularities Policy

Supervisors should be consulted if there are any doubts about what is permissible.

1. STUDENT DETAILS

Student ID Number	1405072	**Title**	Dr.
First Name(s)	Francis Andrew		
Surname/Family Name	Auld		
Programme of Study	Doctor of Philosophy		
LSHTM Email (if this is no longer active, please provide an alternative)	aauld@cdc.gov		

2. TITLE OF THESIS

Title of Thesis	Opportunities to reduce early antiretroviral therapy mortality in sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care

3. DECLARATION

I have read and understood the LSHTM's definition of plagiarism and cheating. I declare that this thesis is my own work, and that I have acknowledged all results and quotations from the published or unpublished work of other people.

I have read and understood the LSHTM's definition and policy on the use of third parties (either paid or unpaid) who have contributed to the preparation of this thesis by providing copy editing and, or, proof reading services. I declare that no changes to the intellectual content or substance of this thesis were made as a result of this advice, and, that I have fully acknowledged all such contributions.

I have exercised reasonable care to ensure that the work is original and does not to the best of my knowledge break any UK law or infringe any third party's copyright or other intellectual property right.

Student Signature	[redacted]
Date	14 July 2020

Abstract

Undiagnosed tuberculosis (TB) remains the most common cause of HIV-related mortality, including among people living with HIV (PLHIV) starting antiretroviral therapy (ART). This thesis explores opportunities for reducing PLHIV mortality in sub-Saharan Africa.

Firstly, a systematic review of eight Xpert MTB/RIF (Xpert) impact trials found that lack of Xpert impact on mortality was mainly due to higher empiric TB treatment rates in microscopy versus Xpert arms.

Secondly, the Botswana XPRES trial evaluated the effect of an intervention package comprising (1) support for intensified TB case finding (ICF), (2) strengthened tracing to support retention, and (3) Xpert replacing sputum-smear microscopy on early (6-month) ART mortality. Strengthened ICF and retention were associated with about 23% lower 6-month mortality. No mortality benefit of Xpert replacing microscopy was observed.

Thirdly, to identify PLHIV at highest risk of early ART mortality, CD4-independent and -dependent scores were derived from XPRES data and externally validated. Sensitivity of CD4-independent score ≥4 in predicting mortality (86%) was twice that of WHO stage alone (48%). Both CD4-independent score ≥4 and CD4-dependent score ≥5 had similar sensitivity but higher specificity than WHO-recommended advanced HIV disease criteria (i.e., CD4 <200/µL or WHO stage III/IV).

Finally, a TB risk score for PLHIV was derived from XPRES data and validated on three external datasets, with the aim of increasing (1) detection of asymptomatic TB, and (2) sensitivity to exclude TB prior to TB preventive therapy. In the external datasets, TB risk score ≥2 had higher sensitivity (87–97%) than the WHO four-symptom screening rule (80–94%) but lower specificity (12–58% versus 16–70%).

In conclusion, strengthening TB screening and retention in care could reduce early ART mortality in sub-Saharan Africa. In addition, early mortality and TB risk scores could help clinicians better detect and differentiate risk and should be further evaluated.

Acknowledgments

I am very grateful to many people without whom this thesis would not have been possible.

Firstly, I need to thank my two primary supervisors Steve Lawn and Alison Grant. I was very privileged and grateful to have been mentored and supervised by Steve Lawn for the first 15 months of the thesis. He was always encouraging, insightful, and generous with his time when providing feedback and advice. His generosity was remarkable given that for most of the 15 months he served as my primary supervisor he was living with, and undergoing treatment for, glioblastoma. He is sorely missed since his death on 23 September 2016.

I am also very grateful to Alison Grant for being willing to take over as my primary supervisor in 2016 and from whom I have learnt a lot in terms of critical thinking, the principles of risk screening discussed in this thesis, and practical approaches to improving care for TB patients in sub-Saharan Africa. I am also very grateful to Katherine Fielding for persisting as a secondary supervisor throughout the process, her patience with my many questions, and her support thinking through the analytic approaches described in this thesis. To both Alison and Katherine I am very grateful for the many hours of teleconferences, excellent feedback, patience, encouragement, and flexibility as I completed this thesis as a part-time student.

I also need to thank my CDC supervisors Tedd Ellerbrock and George Bicego for encouraging me to complete the research, as well as many colleagues in Botswana and Atlanta who worked on the XPRES trial to make it a reality. Specifically, I also thank Tefera Agizew, Anikie Mathoma, Rosanna Boyd, Anand Date, Sherri Pals, Chris Serumola, Unami Mathebula, Heather Alexander, James Shepherd, and Alyssa Finlay. I also thank Botswana Ministry of Health colleagues Dr. Rankgoane-Pono and Dr. Pontsho Pono for their support

for XPRES, as well as the many study participants who sacrificed their time to join the study and generate new evidence.

Finally, I thank my family, especially my mother and father, who continue to teach by example with extraordinary bravery since my father's stroke in April 2016. Finally, I thank my children and my wife Evelyn, who has supported me throughout, and without whom this would not have been possible.

Table of contents

List of tables

Chapter 1

Chapter 2

Chapter 3

No tables

Chapter 4

No tables

Chapter 5

Chapter 6

Chapter 7

Chapter 8

List of figures

Chapter 1

Chapter 2

No figures

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Table of abbreviations

AFB	Acid-fast bacilli
AHR	Adjusted hazard ratio
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
ATT	Anti-tuberculosis treatment
BMI	Body mass index
CD4	$CD4^+$ T-cell count
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CrAg	Cryptococcal antigen
CRP	C-reactive protein
CXR	Chest radiography
ELISA	Enzyme Linked Immunosorbent Assay
GFR	Glomerular filtration rate
HCV	Hepatitis C virus
HIV	Human immune-deficiency virus
HR	Hazard ratio
ICF	Intensified TB case finding
IPT	Isoniazid preventive therapy
IQR	Inter-quartile range
IRB	Institutional review board
LFA	Lateral flow assay
LMIC	Low- and middle-income countries
LSHTM	London School of Hygiene & Tropical Medicine
LTFU	Loss to follow-up
MDR	Multidrug resistant TB
MGIT	Mycobacterium growth indicator tube
MTB	Mycobacterium tuberculosis complex
MUAC	Mid-upper arm circumference
NNS	Number needed to screen
NPV	Negative predictive value
OI	Opportunistic infection
OR	Odds ratio
PHIA	Population-based impact assessment
PLHIV	People living with HIV
POC	Point of care
PPV	Positive predictive value

RNA	Ribonucleic acid
SA	South Africa
SOC	Standard of care
SOP	Standard operational procedure
SSA	Sub-Saharan Africa
TB	Tuberculosis
TBFT	TB Fast Track Trial
TB-LAM	Determine TB-LAM Ag assay
TLR	Toll Like Receptor
TNF	Tissue Necrosis Factor
TPT	TB preventive therapy
UNAIDS	Joint United Nations Programme on HIV/AIDS
VACS	Veterans Aging Cohort Study
WHO	World Health Organisation
Xpert	Xpert MTB/RIF assay
XPHACTOR	Xpert for people attending HIV/AIDS care: test or review?
XPRES	Xpert Package Rollout Evaluation using a Stepped-wedge design
XTEND	Xpert for TB: evaluating a new diagnostic

Chapter 1: Introduction

Globally, about 37.9 million persons are living with HIV, and about 770,000 persons die from HIV-related illnesses annually.[1] The HIV pandemic disproportionately affects sub-Saharan Africa; despite holding only 11% of the global population, sub-Saharan Africa is home to about 25.6 million (68%) of people living with HIV (PLHIV) and accounts for about 470,000 (61%) of all HIV-related deaths.[1] Undiagnosed tuberculosis (TB) or TB diagnosed late in the course of disease remains the most common cause of HIV-related deaths in low- and middle- income countries (LMIC), including sub-Saharan Africa.[2,3] In the most recent meta-analysis of autopsy-confirmed causes of death among hospitalized PLHIV in LMIC, about two-fifths (37%) of deaths were due to TB and in nearly half these deaths (one-fifth of all deaths), TB was undiagnosed *ante mortem*.[3]

Scale-up of antiretroviral therapy (ART) is the most important public health intervention to reduce annual HIV-related mortality.[4-6] Since 2004, when there were 1.7 million HIV-related deaths, the annual number of HIV-related deaths has declined by about 55% as ART coverage increased from <1% to 62%.[7] By 2020, targets of reaching 81% of PLHIV with ART were globally endorsed, but many countries will not reach this target.[8] Two new targets have been proposed for 2030: first, to reach 90% of PLHIV with ART, and second, to reduce HIV-related deaths by 90% compared with 2010.[9] With an estimated 23.3 million PLHIV currently receiving ART in 2020, an additional 12-13 million PLHIV will need to be enrolled on ART over the next 10 years to reach the 2030 targets. In addition, accelerated declines in HIV-related mortality will be needed to reach 90% HIV mortality reduction goals.[10,11] Among all ART enrolees, the highest incidence of mortality for both adults and children is in the first six months of therapy.[12] Therefore, identifying interventions to reduce this early mortality on ART remains an urgent public health priority.[13]

In addition, HIV-associated TB deaths accounted for 251,000 (21%) of the annual 1.2 million TB deaths in 2018, helping to make TB the leading infectious cause of death globally.[14] Following the September 2018 United Nations (UN) General Assembly high-level meeting on TB, world leaders recommitted to ambitious End TB goals, which include reducing annual TB deaths by 90% by 2030.[14] These ambitious goals can only be achieved if significant progress improving TB screening, diagnosis and treatment among PLHIV is made.[15,16]

Therefore, this thesis focuses on evaluating the impact of improved TB screening algorithm implementation among PLHIV during early ART, investigates opportunities to improve on existing WHO standard eligibility criteria for who needs early ART care intensification, and evaluates opportunities to improve TB screening approaches for both ART-naïve and -experienced PLHIV with the goal of contributing to HIV and TB pandemic control goals by 2030.[16]

1.1. Early mortality after ART initiation

1.1.1. Incidence and regional variations of early mortality after ART initiation

In this thesis we define early ART mortality as all-cause mortality within 6 months of ART initiation. However, the time period defining early mortality after ART initiation varies across published papers, ranging from 3,[17] to 6,[18] to 12 months.[19] The most recent meta-analysis of adult (≥15 years old) early mortality on ART in LMIC was published in 2016 and included 58 studies that were published between January 2003 and April 2016 focusing on mortality within 3 months of ART initiation.[17] Key findings were that 3-month mortality rates (unadjusted for under-ascertainment of mortality during loss to follow-up (LTFU)) were 6% (95% confidence interval (CI), 5-7%) overall, but declined from 7% (95% CI, 6-8%) for ART enrolees before 2010 to 4% (95% CI, 3-5%) during or after 2010.[17] Point estimates of mortality within 3 months of ART initiation varied slightly by region, with point estimates highest in sub-Saharan Africa (6.3%) compared with the Caribbean/Latin

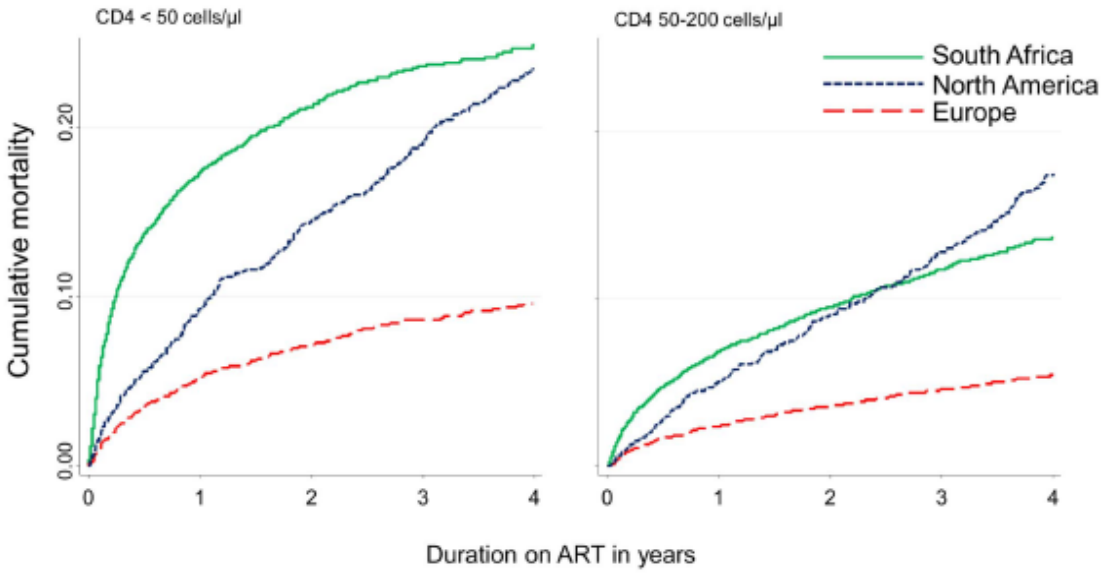
America (6.0%), and Asia (5.3%), although 95% CI's for these point estimates overlapped. Published meta-analyses and recent observational studies show that a substantial percentage of patients who become LTFU during early ART (20-60% according to recent studies)[12,20] are found to have died, usually shortly after the missed appointment. When authors made the assumption that 47% of those LTFU within 3 months of ART would have died by 3 months, overall 3-month mortality estimates nearly doubled from 6.0% to 10.6%.[17] If we assume that about 70% of 6-month ART mortality occurs within 3 months of ART initiation,[21] include adjustment upwards for deaths during LTFU that were not ascertained, and account for declining early ART mortality over time, a reasonable estimate for 6-month mortality on ART for sub-Saharan Africa for the time period 2010–2016 is about 10%, which is high compared with resource-rich countries where 6-month mortality rates under 5% have been reported for the last 10-20 years.[22,23]

The observation that sub-Saharan Africa has higher early mortality on ART than resource-rich settings and other LMIC was also noted in a 2010 systematic review and meta-analysis of 12-month ART mortality in LMIC, which synthesized data from 50 observational cohort studies published during 1996–2010 from program settings in sub-Saharan Africa, Asia, and the Americas.[19] The meta-analysis adjusted crude mortality estimates upwards to account for expected mortality among patients LTFU during the first 12 months of therapy.[12,20] The meta-analysis reported a global 12-month ART mortality estimate for LMIC of 14% (95% CI, 10-20%).[19] The meta-analysis, similar to contemporary multi-cohort analyses,[13,23] reported significant regional variations in 12-month ART mortality, with 12-month mortality highest in sub-Saharan Africa (17%), followed by Asia (11%), and the Americas (7%).

Multiple potential reasons for the higher early mortality on ART in sub-Saharan Africa compared with resource-rich settings have been proposed and explored.[19,21] An analysis by Boulle *et al* showed that reasons for higher early mortality in sub-Saharan Africa are not purely related to a higher prevalence of advanced HIV disease at ART initiation (Figure

1).[22] Boulle and other authors support the interpretation that a combination of factors likely account for higher early mortality on ART in sub-Saharan Africa versus resource-rich settngs.[19,21] A combination of interacting problems including higher disease burden of opportunistic infections (OI) like TB in the general population exacerbated by a higher prevalence of late presentation for ART initiation among PLHIV, which is often driven by socio-economic factors,[23,24] are considered to be some key factors driving higher early mortality on ART in sub-Saharan Africa compared with resource-rich settings.[19,22] These findings are supported by autopsy studies, which show that HIV-associated infections, especially TB, but often multiple concurrent infections, account for the vast majority of early deaths during ART,[2] whereas only 30% of classifiable deaths in Europe and North America are infection-related.[22]

Figure 1.1. Comparison of cumulative incidence of mortality up to four years after ART start by region, corrected in South Africa for mortality under-ascertainment*



*Taken from Boulle, A et al, PloS Med, 2014.[22] Analysis included ART enrolees between 2001-2010. Note: Authors estimate good linkage with mortality registers in South Africa and the United States to determine LTFU outcomes, and less frequent linkage to mortality registers in European cohorts.

1.1.2. Causes of early mortality after ART initiation

The most recent meta-analysis of pathological autopsy studies among persons with HIV in LMIC included persons with HIV regardless of ART status across 36 eligible studies, and

reported on 3,237 autopsies conducted by 2013; TB was reported as the most common cause of death, accounting for 37% of deaths overall.[3] Similarly, the only study focused on describing autopsy-confirmed causes of early mortality after ART initiation in sub-Saharan Africa, which was conducted in South Africa in 2012,[25] reported that mycobacterial infections (usually *Mycobacterium tuberculosis*) were implicated in 69% of early deaths during ART. A more recent autopsy study published in 2016 among PLHIV with CD4 count <150 cells/μL who died within 6 months of enrolling in a cluster-randomised trial in South Africa reported that 47% of cadavers had evidence of TB (about half untreated *antemortem*), 68% had clinically important bacterial infections, and 12% cryptococcal disease, with 59% having two or more infections.[2]

1.1.3. The persistent problem of early mortality after ART initiation in sub-Saharan Africa

Observational cohort studies among ART enrolees have shown that early mortality incidence is largely determined by prevalence of markers for advanced HIV disease at ART initiation, such as low CD4 count (e.g. CD4 <50 cells/μL), advanced disease stage (e.g., WHO stage IV), low body mass index (BMI <18.5), and severe anaemia (haemoglobin <8 g/dL).[19,26,27] Consequently, as the prevalence of advanced HIV disease among ART enrolees has declined over successive annual cohorts in many LMIC due to expanding ART access and lower thresholds for ART initiation,[28,29] early mortality after ART initiation has been reported to decline over time in several countries, including South Africa,[30,31] Botswana,[32] Mozambique,[21] and in the most recent meta-analysis referenced.[17]

The trend of increasing median CD4 count at ART initiation and declining early mortality in LMIC raises the question whether AIDS-related causes of death, including TB, might be declining in importance as a cause of early mortality after ART initiation.[33] A recent meta-analysis to assess the proportion of on-ART mortality due to non-AIDS causes of death (e.g. cardiovascular disease, non-AIDS malignancies, and liver disease) synthesized data from 19 studies conducted across 55 different countries representing both high-income

countries and LMIC.[33] The meta-analysis reported that non-AIDS causes of death among people taking ART accounted for about 53%, 34%, and 19% of deaths in high-income countries, LMIC overall, and sub-Saharan Africa specifically.[33] Authors hypothesized that as median CD4 count at ART initiation has increased among successive annual cohorts of patients in the last 20 years in high-income countries, non-AIDS causes of death have become proportionally more important.[33] Notably, the meta-analysis relied mostly on data from verbal autopsies or medical record review, an important limitation.[3] In addition, the meta-analysis examined causes of death at any time during ART and not only in the first 6-12 months of therapy. However, the author's hypothesis for high-income countries is supported by a multi-country analysis, including data from nearly 50,000 patients in 212 clinics in Europe, Australia, and the United States, which reported that the percentage of deaths due to AIDS-related causes declined from 34% to 22% between 1999 and 2011 as median CD4 count at ART initiation increased.[34]

A separate meta-analysis of autopsy studies, which is not yet published as a peer-reviewed manuscript, included 56 autopsy studies describing over 10,000 autopsies conducted between 1984 and 2015. This meta-analysis evaluated both the trend in proportional mortality due to HIV-associated causes, and among those deaths classified as HIV/AIDS-associated, the percentage due to TB.[35] This meta-analysis showed the percentage of deaths due to HIV-associated causes declined from 87% for studies conducted pre-2005, to 77% for studies conducted during 2005–2010, and to 70% for autopsy studies conducted during 2011–2015.[35] However, TB as a cause of HIV/AIDS-associated death appeared to increase from 24% in 2005 to 36% in 2015. Similarly, in the meta-analysis by Gupta *et al,* which evaluated prevalence of TB in pathologic autopsies among PLHIV who died in hospital, TB prevalence increased over a time of massive ART scale-up rather than decreased.[3] These two meta-analyses suggest that while ART scale-up appears to be reducing mortality due to HIV/AIDS-associated causes, ART scale-up appears to have had a lower *relative* impact on HIV-associated TB mortality.[35] A limitation affecting both meta-analyses is that methods to detect TB at autopsy have improved over

time and the non-standard autopsy methods used across the autopsy studies limit ability to confidently infer understanding of trends over time.[35]

In addition, although most studies in sub-Saharan Africa have reported increases in median CD4 count at ART initiation over the last 15 years as WHO ART eligibility guidelines have shifted towards universal ART eligibility (i.e., test-and-treat), which has been recommended since 2015,[28,36,37] most studies in sub-Saharan Africa continue to report that 15-30% of ART enrolees present late and initiate ART with advanced HIV disease (i.e., a CD4 count <200/µL or WHO stage III/IV). For example, in a recent large multi-year review of CD4 count test results (N=864,389) across four high burden sub-Saharan countries, the percentage of ART enrolees starting ART with advanced HIV disease (CD4 <200/µL) remained fairly consistent: 19.4% (95% CI: 18.8-20.1%) in 2012 compared to 16.1% (95% CI: 16.0-16.3%) in 2016,[38] with the proportion of patients diagnosed as having advanced HIV disease ranging from 14.5% in Uganda to 29.8% in Cameroon.[37,38] This shows that even though WHO now recommends test-and-treat,[4,39] barriers to early HIV testing, linkage to care, and early ART initiation remain in LMIC.[40,41] In addition, recent studies report that persons presenting to ART clinics with advanced HIV disease are more likely to be ART-experienced who have cycled in and out of care with associated fluctuations in immune-competence.[11] Consequently, improved early ART care and improved advanced HIV disease detection and management algorithms, that address risk of co-infections including TB, remain a public health priority if 2030 targets of reducing AIDS-related deaths are to be met.[10,42]

1.2. Current WHO-recommended standards for early ART care

Given the clear evidence that ART initiation at earlier disease stages is essential to optimize early ART outcomes,[4,43] WHO strongly recommends rapid ART initiation for all PLHIV after a confirmed HIV diagnosis and clinical assessment, ideally offering ART on the same day as diagnosis for those ready to start and for whom there is no clinical reason for

delay.[44] However, while significant progress has been made in scaling up access to testing and linkage to treatment, real-world challenges of achieving early HIV diagnosis,[45,46] linkage to treatment,[47] and retention in early pre-ART and ART care,[48] especially in certain population groups like men,[49] youth,[50] and those who are asymptomatic at the time of HIV diagnosis, remain.[51]

For example, a 2020 meta-analysis of progress to 90-90-90 targets (i.e., 90% of PLHIV aware of their status, 90% of those aware on ART, and 90% of those on ART virally suppressed) in sub-Saharan Africa included 92 studies published between 2014 and 2018.[51] Authors did not estimate a pooled estimate of completion of each step in the cascade, but rather used an unweighted median achievement of each treatment cascade step across studies by age and gender. PLHIV 15 to 24 years old had lower median completion of the treatment cascade (60-49-81), as compared to PLHIV ≥25 years (70-63-91). Men also had lower median achievement of the treatment cascade (66-72-85), compared to women (79-76-89).[51] These differences by age and sex in 90-90-90 cascade completion have also been observed across all 13 completed population-based HIV impact assessments (PHIAs), which are large nationally representative cross-sectional surveys of 90-90-90 cascades among PLHIV, in sub-Saharan Africa.[52] These persistent challenges reaching certain population groups in certain geographies with early diagnosis and linkage to treatment, plus the relatively high rates of LTFU during ART in many sub-Saharan African settings, resulting in clients cycling in and out of care,[11] mean that detecting and providing optimal care packages for PLHIV at highest risk of early mortality on ART remains a priority.[11,44]

The WHO currently recommends intensification of care for persons >5 years old starting ART with advanced HIV disease as defined by $CD4^+$ T-cell (CD4) count <200 cells/μL or WHO stage III/IV (Table 1).[44] The intensification of care package, some components of which have been shown to reduce mortality in clinical trials,[53] include tuberculosis (TB) screening with subsequent TB treatment for those diagnosed as having active TB, TB

preventive therapy (TPT) for those who screen negative or are diagnosed as not having active TB,[54] cotrimoxazole prophylaxis,[55,56] cryptococcal antigen (CrAg) screening for those with CD4 count ≤100/µL and pre-emptive therapy for CrAg-positive people with no evidence of meningitis,[57] and enhanced adherence counselling[57] (Table 1).[44]

Table 1.1. WHO-recommended components of the package of care for people with advanced HIV disease for adults and adolescents*

	Intervention	CD4 cell count
Diagnosis	WHO four-symptom TB screening with sputum Xpert MTB/RIF as the first test for TB diagnosis among symptomatic PLHIV	Any
	LF-LAM for TB diagnosis among people with signs and symptoms of TB	<100/µL or at any count if seriously ill
	Cryptococcal antigen screening	<100/µL
Prophylaxis and pre-emptive treatment	Co-trimoxazole prophylaxis	≤350 cells/µL or clinical stage 3 or 4 Any CD4 count in settings with high prevalence of malaria or severe bacterial infections.
	TB preventive therapy (TPT)	Any
	Fluconazole pre-emptive therapy for cryptococcal antigen–positive people without evidence of meningitis	<100/µL
ART initiation	Rapid ART initiation	Any
	Defer initiation if clinical symptoms suggest TB or cryptococcal meningitis	Any
Adapted adherence support	Tailored counselling to ensure optimal adherence to the advanced disease package, including home visits if feasible	<200/µL or at any count if seriously ill

Abbreviations: WHO, World Health Organisation; TB, tuberculosis; PLHIV, people living with HIV; LF-LAM, lateral flow urine lipoarabinomannan assay.
*Taken from the 2017 WHO advanced HIV disease guidelines[44]

1.3. Adherence to WHO-recommended TB screening

In 2008, the WHO launched the three "I"s strategy, namely use of intensified case finding (ICF), isoniazid preventive therapy (IPT), and infection prevention and control, to be scaled

up in tandem with ART to reduce morbidity and mortality from TB.[58] The ICF strategy includes regularly screening all PLHIV for symptoms of TB and implementing TB diagnostic and treatment algorithms for those who screen positive. WHO has recommended a four-symptom screening questionnaire for current cough, fever, weight loss, or night sweats, to be implemented at every clinical visit to identify who requires further TB diagnostic work up since 2011.[59] The four-symptom screen was initially derived from a meta-analysis of 12 studies, including approximately 10,000 PLHIV who were predominantly ART-naïve, which reported screening sensitivity in detecting culture positive TB of 79%.[60] Notably, the goal of this initial meta-analysis was to derive a TB symptom screening rule that maximised sensitivity in detecting sputum culture-positive TB to facilitate ruling out active TB disease and allow subsequent TPT prescription. A more recent meta-analysis also reported good sensitivity (89%) among ART-naïve PLHIV prior to ART initiation.[61]

However, the TB diagnostic cascade that starts with TB symptom screening is not well implemented in LMIC, with loss of patients at all steps of the cascade from TB screening to treatment.[62,63] Reasons for low compliance with TB screening and ICF algorithms are not well understood, but could relate to high patient load making healthcare workers more likely to omit key steps in care algorithms, inadequate training and knowledge of the guidelines, lack of trust in the accuracy of diagnostics, or deficiencies in monitoring and evaluation.[16,63-65] Prior to this research, no trial had yet evaluated impact of improving adherence to WHO-recommended TB symptom screening and ICF cascade guidelines on patient-important outcomes.[66]

1.4. Adherence to WHO-recommended retention interventions

In sub-Saharan Africa, incidence of mortality among those ART enrolees LTFU is 20-fold higher than patients retained on ART, with most mortality occurring soon after the missed appointment.[67] Several trials have also shown high rates of LTFU among PLHIV with microbiologically confirmed TB prior to starting TB treatment,[68-70] or during TB treatment

itself.[68,71-73] WHO recommends intensified counselling and adherence interventions for people with advanced HIV disease during early ART based on these data and evidence from randomized trials, including the REMSTART trial where weekly home visits were provided during the first month of ART.[57] However, pre-emptive home visits are not widely implemented due to resource limitations. WHO also recommends rapid tracing for patients who miss appointments, initially by phone or through home visit if not reachable by phone, which is a retention intervention more widely feasible in sub-Saharan Africa.[44,74,75] However, prior to this research, no trial had yet included active tracing as part of an intervention package to reduce early mortality after ART initiation.[76]

1.5. Role and impact of Xpert MTB/RIF

In 2009, commercial release of the Xpert MTB/RIF® assay for the GeneXpert® platform (Xpert) (Cepheid, Sunnyvale, CA) represented an important advance over sputum smear microscopy in diagnosing HIV-associated TB and drug resistant TB, and therefore became an important component of the three "I"s strategy.[77] With features including a TB diagnostic sensitivity among PLHIV of about 79%,[78,79] significantly superior to smear microscopy (±45%),[78] ability to detect rifampicin resistance-conferring mutations, capacity to provide results from sputum within 100 minutes, and minimal training requirements, Xpert significantly advanced TB diagnostic capability for clinicians managing PLHIV.[80] Consequently, Xpert rollout in high HIV prevalence settings was expected to avert a significant portion of TB-related mortality among PLHIV, including those starting ART.[81,82] In the next chapter (chapter 2), results of a systematic review of trials evaluating Xpert impact on patient-important outcomes are presented.

1.6. Limitations of WHO advanced HIV disease eligibility criteria

WHO defines advanced HIV disease as CD4 count ≤200/µL or WHO stage III/IV.[44] The definition of advanced HIV disease was arrived at through expert consensus and a Delphi

process in developing the 2017 WHO advanced HIV disease guidelines.[83] Following the consensus process, the definition for advanced HIV disease became the WHO-recommended eligibility criterion for accessing the advanced HIV disease package of care.[44] However, no study has systematically attempted to quantify the screening accuracy of the WHO advanced disease eligibility criteria in predicting who is at risk of early mortality after ART initiation, in terms of sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV).[84] A contrasting approach has sometimes been used in resource-rich countries, where internally and externally validated regression-derived mortality risk scores have been used to define levels of mortality risk, and therefore need for intensification of ART care among PLHIV.[85,86] Only one previous study from Haiti, which was not externally validated, has used regression approaches to create a mortality risk score for ART enrolees in a country classified as LMIC.[87] However, validated risk scores to determine who needs ART care intensification in sub-Saharan Africa, the region of the world with the highest early ART mortality rates and the highest number (470,000) and percentage (61%) of global annual HIV-related deaths, have not yet been developed.[1]

Within LMIC, another limitation of the WHO advanced disease eligibility criteria is that the majority of health facilities providing ART lack access to rapid or point-of-care CD4 testing.[44] In these settings, up to half of adults with a CD4 count <100/μL could be categorized as WHO stage I/II, and would be missed by an advanced disease screening algorithm that relied on WHO stage alone.[53] In addition, a screening tool for advanced disease that relies only on CD4 count and WHO disease stage misses the many other demographic and clinical predictors associated with early mortality on ART.[84] Therefore, externally validated clinical screening tools to inform who needs intensification of early ART care are needed.[84]

1.7. Limitations of WHO four-symptom TB screening rule

Currently, the WHO recommends the four-symptom TB screening rule at each clinical visit for PLHIV in LMIC, regardless of expected prevalence of active TB or ART status (ART-naïve or ART-experienced).[88] However, the screening accuracy of the WHO four-symptom screening rule varies by population, setting, and ART status, raising the question whether a "one-size-fits-all" screening rule is appropriate.[89] For example, the most recent meta-analysis observed that while sensitivity of the WHO four-symptom TB screening rule is about 89% among ART-naïve PLHIV, it was only 51% among people on ART due to a higher prevalence of asymptomatic TB among stable ART patients.[61,90,91] At a time when global health donors have committed to reaching over 13 million PLHIV on ART with TPT by 2021,[92] low sensitivity of the WHO four-symptom screening rule for active TB among PLHIV on ART warrants consideration of more sensitive screening approaches in order to achieve a higher NPV.[16]

Although new WHO guidelines recommend adding chest radiography to the screening rule for PLHIV on ART to increase sensitivity, this comes at the expense of specificity, carries significant additional costs and operational challenges, and might hinder rather than expedite TPT scale-up in some LMIC settings.[61,93] Asymptomatic active TB (i.e., absence of self-reported cough, loss of weight, night sweats, and fever) has also been reported in other patient groups, including among severely immune compromised PLHIV,[94,95] and among pre-ART patients without advanced disease in high prevalence settings,[96] among whom missing asymptomatic active TB can have suboptimal health consequences for patients and possibly impede disease control activities.[24] Finally, the WHO four-symptom screening rule does not allow TB risk differentiation into low-, moderate-, and high-risk groups which might inform differentiated models of care. Therefore, improved TB screening tools are urgently needed.[16,97]

1.8. Structure of the thesis

This thesis is structured in research paper style format.

Chapter 2 is the literature review and has three components. The first component is a published systematic literature review of Xpert impact trials (Research paper 1). The second component is a literature review of existing WHO guidelines for evaluating early ART mortality risk and a review of other published risk scores to inform PLHIV mortality risk. The third component is a review of meta-analyses supporting the WHO four-symptom TB screen and other published risk scores to inform understanding of TB risk among PLHIV. All literature reviews summarize existing scientific evidence to clarify gaps in needed research and aim to frame the relevance of thesis research questions and aims presented in Chapter 3.

Chapter 4 describes the methodology of how thesis research aims are addressed, summarizing the XPRES trial protocol, and outlining analytic methods used in the three published research papers in Chapters 5, 6, and 7.

Chapter 5 is the research paper describing XPRES primary objective trial findings, which address the first thesis research question (Research paper 2). Chapter 6 is a research paper describing development of a new early ART mortality risk score for use in sub-Saharan Africa (Research paper 3). Chapter 7 is a research paper describing development of a new TB screening score for use in sub-Saharan Africa (Research paper 4).

Chapter 8 represents the discussion, recommendations and conclusion section of the thesis, where key results, contribution to the literature, implications of findings, limitations, strengths, recommendations and conclusions are provided.

1.9. Role of the candidate

Since 2011, I have played the following roles in the XPRES trial: (1) conceptualized the study; (2) served as a principle investigator (PI) for the study; (3) developed the stepped-wedge trial design with a retrospective baseline component, (4) wrote the protocol (the published version of which is attached as Appendix 1, where I am the first author); (5) created all data collection instruments relevant to the thesis, incorporated co-investigator feedback, and then finalized the instruments; (6) corresponded with the CDC institutional review board (IRB), as well as other ethical oversight bodies; (7) registered the trial at ClinicalTrials.gov (NCT02538952); (8) visited Botswana multiple times in 2011 during study planning; (9) spent six consecutive months in Botswana during training and site initiation for the stepped-wedge trial from May through October 2012; (10) participated in multiple monitoring visits during 2013-2015; (11) coordinated weekly conference calls for the study, documenting minutes, and tracking action items to ensure study quality and protocol compliance; and (12) provided quarterly progress reports to funding oversight bodies (the Associate Director of Science Office) at CDC Atlanta.

I led design of the XPRES trial intervention package. I proposed the need for health system strengthening interventions that support implementation of WHO-recommended TB screening and retention in HIV-TB care to be part of the package of interventions implemented in the XPRES trial. I proposed this intervention package based on prior clinical experience in busy public sector outpatient clinics in South Africa between 2003 and 2007, as well as operational research conducted in sub-Saharan Africa between 2007 and 2011.[62,98,99]

I conducted all analyses included in this thesis. I wrote the initial drafts of the four manuscripts, obtained feedback from co-authors, finalized, and have submitted the manuscripts for peer-review, with two manuscripts published, one provisionally accepted and the final manuscript under peer review. I wrote all sections of the thesis.

1.10. Funding

This research was undertaken while working for the U.S. Centers for Disease Control and Prevention (CDC). XPRES has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the U.S. Centers for Disease Control and Prevention. Disclaimer: The findings and conclusions in this thesis do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

Chapter 2. Literature Review

2.1. Introduction

This thesis explores opportunities to reduce early mortality, and mortality in general, among PLHIV in sub-Saharan Africa through improved TB case finding and retention interventions. The literature review section contains three parts.

Firstly, a systematic review of trials evaluating Xpert impact on patient-important outcomes is presented to frame the contribution for the primary research paper in this thesis. The primary research paper in this thesis evaluates whether a package of health system strengthening interventions supporting implementation of WHO-recommended TB screening and ICF cascades and retention in HIV-TB care, combined with Xpert roll-out can impact early ART mortality compared with standard of care in real-world ART programs in sub-Saharan Africa.

Secondly, a literature review is presented that explores different approaches for determining who is at risk of early mortality on ART, comparing various published approaches with the current WHO-recommended approach.

Thirdly, a literature review is presented that summarizes firstly the meta-analyses supporting the WHO four-symptom TB screening rule and secondly summarizes existing published clinical scores to help identify PLHIV at risk of prevalent active TB, to frame the unique contribution of the TB screening tool presented in this thesis for ART-naïve and ART-experienced PLHIV.

2.2. Systematic review of Xpert impact (Research paper 1)

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	1405072	**Title**	Dr.
First Name(s)	Francis Andrew		
Surname/Family Name	Auld		
Thesis Title	Opportunities to reduce early antiretroviral therapy mortality in sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care		
Primary Supervisor	Prof. 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?	Trans R Soc Trop Med Hyg		
When was the work published?	2016		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	No	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)	The candidate conceived the idea with input from supervisors, developed the search criteria and abstraction tools, conducted the literature review and data abstraction per standard guidance for systematic reviews, and wrote the paper.

SECTION E

Student Signature	[redacted]
Date	14 July 2020

Supervisor Signature	[redacted]
Date	16 July 2020

RightsLink Printable License

OXFORD UNIVERSITY PRESS LICENSE
TERMS AND CONDITIONS

Jul 14, 2020

This Agreement between CDC -- Andrew Auld ("You") and Oxford University Press ("Oxford University Press") consists of your license details and the terms and conditions provided by Oxford University Press and Copyright Clearance Center.

License Number	4867760411550
License date	Jul 14, 2020
Licensed content publisher	Oxford University Press
Licensed content publication	Transactions of The Royal Society of Tropical Medicine and Hygiene
Licensed content title	Xpert MTB/RIF — why the lack of morbidity and mortality impact in intervention trials?
Licensed content author	Auld, Andrew F.; Fielding, Katherine L.
Licensed content date	Sep 23, 2016
Type of Use	Thesis/Dissertation
Institution name	
Title of your work	

	Opportunities to reduce early antiretroviral therapy mortality in Sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care
Publisher of your work	London School of Hygiene & Tropical Medicine
Expected publication date	Jul 2020
Permissions cost	0.00 USD
Value added tax	0.00 USD
Total	0.00 USD
Title	Opportunities to reduce early antiretroviral therapy mortality in Sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care
Institution name	London School of Hygiene & Tropical Medicine
Expected presentation date	Jul 2020
Portions	Pages 1-13
Requestor Location	CDC 2280 Lilongwe Place, Apartment 33 DULLES, VA 20189 United States Attn: CDC
Publisher Tax ID	GB125506730
Total	0.00 USD

Terms and Conditions

STANDARD TERMS AND CONDITIONS FOR REPRODUCTION OF MATERIAL FROM AN OXFORD UNIVERSITY PRESS JOURNAL

1. Use of the material is restricted to the type of use specified in your order details.

2. This permission covers the use of the material in the English language in the following territory: world. If you have requested additional permission to translate this material, the terms and conditions of this reuse will be set out in clause 12.

3. This permission is limited to the particular use authorized in (1) above and does not allow you to sanction its use elsewhere in any other format other than specified above, nor does it apply to quotations, images, artistic works etc that have been reproduced from other sources which may be part of the material to be used.

4. No alteration, omission or addition is made to the material without our written consent. Permission must be re-cleared with Oxford University Press if/when you decide to reprint.

5. The following credit line appears wherever the material is used: author, title, journal, year, volume, issue number, pagination, by permission of Oxford University Press or the sponsoring society if the journal is a society journal. Where a journal is being published on behalf of a learned society, the details of that society must be included in the credit line.

6. For the reproduction of a full article from an Oxford University Press journal for whatever purpose, the corresponding author of the material concerned should be informed of the proposed use. Contact details for the corresponding authors of all Oxford University Press journal contact can be found alongside either the abstract or full text of the article concerned, accessible from www.oxfordjournals.org Should there be a problem clearing these rights, please contact journals.permissions@oup.com

7. If the credit line or acknowledgement in our publication indicates that any of the figures, images or photos was reproduced, drawn or modified from an earlier source it will be necessary for you to clear this permission with the original publisher as well. If this permission has not been obtained, please note that this material cannot be included in your publication/photocopies.

8. While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by Oxford University Press or by Copyright Clearance Center (CCC)) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never

granted. Use of materials as described in a revoked license, as well as any us materials beyond the scope of an unrevoked license, may constitute copyright infringement and Oxford University Press reserves the right to take any and all action to protect its copyright in the materials.

9. rhis license is personal to you and may not be sublicensed, assigned or transferred by you to any other person without Oxford University Press's written permission.

10. Oxford University Press reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

11. You hereby indemnify and agree to hold harmless Oxford University Press and CCC, and their respective officers, directors, employs and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

12. Other rerms and Conditions:

v1.4

Questions? +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Trans R Soc Trop Med Hyg 2016; **00**: 1–13
doi:10.1093/trstmh/trw056

REVIEW

Xpert MTB/RIF — why the lack of morbidity and mortality impact in intervention trials?

Andrew F. Auld[a,*], Katherine L. Fielding[b], Ankur Gupta-Wright[b] and Stephen D. Lawn[b,c,d]

[a]Division of Global HIV & Tuberculosis, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, 30333, USA; [b]Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E7HT, UK; [c]The Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; [d]Department of Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

*Corresponding author: Present address: United States Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, Mailstop-E04, Atlanta, GA 30333, USA; Tel: +1 404 639 8997; E-mail: aauld@cdc.gov

Received 8 June 2016; revised 9 August 2016; accepted 10 August 2016

Compared with smear microscopy, the Xpert MTB/RIF assay (Xpert), with superior accuracy and capacity to diagnose rifampicin resistance, has advanced tuberculosis (TB) diagnostic capability. However, recent trials of Xpert impact have not demonstrated reductions in patient morbidity and mortality. We conducted a narrative review of Xpert impact trials to summarize which patient-relevant outcomes Xpert has improved and explore reasons for no observed morbidity or mortality reductions. We searched PubMed, Google Scholar, Cochrane Library and Embase and identified eight trials meeting inclusion criteria: three individually randomized, three cluster-randomized, and two pre-post trials. In six trials Xpert increased diagnostic yield of bacteriologically-confirmed TB from sputa and in four trials Xpert shortened time to TB treatment. However, all-cause mortality was similar between arms in all six trials reporting this outcome, and the only trial to assess Xpert impact on morbidity reported no impact. Trial characteristics that might explain lack of observed impact on morbidity and mortality include: higher rates of empiric TB treatment in microscopy compared with Xpert arms, enrollment of study populations not comprised exclusively of populations most likely to benefit from Xpert, and health system weaknesses. So far as equipoise exists, future trials that address past limitations are needed to inform Xpert use in resource-limited settings.

Keywords: Clinical trials, Health system weaknesses, Impact, Limitations, Study design, Xpert MTB/RIF

Introduction

In 2009, the commercial release of the Xpert MTB/RIF assay for the GeneXpert platform (Xpert) represented an important breakthrough in the fight against TB. With features including sensitivity to diagnose culture-positive TB from sputum samples among persons living with HIV (PLHIV) of about 79%,[1] significantly superior to smear microscopy (45%),[2] ability to detect rifampicin resistance-conferring mutations, capacity to provide results from sputum within 100 minutes, robustness under varying temperature and humidity conditions, and minimal training requirements, Xpert has advanced TB diagnostic capability for clinicians managing presumptive TB patients in resource-limited settings, especially those with suspected HIV-associated TB and persons with suspected drug-resistant TB.[3]

With ample evidence that undiagnosed TB or TB diagnosed late in the course of disease is an important cause of death among persons with HIV,[4,5] there was optimism that rapid scale-up of Xpert in settings with high HIV prevalence, as recommended by WHO,[6] would significantly impact key patient outcomes like morbidity and all-cause mortality.[7] For example, modelling studies predicted that, compared with the status quo (smear-microscopy), Xpert would avert >100 000 deaths in five sub-Saharan African countries over 10 years.[7] In line with WHO recommendations and published expert opinion,[6,8] several trials set out to evaluate Xpert impact on patient outcomes, including morbidity and mortality.[9–16] Despite optimism, however, published trials of Xpert impact have not yet observed morbidity and mortality reductions. We conducted a narrative review of published Xpert impact trials to answer two questions: what impact has Xpert had on patient-relevant outcomes, and why have Xpert impact trials not demonstrated morbidity and mortality reductions?

© Published by Oxford University Press on behalf of Royal Society of Tropical Medicine and Hygiene 2016. This work is written by (a) US Government employee(s) and is in the public domain in the US.

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

Materials and methods

Search strategy

We conducted a narrative literature review according to published guidelines.[17] We searched PubMed, Google Scholar, the Cochrane Library and Embase from 1 January 2005 to 31 December 2015 for reports published in English with the terms 'Xpert MTB/RIF assay' or 'GeneXpert' or 'Xpert' and 'impact' or 'trial' or 'clinical trial'.

Study selection

Studies that met the following criteria were included: 1. the study was a clinical trial, as defined by the International Committee of Medical Journals (ICMJ) (i.e., 'any research study that prospectively assigned human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes')[18]; 2. the study included Xpert in one of the intervention arms or phases; and 3. a stated primary or secondary aim of the study was to assess Xpert impact on at least one patient-related outcome.

Studies were excluded if there was no direct comparison between patients receiving standard of care (sputum microscopy) and patients receiving an intervention including Xpert; therefore, so-called 'hypothetical trials', where patients received both microcopy and Xpert and investigators hypothesized the impact of Xpert in a scenario where only microscopy was available, were excluded. In addition, studies in which outcomes of patients receiving Xpert were compared with historical national average outcomes when microscopy was standard of care, were excluded.[8] Pre-post trials at the same health facilities, which compared patient outcomes between pre-Xpert microscopy phases and post-Xpert rollout phases, were included in the review, as these trials meet the ICMJ definition of a clinical trial.[18,19]

The titles and abstracts of studies identified in the search were retrieved and assessed by one reviewer who excluded those that were clearly not relevant. The full texts of remaining studies were assessed for inclusion by four reviewers, using the inclusion and exclusion criteria described above.

Data extraction

Data were extracted directly into a spreadsheet that included the following variables: first and second authors, publication year, abbreviated study name, setting, design, randomization level, sample size, study population inclusion criteria, standard of care, intervention and role of Xpert MTB/RIF in the intervention, key questions related to Xpert MTB/RIF impact on patient outcomes, and key results including diagnostic yield, time to TB diagnosis, time to TB treatment, TB treatment initiation rates, empiric TB treatment initiation rates, loss to follow-up (LTFU) before TB treatment, TB treatment outcomes, overall treatment outcomes among all patients enrolled, and predictors of mortality. In addition, trial limitations as they relate to trial design, conduct, or health system weaknesses were either abstracted or postulated based on published data.

Results

Characteristics of studies included

Eight clinical trials, reported in 11 publications, were included (Table 1). Of the eight trials, six were from sub-Saharan Africa, one from Brazil, and one from Indonesia. All eight trials were considered pragmatic (i.e., conducted in routine healthcare settings, with the potential for existing programmatic weaknesses to impact trial outcomes). Six of the eight trials included a randomization component, while two were pre-post trials.[13,16] Of the six randomized trials, all had two arms or phases, three were individually randomized,[9,14,15] and three were cluster-randomized.[10–12] Of the three cluster-randomized trials (CRT), one was a parallel group trial,[10] one a stepped-wedge trial,[11] and one a time-randomized trial at a single clinic,[12] where patients were randomized to receive microscopy or Xpert depending on which week they attended the clinic. For the three individually randomized trials, sample sizes were 242, 424, and 1502 patients. For the three CRTs, the number of clusters were: 51 in the time-randomized trial with 1985 patients enrolled, 14 in the stepped-wedge CRT with 24 227 patients enrolled, and 20 in the parallel group CRT with 4656 patients enrolled.

Study populations varied across the eight trials; six enrolled persons being evaluated for TB,[9–13,15] referred to as presumptive TB patients in this review, one enrolled HIV-positive patients starting antiretroviral therapy (ART) regardless of TB symptoms,[14] and one enrolled presumptive TB patients considered to be at risk for multi-drug resistant (MDR) TB.[16] Six of eight trials were focused on assessing Xpert impact among adults (≥18 years at enrollment),[9,10,12–15] while two included both adults and children.[11,16] In the control arms, sputum smear microscopy was used in six trials,[9–12,14,16] tracheal aspirate smear microscopy in one trial,[15] and sputum fluorescent smear microscopy in one trial.[13]

Xpert impact on TB diagnostic cascade

All eight trials reported diagnostic yield of bacteriologically-confirmed TB (i.e., the percentage of study enrollees providing sputum samples who tested positive for TB via either microscopy or Xpert). In six of eight trials, Xpert achieved higher diagnostic yield than microscopy (Table 2); in these six trials, compared with microscopy, Xpert increased TB diagnostic yield by a factor of about 1.6,[9] 1.2,[10] 1.5,[11] 1.5,[12] 3.0,[15] and 1.2,[16] respectively.

In the four trials that reported time from sample collection to result availability among drug-sensitive TB positive cases, Xpert reduced this time in three trials[9,13,15] (Table 2). In these three trials,[9,13,15] Xpert and microscopy tests were performed on-site (i.e., at the point-of-care), whereas in the trial that showed no difference in time to result availability, both Xpert and microscopy tests were performed off-site at a separate laboratory.[14]

Six of eight trials reported median time from enrollment or sputum collection to standard TB treatment initiation among all patients who started TB treatment, regardless of reason for starting TB treatment; in four of six trials there was either strong evidence the median time to treatment was shorter[9,11,12] or

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

Table 1. Study designs of clinical trials with primary or secondary aims to estimate impact of Xpert on patient outcomes

Study name,[a] Ref no.	Setting	Design	Randomization level	Sample Size	Study population inclusion criteria (main criteria)	Standard of care	Intervention	Patient outcome questions addressed
TB-NEAT[9]	South Africa, Zimbabwe, Zambia and Tanzania	Pragmatic, randomized, two-arm parallel-group, multicenter trial.	Individual	1502 patients with presumptive TB.	Patients: ≥18 years old; presenting to primary care TB clinics; ≥1 TB symptom as defined by WHO (i.e., presumptive TB patient); spontaneous sputum expectoration possible; no prior TB treatment in last 60 days.	Same-day, onsite sputum smear microscopy by laboratory technician (one spot sputum/ patient). One spot for culture.[b]	Nurse-performed point-of-care Xpert at the clinic (one spot sputum per patient). Reference standard was liquid culture.[b]	Primary: TB-related morbidity (measured with the TB score and the Karnofsky performance score (KPS) among culture-positive patients who had begun anti-TB treatment. Other: diagnostic yield, time from sample collection to TB treatment, TB treatment initiation rates, empiric TB treatment, LTFU before TB treatment, TB treatment outcomes, and mortality.
XTEND[10,20,29]	South Africa	Pragmatic, two-arm, parallel, cluster-randomized trial.	Cluster (a TB lab with 2 clinics per lab)	20 labs, 2 clinics per lab, and 4656 patients with presumptive TB.	Laboratories and their clinics: not part of other Xpert evaluations; did not already have GeneXpert; complied with current SOC TB diagnostics; not likely to be closed.Patients: ≥18 years old; not on TB treatment; had been asked to and were able to provide a sputum specimen (i.e., presumptive TB patient); local resident.	Sputum smear microscopy by laboratory technician (two spot sputa/ patient).	Xpert performed at the laboratory by technicians (one spot sputum/ patient).	Primary: Mortality at 6 months from enrollment.Other: diagnostic yield, time from sample collection to TB treatment, TB treatment initiation rates, empiric TB treatment, LTFU before TB treatment.
Brazil stepped wedge[11,21]	Brazil	Stepped-wedge cluster-	Cluster (primary care lab)	14 labs, 24 227 presumptive TB patients among	Primary care labs: all 11 labs in one city (Rio de Janeiro), and 3 labs in	Sputum smear microscopy by laboratory	Xpert performed at the laboratory by technicians (one	Primary: Laboratory-confirmed TB case notification rate; time

Continued

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

Table 1. *Continued*

Study name,[a] Ref no.	Setting	Design	Randomization level	Sample Size	Study population inclusion criteria (main criteria)	Standard of care	Intervention	Patient outcome questions addressed
		randomized trial		whom 4640 patients started TB treatment.	Manaus, purposefully selected with criteria not specified. Patients: All patients who provided sputa for TB diagnostic work up were eligible (i.e., presumptive TB patients).	technician (one or two spot sputa/patient).	spot sputum/ patient).	from sample collection to TB treatment initiationOther: TB treatment initiation rates, empiric TB treatment, TB treatment outcomes.
Zimbabwe RCT[14]	Zimbabwe	Pragmatic, randomized, two-arm parallel-group, trial.	Individual	424 patients starting ART.	Patients: Symptomatic and asymptomatic HIV-infected patients initiating ART; ≥18 years old; no prior ART; not receiving TB treatment; produced at least 1 sputum sample (spontaneous or with induction)	Sputum smear microscopy (two spot sputa/patient).	Xpert performed at the laboratory by technicians (two spot sputa/patient).	Primary: % of patients who died or developed incident TB (composite outcome) during ART within 3 months of randomization. Other: diagnostic yield, time from sample collection to TB treatment, TB treatment initiation rates, empiric TB treatment, TB incidence, LTFU after ART start.
South Africa single clinic CRT[12]	Khayelitsha, South Africa	Single clinic, pragmatic, two-phase, crossover, cluster-randomized trial.	Cluster (one primary healthcare clinic randomized on weekly basis to each arm)	51 weeks randomized; 1985 presumptive TB patients randomized among whom 492 started TB treatment.	Cluster: Purposefully chosen clinic. Patients: Presumptive TB patients; ≥18 years old; not receiving TB treatment for 3 days or more; all presumptive TB patients included in the intention to treat (ITT) while the per protocol analysis excluded 40 of 1985 patients unable to produce sputa.	On site lab sputum smear microscopy (two spot sputa/patient).	On site Xpert (one spot sputum/patient).	Primary outcome: % of bacteriologically-confirmed TB cases not starting TB treatment within 3 months of randomization. Secondary outcomes: diagnostic yield, time from sample collection to TB treatment, TB treatment initiation rates, empiric TB treatment, TB treatment outcomes,

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

								and 6-month mortality.
Uganda pre-post trial[13]	Kampala, Uganda	Single clinic, prospective pre-post study.	Not randomized	477 hospitalized presumptive TB patients among whom 252 started TB treatment.	Patients: ≥18 years old; presumptive TB patient; not receiving TB treatment; patients with insufficient or absent sputa were excluded from analysis (29 of 525 initial enrollees excluded for this reason); patients who died within 3 days of hospital admission, excluded from analysis.	On site lab fluorescent smear microscopy (two spot and one morning sputum/patient). Remainder for culture.[b]	On site Xpert (one spot sputum/patient). One spot and the morning sputum sent for culture.[b]	Primary outcome: Not specified. Other: time from sample collection to TB treatment, TB treatment initiation rates, empiric TB treatment, TB treatment outcomes, LTFU after hospital admission, and 2-month mortality.
South Africa ICU RCT[15]	South Africa, Cape Town	Prospective cohort at 4 ICUs with nested individual RCT sub-study.	Individual	341 ICU patients with presumptive TB, of whom 242 randomized.	Patients: ≥18 years old; presumptive TB patient; mechanically ventilated; tracheal aspirate obtained for all enrollees.RCT sub-study: Enrolled during 2010–12 before Xpert became SOC.	1.5–7.5 mL of tracheal secretions sent for blinded smear microscopy.	1.5–7.5 mL of tracheal secretions sent for blinded Xpert.	Primary: % of culture-positive TB patients started on TB treatment at 48 h after enrolment. Other: Diagnostic yield, time from sample collection to TB treatment, TB treatment initiation rates, empiric TB treatment, and mortality at various time points after randomization.
Indonesia pre-post trial[16]	Java, Indonesia	Pre-post trial at three provincial public hospitals in Indonesia.	Not randomized	975 patients at risk of drug-resistant TB pre-Xpert and 1442 post-Xpert	Patients: Any age, at risk of MDR-TB, according to Indonesian guidelines.	1 sputum for microscopy and 1 for culture. If positive culture, first-line DST.	1 sputum sample sent for Xpert, one sputum sample for culture. If positive culture, first-line DST.	Primary: TB case detection rates (diagnostic yield), RR TB detection rates among TB cases (RR TB diagnostic yield), RR TB treatment initiation rates, and time to RR TB treatment initiation.

ART: antiretroviral therapy; DST: drug susceptibility testing; ICU: intensive care unit; LTFU: loss to follow-up; MTB: *Mycobacterium tuberculosis*; RCT: randomized clinical trial; Xpert: Xpert MTB/RIF; RR: rifampicin resistant; SOC: standard of care.

[a]If the trial did not have an official name, it is referred to by a combination of the country it was conducted in and the trial design.

[b]Diagnostic algorithms varied across studies. Only in two studies, marked with [b] was a specimen supposed to be sent for culture for all enrollees. Chest x-ray was a diagnostic tool available to clinicians if deemed necessary, in all studies.

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

Table 2. Patient outcomes related to the tuberculosis diagnostic cascade from clinical trials of Xpert impact

Study name, Ref no.	Population	Follow-up time (days)	Diagnostic yield (% of study enrollees with either microscopy or Xpert-confirmed TB)[a]			Time from sample collection/enrollment to result (days)			Time from sample collection/ enrollment to TB treatment initiation for any reason (days)			Reported TB treatment initiation risk during follow-up			Empiric TB treatment rates (% of enrollees treated for TB by study end without bacteriologic confirmation)		
			Micro %	Xpert %	p	Micro	Xpert	p	Micro	Xpert	p	Micro %	Xpert %	P	Micro %	Xpert %	p
TB-NEAT[9]	Presumptive TB patients (outpatients)	56	15	24	<0.0001	0 (0–6)	0 (0–0)	0.005	1 (0–4)	0 (0–3)	0.0004	42	43	NS	26	17	0.0001
XTEND[10,20,29]	Presumptive TB patients (outpatients)	182	7.8	9.2	0.05	NA	NA	NA	NA[b]	NA[b]	NA[b]	12.5	10.8	NS	4.4[c]	2.3[c]	NA[c]
Brazil Stepped Wedge[11,21]	Presumptive TB patients (outpatients)	NA	9.7	14.2	<0.001	NA	NA	NA	11.4	8.1	0.040	17.5[d]	20.8[d]	NA[d]	10.4[e]	9.8[e]	NA[e]
Zimbabwe RCT[14]	ART enrollees (outpatients)	182[f]	7	9	NS	6	2	NS	8	5	NS	21	20	NS	15[g]	11[g]	NA[g]
South Africa single clinic CRT[12]	Presumptive TB patients (outpatients)	182	17[h]	26	<0.001	NA	NA	NA	8	4	0.013	23[i]	28[i]	0.013	9.8[i]	5.2[i]	0.0025
Uganda Pre-post trial[13]	Presumptive TB patients (hospitalized)	60	37	43	NS	1	0	<0.001	1	0	0.06	81	85	NS	15	7	0.047
South Africa ICU RCT[15]	Presumptive TB patients (hospitalized – admitted to ICU)	90	6	18	0.012	12.1	0.2	0.0004	0.7	0.3	NS	14	22	NS	7.8[j]	3.6[j]	NA[j]
Indonesia Pre-post trial[16]	Patients with presumptive drug-resistant TB (outpatients)	NA	65.2[k]	80.2[k]	<0.001[k]	75.0[l]	1.0[l]	0.001[l]	88.0[m]	16.0[m]	<0.001[m]	39.3[n]	58.5 [n]	<0.001[n]	NA	NA	NA

ART: antiretroviral therapy; CRT: cluster randomized trial; ICU: intensive care unit; MDR: multidrug resistant; Micro: microscopy; NA: not available; NS: not significant; RCT: randomized controlled trial; RR: rifampicin resistant.
[a]Following study enrollment, sputum samples were obtained from all study enrollees, except in the Zimbabwe RCT where sputa were obtained only from symptomatic ART enrollees. Diagnostic yield represents yield of bacteriologically-confirmed TB from these sputum samples collected soon after study enrollment.
[b]In XTEND, authors reported median time from enrollment to TB treatment only for those who were bacteriologically confirmed as having TB (10 days in microscopy arm vs 7 days in Xpert arm). No p-value was provided but the text suggests the difference was not statistically significant.
[c]Calculated from published data: 102 (4.4%) of 2332 presumptive TB patients in the microscopy arm and 54 (2.3%) of 2324 presumptive TB patients in the Xpert arm started empiric TB treatment. No statistical test was published. However, among TB patients, the percentage with microbiological confirmation was higher in the Xpert than microscopy arms (78.4% vs. 65.0%, p = 0.07).
[d]Calculated from published data: 2050 (17.5%) of 11 705 presumptive TB patients in the microscopy phase and 2610 (20.8%) of 12 522 presumptive TB patients in the Xpert phase started TB treatment. No published statistical test.
[e]Calculated from published data: 906 (7.7%) of 11 705 presumptive TB patients in the microscopy phase and 1 009 (8.1%) of 12 522 presumptive TB patients in the Xpert phase started empiric TB treatment without microbiology results. 313 (2.7%) of 11 705 presumptive TB patients in the microscopy phase and 216 (1.7%) of 12 522 presumptive TB patients in the Xpert phase started empiric TB treatment with negative microbiological results. No published statistical test.
[f]Although follow-up was for 6 months (182 days) after ART initiation, data presented in this table represent diagnostic yield, time-to-diagnosis, time-to-TB-treatment, TB treatment initiation rates and empiric TB treatment rates in the time from study enrollment to ART initiation.
[g]Calculated from published data: among all ART enrollees, the percentage given empiric TB treatment was similar between the microscopy arm (31 (15%) of 210) and Xpert arm (23 (11%) of 214). No published statistical test. However, among TB treatment patients, the % treated on empiric grounds was high in both Xpert (54%) and microscopy (69%) arms, p = 0.12.
[h]In the microscopy arm, limited use of culture contributed to diagnostic yield estimates as some smear-negative patients had positive culture.
[i]Although follow-up was 182 days, TB incidence risk reported in this analysis was over 3 months (90 days).
[j]Calculated from published data: 9 (7.8%) 115 ICU presumptive TB patients in the microscopy arm and 4 (3.6%) of 111 ICU presumptive TB patients in the Xpert arm were prescribed empiric TB treatment. No statistical test published. However, among patients started on TB treatment, empiric TB treatment was higher in the microscopy than Xpert arms (56% vs. 17%, p = 0.015).
[k]Among patients at risk for MDR-TB, diagnostic yield of TB increased from 65.2% pre-intervention to 80.2% in the Xpert phase (p < 0.001).
[l]Time from registration to release of RR TB result declined from 75.0 days to 1.0 days after Xpert implementation, p < 0.001.
[m]Time from registration to initiation of treatment for RR-TB decreased from a median of 88.0 days to 16.0 days, p < 0.001).
[n]The percentage that were considered to have RR TB who started second-line TB treatment increased from 39.3% in the baseline phase to 58.5% in the Xpert phase (p < 0.001).

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

weak evidence it was shorter[13] in the Xpert than microscopy arms (Table 2). In these four trials, Xpert reduced median time to TB treatment by about 1 day,[9] 3.3 days,[11] 4 days[12] and 1 day,[13] respectively.

In the one trial comparing time to diagnosis of rifampicin resistant TB and time to second-line TB treatment between Xpert and culture arms, Xpert reduced time to diagnosis from 75 days to 1 day, and time to second-line TB treatment from 88 days to 16 days (Table 2).[16] Xpert was located on-site in the post-Xpert phase, whereas in the pre-Xpert phase, all culture and drug susceptibility testing occurred at an off-site laboratory.

In the seven trials reporting percentages of enrollees initiating drug-sensitive TB treatment by study end,[9–15] TB treatment initiation rates were only significantly higher in the Xpert arm in one trial.[12] Across the seven trials, among presumptive TB patients, TB treatment initiation rates ranged from 12.5 to 81% in the microscopy arms and from 10.8 to 85% in the Xpert arms (Table 2).

In the one trial reporting the percentage of enrollees initiating second-line TB treatment among presumptive MDR TB patients, the percentage starting second line increased from 39.3% in the microscopy and culture phase to 58.5% in the Xpert phase (Table 2).[16]

Among seven trials reporting the percentage of enrollees receiving empiric TB treatment (i.e., TB treatment based on clinical picture or chest x-ray) by study end, five reported higher percentages of enrollees receiving empiric TB treatment in the microscopy than the Xpert arms; in these five trials, Xpert reduced the percentage of enrollees receiving empiric TB treatment by about 35%,[9] 48%,[10] 47%,[12] 53%,[13] and 54%,[15] respectively (Table 2).

Xpert impact on patient outcomes

In the two trials reporting the percentage of bacteriologically-confirmed TB patients LTFU before TB treatment start,[9,10] one trial reported lower LTFU in the Xpert arm (15 vs 8%, p=0.03)[9] (Table 3). In the one trial reporting the percentage of rifampicin resistant TB patients LTFU before second-line TB treatment, the percentage LTFU before second-line treatment initiation declined from 52.4 to 31.0% after Xpert rollout (p<0.001).[16]

Only one trial compared TB treatment morbidity outcomes, as measured by TB scores and Karnofsky Performance Scores after TB treatment initiation[9]; in this trial morbidity scores were similar between arms (Table 3).

Of eight trials, five reported incidence of unfavorable outcomes following TB treatment initiation (i.e., LTFU, death, TB-attributable death, or some combination of these outcomes).[9,12,13,20,21] Across the five trials the percentage with unfavorable TB treatment outcomes was similar between microscopy and Xpert arms (Table 3). In the one trial that compared incidence of TB-attributable death following TB treatment initiation between microscopy and Xpert arms, TB-attributable deaths were reported to be significantly lower in the Xpert than microscopy phase (2.3 vs 3.8%),[21] but there was considerable LTFU (15.9% in Xpert phase and 16.2% in microscopy phase), limiting ability to interpret this finding (Table 3).

In all six trials that compared all-cause mortality between microscopy and Xpert arms,[9,10,12–15] no difference in all-cause mortality was observed at any time point after enrollment (Table 3). Two trials compared risk of LTFU between Xpert and microscopy arms[13,14] and in one trial LTFU incidence was higher in the microscopy (10%) than Xpert (2%) arms (p<0.001),[13] but sensitivity analysis suggested this did not affect the conclusion of no mortality difference between arms (Table 3).

Predictors of outcomes

Five trials reported multivariable models describing predictors of mortality among trial enrollees. In the four trials that enrolled presumptive TB patients, being HIV-positive vs HIV-negative (two trials), being HIV-positive and not on ART vs HIV-negative (one trial), being HIV-positive with ART status unknown vs being HIV-positive and not on ART (one trial), and not knowing HIV status vs being HIV-negative (two trials), were factors predictive of mortality. In the fourth trial reporting a multivariable model, which enrolled only HIV-positive patients starting ART, CD4 count <100 cells/μL vs ≥100 cells/μL was predictive of mortality.

Discussion

Across the eight trials reviewed, Xpert generally had a beneficial impact early in the TB diagnosis and treatment cascade: six of eight trials reported improvements in yield of bacteriologically-confirmed drug-sensitive TB among patients who provided sputa, three of four trials reported reduced time to drug-sensitive TB-diagnosis, four of six trials reported reduced time to drug-sensitive TB treatment, and five of seven trials reported reduced rates of empiric drug-sensitive TB treatment in the Xpert compared with the microscopy phase or arm. In addition, in the one trial examining impact of Xpert on drug-resistant TB treatment outcomes compared to culture, Xpert achieved remarkable reductions in time from sputum collection to rifampicin resistant TB detection, reductions in time to second-line TB treatment, and reductions in apparent LTFU before second-line TB treatment. However, Xpert had less impact in later stages of the TB diagnosis and treatment cascade; rates of TB treatment initiation were similar between microscopy and Xpert arms in six of seven trials, TB treatment outcomes were similar between arms in all five trials reporting this outcome, and mortality was similar between arms in all six trials reporting this outcome. There are several possible reasons related to trial design, trial conduct, and prevalent health system weaknesses that might help explain why improvements in outcomes early in the diagnostic cascade did not translate into observed improvement in final patient outcomes (Table 4).

Trial design

Higher rates of empiric TB treatment in the microscopy arms

Despite improvements in diagnostic yield of bacteriologically-confirmed TB in Xpert arms in most trials, higher incidence of empiric TB treatment in the microscopy arms meant that likelihood of TB treatment by study end was similar between microscopy and Xpert arms in most trials, with empiric TB treatment of culture-positive smear-negative TB patients in the microscopy arms largely removing any potential for observed Xpert impact

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

Table 3. Treatment outcomes assessed in clinical trials designed to estimate Xpert impact on patient outcomes

Study	LTFU before TB treatment among microbiologically-confirmed TB patients			TB treatment outcomes				LTFU and mortality among all study enrollees				Mortality predictors
	Micro %	Xpert %	p	Outcome	Micro %	Xpert %	p	Outcome	Micro %	Xpert %	p	
TB-NEAT[9]	15	8	0.03	LTFU 2 m TB score[a] 2 m KPS[a]	32 2 80	29 2 90	NS NS NS	3 m Mortality	8	8	NS	Multivariable analysis: – HIV-positive vs HIV-negative – Lower baseline TB score.
XTEND[10,20,29]	14.9[b]	17[b]	NS	Composite[c]	12.5	11.7	NS	6 m Mortality	5.0	3.9	NS	Multivariable analysis: – Known HIV-positive and not on ART vs HIV-negative – Not knowing HIV status vs HIV-negative – BMI <18.5 vs 18.5–24.9 – Age <30 vs ≥50 years – Higher number of TB symptoms
Brazil stepped wedge[11,21]	NA	NA	NA	Composite[d] LTFU TB-mortality	31.7 16.2 3.8	29.6 15.9 2.3	NS[e] NS[f] SS[g]	NA	NA	NA	NA	Multivariable analysis (predictors of unfavorable TB treatment outcome): – Male sex – HIV positive vs HIV-negative – HIV unknown vs, HIV-negative – Rio vs Manaus
Zimbabwe RCT[14]	NA	NA	NA	NA	NA	NA	NA	6 m Mortality 6 m LTFU 6 m TB incidence 6 m Death or TB	10 18 4 12	6 15 3 9	NS NS NS NS	Multivariable analysis: – Male sex – Low CD4 count (<100) vs >100 – TB diagnosed at enrollment before ART start.
South Africa single clinic CRT[12]	NA	NA	NA	Composite[h]	12.5	12.7	NS	6 m Mortality	3.8	3.4	NS	NA
Uganda pre-post Trial[13]	NA	NA	NA	2 m Mortality	17	14	NS	2 m Mortality 2 m LTFU	17 10	17 2	NS <0.001	NA
South Africa ICU RCT[15]	NA	NA	NA	NA	NA	NA	NA	1 m Mortality 3 m Mortality	34 42	27 32	NS NS	Mortality predictors among all ICU enrollees (n=341), not just those randomized: – Age 24–39 vs <24 years – HIV-positive and ART unknown vs HIV-positive not on ART. – Inotrope use – APACHE-II score >25 vs <20.
Indonesia Pre-post trial[16]	52.4[i]	31.0[i]	<0.001[i]	NA	NA	NA	NA	NA	NA	NA	NA	NA

ART: antiretroviral treatment; BMI: body mass index; ICU: intensive care unit; KPS: Karnofsky Performance Score; LTFU: loss to follow-up; m: month; NA: not available; NS: not significant; RR: rifampicin resistant; SS: statistically significant on the basis of an odds ratio with 95 CI excluding 1 (p-value not provided).
[a]Both median TB score (2 vs 2, p = 0.85), and median KPS (80 vs 90, p = 0.23) in culture-positive patients, who had started TB treatment, did not differ at 2 months post randomization, or at 6 months.
[b]In XTEND, the percentages reported here represent those not starting TB treatment by 28 days after bacteriological TB confirmation.
[c]Composite poor outcome was death, LTFU, and treatment failure.
[d]Composite poor outcome was incidence of LTFU, TB-attributable death, other deaths, change of diagnosis, transfer out, or resistance.
[e]p-value was not provided. Instead, the 95% CI was provided and included 1: 29.6 vs 31.7, OR = 0.93; 95 CI = 0.79–1.08.
[f]p-value was not provided. In the text, the paper states 'loss to follow-up was not changed by the intervention (16.2 vs 15.9)'.
[g]No p-value was provided. The text states that 'Adjusted for HIV status, age group and city, the intervention resulted in a 35 decrease in TB-attributed deaths (OR = 0.65, 95CI = 0.44-0.97)'.
[h]Composite poor outcome was LTFU, death, or TB treatment failure.
[i]The percentage of RR TB patients with missing information on RR treatment initiation declined from 52.4 in the baseline phase to 31.0 in the Xpert phase, p < 0.001. No differences in the percentage of enrollees documented to be LTFU before RR treatment (0.9 pre- vs 2.3 post-Xpert, p = 0.30), or documented to be dead before RR treatment (2.4 pre- vs 1.0 post-Xpert, p = 0.50) were noted.

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

Table 4. Limitations of clinical trials designed to estimate Xpert impact on patient outcomes

Study, Ref no.	Design							Conduct	Health system weaknesses				
	Study population not exclusively a WHO priority population (reason)		Restricted to outpatients	Not a randomized trial	No blinding to TB diagnostic used	Higher rates of empiric TB treatment in microscopy arm	Not powered to detect a morbidity or mortality difference	Anticipated morbidity or mortality difference possibly too large (i.e., possibly underpowered)	LTFU of enrollees restricted key outcome ascertainment	% of study enrollees not knowing their HIV status	% of HIV-positive enrollees on ART (ART coverage)	High LTFU of microbiologically-confirmed TB patients before TB treatment[j]	High LTFU of TB patients during TB treatment
TB-NEAT[9]	L	(40% HIV-negative)	L	NL	L	L	NL	L[a]	L[b]	<1[i]	26	L	L
XTEND[10,29]	L	(50–55% HIV-negative)	L	NL	L	L	NL	L[a]	NL[c]	21-27	33	L	NA
Brazil stepped wedge[11,21]	L	(90–92% HIV-negative)	L	NL	L	NL	L	N/A	L[d]	>50	NA	NA	L
Zimbabwe RCT[14]	NL	(100% HIV-positive)	L	NL	L	NL	NL	L[a]	L[e]	0	100	NA	NA
South Africa CRT[12]	L	(40–41% HIV-negative)	L	NL	L	L	L	N/A	NA	18	NA	NA	L
Uganda pre-post[13]	L	(24% HIV-negative)	NL	L	L	L	L	N/A	L[f]	0	NA	NA	L
South Africa ICU trial[15]	L	(70% HIV-negative)	NL	NL	L	L	L	N/A	NL[g]	15	31	NA	NA
Pre-post trial, Indonesia[16]	NL	(100% DR TB suspects)	L	L	L	NL	L	N/A	L[h]	NA	NA	L	NA

CRT: cluster-randomized trials; DR: drug resistant; HTC: HIV testing and counselling; ICU: intensive care unit; L: stated study limitation applies to this study; LTFU: loss to follow-up; NA: not available; N/A: not applicable; NL: study limitation does not apply (see corresponding sub-headings in the Discussion section, i.e., Trial design, Trial conduct, and Health system weaknesses for full discussion of limitations noted in this table); RCT: randomized controlled trial; SOC: standard of care.
[a]See Discussion section under Trial design sub-heading for explanation.
[b]20% LTFU of culture-confirmed TB cases.
[c]Although LTFU before TB treatment was 16% among microbiologically confirmed TB patients, investigators ascertained vital status of nearly all study enrollees by study end.
[d]High incidence of LTFU (about 16% in both SOC and intervention phase).
[e]16.5% (70/424) of ART enrollees LTFU before 6 months.
[f]6.7% of study enrollees (32/477) LTFU before study end.
[g]Less than 4% were LTFU at 90 days.
[h]Missing data on 2nd line treatment initiation was very high both pre-Xpert (52.4%) and post-Xpert (31.0%). Based on available data, missing data on TB treatment initiation seems equivalent to LTFU before second-line TB treatment initiation. Overall missing data (probable LTFU) before second-line TB treatment was 42% (267/634) among rifampicin resistant cases.
[i]Study enrollees were offered HIV testing and counseling at study enrollment.
[j]Data points are presented in Table 3.

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

(Table 4).[22,23] For example, in the TB-NEAT study,[9] of the 68% of patients with smear-negative tuberculosis in the microscopy arm, that were later correctly detected by Xpert, 93% were treated empirically anyway (Table 4). In these trials, an important driver of empiric TB treatment in the microscopy compared with the Xpert arms may have been that clinicians administering the study were not blinded to the diagnostic used and were aware of the study hypothesis. Therefore, in all study settings, clinicians would have known firstly that there was a relatively high pre-test probability of true TB among all patients enrolled, and secondly that the predictive value of a negative test was lower in the microscopy arm than the Xpert arm, resulting in higher empiric TB treatment in the microscopy arm.[22]

Study populations not exclusively focused on priority populations

Of the eight trials, six enrolled presumptive TB patients, one HIV-positive adults starting ART and one patients with presumptive drug-resistant TB. In the six trials enrolling presumptive TB patients, HIV prevalence ranged from 8% to 76% (Table 4). The main advantage of Xpert over smear-microscopy in diagnosing drug-sensitive TB is ability to diagnose culture-positive smear-negative TB, which is more common among PLHIV, especially PLHIV who are significantly immune-compromised,[24,25] since waning immunity is associated with reduced pulmonary immunopathology[25] with liberation of lower concentrations of bacilli into the airways. Therefore, with smear microscopy sensitivity higher among HIV-negative persons (±69%) than among PLHIV (±45%),[2] one would expect Xpert impact on TB diagnostic yield and therefore morbidity and mortality to be higher among exclusively HIV-positive study populations than study populations including HIV-negative persons.[23]

The only study to assess Xpert impact among exclusively HIV-positive persons was by Mupfumi et al. in Zimbabwe. In this study, other limitations (e.g., very high rates of empiric TB treatment in both arms and small sample size [n=424]), might explain lack of observed impact. The high rates of empiric TB treatment observed in the Zimbabwe trial raise the issue that, although Xpert should increase diagnostic yield of bacteriologically confirmed TB to a greater extent in HIV-positive than HIV-negative populations, rates of empiric TB treatment are also likely to be higher in HIV-positive than HIV-negative populations.[23] Consequently, restriction of Xpert impact trials to exclusively HIV-positive outpatient populations might not, by default, increase probability of observing Xpert impact on mortality.[23]

Only one pre-post trial from Indonesia enrolled patients considered at high risk of MDR TB.[16] This trial showed remarkable impact of Xpert in reducing median time to rifampicin resistant TB diagnosis (from 75 to 1 day) and in median time to second-line TB treatment (from 88 to 16 days). In addition, there was a reduction in LTFU of rifampicin resistant TB patients before second-line TB treatment initiation. Although there are limited data on Xpert impact among patients at risk for MDR TB,[26] the reduction in time to diagnosis and appropriate treatment of rifampicin resistant and MDR TB has potential to reduce transmission and mortality from MDR TB and prevent emergence of extensively drug-resistant TB.[7,26,27] However, patterns of empiric initiation of second-line TB treatment among patients at risk of MDR TB also need to be considered when evaluating Xpert impact.[23] It is perhaps surprising that only two of the eight trials examined Xpert impact among exclusive priority populations for Xpert rollout (i.e., HIV-positive patients at high risk for TB and presumptive MDR TB patients).[6]

Most trials evaluated Xpert impact among outpatients

A recent meta-analysis of autopsy studies in resource-limited settings showed that the majority of deaths among HIV-infected inpatients that were due to TB (37%), involved disseminated TB (>85%).[4] Although the autopsy meta-analysis among hospitalized patients is not representative of all deaths among PLHIV, this finding suggests that disseminated TB is a common precursor to death among patients who die from TB.[4] Therefore, restricting trial enrollees to healthier outpatients, who are unlikely to have disseminated TB, in six of eight trials (Table 4), through study exclusion criteria,[9] enrollment at outpatient primary healthcare clinics (PHCs),[10–12] or because study enrollment required sputum production,[9–14] might have excluded many patients likely to benefit from early accurate TB diagnosis with Xpert. In these outpatient study populations, the improvements in time to diagnosis and time to TB treatment through earlier confirmation of bacteriologically-confirmed TB with Xpert, might not have resulted in significant improvements in patient morbidity or mortality outcomes.[23]

In the two trials that did enroll presumptive TB patients admitted to hospital (i.e., very ill patients),[13,15] other study limitations restrict ability to detect Xpert impact on patient outcomes. For example, in the Uganda pre-post trial,[13] sample size was small (n=477), empiric TB treatment was higher in the microscopy phase, a higher proportion of enrollees in the Xpert phase had ≥1 danger sign, Xpert sensitivity was surprisingly low among smear-negative TB patients (42%), and patients who died within three days of admission were excluded from analysis. In the South African study enrolling intensive care unit presumptive TB patients,[15] only 30% of enrollees were HIV-positive, and there were higher rates of empiric TB treatment in the microscopy than Xpert arms.

It should also be noted that, compared with healthier outpatients, sicker patients admitted to hospital with TB symptoms have higher rates of empiric TB treatment.[23,28] Therefore, among sicker, hospitalized patients, empiric TB treatment might again replace any benefit associated with Xpert's improved diagnostic sensitivity.[23] If future trials were to evaluate Xpert impact on mortality among hospitalized HIV-positive patients, recent data suggest that rapid Xpert testing for disseminated TB, especially Xpert testing of urine, in the intervention arm would be important in addition to Xpert testing of sputum, which has become standard of care in many settings.[4,28,29]

Either not powered to detect a morbidity or mortality outcome or under-powered to detect these outcomes

Of the eight trials reviewed, only three had primary study aims of assessing Xpert impact on morbidity or mortality outcomes (Table 4).[9,10,14] TB-NEAT aimed to assess Xpert impact on 2- and 6-month morbidity scores among culture-positive patients who started TB treatment.[9] As described above, the higher incidence of empiric TB treatment in the microcopy arm probably explains lack of observed impact of Xpert on morbidity.[23] However,

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

another possible explanation is that, among relatively healthy outpatients with presumptive TB, a very large sample size would be needed to detect differences in morbidity scores at 2 and 6 months of TB treatment, raising the possibility, in retrospect, that the study may have been under-powered.

The XTEND trial aimed to detect a 50% reduction in 6-month mortality in the Xpert compared with the microscopy arms, based on preliminary data from a pilot study at two PHCs showing a potential 74% reduction in mortality.[10] However, a recent meta-analysis of autopsy-confirmed causes of death among PLHIV suggests that among hospitalized PLHIV, about two-fifths (37%) of deaths were due to TB, and in nearly half of TB deaths (one-fifth of all deaths), TB was undiagnosed antemortem. Although the study population in the autopsy meta-analysis is different to the XTEND study population, the autopsy meta-analysis suggests that a more accurate and rapid TB diagnostic like Xpert, through early, accurate TB diagnosis and subsequent treatment antemortem, might avert about one-fifth (20%) of deaths among persons with advanced HIV. Among mixed HIV-positive and negative populations Xpert impact might be lower. More recent trials of TB diagnostics have assumed 20% reductions in mortality for power calculations.[30] Therefore, XTEND may have over-estimated Xpert impact mortality in the study population.

In power calculations for the randomized trial from Zimbabwe,[14] investigators assumed a 67% reduction in mortality and a 58% reduction in TB incidence giving an overall 61% reduction in mortality or TB in the first six months of ART. Again, the autopsy meta-analysis suggests the study may have over-estimated Xpert impact on all-cause mortality in power calculations.[4]

Trial conduct

All trials were conducted in a programmatic setting where LTFU of enrollees is a problem; however, in five of eight trials LTFU restricted ability to fully interpret key outcomes (Table 4). LTFU among study enrollees can result in non-differential or differential outcome ascertainment error between arms. Non-differential error in outcome ascertainment between arms can result in reduced power to detect true differences in morbidity and mortality, while differential outcome misclassification between arms can bias study outcomes. It is possible, as was done for the XTEND trial, to monitor and report LTFU in both arms, and then through subsequent tracing activities ascertain final vital status. Such tracing activities help to reduce impact of LTFU on ability to interpret study outcomes.[31]

Health system weaknesses

In all the trials reviewed, certain health system weaknesses probably blunted ability to detect Xpert impact on patient outcomes (Table 4). Common health system weaknesses included high prevalence of unknown HIV status at enrollment in four of five trials reporting this variable (≥15%),[10–12,15] and low ART coverage among known HIV-positive persons in all three trials reporting this variable (26–31%).[9,10,15] Notably, sub-optimal HIV management (i.e., unknown HIV status, HIV-positive and not on ART, or HIV-positive with unknown ART status) was predictive of poor final outcomes in four trials.[9,10,15,21] Recent data show that all PLHIV regardless of CD4 count or disease stage should start ART to reduce mortality risk.[32] Therefore, in an HIV-positive person not on ART, earlier TB diagnosis and TB treatment through Xpert rather than microscopy may carry lower health benefit.[23]

Another health system-related weakness was high LTFU before TB treatment in three trials[9,10,16] and high LTFU following TB treatment initiation in four trials.[9,12,13,21] LTFU of patients before they can fully benefit from TB treatment indicated by an accurate TB diagnosis diminishes Xpert impact. In XTEND, secondary analysis of trial data showed low compliance with clinical care algorithms following a negative Xpert or microcopy test (i.e., low adherence to a care plan including chest x-ray, sputum culture, or hospital referral within 2 weeks of a negative test).[33] Improving compliance with the algorithm following a negative Xpert test might increase impact of the whole Xpert algorithm on mortality.[33] Operational research to identify health system strengthening interventions that should be implemented in conjunction with Xpert rollout to maximize Xpert impact could inform best practices for Xpert scale-up.[10]

Conclusions

In conclusion, despite improvements in diagnostic yield among patients who can produce sputa, reductions in time to diagnosis, and reductions in time to TB treatment in Xpert compared with microscopy arms in most trials, Xpert was not shown to impact the all-important patient outcomes of interest (patient morbidity or mortality). Trial characteristics related to trial design, trial conduct, and health system weaknesses might explain lack of observed impact. The higher rates of empiric TB treatment in microcopy compared with Xpert arms was a key feature in most trials that contributed to lack of observed Xpert impact on mortality. This suggests empiric TB treatment remains an important strategy for clinicians, especially where Xpert is not available. So far as equipoise exists, future trials of Xpert impact, that take into account past trial limitations, would be helpful to inform Xpert use in resource-limited settings.

Authors' disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Authors' contributions: SDL and AFA conceived the study. AFA led development of the search criteria, abstraction tools, abstraction exercise, and prepared the first draft of the manuscript. All authors contributed to the literature review, data abstraction, writing the manuscript, and critically revising the manuscript for intellectual content. All authors have read and approved the final manuscript. AFA is the guarantor of the paper.

Funding: This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the U.S. Centers for Disease Control and Prevention.

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

Competing interests: None declared.

Ethical approval: Not required.

References

1 Steingart KR, Schiller I, Horne DJ et al. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014;1:CD009593.

2 Boehme CC, Nicol MP, Nabeta P et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 2011;377:1495–505.

3 Lawn SD, Meintjes G, McIlleron H et al. Management of HIV-associated tuberculosis in resource-limited settings: a state-of-the-art review. BMC Med 2013;11:253.

4 Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS 2015;29:1987–2002.

5 Ansari NA, Kombe AH, Kenyon TA et al. Pathology and causes of death in a group of 128 predominantly HIV-positive patients in Botswana, 1997–1998. Int J Tuberc Lung Dis 2002;6:55–63.

6 WHO. Rapid Implementation of the Xpert MTB/RIF diagnostic test. Geneva: World Health Organization; 2011. http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf [accessed 9 March 2016].

7 Menzies NA, Cohen T, Lin HH et al. Population health impact and cost-effectiveness of tuberculosis diagnosis with Xpert MTB/RIF: a dynamic simulation and economic evaluation. PLoS Med 2012;9:e1001347.

8 Lessells RJ, Cooke GS, Newell ML, Godfrey-Faussett P. Evaluation of tuberculosis diagnostics: establishing an evidence base around the public health impact. J Infect Dis 2011;204(Suppl 4):S1187–95.

9 Theron G, Zijenah L, Chanda D et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet 2014;383:424–35.

10 Churchyard GJ, Stevens WS, Mametja LD et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. Lancet Global health 2015;3:e450–7.

11 Durovni B, Saraceni V, van den Hof S et al. Impact of replacing smear microscopy with Xpert MTB/RIF for diagnosing tuberculosis in Brazil: a stepped-wedge cluster-randomized trial. PLoS Med 2014;11:e1001766.

12 Cox HS, Mbhele S, Mohess N et al. Impact of Xpert MTB/RIF for TB diagnosis in a primary care clinic with high TB and HIV prevalence in South Africa: a pragmatic randomised trial. PLoS Med 2014;11:e1001760.

13 Yoon C, Cattamanchi A, Davis JL et al. Impact of Xpert MTB/RIF testing on tuberculosis management and outcomes in hospitalized patients in Uganda. PLoS One 2012;7:e48599.

14 Mupfumi L, Makamure B, Chirehwa M et al. Impact of Xpert MTB/RIF on antiretroviral therapy-associated tuberculosis and mortality: a pragmatic randomized controlled trial. Open Forum Infect Dis 2014;1:Ofu038.

15 Calligaro GL, Theron G, Khalfey H et al. Burden of tuberculosis in intensive care units in Cape Town, South Africa, and assessment of the accuracy and effect on patient outcomes of the Xpert MTB/RIF test on tracheal aspirate samples for diagnosis of pulmonary tuberculosis: a prospective burden of disease study with a nested randomised controlled trial. Lancet Respir Med 2015;3:621–30.

16 van Kampen SC, Susanto NH, Simon S et al. Effects of introducing Xpert MTB/RIF on diagnosis and treatment of drug-resistant tuberculosis patients in Indonesia: a pre-post intervention study. PLoS One 2015;10:e0123536.

17 Green BN, Johnson CD, Adams A. Writing narrative literature reviews for peer-reviewed journals: secrets of the trade. J Chiropr Med 2006;5:101–17.

18 International Committee of Medical Journals (ICMJ). Clinical Trials Registration. http://www.icmje.org/about-icmje/faqs/clinical-trials-registration/ [accessed 9 March 2016].

19 National Institutes of Health (NIH). NIH Definition of Clinical Trial Decision Tree. https://auth.osp.od.nih.gov/sites/default/files/NIH%20Definition%20of%20%20Clinical%20Trial%20Decision%20Tree-%20UPDATED.pdf [accessed 9 March 2016].

20 Fielding K, Mccarthy K, Ginindza S et al. TB treatment outcomes among participants in the XTEND trial (Abstract OA–386–05). 46th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union). Cape Town, South Africa, 2–6 December 2015. http://capetown.worldlunghealth.org/Abstract_Book_2015-Web.pdf [accessed 9 March 2016].

21 Trajman A, Durovni B, Saraceni V et al. Impact on patients' treatment outcomes of XpertMTB/RIF implementation for the diagnosis of tuberculosis: follow-up of a stepped-wedge randomized clinical trial. PLoS One 2015;10:e0123252.

22 Lawn SD, Nicol MP, Corbett EL. Effect of empirical treatment on outcomes of clinical trials of diagnostic assays for tuberculosis. Lancet Infect Dis 2015;15:17–8.

23 Theron G, Peter J, Dowdy D et al. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? Lancet Infect Dis 2014;14:527–32.

24 Gupta RK, Lawn SD, Bekker LG et al. Impact of human immunodeficiency virus and CD4 count on tuberculosis diagnosis: analysis of city-wide data from Cape Town, South Africa. Int J Tuberc Lung Dis 2013;17:1014–22.

25 Lawn SD, Butera ST, Shinnick TM. Tuberculosis unleashed: the impact of human immunodeficiency virus infection on the host granulomatous response to *Mycobacterium tuberculosis*. Microbes Infect 2002;4:635–46.

26 Lawn SD, Mwaba P, Bates M et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis 2013;13:349–61.

27 Cox HS, Daniels JF, Muller O et al. Impact of decentralized care and the Xpert MTB/RIF test on rifampicin-resistant tuberculosis treatment initiation in Khayelitsha, South Africa. Open Forum Infect Dis 2015;2:ofv014.

28 Peter JG, Zijenah LS, Chanda D et al. Effect on mortality of point-of-care, urine-based lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-positive hospital inpatients: a pragmatic, parallel-group, multicountry, open-label, randomised controlled trial. Lancet 2016;387:1187–97.

29 Lawn SD, Kerkhoff AD, Burton R et al. Rapid microbiological screening for tuberculosis in HIV-positive patients on the first day of acute hospital admission by systematic testing of urine samples using Xpert MTB/RIF: a prospective cohort in South Africa. BMC Med 2015;13:192.

30 STAMP. Rapid urine-based screening for tuberculosis to reduce AIDS-related mortality in hospitalized patients in Africa (STAMP) trial:

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

ISRCTN71603869. http://www.isrctn.com/ISRCTN71603869 [accessed 9 March 2016].

31 Geng EH, Odeny TA, Lyamuya RE et al. Estimation of mortality among HIV-infected people on antiretroviral treatment in east Africa: a sampling based approach in an observational, multisite, cohort study. Lancet HIV 2015;2:e107–16.

32 Group ISS, Lundgren JD, Babiker AG et al. Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med 2015;373:795–807.

33 McCarthy K, Grant AD, Chihota V et al. What happens after a negative test for tuberculosis? Evaluating adherence to TB diagnostic algorithms in South African Primary Health Clinics. J Acquir Immune Defic Syndr 2015 [Epub ahead of print].

Downloaded from http://trstmh.oxfordjournals.org/ at Stephen B. Thacker CDC Library on September 19, 2016

2.3. Literature review of screening approaches to inform intensification of early ART care

2.3.1. Introduction: WHO criteria vs. risk score approaches

Although resource-rich countries in North America and Europe have used regression-derived and externally validated risk scores to help define mortality risk and therefore need for ART intensification among PLHIV,[86,100] WHO has not yet recommended or used this approach to inform eligibility for early ART care intensification packages for LMIC.[83] During the 2016 WHO guideline development process for early ART care intensification packages for LMIC, the focus was first on defining "advanced HIV disease" through a literature review and Delphi process,[101] and secondly on using this advanced HIV disease definition as the eligibility criterion for accessing an advanced disease package of care.[83]

To define advanced HIV disease, the WHO guideline development committee used a two-stage process. Firstly, a systematic review was conducted to summarize published definitions of advanced HIV disease. This review revealed a wide range of definitions of advanced HIV disease, with CD4 cell count cut-offs ranging from <50 cells/μL to <350 cells/μL, and a wide range of disease staging classifications (e.g., WHO stage definitions vs. CDC stage definitions) and staging cut-offs used.[83] Notably, 11 of the 12 studies included in the systematic review were from resource-rich countries.[83] After the systematic review, three rounds of questionnaires were sent to 73 expert respondents in 28 countries. Notably, the response rate and percentage of the respondents who agreed with the final WHO-recommended definition of advanced HIV disease is not presented in the publication, although this percentage should ideally be reported when reporting Delphi consensus studies.[83,101]

Using the advanced HIV disease definition as an eligibility criterion for accessing the advanced disease package of care in LMIC has a number of limitations including: (1) WHO

stage and CD4 count are not the only determinants of early ART mortality, (2) the screening accuracy characteristics (e.g., sensitivity and specificity) of the WHO advanced disease eligibility criteria in terms of predicting early ART mortality have not been systematically assessed, although both advanced WHO stage and low CD4 count are known to be strong predictors of early mortality, and (3) rapid CD4 count testing is not available in most LMIC clinic settings, with some clinics choosing to prioritise spending of limited resources on other tests (e.g., HIV viral load testing during ART follow-up) rather than CD4 testing before ART initiation.[102] A regression-based risk score development approach to developing practical clinical scores could address all three limitations.[100,103-105]

This section contains a literature review of published regression-based risk scores for PLHIV mortality, assesses the strengths and weaknesses of the published studies, and discusses the need for externally validated mortality risk scores for PLHIV accessing ART in sub-Saharan Africa and in LMIC in general.

2.3.2. Methods: literature search strategy

Search strategy

MEDLINE®, EMBASE, and the Cochrane library were searched over the time period January 1, 2005, to May 31, 2020 for reports published in English with the terms “HIV” or “AIDS” or “acquired immune deficiency syndrome”, and “antiretroviral therapy” or “treatment”, and “mortality” or “death”, and “risk score” or “clinical score”, and “predict”. This literature search was not conducted as a formal systematic review according to published Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, which for example, recommend a protocol and multiple reviewers examining and abstracting data.[106]

Study selection

Studies that met the following criteria were included: (1) the study population represented adult or adolescent PLHIV >12 years old, and (2) all-cause mortality was the primary outcome of interest or part of a composite primary outcome, and (3) the risk score was developed using a statistical approach (e.g., regression-based or machine-learning approach) rather than other more subjective approaches (e.g., based on author opinion on which variables to include in the score).[107-109]

2.3.3. Results

Characteristics of studies included

From a total of 316 published studies that met search criteria, a total of four regression-based risk score development studies were identified for inclusion based on the study inclusion criteria (Table 2.5.).[85-87,100] Two studies describe the Veterans Aging Cohort Study (VACS) score development. The VACS score was initially published in 2013[100] and updated in 2019 (VACS 2.0).[85] Both VACS and VACS 2.0 were derived in the U.S.-based VACS cohort. VACS was validated in six and VACS 2.0 in nine appended cohorts from the ART Cohort Collaboration (ART-CC) representing PLHIV in care in North America and Europe (Table 2.5.). One study reports the development of the EuroSIDA score, which was developed in the EuroSIDA collaboration of 94 health centres in 31 resource-rich countries, and validated in the Swiss HIV Cohort Study.[86] The only PLHIV mortality risk score developed for adults or adolescents in a LMIC, was developed in Haiti from attendees in six clinics, with validation among attendees in a single clinic also in Haiti.[87]

Table 2.5. Summary of mortality prediction risk score studies among PLHIV — 2005–2020

Papers included in Review	Name of screening tool	Country/Region: Derivation Cohort	Country/Region: Validation	Calendar time period of ART cohort (either testing or validation)	Study Population: Derivation	Study Population: Validation	Sample Size: Derivation Cohort	Sample Size: Validation
Tate et al, 2013[100]	VACS	United States - VACS	ART Cohort Collaboration (ART-CC) (North America and Europe)*	2000-2007	• Veterans Affairs Aging Cohort (VACS) represents HIV-positive U.S. Veterans. • To be eligible for cohort inclusion, required at least one year of ART completed plus complete covariates. • Among 13,582 men initiating ART in VACS between 2000 and 2007, 7823 had a CD4 cell count at ART initiation as well as HIV-1 viral load >500 copies/ml in the 3 months prior to ART initiation. Among these 7,823, 6,324 (81%) had complete biomarker measurements for regression inclusion. At 1 year, complete measurements were available for 4,932 (85%) of 5794 VACS patients who were alive and not lost to follow-up. • Among patients included: o 100% of VACS patients were men. o 46% were older than 50. o 69% had a CD4 >200. o 32% were Hep C co-infected. o 45% self-identified as black.	• Six combined cohorts of ART-CC were appended. • Eligible cohorts needed to have laboratory values of interest and report at least 25 deaths in patients who met the following criteria: HIV-positive, ART-naïve, >=18 years old, enrolled 2000-2007. • Of 5127 ART-CC patients meeting inclusion criteria 3,747 (73%) had complete measurements at ART initiation, varying from 61 to 92% by cohort. At 1 year, complete measurements were available for 3,146 (92%) of 3,434 patients alive and not lost to follow-up. • Among patients included: o 72% were men. o 15% were older than 50. o 84% had a CD4>200. o 12% were Hep C co-infected. o 19% self-identified as black.	4,932	3,146

Tate et al, 2019[85]	VACS 2.0	United States - VACS	ART Cohort Collaboration (ART-CC) (North America and Europe)*	2000-2014	• Veterans Affairs Aging Cohort (VACS) represents aging HIV-positive U.S. Veterans. • To be eligible for cohort inclusion: patients were ≥18 years old, initiated ART between 1996 and 2014, and had a visit between 2000 and 2014. Excluded 2782 individuals who had negative HCV RNA (at any time during study period) after previously having detectable HCV RNA. ≥1 year of ART completed. Any post 1-year visit with complete data eligible as starting point of follow-up. 75% of visits had complete covariate data. • Among patients included: o 98% were male. o Median age was 53. o Median CD4 was 435. o Median time on ART at randomly selected visit was 4.2 years.	• Nine combined cohorts of ART-CC were appended (named: A-I for anonymity). • To be eligible for cohort inclusion: the cohort needed to have laboratory values of interest and report at least 40 deaths in patients. From eligible cohorts, the proportion of visit dates with complete information varied between 5 and 82%. Unclear if any patients excluded from eligible cohorts. • Among patients included: o 74% were male. o Median age was 43. o Median CD4 was 500. o Median time on ART was 4.2 years.	28,390	12,109
Mocroft et al, 2007[86]	EuroSIDA risk-score	Eurosida**	Swiss HIV Cohort Study	1997-2007	• EuroSIDA is a cohort of >14,000 patients with HIV-1 infection in 94 centres from 31 countries in Europe, Israel and Argentina. • Of 14 274 patients within EuroSIDA: 9049 started ART; 5402 had a CD4 cell count and viral load measured 6 months prior to starting ART; 5302 had the potential to calculate the CD4 cell slope prior to starting ART; and 4169 had haemoglobin and BMI measured during follow-up. • Among patients included: o 75% Male. o Median age 38. o 36% ART-naive. o Median CD4 230. o 14% non-white.	• The Swiss HIV Cohort study is a prospective population-based cohort in Switzerland. • Eligible to participate if: HIV-positive, aged >16 years. No other data on study inclusion data provided. Current demographic data provided here: http://www.shcs.ch/ • Among participants included: o 70% male. o Median age 37. o 61% ART-naïve. o Median CD4 209. o 23% non-white (in 2018)	4,169	5,150

					• Derivation cohort: adults ages 15 to 70 years starting ART at 6 non-randomly selected health facilities in Haiti between 2007 and 2013. ART initiation was based on WHO guidelines at the time.†	• Validation cohort: All adults age 15 to 70 years starting ART in 2012 at one non-randomly selected facility. ART initiation was based on WHO guidelines.†		
McNairy et al, 2018[87]	Haiti risk score	Haiti - 6 clinics	Haiti - 1 clinic (enrolled during 2012)	2007-2013	• Patients with documented pregnancy at time of ART initiation were excluded. Multiple imputation was used to account for missing covariate and outcome data. • Among patients included: ○ 63% female. ○ Median age 36. ○ Median CD4 was 248.	• Patients with documented pregnancy at time of ART initiation were excluded. Multiple imputation was used to account for missing covariate and outcome data. • Among patients included: ○ 60% Female. ○ Median age 37 ○ Median CD4 was 259	7,031	1,835

Abbreviations: VACS, Veterans Aging Cohort Study; HCV, Hepatitis C; ART, antiretroviral therapy; Hep B, Hepatitis B, BMI, Body Mass Index

*the AIDS Therapy Evaluation Project Netherlands (ATHENA); Cologne-Bonn Cohort, Germany; Royal Free Hospital Cohort, London United Kingdom; Swiss HIV Cohort Study; Vanderbilt-Meharry Center for AIDS Research Cohort; and the University of Washington HIV Cohort, Seattle, USA]

**94 health centres across 31 resource rich countries

†WHO Stage IV or CD4 count <200 cells/uL (for years 2007±2009), and WHO stage III or IV or CD4 count <350 cells/uL (for years 2009±2013).

Study populations

The study populations included in the VACS, VACS 2.0, and EuroSIDA risk score development and validation approaches were different to the population included in the Haiti score. The majority of VACS, ART-CC, and EuroSIDA study enrolees were male (70-100%) and many were Caucasian (26%-86%), whereas in Haiti only 37-40% were male and <3% were Caucasian. Notably both the VACS and VACS 2.0 scores were derived from PLHIV who had completed 1 year of ART. EuroSIDA included observation time before ART. Only the Haiti score was derived using covariates present at ART initiation. Median CD4 count for participants included in the VACS score derivation was 307/μL, VACS 2.0 derivation was 435/μL, EuroSIDA derivation was 230/μL, and Haiti score derivation was 259/μL. In North American and European cohorts, Hepatitis C co-infection was an important comorbidity with prevalence ranging from 18-32%. Hepatitis C co-infection rates are not reported in the Haiti analysis.

Covariate data completeness

In VACS development only 4,932 (36%) of 13,582 potentially eligible cohort enrolees were included in the final regression due to either LTFU or death during the first year of ART or missing covariate data. In development of VACS 2.0, the starting point for ART observation for each ART patient was a randomly selected visit with complete covariate data with about 75% of visits having complete data. Overall, 28,390 individuals were included in VACS 2.0 development. In EuroSIDA risk score development, only 4,169 (29%) of 14,262 potentially eligible patients were included in the derivation dataset regression model due to missing covariate data. In the Haiti risk score development, 35 bootstrapped imputations were used to achieve complete covariate data based on the rule of thumb of one imputation for every percentage point of missing values for the variable with the most missing values. This implies >35% of observations would have been excluded from the regression if a complete case analysis was used.

Outcome and outcome completeness

In VACS and VACS 2.0, mortality ascertainment is reported as excellent based on a well-functioning national mortality register. The outcome of the EuroSIDA risk score was AIDS or death but the mechanism of death ascertainment was not described in the EuroSIDA publication. In Haiti, in the derivation cohort, 21.6% had missing vital status at 1 year; the death outcome was reported to the clinic passively by friends or family. In the validation clinic, 45.3% of patients at 1 year had missing vital status. The authors reported that missing vital status was imputed in both derivation and validation datasets because vital status was not missing completely at random.

Covariates included in the final scores

The initial VACS analysis developed a restricted and final VACS score (Table 2.6). The restricted score included only age, CD4 count, and viral load. The full VACS score included 11 variables to create 7 score components: age, CD4, HIV viral load, haemoglobin concentration, FIB-4 (a four-variable composite score of hepatic function requiring age, AST, ALT, and platelet concentration), the estimated Glomerular Filtration Rate (eGFR) (a four-variable score requiring creatinine, ethnicity, age, and gender), hepatitis C co-infection for maximum potential scores of 115 or 164 for the restricted and full VACS scores, respectively. The VACS 2.0 included four additional variables for a total of 15 variables and 10 score components by adding albumin concentration, white blood cell count (WBC), and body mass index (BMI) (height/weight^2) to the score. In addition, VACS 2.0 maintained all the originally continuous variables in a continuous linear form or transformed form appropriate for the non-linear relationship with the outcome. The EuroSIDA score included 10 variables for nine score components: age, prior CDC-defined AIDS diagnosis, CD4 count, CD4 slope, anaemia category, BMI, prior ART use, whether currently taking ART, and Injection Drug Use (IDU) history for a total possible score of 6.43. The Haiti score included five variables and score components: sex, WHO stage, CD4 count, current TB co-infection, and weight with a total score of 9.

Table 2.6. Comparison of published risk scores for mortality among PLHIV

	Tate et al, 2013			Tate et al, 2019	Mocroft et al		McNairy et al	
	Restricted† scores		VACS scores	VACS 2.0 scores	EuroSIDA scores		Haiti scores	
Time period that covariates represent	Variables closest to 1 year of completed ART			Randomly selected clinic visit >1 year ART	Time updated variables		Variables at ART initiation	
Sex							Female	0
							Male	1
Age (years)	<50	0	0	Continuous (age 30-75 years = 32-59 points		0.027/year		
	50-64	23	12					
	>65	44	27					
Prior Diagnoses of AIDS					No	0	WHO st.1-3	0
					Yes**	0.19	WHO st.4	2
CD4 (cells/μL)	>500	0	0	Continuous (10-900 cells/μL = 55-32 points)	>=350	0	>250	0
	350-499	10	6					
	200-349	10	6		201-350	0.62		
	100-199	19	10				101-250	1
	50-99	40	28		51-200	1.46	51-100	2
	<50	46	29		<=50	2.44	<=50	3
CD4 slope at time point of interest					<-25	0.49		
					-25 to +25	0		
					>25	0.18		
HIV-1 RNA (copes/ml)	<500	0	0	Continuous (1.3-5.0 log copies/ml = 37-55 points)				
	500-100,000	11	7					
	>100,000	25	14					
Haemoglobin (g/dL)	>14		0	Continuous (9-16 g/dL = 58-42 points)	No anaemia	0		
	12-13.9		10		Mild	0.68		
	10-11.9		22		Severe	1.02		
	<10		38					
FIB-4*	<1.45		0	Continuous (0.5-7.5 = 41-61 points)				
	1.45-3.25		6					
	>3.25		25					
eGFR (ml/min)*	>60		0	Continuous (0-100 = 53-44 points; 120-180 = 46-60 points)				
	45-59.9		6					
	30-44.9		8					
	<30		26					
Hepatitis C co-infection (current)			5	51 if yes				
Active TB disease							No	0
							Yes	2
Albumin				Continuous (2-5g/dL = 65-39 points)				
White Blood Cell Count (k/ml)				Continuous (2.5-11 = 43-55 points)				
BMI				Continuous (15-35 = 62-41 points)	<18	0.8		
					18.1-25	0		
					>25	minus 0.29		
Weight							<50	3
							50-60	1
							>60	0

ART-experienced			Yes	0	
			No	minus 0.39	
Taking ART			Any	0	
			None	1.24	
Route of presumed HIV Acquisition			Any except IDU	0	
			IDU	0.25	
Theoretical maximum score	115	164		6.43	9

Abbreviations: eGFR, estimated glomerular filtration rate; VACS, Veterans Aging Cohort Study; BMI, body mass index; ART, antiretroviral therapy; TB, tuberculosis; AIDS, Acquired Immune Deficiency Syndrome.
*FIB-4: (years of age * AST)/(platelets in 109/l * square root of ALT); eGFR: 186.3 *(serum creatinine ^-1.154) * (age^-0.203) * (0.742 for women) * (1.21 if black).
**CDC criteria from 1993 MMWR (Center for Disease Control. 1993 revised classification system)
†The restricted score was a shorter version of the full VACS score generated to assess improved feasibility of a less complex score

AUROC curve values for final risk score

The area under the receiver operating characteristic (AUROC) curve values for the restricted VACS, full VACS score, VACS 2.0, and Haiti scores in the validation datasets were 0.78, 0.82, 0.83, and 0.69, indicating acceptable (0.7-0.79), good (0.8-0.89), or poor (<0.7) discrimination respectively (Table 2.7). The EuroSIDA analysis did not provide AUROC values. Specific cut-offs for early ART care intensification, and associated sensitivity, specificity, NPV, and PPV were not suggested.

Table 2.7. Discrimination of risk score in derivation and validation datasets

	Tate et al, 2013		**Tate et al, 2019**	**Mocroft et al**	**McNairy et al**
	Restricted	VACS	VACS 2.0	EuroSIDA	Haiti
Number of primary outcomes					
Derivation	656	656	7,293	658*	242†
Validation	86	86	722	897**	50†
AUROC					
Derivation	0.72	0.78	0.805	Not provided	0.73
Validation	0.78	0.82	0.831	Not provided	0.69

Abbreviations: AUROC, Area under the receiver operating characteristic
*388 of the events were new AIDS defining illnesses (59.0%) and 270 patients (41.0%) died
**Patients progressed to a new AIDS-defining illness or death (17.4%), of which 494 were new AIDS-defining events (55.1%)
†Prior to imputation

2.3.4. Discussion

Based on this literature review, no externally validated risk scores for early ART mortality in LMIC have been published. The only internally validated score developed was from Haiti. No regression-based risk scores have been developed for sub-Saharan Africa, the region of the world with the highest early ART mortality and largest annual number of HIV-related deaths.[84]

Lack of mortality risk scores for LMIC, including sub-Saharan Africa

The seven-clinic study from Haiti is the only LMIC regression-based study published to date reporting early ART mortality score development and internal validation among PLHIV.[87] However, the Haiti study has several limitations including: (1) the score has not been validated widely within Haiti (1 clinic) or outside Haiti, (2) the score was developed using a routine electronic medical record (EMR) system with significant missing covariate data (>35%), and (3) the primary outcome of early mortality on ART was not well-ascertained and is therefore falsely low (due to unascertained mortality among PLHIV LTFU), introducing outcome ascertainment error, with 1-year vital status in the derivation and validation datasets missing in 21.6% and 45.3%, respectively.[87]

In addition, the authors used multiple imputation to address missing covariate and outcome data, which introduces at least non-differential measurement and outcome ascertainment error. Because the authors did not describe the patterns of missing data, it is challenging to understand why the missing at random assumption was considered reasonable. Multiple imputation is only acceptable if the missing at random or missing completely at random assumptions hold. If data were missing not at random (i.e., the propensity for data to be missing were related to the value of the data points that were missing), multiple imputation could have led to differential measurement error (i.e., biased or falsely imputed results).[110] Therefore, for example, if low CD4 counts were more

likely to be missing than high CD4 counts the missing at random assumption would not hold and multiple imputation would be inappropriate.[110]

One small study in which a non-regression-based clinical score was piloted in 2 clinics in Zimbabwe between 2008 and 2010[111] is not summarized in detail here because it does not meet inclusion criteria, deviates from standard risk score development approaches,[107-109] and was not used again either within Zimbabwe or outside to our knowledge.[111] In this analysis, authors implemented a literature review to obtain adjusted hazard ratios for author-selected risk factors for mortality during ART, and then used the median adjusted hazard ratio across studies identified as the clinical score; however, neither the literature review methodology nor the rationale for this approach is clearly presented.[111]

Limitations of clinical scores generated for resource-rich settings

The VACS, VACS 2.0, and EuroSIDA scores have a number of strengths including: (1) development using a regression-based approach, (2) wide external validation in cohorts from resource-rich settings outside the cohorts in which they were developed, (3) well-described methodology, (4) large sample size, and (5) in the case of the VACS scores, accurate mortality ascertainment. However, both scores also have several limitations. Firstly, the scores were generated for cohorts with demographic and clinical characteristics that are currently markedly different from all or almost all LMIC settings, with participants tending to be older, male, self-identify as white, live in resource-rich settings, and have additional comorbidities such as hepatitis C co-infection. Such characteristics are different to the majority of ART enrolees in LMIC who are almost exclusively non-white, more likely to be female, live in resource-poor settings, and have different comorbidities (e.g., are more likely overall to have TB disease instead of Hepatitis C co-infection). While the VACS score has been validated in multiple resource-rich cohorts such as ART-CC,[85,100] a younger, healthier population of PLHIV enrolled in the U.S. Military HIV Natural History Study (NHS),[104] female, multi-race/ethnic ART-experienced PLHIV in 2005,[112] a population of PLHIV living with multi-drug resistant HIV in the U.S.,[113] VACS has

not been validated as a predictor of mortality outside resource-rich settings like the U.S. and Europe. A Kenyan study did report that VACS was correlated with biomarkers of inflammation among hospitalized PLHIV in Kenya, however, there was no attempt to validate VACS as a predictor of mortality in this setting.[114]

Other limitations of VACS, VACS 2.0, and EuroSIDA include: (1) none of these scores is focused on the time period after ART initiation or re-initiation, with VACS score development starting after 1 year of ART and EuroSIDA including the pre-ART time period; (2) the large percentage of potentially eligible PLHIV that were dropped from the derivation regression (>50%) due to either death or LTFU in the first year of ART (VACS), or missing covariate data (VACS and EuroSIDA); (3) the outcome of the EuroSIDA score was a combination score of both death or AIDS (CDC 1993 definition), and (4) complexity of the score, with VACS, VACS 2.0, and EuroSIDA requiring ≥10 variables, including some complicated joint scores (e.g., FIB-4 and eGFR), in the case of VACS 2.0, use of continuous variables, and in the case of EuroSIDA, use of scores with decimal points. The complexity of these scores would require an online calculator or built-in electronic medical record algorithm that is not yet widely available in LMIC.[85,86,100]

Proof of concept

Despite the limitations of VACS, VACS 2.0, EuroSIDA, and Haiti scores, these scores do raise the important point that mortality risk is not determined solely by a limited number of HIV biomarkers (such as CD4 count and WHO stage).[84,105] In the VACS score the demographic variable of older age, and in the Haiti score, the demographic variable of male gender, carry important additional risk information for the clinician managing the patient. As McNairy *et al* note, many clinicians in LMIC know that male gender and other non-HIV-specific biomarkers like anaemia are important predictors of death and morbidity, but importantly are not prompted by existing HIV care algorithms to use this information to intervene.[84] Per WHO guidelines, the focus remains on WHO stage and CD4 count, with CD4 count still not widely available in LMIC, although less expensive and

easier to use lateral flow assays are in development.[44] As the WHO guidelines and published reports suggest, up to 50% of those with a CD4 count <100 cells/μL could be classified as having WHO stage I or II and therefore missed by an advanced HIV disease eligibility guideline relying on WHO stage alone.[44,53]

2.3.5. Conclusion

In summary, these data and prior proof-of-concept mortality risk scores suggest that either through absent CD4 testing, or through lack of clinical scores that incorporate wholistic risk factors for on-ART mortality in sub-Saharan Africa, such as male gender and haemoglobin concentration, clinicians are likely missing a substantial percentage of clients requiring intensification of early ART care. Therefore, a clinical scoring system suitable for sub-Saharan Africa that: (1) takes into account both HIV and non-HIV-specific risk factors for mortality, allowing a more precise approach to early ART care, (2) is feasible and easy to use in settings that both have or lack CD4 testing capacity, and (3) has similar or superior screening accuracy (i.e., sensitivity, specificity) in predicting early mortality on ART compared with current WHO advanced HIV disease eligibility guidelines, is needed to facilitate provision of effective interventions to clients who need intensified care.[84]

2.4. Literature review of TB screening approaches for HIV-positive people

2.4.1. Introduction: WHO four-symptom TB screen vs. clinical score approach

WHO currently recommends a four-symptom TB screening rule (i.e., for cough, weight loss, night sweats or fever) to determine which PLHIV need investigation for active TB and which are eligible for immediate TB preventive therapy (TPT).[88] The WHO four-symptom TB screening rule is recommended for LMIC regardless of expected prevalence of active TB, setting (e.g., high or low TB burden settings), or ART status (ART-naïve or ART-experienced).[88] This introductory section describes briefly the development process for the WHO four-symptom TB screening rule and its strengths and limitations. Subsequently, a literature review of alternative approaches to TB screening among PLHIV using clinical scores is provided to summarize existing literature and assess gaps in needed research.

WHO four-symptom screening rule

WHO has recommended the four-symptoms TB screening rule since 2011 following the individual patient data meta-analysis by Getahun *et al,* which included studies published up to and including 2008. A new meta-analysis of studies examining screening accuracy of the four-symptom rule was conducted by Hamada *et al* in 2018 including published studies during January 1, 2011 and March 12, 2018. Both meta-analyses also assessed the added value of chest radiography (CXR) in terms of TB symptom screening rule accuracy. The study designs of the meta-analyses and key findings are shown in tables 2.8 and 2.9 below.

Table 2.8. Comparison of WHO TB symptom screening rule meta-analyses from 2011 and 2018

	Getahun et al, 2011 - **An individual patient data meta-analysis**	**Hamada et al, 2018** - **A meta-analysis of published summary data**
Study aim	To find the screening rule with the highest possible sensitivity and lowest possible negative likelihood ratio for ruling out TB disease (without any predetermined cut-off points).	To assess screening accuracy of the WHO four-symptom TB screen by ART status and effect of adding CXR on screening accuracy.
Eligibility criteria for inclusion	(1) collected sputum specimens from PLHIV regardless of signs or symptoms; (2) used mycobacterial culture of at least one specimen to diagnose TB; and (3) collected data about signs and symptoms.	(1) collected sputum or any specimens (e.g., urine, blood, or fine-needle aspirates from lymph nodes) from PLHIV regardless of signs or symptoms; (2) excluded case-control studies
Calendar time represented	2000-2008	Jan 1, 2011 through March 12, 2018
Number of studies considered	2119 screened, 53 full text reviewed, 14 met inclusion criteria (6 published, 8 unpublished)	4615 screened, 195 reviewed for eligibility, 21 included in review
Numbers of studies included	12 included in final meta-analysis	18 studies in final meta-analysis (7 with disaggregated ART status; 11 were among PLHIV not on ART)
Number of candidate variables	159 (159 different symptoms were included in at least 2 of 12 studies**)**	Only the 4 WHO-recommended symptoms were examined.
Variables/symptoms considered in meta-analysis	5 symptoms: current cough, haemoptysis, fever, night sweats, and weight loss.	Only the 4 WHO-recommended symptoms
Primary outcome definition	TB patient: any PLHIV with >=one sputum specimen culture positive for MTB. No TB: sputum cultures were negative for MTB and participants were judged not to have TB in original study. Excluded from the analysis: (1) patients receiving ATT or TPT at enrolment; (2) patients AFB smear positive, but culture grew nontuberculous mycobacteria; and (3) patients AFB smear positive or scanty, but sputum culture negative.	TB patient: Active TB was defined as tuberculosis diagnosed with bacteriological confirmation by use of culture or Xpert MTB/RIF of any specimens.
Type of populations	2 studies: SA gold miners; 5 studies: community TB prevalence survey; 5 studies clinic population (3 were among pre-screened PLHIV).	20 studies in clinic settings; 1 in prisons; none in community; none in miners*
TB case finding approach	3 studies exclusively used liquid media to culture specimens, two studies used both solid and liquid media, and seven studies exclusively used solid media.	All studies collected sputum specimens. Four studies collected additional specimens: fine-needle aspirates from individuals with enlarged peripheral lymph nodes; non-respiratory samples (e.g., ascitic, cerebrospinal and pleural fluid, blood, stool, fine-needle aspirate, and bone marrow) as clinically indicated; and blood from all participants. 16 of the included studies used culture alone, and the other studies used both culture and Xpert MTB/RIF.

Chest radiography	Data from 4 studies used to assess effect on accuracy	Data from 12 studies used to assess effect on accuracy
Countries represented	Zambia, Zimbabwe (2 studies), Cambodia (3 studies), Thailand, Vietnam, South Africa (5 studies), Ethiopia,	Malaysia, South Africa (10 studies), Ethiopia, Ghana, Swaziland, Zambia, Kenya, Cameroon, Vietnam, Uganda.
Analytic approach	1-of-n rules: 23 candidate rules identified. M-of-n not considered because sensitivity was key feature prioritized.	Main aim was to assess the performance of the 4-symptom screen, not to assess candidate variables
Statistics	Bivariate random-effects meta-analysis (BREMA) and the hierarchical summary relative operating characteristic (HSROC) curve.	When at least four studies were available, bivariate random-effects models were used to estimate both pooled sensitivity and specificity together. If <4 studies were available, meta-analyses using univariate random-effects logistic-regression models were used separately for sensitivity and specificity.
Stratified analyses	To examine how (1) setting, and (2) individual factors affect screening accuracy. The study examined variations in accuracy parameters and used odds ratios to compare relative likelihood of true positives and true negatives between groups.	To examine variation in accuracy by ART status and pregnancy status. 10 studies included people on ART (range: 0.14%-92.3%); 7 gave results stratified by ART status. 11 studies - no ART enrolees.
Number needed to screen (NNS)	Evaluated NNS in hypothetical populations with varying TB prevalence rates of size 1000.	Not provided.
Missing data	Complete case analysis. About 15% observations missing covariate data.	Complete case analysis. >30% who underwent symptom screening were excluded because of their inability to produce sputum or missing values.
Overall characteristics of patients included	Median age 34 (27-41). Overall median CD4 among 9,626 PLHIV was 248 (IQR: 107-409). Among 8,148 PLHIV included in meta-analysis, median CD4 was 94 (IQR 33-215) in those with TB vs. 229 (IQR 94-391) in those with no TB.	Median CD4 was 272 cells per µL (IQR 202–337) in 21 studies included in the review. Median CD4 across 18 studies included in the meta-analysis not provided.
Sample size (n)	8,148 PLHIV of 10,057 total PLHIV, taken from 9 of 12 studies had outcome and 5 symptoms of interest.	15,427 PLHIV in 18 studies included in the final meta-analysis.
Prevalence of TB	557 (5.8%) of 9,626 PLHIV	1559 (10.1%) of 15,427

Abbreviations: PLHIV, people living with HIV; ART, antiretroviral therapy; TB, tuberculosis; ATT, anti-tuberculosis treatment; TPT, TB preventive therapy; AFB, acid-fast bacilli; CXR, chest radiography; WHO, world health organisation; NNS, number needed to screen to detect one TB case; IQR, inter-quartile range; MTB, *Mycobacterium tuberculosis*

*One study was considered to be at high risk of selection bias because it had included only people with HIV who required acute admission to hospital. Seven studies were considered to be of high concern for applicability, three of which included only pregnant or lactating women, one of which included only patients admitted to hospital, and three of which included only participants with low CD4 cell counts or those with advanced HIV clinical disease (WHO stage 3 or 4). Three studies were considered to be at risk of bias either because types of specimens obtained for tuberculosis diagnosis differed between patients, or because a substantial proportion of participants who underwent symptom screening were excluded because of their inability to produce sputum or had missing values, or because Xpert MTB/RIF was initially used only for selected participants with a high risk of developing TB.

Notably, the Getahun meta-analysis was designed to first derive the four-symptom screening rule and then assess the screening rule accuracy with and without chest radiograph in a variety of settings, whereas the key purpose of the Hamada meta-analysis was to assess the screening accuracy of the four-symptom screening rule and changes if CXR is added to the screening algorithm. Both studies are well-described with large sample sizes and wide representation from LMIC. However, two weaknesses of the Getahun analysis are that: (1) the TB outcome was dependent on a sputum culture and this relies on (a) ability to produce sputum, and (b) the assumption that the screening rule is designed to detect pulmonary TB, and (2) the screening accuracy is not stratified by ART status because all participants were pre-ART. In contrast, in the Hamada meta-analysis: (1) TB diagnosis was not purely reliant on sputum culture although most studies still required patients to produce sputum, and (2) screening accuracy results were stratified by ART status. Missing covariate data of 15% in the Getahun analysis, and missing covariate or outcome data (>30%) in the Hamada analysis are limitations of both studies.

However, the three biggest limitations of both meta-analyses and by definition the WHO-recommended four-symptom TB screening rule are that: (1) by developing a screening tool that relies purely on symptoms, all asymptomatic TB will be missed, (2) screening accuracy characteristics (e.g., sensitivity and specificity) vary by setting and patient population, and yet the screening rule derived (either positive or negative for the symptom screen) is a one-size fits all approach regardless of setting, PLHIV population, or use-case scenario, and (3) by stratifying risk into two categories, this does not allow opportunity to derive differentiated TB-HIV algorithms more precisely suitable to the continuum of risk present in the PLHIV population served.

The importance of asymptomatic TB

Asymptomatic TB, as shown by national TB prevalence surveys[115] as well as observational cohorts studies,[61] is relatively common in several settings and populations. Firstly, as is illustrated by the Hamada meta-analysis and has been previously reported from other

settings, asymptomatic TB is common among PLHIV on ART and therefore a WHO symptom screen will not be a sensitive screening tool in this population.[61,90,91] Notably, sensitivity of the WHO four-symptom screen was only 51% among PLHIV on ART versus 89% among ART-naïve PLHIV in the Hamada meta-analysis (Table 2.9). There are at least two key reasons why asymptomatic TB was relatively more common among ART-experienced versus ART-naïve PLHIV who were included in the Hamada meta-analysis. Firstly, PLHIV on ART represent a pre-screened population, where the four WHO symptoms should have been assessed at each pre-ART and ART visit over the course of care, progressively reducing the relative contribution of undiagnosed symptomatic TB and increasing the relative contribution of undiagnosed asymptomatic TB in the population screened.[61] This is well illustrated by the Getahun analysis where sensitivity of the WHO four-symptom TB screen in previously unscreened PLHIV was 88% but dropped to 41% among those who had been previously screened (Table 2.9).[60] Secondly, PLHIV who are stable on ART are likely to have higher CD4 counts with higher immune competence and are more likely to have an indolent course of disease possibly with intermittent symptoms.[90] The lower sensitivity of the WHO four-symptom screening rule among PLHIV with higher CD4 counts is also well-illustrated by the Getahun meta-analysis where odds of a true positive screen result using the WHO four-symptom screening rule was about 6-fold lower in PLHIV with CD4 ≥200 than in PLHIV with CD4 <200 (Table 2.9).[60]

Table 2.9. Summary of key results from the 2011 and 2018 meta-analyses informing four-symptoms screening rule recommendations

		Getahun et al, 2011	Hamada et al, 2018
TB prevalence		5.8% (557/9,626)	1559 (10.1%) of 15,427
% Patients WHO four-symptom screening rule +ve		49.80%	Median 68.0% (IQR 37.0–86.4)
Not on ART		49.80%	71.2% (IQR: 46.7–87.1)
On ART		N/A	29.7% (IQR: 14.3–45.7)
WHO four -symptom screening rule accuracy			
Not on ART*			
Pooled sensitivity (95% CI)**		78.9 (58.3–90.9)	89.4% (83.0–93.5)
Pooled specificity (95% CI)**		49.6 (29.2–70.1)	28.1% (18.6–40.1)
On ART			
Pooled sensitivity (95% CI)		N/A	51.0% (28.4–73.2)
Pooled specificity (95% CI)		N/A	70.7% (47.8–86.4)
WHO four-symptom screening rule or CXR +ve accuracy			
Not on ART			
Pooled sensitivity (95% CI)		90.6% (66.7–97.9)	94.3% (76.2–98.8)
Pooled specificity (95% CI)		38.9% (12.8–73.3)	20.1% (7.6–43.8)
On ART			
Pooled sensitivity (95% CI)		N/A	84.6% (69.7–92.9)
Pooled specificity (95% CI)		N/A	29.8% (26.3–33.6)
Variations by setting			
Screened in clinical setting			
Pooled sensitivity (95% CI)		90.1% (76.3–96.2)	81.7% (61.2-92.7)§
Pooled specificity (95% CI)		N/A	38.8% (18.4-64.1)§
Not previously screened			
Pooled sensitivity (95% CI)		88.0% (76.1–94.4)	N/A
Pooled specificity (95% CI)		N/A	N/A
Screened in community setting			
Pooled sensitivity (95% CI)		67.1% (41.7–85.3)	N/A
Pooled specificity (95% CI)		N/A	N/A
Previously screened for TB			
Pooled sensitivity (95% CI)		40.5% (16.6–69.9)	N/A
Pooled specificity (95% CI)		N/A	N/A
Variations at the patient-level		Age >=33 (1.43);	
Individual level factors predictive of increased sensitivity (odds ratio for true positive screen)		Male (1.26), CD4 <200 (6.38); CXR abnormal (1.36)	N/A
Changes in NPV by TB prevalence		WHO symptom rule only	WHO symptom rule only (plus CXR)†
Not on ART	1% TB prevalence	99.60%	99.6% (99.7%)
	5% TB prevalence	97.70%	98.0% (98.5%)
	10% TB prevalence	95.30%	96.0% (97.0%)
	20% TB prevalence	90.00%	91.4% (93.4%)
On ART	1% TB prevalence	N/A	99.3% (99.5%)
	5% TB prevalence	N/A	96.5% (97.4%)
	10% TB prevalence	N/A	92.8% (94.6%)
	20% TB prevalence	N/A	85.2% (88.6%)
Changes in NNS by TB prevalence	1% TB prevalence	62	
	5% TB prevalence	12	
	10% TB prevalence	6	
	20% TB prevalence	3	

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; NPV, negative predictive value; CXR, chest radiography; NNS, number needed to screen to detect one case

*Getahun analysis no participants were on ART

**Significant heterogeneity between studies in the sensitivity and specificity of the four-symptoms screen

† In this table, the figure in brackets indicates the NPV when CXR is added. Overall NPV increased 0.9% with addition of CXR.

††Participants with CD4 cell count ,200 cells/ml had 6.4 times the odds of a true-positive screen (95% CI 2.9–14.2).

§Regardless of ART status with estimates taken from five studies in the Hamada meta-analysis

In addition, asymptomatic active TB can be present among severely immune compromised PLHIV,[94,95] and among pre-ART patients without advanced disease in high prevalence settings,[96] among whom missing asymptomatic active TB can have suboptimal health consequences for patients and impede disease control activities.[24]

Secondly, both meta-analyses reported significant heterogeneity in WHO four-symptom screening rule by study, which is a proxy for setting. The variations by setting are well illustrated in the Getahun meta-analysis, where the WHO four-symptom screening rule sensitivity varied from 90.1% in clinical settings to 67.1% in community settings, because at the time the study was published prevalence of healthier PLHIV and therefore asymptomatic TB disease was more common in community settings. Similarly, sensitivity varied by pre-screened versus screened populations and by CD4 count as described above. By definition, key parameters like number needed to screen (NNS) and NPV depend on the underlying prevalence of TB. Notably, NPV of the WHO four-symptom screening rule when a 20% TB prevalence was assumed was only 91.4% and 85.2% among ART-naïve and ART-experienced PLHIV, respectively. This indicates the challenge that in high prevalence settings, higher numbers of PLHIV with active TB could potentially be prescribed TB preventive therapy, when a full course of TB therapy is required, with implications for emerging TB drug resistance.[16] Although Hamada *et al* and new WHO guidelines recommend possibly adding chest radiography to the screening rule for PLHIV on ART to increase sensitivity and NPV, this comes at the expense of lower specificity, carries significant additional costs and operational challenges, provides only modest overall NPV increase (0.2% at 1% TB prevalence), and therefore might not facilitate TPT scale-up in many LMIC settings.[61,93]

Thirdly, a screening rule that only classifies patients into two categories (WHO screen positive versus negative), does not allow more precise understanding of TB risk, which could inform differentiated models of TB-HIV care.[116,117]

One approach to overcoming all three limitations might be to derive a clinical score based on easily available variables in LMIC clinic settings that are not purely dependent on TB symptoms and for which cut-offs can be tailored to setting, patient population, use-case scenario, and to inform differentiated TB-HIV care. Therefore, we searched the literature to better understand the availability of clinical TB screening rules and their potential suitability for LMIC, especially in sub-Saharan Africa.

2.4.2. Methods: literature search strategy

Search strategy

MEDLINE®, EMBASE, and the Cochrane library were searched over the time period January 1, 2005, to May 31, 2020 for reports published in English with the terms "Tuberculosis" or "tuberculosis" or "TB", and "HIV" or "acquired immune deficiency" or "AIDS", and "score" or "clinical score", and "screen", and "validate" or "validation" or "predict" or "risk" or "prognostic".

Study selection

Published peer-reviewed studies that met the following criteria were included: (1) the study population represented adult or adolescent PLHIV >12 years old, and (2) active TB disease was the outcome of interest, whether microbiologically confirmed or clinically determined, and regardless of the TB case finding approach to determine presence of active TB.[107-109]

2.4.3. Results

Characteristics of studies included

A total of six studies describing six clinical scores for TB risk among PLHIV were identified that met inclusion criteria from five countries: Thailand, Vietnam, Ethiopia, Guinea Bissau, and South Africa. Three scores were derived for use as the initial screening test, three

scores as the second screening test among patients initially screening positive with the WHO four-symptom TB-screen, and one score (TBScore) was trialled as both an initial screen and second-step screen in two separate studies. The time period of the studies covered 2009-2014 (Table 2.10).

Table 2.10. Characteristics of studies included in the literature review

Papers included in Systematic Review	Country	First or second screening step	Calendar time period of study	Study Population/Screened Population	Sample size (n)	Primary Outcome Definition	TB case finding approach	Prevalence of TB in pop. screened	Data completeness	Regression approach	Score approach
Balcha et al, 2014[118]	Ethiopia	second-step after WHO screen +ve	2011-2013	5 health centres in Oromia region, Ethiopia. All PLHIV study enrolees were ART-naïve, >=18 yo, submitted >=1 sputum sample at enrolment, provided written consent, were not already on ATT for >2 weeks, and were WHO screen +ve. All PLHIV were pre-ART. Median CD4 was 212 (119–321).	625	TB cases were defined as subjects with bacteriologically confirmed pulmonary and/or extrapulmonary TB (at baseline or within 3 months after enrolment). Participants with clinically diagnosed TB (defined as subjects who were prescribed ATT without microbiological confirmation) at baseline or within 3 months of enrolment were excluded from statistical analysis.	At inclusion, participants were requested to submit 2 pairs of spontaneously expectorated morning sputum samples, tested with culture, Xpert, Smear microscopy.	20.2% among those WHO screen +ve	Only 7 eligible persons were excluded because of incomplete covariates	Variables significant at p<0.3 were considered candidate variables. Backward stepwise regression cut-off at p<0.05.	1 point for each significant variable level
Nanta et al, 2011[119]	Thailand	first step	2009-2011	2 hospitals in a region of Thailand, with the PLHIV patient population drawn from outpatient ART and TB clinics and inpatient wards. PLHIV were >=18 yo. Not eligible if had IPT or ATT within 1y. 66.5% were on ART. Enrolled also from TB clinics, implying many were referred for TB investigation. 29% had CD4<200.	257	TB defined as any of: smear +ve, culture +ve, compatible histology, clinical or radiological response to TB Rx. TB investigations were part of routine care through 2 months of follow-up.	Available TB diagnostic procedures were performed, including chest radiographs, ultrasound examinations, CT scans, three consecutive sputum collections for AFB smear and sputum cultures	25.7%	Missing data not reported	Variables significant at p<0.25 = candidate variables. Multivariable logistic regression with backward elimination was used for second stage analysis to identify independent predictors. Only variables which reached statistical significance (p<0.05)	Coefficient derived
Nguyen et al, 2011[120]	Vietnam	first step	2009-2010	1 HIV clinic in Vietnam. Cross sectional. PLHIV outpatients were consecutively enrolled, >=15, and lived in District 6. PLHIV were excluded if screened in last 3 m or on ATT. 57.9% were on ART. 22% with CD4<200.	397	Pulmonary TB (PTB) case was defined as a subject with at least one sputum culture positive for M. tuberculosis.	Two sputum samples (home and clinic), for culture, smear, DST. Chest x-rays and TST performed but did not inform primary outcome.	7.0%	36 patients did not complete study	Conducted a univariate logistic regression. Retained statistically significant variables with clinical significance in multivariable model. Examined combinations of chosen covariates and their accuracy.	No score generated

Rudolph et al, 2014[121]	Guinea-Bissau (TBScore + TBScore II)	second-step after WHO screen +ve	2010-2012	PLHIV enrolled were >15 years old, patients seeking health care for cough, weight loss and/or expectoration within the Bandim Health Project (health + demographic surveillance area). PLHIV were pre-ART. CD4 data not provided. 17% of HIV+ had BMI <18.	164	Patients were smear +ve PTB if ≥1 sputum smear was +ve for AFB. Patients with a -ve sputum smear, but with clinical signs, symptoms and CXR changes considered TB-related, were classified as smear -ve PTB if no improvement with antibiotics and the physician initiated ATT.	Sputum collection for AFB microscopy, chest radiography	16.5%	Complete	Not provided - score previously generated.	TBScore or TBSCore II with 1 point assigned to each variable
Aunsborg et al, 2020[122]	Guinea-Bissau (TBScore)	first step	2014	HIV Clinic of Hospital Nacional Simão Mendes in Bissau, Guinea-Bissau. PLHIV enrolled were newly diagnosed; >=15; not pregnant and not on TB treatment in last year. Median CD4 183 among TB patients and 300 among non-TB patients. About one third with CD4<200.	164	The final TB diagnosis was established based on the following: TB = sputum positive for AFB OR sputum positive for Xpert OR clinical judgement OR X-ray findings not resolved after a short course of antibiotics, according to WHO guidelines. (59% microbiologically diagnosed).	Newly diagnosed HIV patients with a Tbscore >=2 were asked to produce sputum if they were able to for Xpert, smear microscopy and CXR. Newly diagnosed HIV patients with score <2 + TST>5mm had CXR, +- Antibiotic trial, + second CXR.	13.4%	47 of 231 patients eligible but not included due to Ebola outbreak and logistical reasons. 31 of 164 lost to follow-up/screening.	Not applicable since the score was already developed	Same as TBScore for Rudolf, 2014
Hanifa et al, 2017[123]	South Africa	second-step after WHO screen +ve	2012	Prospective cohort at 2 hospital-based and 2 community health centre clinics. PLHIV enrolled were: ≥18 years old, included three groups (newly HIV diagnosed, pre-ART care, or on-ART groups); had not received ATT in previous 3 months; and there were no CD4 count criteria for enrolment. Median CD4: 378 (IQR 228-543). About one quarter with CD4<200.	1048 (515 derivation internal; 533 validation internal)	Clinical or microbiologically confirmed TB within 6 months of enrolment visit.	≥1 Spot sputum sample for all enrolees regardless of symptoms at enrolment. Spot sputum samples collected at subsequent visits if "high risk"**; Xpert for all sputum samples; CXR per national guidelines	10.5%	Complete	Stepwise backward regression with significant variables at p<0.05 selected	Rescaled multivariable beta coefficients from logistic regression

Abbreviations: ART, antiretroviral therapy; TB, tuberculosis; WHO, world health organisation; SA, South Africa; AFB, acid fast bacilli; CXR, chest radiography; PTB, pulmonary TB; TST, tuberculin skin test; ATT, anti-tuberculosis treatment; Rx, treatment; yo, year-old; +ve, positive; -ve, negative; BMI, body mass index; DST, drug susceptibility testing.

Study population

Among the six study populations, three (Ethiopia and the two TBScore studies from Bissau) included only pre-ART[118,121,122] adult patients and three (Thailand, Vietnam, and South Africa studies) included a mix of both pre-ART and ART patients.[119,120,123] Some measure of CD4 count distribution among PLHIV was provided in five studies, with 22% to 50% of PLHIV having a CD4<200/µL. Sample size ranged from 164 to 1,048. The prevalence of active TB in the populations screened ranged from 7.0% to 25.7% (Table 2.10).

TB outcome and case finding approach

The TB outcome definition varied across the studies; four (Thailand, two TBScore studies from Bissau, and South Africa studies) used either a clinical or microbiologically confirmed diagnosis of TB as the outcome of interest, while two (Ethiopia and Vietnam studies)[118,120] used only microbiologically confirmed TB from sputum and other samples. Five studies focused on pulmonary TB and only one (Ethiopia study) focused on either pulmonary or extra-pulmonary TB.[118] Four studies (Ethiopia, Vietnam, initial TBScore study from Bissau, and South Africa study) collected sputum from all enrolees; in the follow-up TBScore study from Bissau, study enrolees only provided sputum if the TBScore was >=2;[122] and in one study (Thailand study) it is unclear whether all enrolees provided a sputum for microbiological assessment as TB diagnosis was done in routine settings.[119]

Covariates assessed

Across the six studies and scores, a large number of potential predictors of prevalent active TB were considered as candidate predictors, with a total of 39 categories of symptoms across the studies evaluated (Table 2.11). Some categories (e.g., WHO TB symptoms and comorbidities), had multiple levels (e.g., 14 symptoms were considered in the Thailand study and 11 in the Vietnam study, while five comorbidities in addition to HIV infection were considered in the Thailand study). Approaches to categorizing the continuous variables was not consistent. For example, BMI was made binary (<19 vs. ≥19)

in the Thailand study, (categorized as <18 and <16 in the TBscore studies from Bissau), and categorized as <18.5, 18.5-24.9, and ≥25 in the South Africa study. Sometimes predictors in one study were considered part of the outcome definition in other studies. For example, a CXR suggestive of TB was considered a predictor in the Vietnam study, but was considered to define clinical TB in the three studies where a clinical diagnosis of TB was part of the outcome definition (i.e., Thailand, TBScore studies from Bissau, and South Africa study).

Table 2.11. Summary of covariates assessed in published clinical TB risk scores among PLHIV

	Balcha et al 2014			Nanta et al, 2011			Nguyen et al, 2011			Rudolf, 2014 & Aunsborg et al, 2020					Hanifa et al, 2017		
	Candidate	AOR	Score	Candidate	AOR	Score	Candidate	AOR	Score	Candidate	AOR*	TBScore	TBScore II	TBScore	Candidate	AOR	Score
Age	Yes			Yes			Yes								Yes		
Sex																	
Male	Yes			Yes			Yes			No					Yes		
Female																	
Residence																	
Urban	Yes			No			No			No					No		
Rural																	
Permanent Home																	
Yes	Yes			No			No			No					No		
No																	
Previous TB																	
Yes	Yes			Yes	9.31	3	Yes	0.89	NP	No					Yes		
No																	
Household TB contact																	
Yes	Yes			Yes			No			No					No		
No																	
Prior TB in HH																	
Yes	Yes			No			No			No					No		
No																	
Household size	No						Yes			No					No		
Smoking																	
Yes	Yes			No			Yes			No					Yes		
No																	
Alcohol																	
Yes	Yes			No			Yes			No					Yes		
No																	
IDU																	
Yes	No			No			Yes			No					No		
No																	
Khat																	
Yes	Yes			No			No			No					No		
No																	
Employment																	
Yes	No			Yes			Yes			No					No		
No																	
Prior incarceration																	
Yes	No			No			Yes			No					No		
no																	
HIV care																	
New to care (pre-ART)	Yes			No			No			No					Yes		
Longer term pre-ART																	
WHO stage																	
1 or 2	Yes			No			No			No					No		
3 or 4																	
WHO TB symptom score				Cough >2 w													
Cough	Yes	**2.1**	**1**		6.16	3	Yes	0.7	NP	Yes		1	1	1	Yes		
Fever				chills >=1w	6.95	3	Yes	2.64	NP	No					Yes		
Loss of weight							Yes	1.22	NP	No					Yes		
Night sweats							Yes	1.14	NP	Yes		1		1	Yes		
Other				12 others considered			Appetite loss	1.93	NP	Haemoptysis		1		1			
							fatigue	0.94	NP	Dyspnoea		1	1	1			
							Chills	1.98	NP								
							Chest pain	1.36	NP	Chest pain		1	1	1			
							4 others considered								Yes >1 Symp.	3.59	4

Duration of Symptoms	No			No			No			No					Yes		
Comorbidities																	
Diabetes																	
Hypertension	No			Yes			No			No					No		
Liver disease																	
Lung disease																	
Other																	
Clinical chest features	No			Yes			No			Yes (Auscultation)		1		1			
On IPT																	
Yes	Yes			No			No			No					Yes		
No																	
On CPT																	
Yes	Yes			Yes			No			No					Yes		
No																	
ART status																	
On ART				Yes			Yes			No					Yes		
Not on ART					6.37	3										2.34	3
Hospitalization																	
Yes	Yes			No			No			No					No		
No																	
Peripheral Lymph nodes	Yes	**7.3**	1	Yes			No			No					No		
Pulse							No			Yes (>90/min)		1		1	No		
Temperature	No			No			No			Yes (>37)		1		1	No		
Karnofsky score	Yes (<=80)	**2.8**	**1**	No			No			No					No		
MUAC	Yes (<20cm)	**2.0**	1	No			No			Yes (<22cm)		1	1	1	No		
										Yes (<20cm)		1	1	1	No		
BMI	Yes			Yes (<19 kg/m2)	3.99	2	No			Yes (<18)		1	1	1	Yes 18.5-24.9	2.23	2
										Yes (<16)		1	1	1	Yes (<18.5)	6.79	6
TST							Yes	2.72	NP						No		
Haemoglobin	Yes (<10g/dL)	2.9	1	Yes			No			Yes (Anaemic eyes)		1	1	1	No		
CD4 count	Yes			Yes (<200/µL)	3.79	2	Yes (<200/µL)	3.17	NP	No					Yes (200-349 µL)	1.4	1
Haematocrit	No			Yes			No			No					No		
Platelet count	No			Yes			No			No					No		
WBC	No			Yes			No			No					No		
AFB +ve	No			No			Yes	32.04	NP	No					No		
TB-CXR	No			No			Yes	4.28	NP	No					No		

Abbreviations: AOR, adjusted odds ratio; TB, tuberculosis, IDU, injection drug use; ART, antiretroviral therapy; IPT, isoniazid preventive therapy; CPT, co-trimoxazole preventive therapy; MUAC, mid-upper arm circumference; BMI, body mass index; TST, tuberculin skin test; WBC, white blood cell count; AFB, acid fast bacilli; CXR, chest x-ray; PLHIV, people living with HIV; symp., symptoms

*AORs not provided for the TBScore analyses.

Statistical approaches to score development

Four of the six studies (Ethiopia, Thailand, Vietnam, and South Africa) used a logistic regression approach to identify predictors in univariate analysis (Table 2.10). Two of these four studies indicated that candidate predictor variables with $p<0.3$ (Ethiopia) and $p<0.25$ (Thailand study) in univariate analysis were eliminated as candidate variables. Of these four studies, three used a backward multivariable stepwise regression approach as the final step to select chosen variables for the score, sequentially eliminating variables with the highest p-value until only those with $p<0.05$ were retained. Five of six studies generated numeric scores for TB risk, whereas one (Vietnam) only provided screening accuracy for a variety of combinations of variables. Among the five studies with numeric scores, one (Ethiopia) assigned a single point for each selected variable regardless of coefficient, two re-scaled multivariable model coefficients (Thailand and South Africa), and two (the TBScore studies from Bissau) did not present the regression approach and assigned a single point to each of the final chosen variables. Only the South Africa study assessed the possibility of non-linear relationships between continuous predictors and log odds outcome.

Screening accuracy and validation approaches

None of the six studies and scores included an external validation component. Only the South Africa study used a temporal internal validation approach, splitting the dataset into derivation and validation components based on the median enrolment date and showing score performance in both derivation and validation datasets.

Predictive accuracy

AUROC of the first-step screening scores were 0.58-0.62, 0.77 (at TBScore II ≥3), and 0.92 in the Vietnam, follow-up TBScore study from Bissau, and Thailand studies, respectively, indicating poor (<0.7), acceptable (0.7-0.79), or outstanding (>0.9) discrimination, respectively (Table 2.12). AUROC of the second step screening scores were 0.52 (at TBScore ≥3), 0.5 (at TBScore II ≥2), 0.631 (at South Africa score ≥3), and 0.75, in the Bissau,

South Africa, and Ethiopia, studies, respectively, indicating poor (<0.7) or acceptable (0.7-0.79) discrimination. Only the Ethiopian second-step score and Vietnam first-step score compared discrimination and screening accuracy of the score versus the WHO standard, with similar or slightly better discrimination of the score versus the WHO standard. In the Vietnam first step screening score, the sensitivity could be increased from the WHO standard of 50% to 100% by adding additional screening variables, and NPV from 94% with the WHO score to 100%. By definition, any second step score is going to have lower sensitivity and lower NPV compared with the WHO screening criteria.

Table 2.12. Screening accuracy of the TB risk scores

	Balcha et al, 2014		**Nanta et al, 2011**	**Nguyen et al, 2011**		**Rudolf et al, 2014 (among PLHIV)**		**Aunsborg et al, 2020**		**Hanifa et al 2017**
	Ethiopia Score	WHO score	Thailand Score	Vietnam Score	WHO score	Bissau TBScore	TBScore II	TB Score	WHO Score	SA score
	*Second step		**First step	First step**		Second step*		First step**	First Step**	Second step*
Number of primary outcomes	137	137	66	28	28	27	27	22	22	110
Characteristics										
AUROC	0.75 (WHO+ and score >=4)	0.70 if WHO screen alone	0.92	0.58-0.62	0.57	>=3: 0.52 >=4: 0.53	>=2: 0.50 >=3: 0.62	>=3: 0.77	NP	>=3: 0.631 >=7: 0.701
Sensitivity	NP	NP	NP	43-100%	50%	>=3: 93% >=4: 78%	>=2: 85% >=3: 74%	>=3: 96%	100%	>=3: 92% >=7: 67%
Specificity	NP	NP	NP	24-79%	64%	>=3:12% >=4: 29%	>=2: 15% >=3: 50%	>=3: 37%	15%	>=3: 34% >=7: 74%
PPV	≤1: PPV (7.8%); 2-3: PPV (27.5%); ≥4: PPV (55.9%)	20.2% among those WHO screen +ve	NP	6-11%	10%			>=3: 23%	NP	
NPV	92% at <=1; 72% at 2-3, and 44% at >=4	94%	NP	91-100%	94%	>=3: 89% >=4: 87%	>=2: 84% >=3: 91%	>=3: 98%	NP	>=3: 97% >=7: 95%

Abbreviations: NP, not provided by the referenced study; AUROC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value; WHO, World Health Organisation; SA, South Africa

*Second step screen is an additional screening rule after a PLHIV has screened positive for one of the four WHO TB symptoms

**A first step screen replaces or supplements the WHO four-symptom TB screen

Simplicity and feasibility of score in LMIC

In general, all the scores included available variables in LMIC clinics such as TB symptoms, clinical measures (e.g., Karnofsky score), temperature, anthropometric measurements (e.g., body mass index, mid-upper arm circumference), and widely available blood tests (e.g., haemoglobin concentration measurements). However, some scores included variables that were more subjective and open to interpretation (e.g., TBScore and TBScore II included findings from chest auscultation), and the other four scores (Ethiopia, Thailand, Vietnam, and South Africa) included the need for CD4 testing, which is not yet widely available in LMIC clinic settings.

2.4.4. Discussion

Overall, the six studies and six clinical TB risk scores had a number of limitations that mean the scores are not easily generalizable outside the study populations included. Three main limitations are common across the studies: (1) lack of external validation, (2) the potential for biased score generation, and (3) use of either variables that are subjective or not rapidly available in LMIC settings.

The need for external validation

Per published guidelines for generating predictive models and scores, external validation in cohorts different to the cohort in which the model and score were developed is important to assess the generalizability of the predictive model and score and therefore utility in settings outside of that in which it was developed.[124] Only one study (the South Africa study by Hanifa *et al*) used a temporal internal validation approach to build confidence in the model and clinical score, but this falls short of external validation in separate settings and cohorts.[124]

The potential for generating biased scores

The study population used for clinical score generation plays an important role in determining which variables will be selected for the clinical score and also the screening accuracy characteristics. For example, two studies from South East Asia (Thailand and Vietnam studies), at initial glance have similar study populations (adult PLHIV outpatients, some of whom are on ART, with 22-29% having a CD4 count <200/μL). However, the prevalence of active TB was markedly different (25.7% in the Thailand study and 7% in the Vietnam study). On deeper examination it is clearer that those enrolled in the Thailand study were already suspected of having TB and had been referred for investigation, whereas those in the Vietnam study represented a less biased study population. Therefore, although the Thailand study is presented as a first-step screening tool, it is in fact a second step screening tool for prevalent active TB among those already reporting symptoms of TB. If the Thailand screening tool were applied as a first screening step in another population of PLHIV, it would possibly not perform as well as it did in the somewhat biased primary study population. In particular, whether the Thailand screening tool is an accurate screening tool in a population of both symptomatic and asymptomatic individuals cannot be ascertained from the data presented.

Variable inclusion suitable and feasible in LMIC

Another limitation of the studies is use of either subjective or difficult to obtain variables in LMIC for the clinical score. The initial and follow-up TBScore studies from Bissau used a 13 variable score (TBScore I) with two main challenges. Firstly, a 13-variable score represents a large number of variables to ask a busy HCW from a LMIC setting to gather during routine care. Currently, it is challenging to support HCWs to screen for just the four WHO TB symptoms in LMIC. For example, the percentage of eligible PLHIV appropriately screened for all four WHO TB symptoms has been reported as 59% in SA,[63] 61% in Mozambique,[62] 4% in Kenya (4%),[125] and 36% in Cote d'Ivoire[98] in recent years. Reasons for low adherence with TB screening protocols are not well understood, but could relate to high patient-to-provider ratios, limited on-the-job training and mentorship, lack

of access to monitoring data, and possibly lack of prompts, either as part of an electronic medical record or on a routine paper form, to screen for TB. Overall, the lesson learned is that the screening tool needs to be very simple. Secondly, the TBScore includes more subjective variables like abnormal findings on auscultation and "anaemic eyes", which leave room for non-standard capture of variables for the risk score. The other four scores (Ethiopia, Thailand, Vietnam, and South Africa scores) all require a CD4 variable, with CD4 not widely available yet in LMIC settings, although a lower cost lateral flow assay to determine CD4 <200 cells/μL is in development.

First step vs. second step screening

Recent WHO guidelines,[88] the recent WHO TB symptom screening meta-analyses,[61] and recent expert opinion[16] all express some level of concern about inability of the WHO TB symptom screen to detect asymptomatic TB and therefore increasingly sub-optimal sensitivity and NPV, especially among PLHIV on ART. By definition, any second step screening approach after the initial WHO four-symptom TB screen is going to have a lower sensitivity and lower NPV than the WHO four-symptom screening rule alone, which does not alleviate the currently most pressing concern emerging in TB screening literature. Of the three first-step screening scores, none are suitable for wide scale-up for reasons already discussed: related to the Thailand score, lack of external score validation, potentially biased derivation study population, and dependence on CD4 limit its use; related to the Vietnam score, lack of external validation and inclusion of CD4 and chest x-ray as potential variables in the score; and related to the TBScore study, lack of external validation, use of a complex 13-level score, inclusion of more subjective variables (auscultation and anaemic eyes) in the score.

Statistical approach

Notably only one study (South Africa study) examined the potential for non-linear relationships between continuous predictors and the log odds of outcome using a fractional polynomial approach per best practice.[126] The other studies used published or

novel cut-offs for continuous variables not derived from the data. Recently, the superior ability of machine learning models to account for non-linear relationships between the continuous predictors and the outcome of interest has been highlighted as a potential advantage of machine learning approaches versus traditional generalised linear regression models. No clinical TB score has yet been derived using the advantages of machine learning approaches, which is a potential area for future research.[127,128]

Other TB screening approaches

Other TB screening approaches, such as use of blood tests for C-reactive protein (CRP) in the initial[129] or second-step screening approach after a positive WHO symptom screen and trial of antibiotics[130] have also been published. Most attempts at using CRP as the initial TB screening test have shown high sensitivity. For example, in one study from KwaZulu-Natal South Africa, sensitivity of both the CRP screening test (>5 vs ≤5) and WHO four-symptom TB screen was 91%,[129] but the CRP test was more specific than the WHO four-symptom TB screen (59% vs. 37%). However, while other studies have reported that CRP as an initial screening test (>10 vs ≤10) had high sensitivity, in some of these prior studies, sensitivity of the CRP screening test was lower than the WHO four-symptom screen standard of care,[131] and in other studies CRP was considered to have little benefit as a screening tool versus the four-symptom screening tool due to no improvement in accuracy or discrimination.[132] One study suggested CRP added value as a second step screening tool after a positive WHO four-symptom screen and trial of antibiotics, but not as an initial screening test.[130] While lower cost point-of-care CRP screening tests are available, this test is not yet widely available in LMIC.[131] In addition, debate about the CRP cut-off and location in the TB screening algorithm continues,[130] and most studies have advocated use of CRP to increase specificity rather than sensitivity.[131] Future inclusion of CRP in clinical scores could yield important additional information rather than using CRP as a single positive or negative screening tool.

2.4.5. Conclusion

In summary, there is no externally validated, feasible, and flexible initial clinical score for TB among PLHIV, including those on ART, which can be used to either improve sensitivity and NPV, or increase specificity and PPV, compared with the WHO four-symptom TB screening rule, depending on the use-case scenario. Use-case scenarios where improved sensitivity in detecting asymptomatic TB and high NPV would be prioritized include: (1) at the point of HIV care or ART enrolment where undiagnosed TB prevalence is relatively high and intensified TB case finding to reduce TB-related mortality is the focus, and (2) prior to provision of TPT (i.e., among ART-naïve or ART-experienced PLHIV who have not yet received TPT).[16] Use-case scenarios where higher specificity and PPV might be prioritized would be among those PLHIV stable on ART who have received TPT and among whom improving cost-effectiveness of TB screening approaches (i.e., lowering the NNS) would be a higher public health priority. In addition, there has been little focus stratifying TB risk among PLHIV to inform differentiated TB-HIV care algorithms for sub-Saharan Africa. A new externally validated screening tool that addresses this gap could be very useful in sub-Saharan Africa, where early ART mortality due to undiagnosed TB remains high, global efforts to scale-up TPT to >13 million PLHIV are ongoing, and resource-limitations demand investigation of opportunities to improve efficiency through differentiated service delivery models.

Chapter 3. Thesis aim, research questions, and study setting

3.1. Aim

The overarching aim of this thesis was to investigate opportunities to reduce early mortality on ART specifically, and HIV-TB mortality more broadly, among PLHIV in sub-Saharan Africa, through improved approaches to TB screening, diagnosis, and retention in HIV care.

3.2. Specific research questions

To meet this over-arching aim, I specifically aimed to address the following research questions:

1. Compared with standard of care in Botswana, what is the impact of the following package of interventions on early (6-month) adult ART mortality rates: (1) additional support for ICF, (2) intensified tracing for patients missing clinic appointments to return them to care, and (3) Xpert replacing sputum-smear microscopy.

Note: this was a co-primary objective of the Botswana Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES) trial (ClinicalTrials.gov: NCT02538952).[18]

2. Compared with the current WHO advanced disease eligibility criteria for early ART care intensification, can predictive clinical scores tailored for settings that (a) do not have access to rapid CD4 count testing, and (b) do have access to rapid CD4 count testing, better predict who is at risk for early (6-month) ART mortality and therefore in need of early ART care intensification?

3. Can a predictive clinical score, developed using easily available covariates in resource-constrained clinic settings in sub-Saharan Africa, predict TB risk among PLHIV better than the current WHO four-symptom screening rule?

3.3. Setting the scene

3.3.1. Botswana

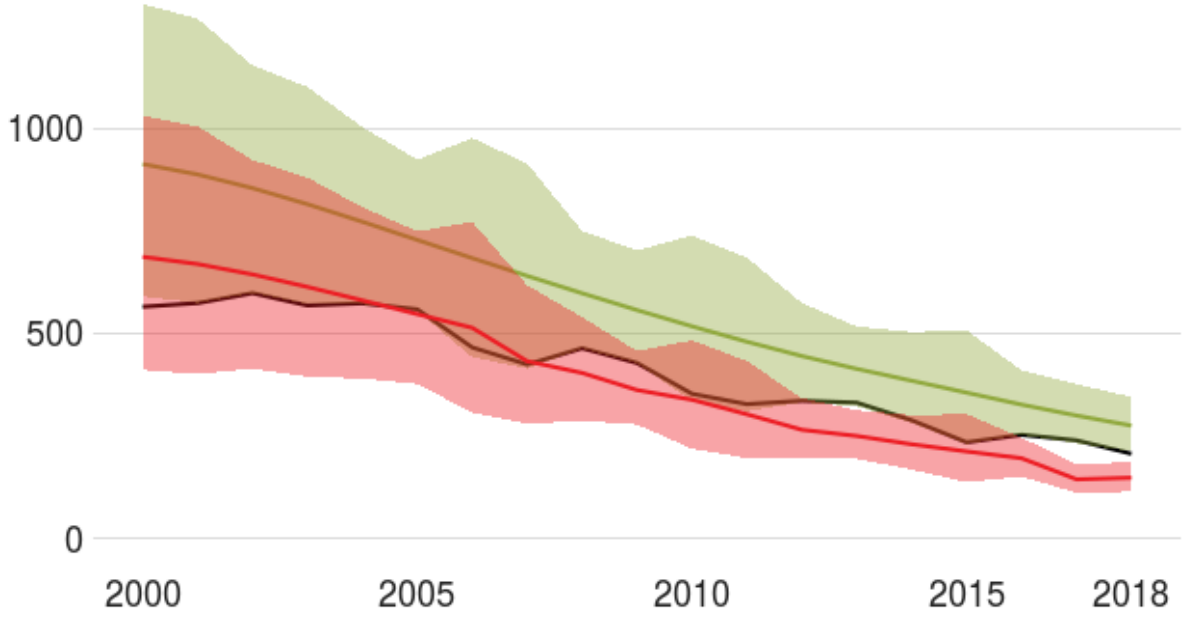
In 2018, Botswana was ranked 94 out of 189 countries on the human development index (HDI),[133] with a Gross Domestic Product (GDP) per capita of about $15,000 making it an upper middle-income country, with economic growth since independence in 1966 fuelled by significant mineral (diamond) wealth, good governance, prudent economic management, and a relatively small population of slightly more than two million.[134] Most of the population (71%) reside in urban centers and life expectancy in 2020 was estimated at 69.9 years.[135]

Botswana has the third highest prevalence of HIV-infection among adults aged 15-49 globally (22%), however comprehensive and effective treatment programs have reduced HIV/AIDS-related deaths substantially from 17,000/year in 2004 to 4,800/year in 2018.[136] In 2020, Botswana's progress to 90-90-90 is estimated at 91-92-95, making it one of the first countries in the region estimated to have reached 90-90-90 targets.[1]

In 2018, overall TB incidence was estimated at 275 (95% CI, 213-345) per 100,000 population and incidence of HIV-associated TB in the total population was estimated at 148 (95% CI, 114-186) per 100,000 population, with incidence rates steadily declining since 2000 (Figure 1). Therefore, about half of TB cases in Botswana are estimated to be among PLHIV. Multi-drug resistant TB is estimated to be low (13/100,000 population) but increasing in prevalence among TB cases over time (from 0.2% in 1996 to 2.5% in

2008).[16,137] Annually there are an estimated 560 deaths from TB among HIV-negative persons and 1,200 deaths from TB among HIV-positive persons.

Figure 3.1. Estimated total TB incidence (green), new and relapse TB cases notified (black), and HIV-positive TB incidence (red) in Botswana — 2000–2018*



*Taken from the most recent WHO Botswana Country Profile report.[138]

The Botswana National TB Programme was established in 1975. National guidelines since 2011 have placed significant emphasis on coordinated TB-HIV services, although in most cases TB treatment clinics are run separately from HIV treatment clinics in terms of location of the clinic or timing of the clinic if the TB and HIV treatment clinics are co-located on the same health facility grounds. Laboratory diagnosis using sputum smear microscopy was the mainstay of TB identification for PLHIV prior to rollout of Xpert as the first line TB diagnostic starting in 2012. Although TB culture using mycobacterial growth indicator tubes was available at the central National TB Reference Laboratory in Gaborone, culture was seldom requested in practice for PLHIV suspected of having TB (i.e., those PLHIV who screened positive for one of cough, fever, loss of weight, or night sweats).[18] Chest radiography was recommended for TB-symptomatic PLHIV who tested negative for TB using either sputum-smear microscopy, or Xpert after Xpert was rolled out

in 2012. Our XPRES study activated the first 13 Xpert devices in service of PLHIV in a phased manner as described in Chapter 4. Nurse practitioners, in addition to medical doctors, are allowed to initiate and monitor TB treatment. Since 1993 Botswana adopted directly observed therapy for all patients for the entire TB treatment period, with community healthcare workers supporting direct observed therapy implementation where possible and implementation of direct observed therapy affected by logistical challenges (e.g., cost of transport for the patients, work load for healthcare providers).[139]

3.3.2. South Africa

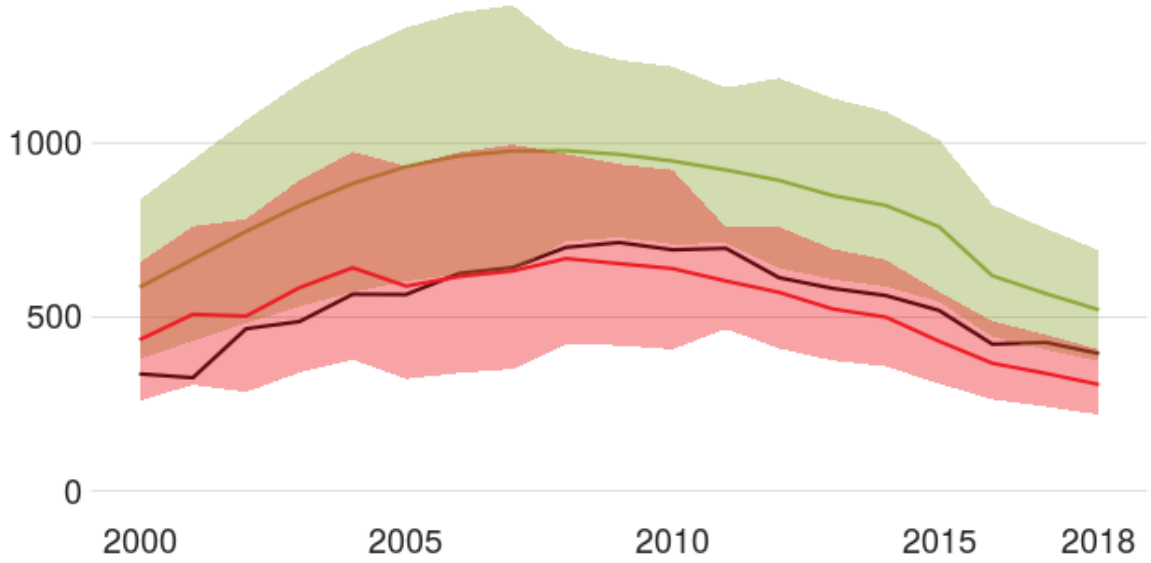
For the second and third thesis research questions, data from three prospective clinical cohorts from South Africa were used to validate the clinical scores derived from the XPRES dataset. The three cohorts are: (1) TB Fast Track (TBFT) trial enrolees from Gauteng, Limpopo, and North West Provinces in South Africa, which represents a homogenous ART-naïve population with advanced HIV disease;[117] (2) prospective cohort data for XPHACTOR enrolees from Gauteng province, South Africa, which represents a predominantly stable, long-term ART population;[123] and (3) prospective cohort data from the Western Cape, South Africa, which represents an ART-naïve population in a very high TB incidence setting.[140]

In 2018, South Africa was ranked 113 out of 189 countries on the human development index (HDI), with a Gross Domestic Product (GDP) per capita of about $12,000 making it a middle-income country.[141] Economic growth has stagnated recently with rising unemployment (27%) and significant socio-demographic inequalities in wealth across the population of about 57.8 million. Most of the population (67%) resides in urban centers and life expectancy in 2020 was estimated at 63.9 years.[142]

In 2018, overall TB incidence was estimated at 520 (95% CI, 373-691) per 100,000 population with HIV-positive TB incidence at 306 (95% CI, 219-406) per 100,000

population, with incidence rates rising between 2000 and 2008 and then declining since 2009.[138] Over half of TB cases in South Africa are estimated to be among PLHIV. Multi-drug resistant TB is estimated to be 19/100,000 population. Annually there are an estimated 21,000 HIV-negative TB deaths and 42,000 HIV-positive TB deaths.

Figure 3.2. Estimated total TB incidence (green), new and relapse TB cases notified (black), and HIV-positive TB incidence (red) in South Africa — 2000–2018*



3.4. Key challenges facing the Botswana national ART programme at the time of XPRES

As described above, Botswana is one of the worst affected countries by the HIV pandemic with nearly one in four adults living with HIV. To respond to the national HIV epidemic, the Government of Botswana launched its national antiretroviral therapy (ART) program called "Masa", which means "a new dawn" in Setswana, on January 21, 2002. After 12 years of scale-up, by the end of 2014, the Masa program had expanded to more than 302 healthcare facilities and the number of patients ever initiated on ART had reached 247,856, of which 229,133 were in the public sector. At the time of XPRES trial initiation in 2012, early mortality on ART especially due to undiagnosed TB,[143] and loss to follow-up

during ART and from TB-HIV care cascades were considered important challenges facing the Masa program.[32] For example, in an observational cohort study of 226,030 adult patients starting ART during 2002–2013, mortality (uncorrected for mortality among ART enrolees LTFU) during the first three months of ART was 11.8 per 100 person-years (95% CI, 0.98-1.04), but dropped to 1.0 per 100 person-years (95% CI, 0.98-1.04) among PLHIV in the time period after one year of completed ART.[32] In addition, LTFU during the first year of ART was 14.9 per 100 person-years (95% CI, 14.7-15.1) overall, and LTFU rates among ART enrolees was observed to have increased from 7.7 per 100 person-years for ART enrolees in 2003 to 22.5 per 100 person-years for ART enrolees in 2011.[32] In addition, in the only pathological autopsy evaluating causes of death among adult PLHIV in Botswana in 2002, 38% of deaths were due to TB, and 90% of TB cases had both pulmonary and extra-pulmonary disseminated TB.[143] These challenges were important in informing the XPRES trial intervention package which is described in Chapter 4.

Chapter 4. Methods

This Chapter describes methodology used to answer the three thesis research questions described in Chapter 3. A summary of the XPRES trial methods is provided in section 4.1. with additional details provided in the published manuscript in Chapter 5 and the published protocol, which can be found as Appendix 1. An overview of analytic methods used to develop and validate the early ART mortality risk scores is provided in section 4.2., and analytic methods used to develop and validate the TB risk score for PLHIV in section 4.3.

4.1. XPRES trial summary

4.1.1. Primary trial objectives

In 2012, as a pilot for Botswana's national Xpert MTB/RIF (Xpert) rollout plans, I designed a study called the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES) trial.[18] XPRES had two co-primary objectives. The XPRES co-primary objective reported in this thesis, and the first key research question for this thesis as described in Chapter 3, was to evaluate the impact of a package of interventions comprising (1) additional support for TB screening and intensified TB case finding (ICF) algorithms, (2) active tracing for patients missing clinic appointments to support retention, and (3) Xpert replacing sputum-smear microscopy, on early (6-month) antiretroviral therapy (ART) mortality.[18] The other XPRES co-primary objective, which aimed to compare diagnostic sensitivity of the new Xpert-based TB diagnostic algorithm with that of the sputum-smear-microscopy-based algorithm is not part of this thesis.

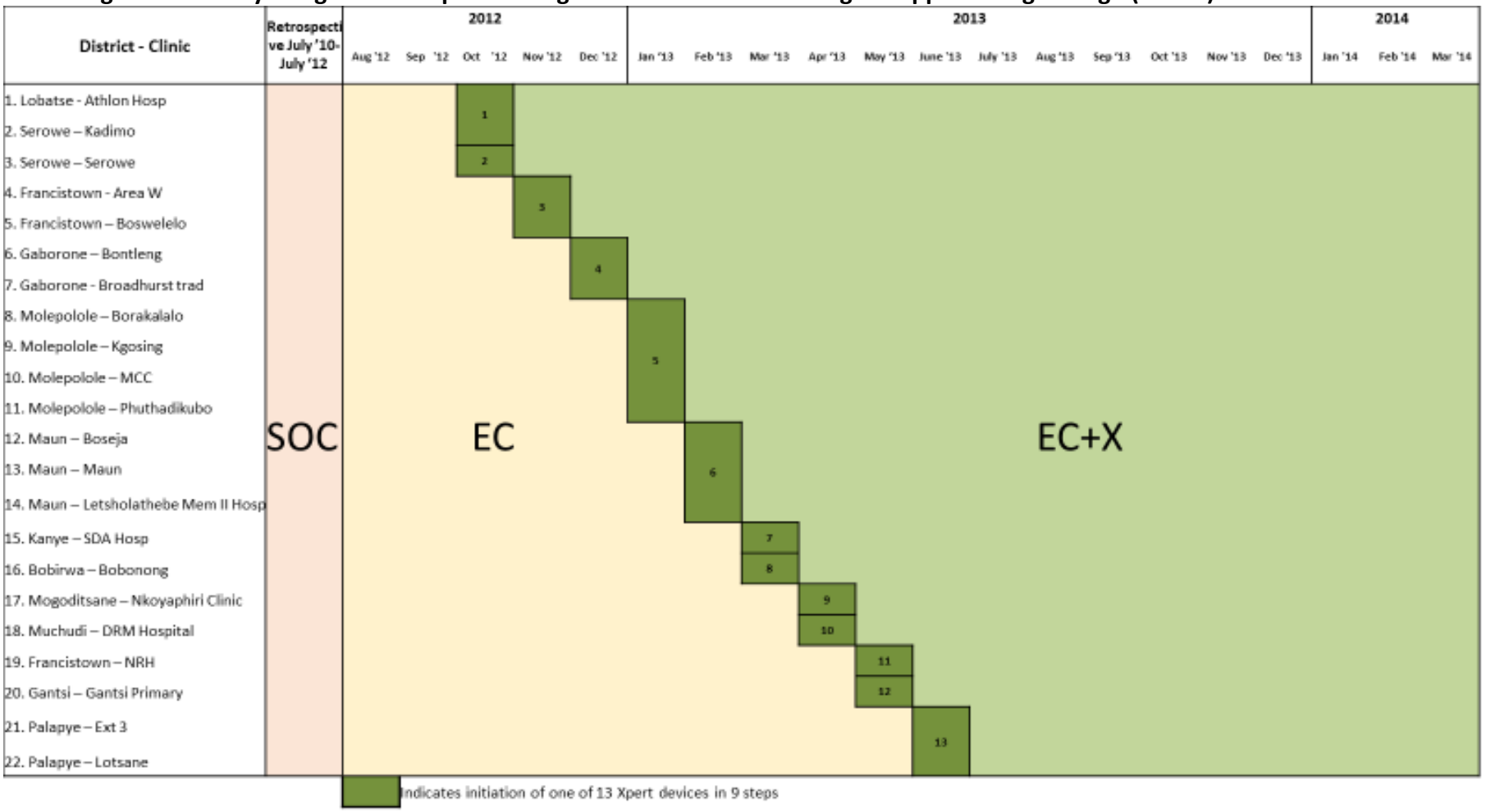
4.1.2. Secondary trial objectives

Two secondary XPRES trial objectives are also reported in this thesis as follows: (1) to evaluate impact of the TB screening, retention, and Xpert package on 12-month ART mortality compared with standard of care, and (2) to evaluate impact of Xpert compared with smear microscopy on all-cause, adult, 6-month ART mortality.

4.1.3. XPRES Study design

The XPRES stepped-wedge cluster randomised trial (CRT) design with a retrospective baseline component is illustrated in Figure 4.1. XPRES was conducted at 22 clinics purposively chosen to be representative of HIV care and treatment clinics in Botswana. All HIV clinic enrolees >12 years old, except for PLHIV who were incarcerated, were eligible for inclusion in the study in three phases: a retrospective standard of care (SOC), prospective enhanced care (EC), and prospective EC plus Xpert (EC+X) phase (Figure 4.1.). EC and EC+X phases were implemented as a stepped-wedge trial. Participants in the EC phase received SOC plus components 1 (strengthened ICF) and 2 (active tracing) of the intervention package, and participants in the EC+X phase received SOC plus all three intervention package components.

Figure 4.1. Study design for the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES)



Abbreviations: SOC, Standard of Care; EC, Enhanced Care; EC+X, Enhanced Care plus Xpert

4.1.4. Study design rationale

A pragmatic stepped-wedge cluster-randomised trial design with a retrospective component was chosen for the following reasons. A cluster-randomised design (i.e., for the EC vs. EC+X comparison) was chosen because Xpert device activation was most feasibly achieved for an entire district TB laboratory, which often served more than one health facility; this fact made an individually randomised controlled trial design less desirable.[144] A stepped-wedge rather than parallel-group design was chosen because: (1) at the time, according to WHO guidance[77] and Ministry of Health guidelines,[137] the Xpert device was expected to be beneficial for both patients and providers, and therefore it was considered ethically sub-optimal to implement a parallel group cluster-randomised trial, where certain district TB laboratories and their associated clinics were denied access to Xpert for an extended period of time,[78,145] (2) the phased rollout of Xpert provided logistical advantages, because it meant that a single site activation team, in charge of training and activation of the Xpert device, could sequentially initiate all study sites,[145] (3) the need for only a single site activation team reduced projected study cost, and (4) in a real-world setting, the sequential rollout of an intervention allows lessons learned during earlier steps to be applied during later steps.

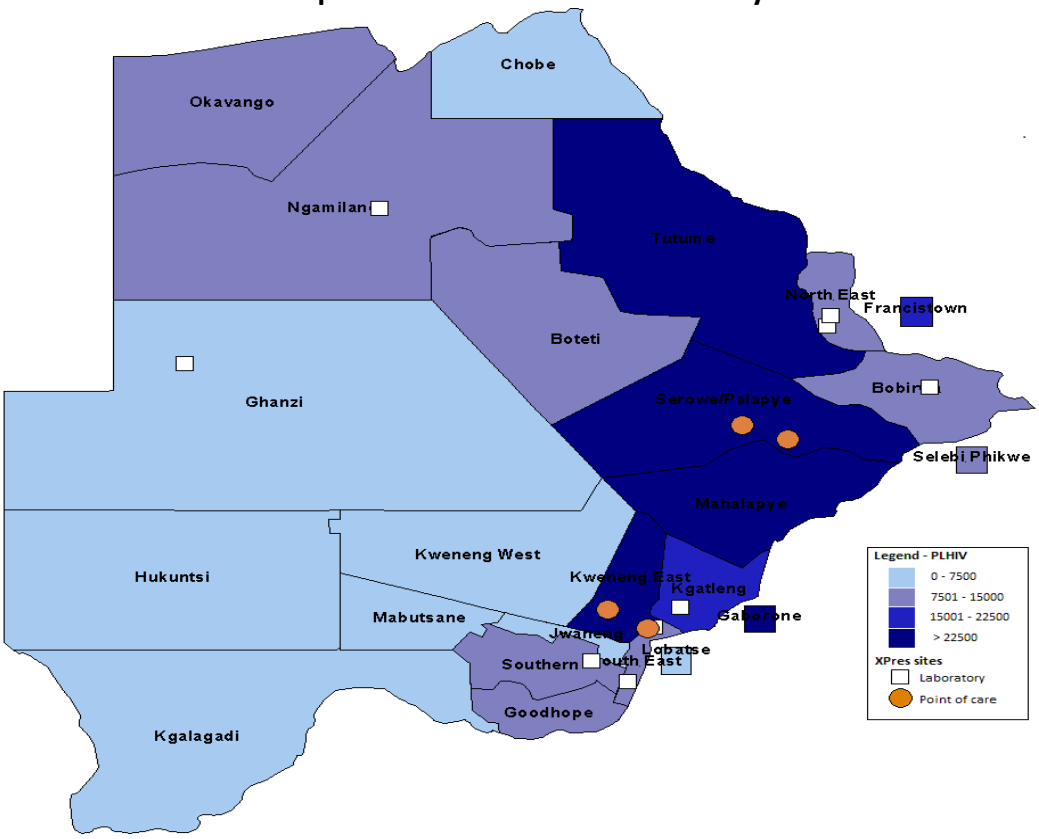
The primary study question, however, was addressed through a pre versus post comparison (i.e., a comparison of 6-month ART mortality between SOC and EC+X phases). The main reasons a pre- versus post-design was chosen related to sample size, Ministry of Health preferences concerning speed of Xpert rollout, funding availability, desire for a more pragmatic study design to increase generalizability of findings related to the primary study question, and opportunity to address a different study question than other Xpert trials.[146] As described in the sample size section below (4.1.11), we estimated a possible 40% impact of the Xpert package versus standard of care on early ART mortality. To observe a 40% difference in 6-month mortality rates within a stepped-wedge trial would have required a very large and lengthy EC and EC+X prospective cohort enrolment. This was not feasible because: (1) the MOH preferred the 13 Xpert devices be rolled out as soon as possible due to Xpert superiority over smear microscopy in terms of diagnostic

accuracy;[81] and (2) there was insufficient funding for a stepped-wedge trial of the needed size to detect a >40% reduction in all-cause mortality (>10,000 prospective trial enrolees). In addition, since investigators wanted the study findings related to intervention package impact on mortality to be generalizable to other settings in sub-Saharan Africa, a pragmatic design was needed and the pre-requisite for a pragmatic trial is that the standard of care arm or phase needs to reflect true standard of care.[147] Prospectively enrolling the standard of care cohort would likely alter the true standard of care as it did in many other Xpert impact evaluation trials.[148] In Chapter 8, Section 8.2.2., the thesis provides more detail on why investigators consider the XPRES trial design a pragmatic design. In addition, by implementing three phases, XPRES was positioned to answer a question other Xpert trials were not expressly designed to answer, namely what is the impact of the Xpert package of interventions (i.e., strengthened TB screening, retention, and Xpert rollout) on early ART mortality?[18] As a secondary trial objective, the trial aimed to examine, using data from the stepped-wedge portion of the trial, representing a controlled, strengthened health system with high completion of both smear microscopy and Xpert diagnostic algorithms, whether Xpert provided additional benefit in terms of patient-important outcomes. However, similar to most trials,[149,150] XPRES was powered to meet the primary study objective (i.e., the pre versus post comparison of 6-month ART mortality between SOC and EC+X phases) and was not powered to answer this secondary objective due to the timeline and funding limitations described above.[149,150]

4.1.5. Cluster eligibility criteria

A cluster was defined as an HIV care and treatment clinic. Twenty-two clusters, located at five district hospitals and 17 primary healthcare facilities, were purposively selected to: (1) be representative of HIV treatment clinics in Botswana, and (2) have new ART initiation rates sufficient to meet sample size requirements (all clinics had $\geq$8 new ART enrolees per month, range 8-46/month according to routine programme data).

Figure 4.2. Location of 13 Xpert devices in service of 22 study clinics



4.1.6. Study enrolee eligibility criteria

At these 22 clusters, individual patients were eligible for study enrolment if they were new HIV clinic attendees, regardless of TB treatment status, and not prisoners at the time of the first HIV clinic visit. For the XPRES primary outcome trial analysis, only those study enrolees who newly started ART at or after study enrolment and were >12 years old at ART initiation were included.[18]

4.1.7. Randomisation and masking

The selected 22 clusters received TB diagnostic services from 13 laboratories (Figure 4.1.). Because some of the study clinics used the same TB diagnostic laboratory, full Xpert, ICF, and retention package activation was planned to be simultaneous for these clinic consortiums (Figure 4.11). After obtaining ethical approvals and agreement to participate in the study from MOH at a central level and MOH management at the selected facilities,

the study statistician randomly selected one of the rollout permutations.[18] Participants, investigators and health facilities were not blinded to their phase of enrolment because it was considered unfeasible and also ethically sub-optimal to blind health care providers (who might make different empiric TB treatment decisions depending on the diagnostic used).[137,151]

4.1.8. Procedures

At the 22 clusters, per Botswana national guidelines during the time period of the study (July 2010 through June 2015), all study participants in all phases were eligible for ART initiation if they had a CD4 count ≤350 cells/μL, were diagnosed as having WHO stage III/IV, or were pregnant or breastfeeding.[152] All study participants received clinical care and follow-up appointments according to MOH guidelines, with follow-up schedules described as Additional File 2 of the trial manuscript in Chapter 5.

Standard of care phase

Enrolment in the retrospective SOC phase was through chart abstraction of eligible adult patients who started ART between July 2010 and the end of July 2012 (Figure 4.1.) (Appendix 1 and 2).[18] The SOC phase enrolees received HIV care according to national guidelines, limited ICF, infrequent active tracing due to resource limitations, and sputum-smear microscopy for presumptive TB patients.

Intervention phases EC and EC+X

Prospective EC enrolment started in August 2012 and was complete by January 2013. Prospective EC+X enrolment occurred from October 2012 through March 2014 according to the stepped-wedge design (Figure 4.1.). At enrolment, after written informed consent procedures were followed, standard baseline and follow-up questionnaires were completed as described in Appendices 1 and 2. EC phase participants received SOC supplemented by two components of the Xpert, ICF, and retention package (i.e., additional support for ICF and intensified tracing) combined with sputum-smear microscopy (Figure 4.3.). EC+X phase participants received SOC supplemented by all three

components of the Xpert, ICF, and retention package (i.e., additional support for ICF, intensified tracing, and Xpert in place of sputum-smear microscopy) (Figure 4.3.). All interventions were activated at the cluster-level for the benefit of all clients receiving care at the clinic. Figure 4.3 below summarises the key differences between the SOC and intervention phases.

Figure 4.3. Comparison of interventions introduced in the EC and EC+X phases

Intervention component	Standard of Care (SOC)*	Enhanced Care (EC)	EC + Xpert (EC+X)
TB screening and ICF	Weak	Strengthened	Strengthened
Active tracing for missed appointments to support retention	Infrequent	Strengthened	Strengthened
TB Diagnostic	Smear microscopy	Smear microscopy	Xpert

Abbreviations: ICF, intensified TB case finding

EC and EC+X participants were followed for 12 months, or until the end of TB treatment, whichever was later. The final follow-up visits for EC+X enrolees were in June 2015.

Interventions

The ICF and active tracing interventions were strengthened through four key mechanisms: (1) additional human resources (study nurses) to support implementation, (2) additional training for clinic and laboratory personnel, (3) use of checklists and job aids to standardize implementation, and (4) regular supervisory visits to track adherence to ICF and tracing checklists.

Regarding the ICF intervention, implementation of the WHO 4-symptom TB screening rule (i.e., screening for cough of any duration, fever, loss of weight, and night sweats) was recommended for all enrolees at each clinic visit in the SOC, EC, and EC+X phases, but implementation was strengthened in the EC and EC+X phases. In all phases, clients were considered symptomatic if they screened positive for one or more of the four TB symptoms. In all phases, at least two same-day, on-the-spot (spot) sputum samples were recommended for collection from symptomatic clients. As part of strengthened ICF in the EC and EC+X phases, a previously published job-aid was used by study nurses to inform the patient how to collect quality sputum samples. Prior to the EC phase, laboratory personnel at the 13 laboratories serving the 22 clusters received refresher training on Ziehl-Neelsen staining for sputum-smear microscopy and, prior to the EC+X phase, laboratory personnel were trained for Xpert implementation. In all phases, sputum test results were returned to the clinics, with clinicians responsible for informing the patients. In the SOC phase, the patient was informed of a TB diagnosis at the next scheduled clinic appointment. In the EC and EC+X phases, study nurses were trained to work with laboratories to ensure the turnaround time from sample collection to result return to the clinic was ≤4 days for sputum-smear microscopy and ≤2 days for Xpert testing. In the EC and EC+X phases, nurses were trained to inform patients of positive TB diagnoses the same day via phone, or if unreachable by phone, by active tracing to the household. Indicators monitoring implementation of the ICF cascade were collected and used to inform supervision visits (see Additional file 3 of Chapter 5, a table summarizing the indicators)

Per national guidelines, clients ≥1 day late for an HIV clinic appointment should be traced through phone and home visit starting the day after the missed visit. However, programme reports showed this tracing was infrequently implemented in the SOC phase due to lack of human and financial resources. Implementation of the active tracing policy was strengthened in the EC and EC+X cohorts. In the EC and EC+X phases, a patient locator form was used to document telephone numbers and home addresses for intensified tracing activities to support retention. Up to five telephone calls and two

home visits, facilitated by checklists, were used in attempts to return clients, who had missed clinic appointments, to care. The key HIV care retention indicator used for monitoring purposes was the rate of loss to follow-up (LTFU) per 100 person-years (see Additional file 3 of Chapter 5, a table summarizing the indicators). LTFU was defined as being >60 days late for a scheduled appointment, per Botswana guidelines.

4.1.9. Primary trial outcome

In XPRES, intensive efforts were implemented to ascertain true mortality outcomes among trial participants. All-cause mortality was either reported passively to clinics by friends or family of the decedents or, if a client was considered lost to follow-up, telephonic tracing, home visits, and if tracing was unsuccessful, review of the national mortality register, were conducted, with further details provided in Chapter 5 and in the published protocol (Appendix 1).

4.1.10. Sample size

To estimate power for the comparison of all-cause 6-month mortality in the SOC versus EC+X cohort, the approach of Moulton *et al*, suitable for stepped-wedge trial designs, was chosen because these power estimates were more conservative than those derived from a pre-post sample size calculation.[149] Per this approach, published formulae for the comparison of two rates in an unmatched parallel group CRT[153] were adapted to the stepped-wedge design as follows:

$$Z_\beta = \sqrt{\frac{(c-1)(r_c - r_t)^2}{[r_0/y_c + r_1/y_t + k^2(r_0^2 + r_1^2)]}} - Z_{\alpha/2}$$

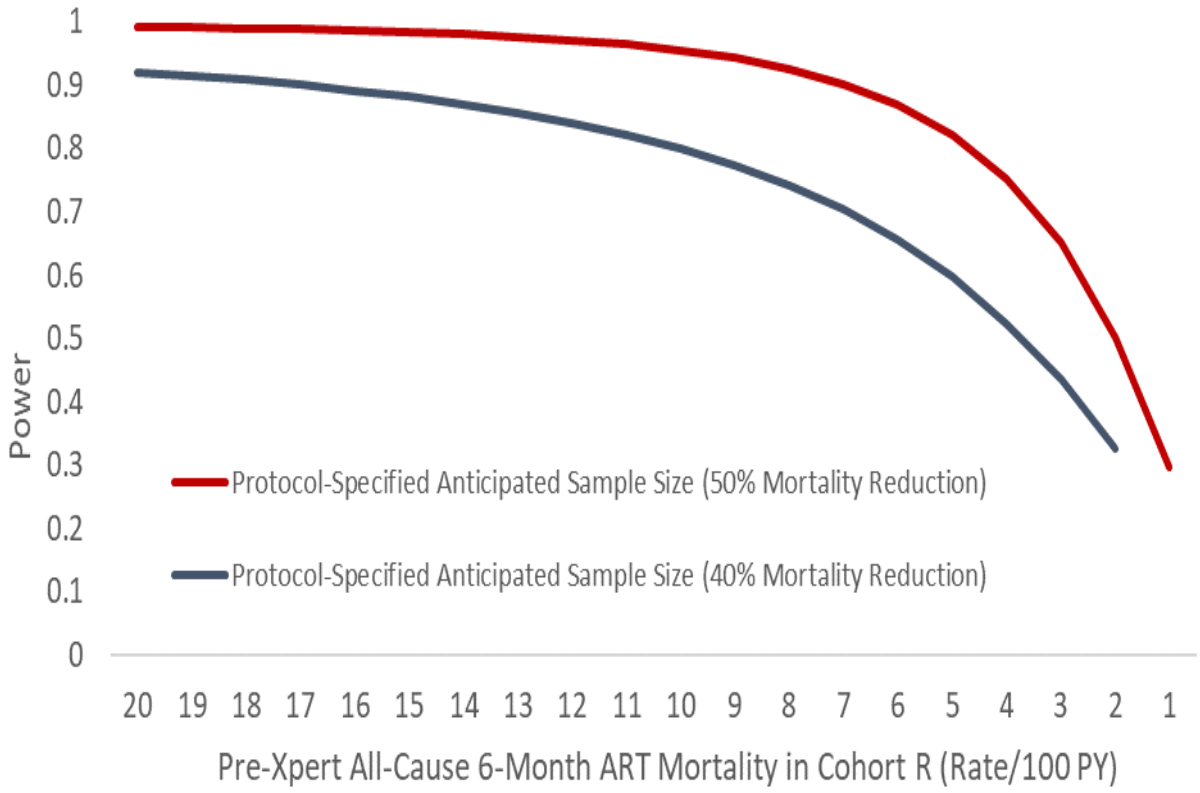
where Z_β is the standard normal deviate corresponding to the upper tail probability of β and β is the probability of a Type II error; c is the number of clusters (study facilities) per arm, where, since this is a stepped-wedge trial involving 22 clinics, 22/2 was used;[149] r_c is the estimated true 6-month ART mortality rate in the SOC phase; r_t is the estimated true mortality rate in the EC+X phase; y_c is the average number of person-years per clinic in the SOC phase, estimated as the average retrospective cohort size per clinic (552) divided by

two since each patient commits 6 months of follow-up time to the analysis; y_t is average number of person-years per clinic in the intervention phase, conservatively estimated as the harmonic mean of person-years contributed by each study site in EC+X, again assuming 6 months of follow-up time per participant;[149] k is the estimated between-cluster coefficient of variation of the true rates in both the SOC and EC+X phases, estimated as 0.2;[149] $Z_{\alpha/2}$ is the standard normal deviate corresponding to the upper tail probability of $\alpha/2$ where α is the probability of a Type I error.

Since a log-rank test statistic for intervention effect calculated for a simulated stepped-wedge trial (Z_{SW}) will generally always be lower than the corresponding statistic (Z_E) for a parallel group trial, because allocation ratios of patients to intervention or control status for parallel group trials remain equal while for stepped-wedge trials they are usually unequal, except at the mid-point of the stepped-wedge design, the z-score in the stepped-wedge trial formula (Z_β above) was divided by a published estimate of Z_E/Z_{SW} (i.e., 1.2) prior to extrapolating the z-score to a power estimate.[149] Similarly, for Type 1 error of 5%, instead of assuming a $Z_{\alpha/2}$ of 1.96, an inflated estimate of 2.352 was used, per published precedent.[149]

Prior to study start, available data from Botswana suggested that the documented all-cause early mortality rates in the first 6 months of ART among adults were about 15 deaths per 100 person-years,[154] which was similar to estimates from a meta-analyses of 18 programs in LMIC with active tracing programs (14.7/100 PY).[23] Since Botswana data and available meta-analyses suggested about 40% of deaths among PLHIV were due to undiagnosed TB or TB diagnosed late, and given that interrupting ART during the first 6 months of therapy by missing clinic appointments increases mortality risk;[67,155] it was considered reasonable that the Xpert package plus the tracing intervention might reduce mortality by about 40%.[143,156] To provide >80% power to detect a ≥40% reduction in all-cause 6-month ART mortality between the two SOC and EC+X groups, assuming SOC mortality was ≥10/100 person-years, a 24-month SOC phase enrolment period (N=12,144) and an 18 month EC+X phase enrolment period (N=6,348) were chosen.

Figure 4.4. Power to detect a 40% and 50% difference in all-cause 6-month ART mortality between SOC and EC+X cohorts over a range of pre-ART SOC mortality rates



Abbreviations: ART, antiretroviral therapy; SOC, standard of care; EC+X, enhanced care plus Xpert

4.1.11. Laboratory procedures

Sputum collection

Implementation of the WHO four-symptom TB screening rule (i.e., screening for cough of any duration, fever, loss of weight, and night sweats)[60] was recommended for all enrolees at each clinic visit in the SOC, EC, and EC+X phases, but implementation was strengthened in the EC and EC+X phases. Implementation of four-symptom TB screening and all components of the ICF cascade were strengthened by situating one additional study nurse at each study facility, additional training for the health facility in ICF cascades, provision of checklists and job aides, and supportive supervision and mentorship. In all phases, clients were considered symptomatic if they screened positive for one or more of the four TB symptoms. In all phases, at least two same-day, on-the-spot (spot) sputum samples were

recommended for collection from symptomatic clients. As part of strengthened ICF in the EC and EC+X phases, a previously published job-aid was used by study nurses to inform the patient how to collect quality sputum samples.[18] In addition, where feasible, EC and EC+X enrolees were asked to return to the clinic on the second day after screening symptom-positive for TB to provide a morning sputum sample and third on-the-spot sputum sample primarily to meet other study objectives not covered in this thesis.

Laboratory procedures

Prior to the EC phase, laboratory personnel at the 13 laboratories serving the 22 clusters received refresher training on Ziehl-Neelsen staining for sputum-smear microscopy and, prior to the EC+X phase, laboratory personnel were trained for Xpert implementation. Two spot sputa were sent to the on-site or peripheral district TB lab for: (1) smear microscopy, and (2) Xpert, if the Xpert device had been activated by that time. The other sputum samples were sent to the national TB reference lab to meet other study objectives not covered in this thesis (Appendix 1).

Xpert activation

Training for GeneXpert operators consisted of a three-day curriculum. The training covered the theoretical basis of the Xpert test, how to operate the instrument, interpretation of results, troubleshooting, and GeneXpert maintenance (daily, weekly, and monthly). GeneXpert operators were provided a standard operating procedure (SOP) manual to serve as a reference for GeneXpert operation. The third day of the training was hands-on operational training and all trainees had to pass a competency test before testing patient specimens from study sites. The GeneXpert was initially installed by a local GeneXpert vendor who provided calibration and maintenance services during the study, Xpert cartridge sales, and cartridge delivery services.

Air conditioning units were also installed with each GeneXpert to ensure control of room temperature. Uninterruptible power supply (UPS) systems were installed with each GeneXpert to ensure sufficient electrical power to allow completion of in-process sample

analysis during power grid outages. Xpert cartridges were procured through the same vendor at $18 per cartridge at the time, which included the cost of the cartridge, central warehousing, and delivery to sites when requested.[157]

Turn-around times

In all phases, sputum test results were returned to the clinics, with clinicians responsible for informing the patients. In the SOC phase, the patient was informed of a TB diagnosis at the next scheduled clinic appointment. In the EC and EC+X phases, study nurses were trained to work with laboratories to ensure the turnaround time from sample collection to result return to the clinic was ≤4 days for sputum-smear microscopy and ≤2 days for Xpert testing. In the EC and EC+X phases, nurses were trained to inform patients of positive TB diagnoses the same day via phone, or if unreachable by phone, by active tracing to the household.

4.1.12. Data collection and management

Paper trial data collection forms (Appendix 2) were completed by study nurses, evaluated for completeness and consistency by study nurse supervisors, and then transported securely to Gaborone CDC Botswana offices, where data were double-entered into Clindex Clinical Trial Software (Fortress Medical Systems, Inc). The data entry software included completeness and consistency checks. Missing data and inconsistencies were corrected where possible through liaison with study nurses at the sites, with corrections made to paper forms, and corrections signed by both study nurses and study nurse supervisors.

4.1.13. Trial statistical analysis

For the primary outcome analysis, time at risk for ART enrolees started on the day of ART initiation and ended at 6 months of follow-up after ART initiation, or at the time of death, LTFU, or transfer out if these events were before 6 months of ART follow-up. Crude and multivariable Cox proportional hazards regression models, with a random effect for clinic, were used to assess the effect of intervention status (SOC versus EC+X) on time to death.[18]

Secondary objective analyses were conducted to: (1) compare 12-month ART mortality between SOC and EC+X phases, and (2) compare 6-month ART mortality rates between cohorts EC and EC+X.[18] For the latter I used analytic methods described by Moulton *et al*, fitting Cox proportional hazards models to the data with the underlying time frame being time since August 2012 (initiation month for the stepped-wedge component of the trial), fixed effect for intervention arm (Xpert device activation), and a random effect for clinic.[149] I also implemented several sensitivity analyses to assess robustness of primary trial findings to different analytic approaches which are described in the trial manuscript in Chapter 5.

4.1.14. Ethical considerations

Ethical approvals for this study were obtained from the U.S. Centers for Disease Control and Prevention (CDC) Institutional Review Board (IRB) C, the Health Research and Development Division of the Health Research and Development Committee (HRDC) in Botswana, the University of Pennsylvania IRB No.4, and the London School of Hygiene & Tropical Medicine (LSHTM) Observational/Interventions Research Ethics Committee (LSHTM ethics reference number: 11779). All ethical approvals are provided as Appendix 3. All consent procedures were approved by the ethical review committees. For the SOC cohort, a waiver of informed consent for chart abstraction was granted in accordance with 45CFR 46.116 (d). Written informed consent was obtained from all EC and EC+X enrolees. XPRES is registered at ClinicalTrials.gov (trial registration no. NCT02538952).

4.2. Early ART mortality risk score development

I used data from the EC and EC+X phases of the XPRES trial to derive two clinical scores to help clinicians identify those at highest risk of early ART mortality and therefore in need of ART care intensification.[18] The first clinical score assumes CD4 is unavailable at ART initiation (i.e., a CD4-independent score) and the second clinical score assumes CD4 count is available (i.e., a CD4-dependent score). I considered the XPRES EC and EC+X cohorts as a single cohort for this analysis because there were no differences in 6-month ART

mortality or major differences in other cohort characteristics between EC and EC+X phases as described in Chapter 5. I split the cohort temporally at the mid-point date of ART initiation and used the first 50% of XPRES EC and EC+X cohort enrolees to derive a parsimonious, multivariable, logistic regression prognostic model for 6-month all-cause ART mortality, and the second 50% to internally validate the model.[124,126] I used data from the TB Fast Track (TBFT) trial in South Africa (SA) to externally validate the derived clinical scores.[117] I then compared screening accuracy in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the derived clinical scores with existing WHO eligibility criteria for advanced disease and ART care intensification (i.e., CD4 <200/µL or WHO stage III/IV). Full details of the analytic approach are provided in the manuscript in Chapter 6.

4.3. Development of a risk score for TB among PLHIV

I again used data from the from the EC and EC+X phases of the XPRES trial to derive the TB clinical score.[18] The outcome of interest was prevalent active TB, defined as a new clinical or microbiological diagnosis of TB within the first 6 months after HIV clinic and XPRES trial enrolment.[123] In this analysis, I split the XPRES cohort data into 11 southern and 11 northern clinics to serve as an internal derivation and validation datasets, respectively. I used a geographical split rather than a temporal split because, although there was no difference in overall new TB case finding between EC (5%) and EC+X (6%) phases, the percentage of TB diagnoses that were microbiologically confirmed was higher in the EC+X (65%) than EC (51%) phases. I used two different but complementary modelling approaches to generate a parsimonious TB clinical risk score comprised of variables easily available in a resource-constrained clinic setting: (1) logistic regression models, and (2) random forest machine learning models. Random forest machine learning models are particularly useful for identifying important non-linear associations between predictors and outcomes.[127] Having derived the clinical score, I then used data from three other settings to validate the derived clinical score: (1) prospective cohort data for XPHACTOR study enrolees from Gauteng province, South Africa, which represents a predominantly stable, long-term ART population;[123] (2) trial data from the TB Fast Track (TBFT) trial from

Gauteng, Limpopo, and North West Provinces in South Africa, which represents a population with advanced HIV disease not taking ART;[117] and (3) prospective cohort data from the Western Cape, SA, which represents an ART-naïve population in a very high TB incidence setting.[140] I compared screening accuracy of our derived clinical scores with existing WHO TB symptom screening criteria for active TB among PLHIV in each of these populations.

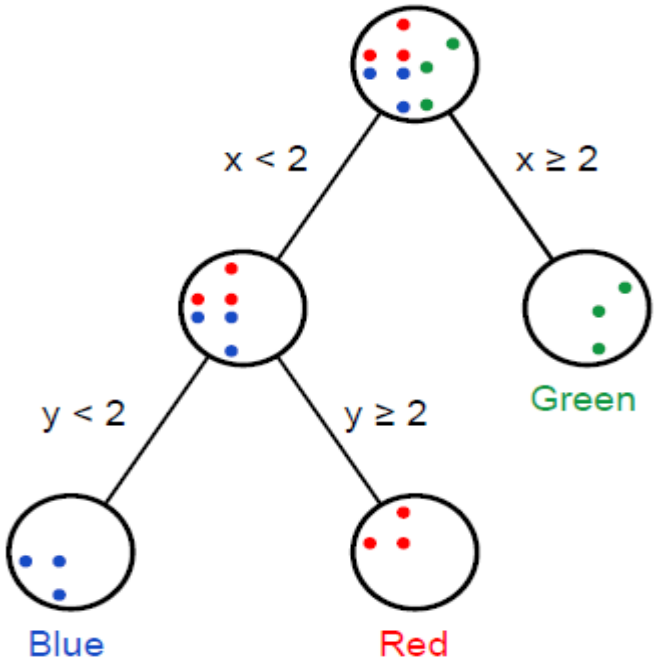
4.4. Random forest model

A random forest modelling approach, which is a type of machine learning, was used to supplement the traditional logistic regression approach to score development because of its relative strength in ability to identify important non-linear relationships between covariates and categorical outcomes.[127,158]

A random forest model is built from many decision trees

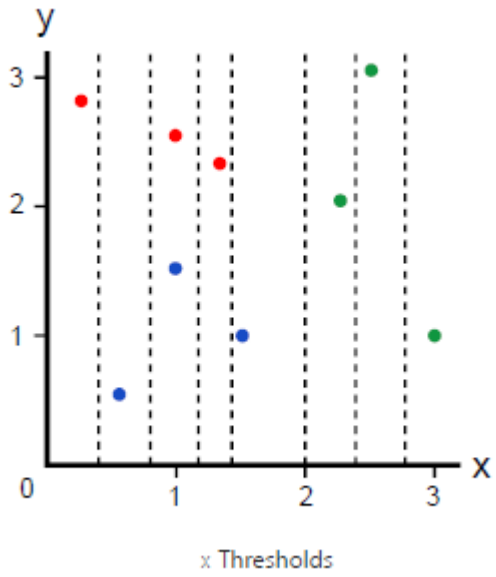
The random forest model consists of multiple decision trees that attempt to sequentially classify data into homogenous groups similar to the figure below (Figure 4.5.).

Figure 4.5. A decision tree with two nodes with classification informed by covariates x and y (taken from Zhou et al)[159]



To develop a single decision tree, the best split at each node is assessed by evaluating which cut-off gives the most homogenous classifications (i.e., lowest Gini impurity or highest Gini Gain according to published formulae).[159] Random forest packages available in R software (R Core Team (2017). R Foundation for Statistical Computing, Vienna, Austria) try every possible split to find the split that gives the least Gini impurity or greatest Gini Gain (Figure 4.6.)

Figure 4.6. Example of splits for covariate x tried to evaluate "best" split with lowest Gini impurity



In training a decision tree, multiple nodes are formed until it is no longer possible to further split the homogenous group based on available data. This occurs when all possible splits are equally good and have a Gini Gain of 0. The "leaves" of the decision tree can then be classified (e.g., green, red, and blue in Figure 4.5).

Bootstrap aggregating or bagging

A random forest model combines predictions from multiple trees through a process of sampling with replacement a certain number of training datasets, with each sample representing a random sample of two-thirds of all observations from the full dataset. A decision tree is then trained on each sampled dataset as described earlier. This process is repeated a certain number of times to create a certain number of trees (usually 200-1000 trees).[160] Finally, the predictions from the individual decision trees are aggregated into either a "majority vote" (if the outcome is categorical like the TB outcome), or an average (if the outcome is a continuous variable).

Feature bagging

Random forests also have a parameter that controls how many covariates to try when assessing splits within each decision tree. Random forest software packages include

algorithms that help modelers identify the right number of covariates (referred to as the *mtry* feature in Chapter 7) to randomly select and use for decision tree formation. By using subsets of the covariates rather than all covariates, this injects randomness that makes the individual trees more unique and reduces correlations between the trees, which improves the random forest predictions overall on validation datasets. In this way random forests are designed to reduce the problem of over-fitting on the training dataset that is inherent in single decision tree modelling approaches.

Variable importance

A key reason I supplemented standard logistic regression modelling with the random forest machine learning approach was to understand the importance of each covariate in its ability to split patients into homogenous groups (i.e., those with TB versus those without). Random forest models allow this evaluation of variable importance by assessing the mean decrease in Gini impurity associated with each variable included in a random forest model. The mean decrease in impurity is the average of a variable's total decrease in node impurity, weighted by the proportion of samples reaching that node in each individual tree in the random forest. Therefore, high mean decrease in Gini indicates higher variable importance (i.e., the variable was on average important in splitting nodes into groups that had TB versus did not have TB).[158] Further description of this modelling approach is provided in the methods section of Chapter 7.

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	1405072	**Title**	Dr.
First Name(s)	Francis Andrew		
Surname/Family Name	Auld		
Thesis Title	Opportunities to reduce early antiretroviral therapy mortality in sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care		
Primary Supervisor	Prof. 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?	BMC Medicine		
When was the work published?	2020		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**No**	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)	The candidate conceived the idea for the study, wrote the protocol, served as a principle investigator, designed the data collection forms, co-managed the study, conducted the data analysis, and wrote the paper.

SECTION E

Student Signature	[redacted]
Date	14 July 2020

Supervisor Signature	[redacted]
Date	16 July 2020

Copyright Clearance Center RightsLink®

Help Email Support

SPRINGER NATURE

Effect of tuberculosis screening and retention interventions on early antiretroviral therapy mortality in Botswana: a stepped-wedge cluster randomized trial

Author: Andrew F. Auld et al

Publication: BMC Medicine

Publisher: Springer Nature

Date: Feb 11, 2020

Copyright © 2020, Springer Nature

Creative Commons

This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

You are not required to obtain permission to reuse this article.

CC0 applies for supplementary material related to this article and attribution is not required.

© 2020 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Terms and Conditions

Comments? We would like to hear from you. E-mail us at customercare@copyright.com

Auld et al. BMC Medicine (2020) 18:19
https://doi.org/10.1186/s12916-019-1489-0

BMC Medicine

RESEARCH ARTICLE Open Access

Effect of tuberculosis screening and retention interventions on early antiretroviral therapy mortality in Botswana: a stepped-wedge cluster randomized trial

Andrew F. Auld[1,2*], Tefera Agizew[3], Anikie Mathoma[3], Rosanna Boyd[3], Anand Date[1], Sherri L. Pals[1], Christopher Serumola[3], Unami Mathebula[3], Heather Alexander[1], Tedd V. Ellerbrock[1], Goabaone Rankgoane-Pono[4], Pontsho Pono[4], James C. Shepherd[3,5], Katherine Fielding[6,7], Alison D. Grant[6,7,8] and Alyssa Finlay[3]

Abstract

Background: Undiagnosed tuberculosis (TB) remains the most common cause of HIV-related mortality. Xpert MTB/RIF (Xpert) is being rolled out globally to improve TB diagnostic capacity. However, previous Xpert impact trials have reported that health system weaknesses blunted impact of this improved diagnostic tool. During phased Xpert rollout in Botswana, we evaluated the impact of a package of interventions comprising (1) additional support for intensified TB case finding (ICF), (2) active tracing for patients missing clinic appointments to support retention, and (3) Xpert replacing sputum-smear microscopy, on early (6-month) antiretroviral therapy (ART) mortality.

Methods: At 22 clinics, ART enrollees > 12 years old were eligible for inclusion in three phases: a retrospective standard of care (SOC), prospective enhanced care (EC), and prospective EC plus Xpert (EC+X) phase. EC and EC+X phases were implemented as a stepped-wedge trial. Participants in the EC phase received SOC plus components 1 (strengthened ICF) and 2 (active tracing) of the intervention package, and participants in the EC+X phase received SOC plus all three intervention package components. Primary and secondary objectives were to compare all-cause 6-month ART mortality between SOC and EC+X and between EC and EC+X phases, respectively. We used adjusted analyses, appropriate for study design, to control for baseline differences in individual-level factors and intra-facility correlation.

Results: We enrolled 14,963 eligible patients: 8980 in SOC, 1768 in EC, and 4215 in EC+X phases. Median age of ART enrollees was 35 and 64% were female. Median CD4 cell count was lower in SOC than subsequent phases (184/μL in SOC, 246/μL in EC, and 241/μL in EC+X). By 6 months of ART, 461 (5.3%) of SOC, 54 (3.2%) of EC, and 121 (3.0%) of EC+X enrollees had died. Compared with SOC, 6-month mortality was lower in the EC+X phase (adjusted hazard ratio, 0.77; 95% confidence interval, 0.61–0.97, $p = 0.029$). Compared with EC enrollees, 6-month mortality was similar among EC+X enrollees.

(Continued on next page)

* Correspondence: aauld@cdc.gov
Presented in part at the Conference on Retroviruses and Opportunistic Infections (CROI), Boston, 4–7 March, 2018 (Oral Abstract # 31, session O-03)
[1]Division of Global HIV & TB, Centers for Disease Control and Prevention, Atlanta, USA
[2]Center for Global Health, U.S. Centers for Disease Control and Prevention (CDC), Lilongwe, Malawi
Full list of author information is available at the end of the article

© The Author(s). 2020 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

(Continued from previous page)
Conclusions: Interventions to strengthen ICF and retention were associated with lower early ART mortality. This new evidence highlights the need to strengthen ICF and retention in many similar settings. Similar to other trials, no additional mortality benefit of replacing sputum-smear microscopy with Xpert was observed.

Trial registration: Retrospectively registered: ClinicalTrials.gov (NCT02538952)

Keywords: Tuberculosis, Xpert MTB/RIF, Intensified tuberculosis case finding, Mortality

Background

In resource-limited settings, tuberculosis (TB) remains the most common cause of death among people living with HIV (PLHIV), including those starting antiretroviral therapy (ART), and is commonly undiagnosed at the time of death [1, 2]. Death from undiagnosed TB or TB diagnosed late is a key reason early (6-month) ART mortality rates remain significantly higher in sub-Saharan Africa (SSA) than resource-rich settings [2–4]. All data point towards a critical need to improve TB case finding among PLHIV starting ART.

In 2011, following World Health Organization (WHO) endorsement of Xpert MTB/RIF® (Xpert) as the first-line TB diagnostic test for symptomatic PLHIV [5], the Botswana Ministry of Health (MOH) and partners initiated planning for a phased national Xpert rollout [6]. Review of available program data for new HIV care enrollees showed that many components of the intensified TB case finding (ICF) cascade, especially compliance with the WHO-recommended 4-symptom TB screening rule, and early retention in HIV care, should be strengthened in order for Xpert to have maximum benefit [7]. Weaknesses in the health system that have resulted in poor completion of the TB diagnostic and treatment cascade and sub-optimal retention in HIV care, have been cited as important reasons for lack of observed Xpert impact on PLHIV mortality in similar settings [8, 9]. Therefore, Botswana used the Xpert rollout as an opportunity to strengthen ICF and retention in early HIV care through rollout of a package of services [6]. The intervention package has three components: (1) additional support for ICF, (2) intensified tracing for patients missing clinic appointments to return them to care, and (3) Xpert replacing sputum-smear microscopy.

No trial has yet evaluated impact of Xpert combined with strengthened health systems on mortality [8–10]. We evaluated impact of the Xpert, ICF, and retention package versus standard of care on early ART patient mortality.

Methods

Study design

We conducted a multi-center, stepped-wedge cluster randomized trial (CRT) with a retrospective baseline component called the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES) trial. A stepped-wedge rather than parallel group design was chosen because the Xpert, ICF, and retention package was expected to be beneficial for patients and the trial was part of a national rollout [6].

Participants

A cluster was defined as an HIV care and treatment clinic. Twenty-two clusters, located at five district hospitals and 17 primary healthcare facilities, were purposively selected to (1) be representative of HIV treatment clinics in Botswana and (2) have new ART initiation rates sufficient to meet sample size requirements (see Additional file 1, providing text on clinic selection criteria). At these 22 clusters, individual patients were eligible for study enrollment if they were new HIV clinic attendees, regardless of TB treatment status, and not prisoners at the time of the first HIV clinic visit. The study aimed to enroll or offer enrollment to all eligible HIV clinic attendees in three consecutive phases: (1) a retrospective standard of care (SOC) phase, (2) a prospective enhanced care (EC) phase, and (3) a prospective EC plus Xpert (EC+X) phase (Fig. 1). For this predefined protocol analysis, only those study enrollees who newly started ART at or after study enrollment and were ≥ 12 years old at ART initiation were included [6].

Randomization and masking

The selected 22 clusters received TB diagnostic services from 13 laboratories (Fig. 1). Because some of the study clinics used the same TB diagnostic laboratory, full Xpert, ICF, and retention package activation was planned to be simultaneous for these clinic consortiums (Fig. 1). After obtaining ethical approvals and agreement to participate in the study from MOH at a central level and MOH management at the selected facilities, the study statistician randomly selected one of the rollout permutations [6].

Procedures

At the 22 clusters, per Botswana national guidelines during the time period of the study (July 2010 through June 2015), all study participants in all phases were eligible for ART initiation if they had a CD4 count ≤ 350 cells/μL, were diagnosed as having WHO stage III/IV, or were pregnant or breastfeeding [11]. All study participants

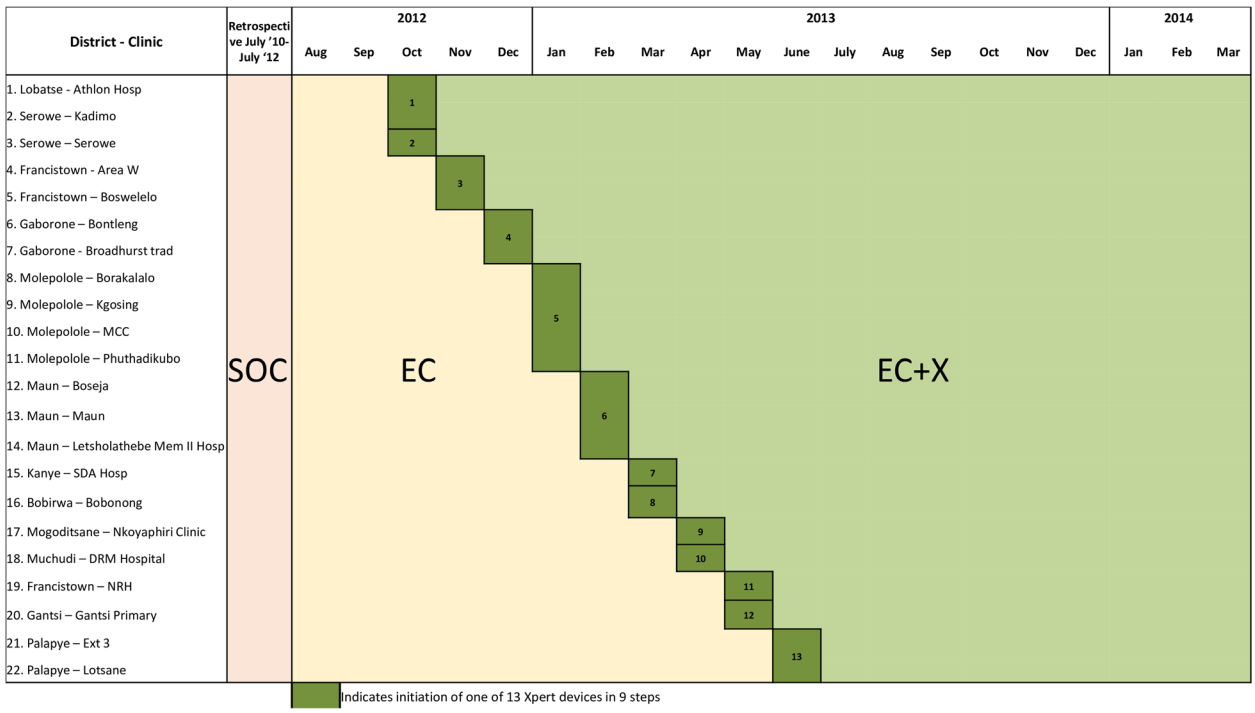


Fig. 1 Study design for the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES). Abbreviations: SOC, standard of care phase; EC, enhanced care phase; EC+X, enhanced care plus Xpert phase

received clinical care and follow-up appointments according to MOH guidelines (see Additional file 2, a table summarizing standard clinical care follow-up).

Standard of care phase

Enrollment in the retrospective SOC phase was through chart abstraction of eligible adult patients who started ART between July 2010 and the end of July 2012 (Fig. 1) [6]. The SOC phase enrollees received HIV care according to national guidelines, limited ICF, infrequent active tracing due to resource limitations, and sputum-smear microscopy for presumptive TB patients.

Intervention phases EC and EC+X

Prospective EC enrollment started in August 2012 and was complete by January 2013. Prospective EC+X enrollment occurred from October 2012 through March 2014 according to the stepped-wedge design (Fig. 1). EC phase participants received SOC supplemented by two components of the Xpert, ICF, and retention package (i.e., additional support for ICF and intensified tracing) combined with sputum-smear microscopy. EC+X phase participants received SOC supplemented by all three components of the Xpert, ICF, and retention package (i.e., additional support for ICF, intensified tracing, and Xpert in place of sputum-smear microscopy). All interventions were activated at the cluster-level for the benefit of all clients receiving care at the clinic. EC and EC+X participants were followed for 12 months, or until the end of TB treatment, whichever was later. The final follow-up visits for EC+X enrollees were in June 2015.

Interventions

The ICF and active tracing interventions were strengthened through four key mechanisms: (1) additional human resources (study nurses) to support implementation, (2) additional training for clinic and laboratory personnel, (3) use of checklists and job aids to standardize implementation, and (4) regular supervisory visits to track adherence to ICF and tracing checklists.

ICF intervention

Implementation of the WHO 4-symptom TB screening rule (i.e., screening for cough of any duration, fever, loss of weight, and night sweats) [12] was recommended for all enrollees at each clinic visit in the SOC, EC, and EC+X phases, but implementation was strengthened in the EC and EC+X phases. In all phases, clients were considered symptomatic if they screened positive for one or more of the four TB symptoms. In all phases, at least two same-day, on-the-spot (spot) sputum samples were recommended for collection from symptomatic clients. As part of strengthened ICF in the EC and EC+X phases, a previously published job-aid was used by study nurses to inform the patient how to collect quality sputum samples [6]. Prior to the EC phase, laboratory personnel at

the 13 laboratories serving the 22 clusters received refresher training on Ziehl-Neelsen staining for sputum-smear microscopy, and prior to the EC+X phase, laboratory personnel were trained for Xpert implementation. In all phases, sputum test results were returned to the clinics, with clinicians responsible for informing the patients. In the SOC phase, the patient was informed of a TB diagnosis at the next scheduled clinic appointment. In the EC and EC+X phases, study nurses were trained to work with laboratories to ensure the turnaround time from sample collection to result return to the clinic was ≤ 4 days for sputum-smear microscopy and ≤ 2 days for Xpert testing. In the EC and EC+X phases, nurses were trained to inform patients of positive TB diagnoses the same day via phone, or if unreachable by phone, by active tracing to the household. Indicators monitoring implementation of the ICF cascade were collected and used to inform supervision visits (see Additional file 3, a table summarizing the indicators) [7].

Active tracing intervention

Per national guidelines, clients ≥ 1 day late for an HIV clinic appointment should be traced through phone and home visit starting the day after the missed visit. However, program reports showed this tracing was infrequently implemented in the SOC phase due to lack of human and financial resources. Implementation of the active tracing policy was strengthened in the EC and EC+X cohorts. In the EC and EC+X phases, a patient locator form was used to document telephone numbers and home addresses for intensified tracing activities to support retention. Up to five telephone calls and two home visits, facilitated by checklists, were used in attempts to return clients, who had missed clinic appointments, to care. The key HIV care retention indicator used for monitoring purposes was the rate of loss to follow-up (LTFU) per 100 person-years (see Additional file 3, a table summarizing the indicators). LTFU was defined as being > 60 days late for a scheduled appointment, per Botswana guidelines.

Objectives and outcomes

The study had two primary objectives. The primary objective reported here is the non-randomized comparison of all-cause 6-month ART mortality among adult ART enrollees (≥12 years old) between the SOC and EC+X phases [6]. The second primary objective, which aimed to compare diagnostic sensitivity of the new Xpert-based TB diagnostic algorithm with that of the sputum-smear-microcopy-based algorithm, will be reported separately according to diagnostic accuracy study reporting guidelines.

Secondary objectives reported in this paper include (1) the comparison of 12-month ART mortality between SOC and EC+X phases and (2), within the randomized stepped-wedge trial, the comparison of all-cause, adult, 6-month ART mortality between the EC and EC+X phases.

We implemented intensive efforts to ascertain true mortality outcomes among participants. Deaths and date of death were either passively reported to the clinic by friends or relatives of the deceased participant, or actively ascertained if the client had missed an appointment or was considered LTFU [13]. Initial efforts to ascertain outcomes of clients who missed an appointment or were LTFU included phone outreach to the client or contact and home visits. For participants in the SOC phase, these efforts started after data entry was complete which was always > 12 months after ART initiation. In the EC and EC+X phases, this outreach started immediately after the missed appointment, in an attempt to return the client to care. For all clients unreachable by phone or home visit who met the LTFU definition, vital status was ascertained through national Death Registry review. By law, since 1969, all deaths need to be registered in the Death Registry, which is maintained by the Civil and National Registration Office.

Sample size

As described previously [6], to obtain conservative sample size estimates, we used the approach of Moulton et al., suitable for stepped-wedge trial designs, to estimate required sample sizes to meet the primary study objective comparing 6-month ART mortality rates between SOC and EC+X phases [14]. Funding limitations restricted the number of clinics that could be included in the study to 22. A between-cluster coefficient of variation of 0.2 was used based on review of the literature of similar stepped-wedge trials [14]. Monthly HIV clinic (cluster) size was derived from reported program ART enrollment rates in the SOC phase and varied between clinics (average, 23 ART enrollees/month; range, 8–46/month). Prior to study start, available data from Botswana suggested that all-cause, adult, 6-month ART mortality rates were about 15 deaths per 100 person-years [3, 15]. To provide > 80% power to detect a ≥ 40% reduction in all-cause 6-month ART mortality between the two groups, assuming SOC mortality was ≥ 10/100 person-years, a 24-month SOC phase enrollment period (N = 12,144) and an 18 month EC+X phase enrollment period (N = 6348) were chosen.

Statistical analysis

For the primary outcome analysis, time at risk for ART enrollees started on the day of ART initiation and ended at 6 months of follow-up after ART initiation, or at the time of death, LTFU, or transfer out if these events were before 6 months of ART follow-up. Crude and multivariable Cox proportional hazards regression models, with a random effect for clinic, were used to assess the effect of

intervention status (SOC vs EC+X) on time to death [6]. Per a pre-specified analysis plan, age at ART initiation, sex, pregnancy status, and baseline CD4 count were a priori covariates to be included in the multivariable model. Hemoglobin at ART initiation [16], ART regimen [17], and weight at ART initiation [16] were included in the multivariable model because of their importance as predictors of mortality in this and other analyses.

Pre-specified secondary analyses were conducted to (1) compare 12-month ART mortality between SOC and EC+X phases and (2) compare 6-month ART mortality rates between cohorts EC and EC+X [6]. For the latter, we used analytic methods described by Moulton et al., fitting Cox proportional hazards models to the data with the underlying time frame being time since August 2012 (initiation month for the stepped-wedge component of the trial), fixed effect for intervention arm (Xpert device activation), and a random effect for clinic [14]. The proportionality assumption was checked using visual methods and the Grambsch and Therneau test.

Per the pre-specified analysis plan, plausible interactions between the intervention effect and other covariates, including CD4 count at ART initiation, were examined by comparing models with and without interactions using the likelihood ratio test. Per the pre-specified analysis plan, the primary time-to-event analytic approaches comparing SOC versus EC+X and EC versus EC+X mortality rates assigned follow-up time to the phase in which the participant started ART because the interventions were expected to have maximum impact around the time of ART initiation. However, two pre-specified sensitivity analyses of this approach were planned. The first sensitivity analysis censors follow-up time for ART enrollees at the time of cross-over between phases, while the second assigns follow-up time to contemporary intervention phases when cross-over occurs, through use of a time-dependent covariate [18]. In addition, per a third pre-specified sensitivity analysis, an inverse probability weighting approach was used to account for non-enrollment in the EC and EC+X phases of the study. Separate adjusted logistic regression models for hospital versus clinic enrollees were used to predict the probability of being enrolled in the study. Patients consenting to enrollment were up-weighted by the inverse of the calculated enrollment probability. An adjusted logistic regression approach was used to estimate inverse probability weights to lower the likelihood of bias given the possibility of non-random enrollment in the EC and EC+X phases [19]. All analyses were conducted using STATA 14 or 16 (StataCorp, 2009, Stata Statistical Software, Release 14 and 16, College Station, TX). XPRES is registered at ClinicalTrials.gov (trial registration no. NCT02538952).

Results

Enrollment

Across the 22 study clinics, there were 528 months of enrollment in the SOC phase (mean 24/clinic), 120 months in the EC phase (mean 5.5/clinic), and 299 months of enrollment in the EC+X phase (mean 13.6 months/clinic) (Fig. 2). All 10,047 eligible patients for the SOC phase were enrolled. Among the 2703 and 5834 patients eligible for the EC and EC+X phases, respectively, 1794 (66%) and 4247 (73%) consented to enrollment. The main reason eligible clients were not enrolled prospectively is that they left the clinic before they could be offered enrollment. The demographic and clinical characteristics of clients consenting to enrollment were very similar to the characteristics of clients not enrolled (see Additional file 4, a table comparing characteristics of those enrolled versus not enrolled). We excluded from this analysis patients who transferred into the clinic on ART ($n = 1067$), were < 12 years old at ART initiation ($n = 22$), or did not start ART during follow-up ($n = 36$) (Fig. 2). In total, 8980, 1768, and 4215 patients were included in the SOC, EC, and EC+X phases for analysis, respectively.

Baseline characteristics

Among all study enrollees included in the analysis, median age was 35 (interquartile range (IQR) 29–42) at ART initiation and the percentage female was 64% and these characteristics were similar between phases (Table 1). Among female enrollees, the percentage who were pregnant at the time of ART initiation was lower in the SOC phase (16%) than EC (23%) and EC+X (32%) phases. Among all enrollees, median weight (58.4 kg) and median hemoglobin (11.7 g/dL) were similar between phases. However, median CD4 count at ART initiation was lower in the SOC phase (184 cells/μL) than in the EC (246 cells/μL) and EC+X (241 cells/μL) phases. In addition, the percentage of enrollees with mild or moderate anemia per WHO criteria was higher in the SOC phase (56%) than EC (48%) and EC+X phases (46%). Tenofovir (combined with lamivudine or emtricitabine and efavirenz or nevirapine) was less commonly prescribed as first-line ART in the SOC (78%) compared with the EC (93%) and EC+X (96%) phases.

Primary outcome: 6-month ART mortality in SOC versus EC+X

By 6 months after ART initiation, 461 (5.3%) of enrollees in the SOC phase had died compared with 121 (3.0%) of enrollees in the EC+X phase. Six-month ART mortality rates were 11.4 deaths per 100 person-years in the SOC phase versus 6.3 deaths per 100 person-years in the EC+X phase (Table 2). Compared with the SOC phase, 6-month mortality was lower in the EC+X phase in

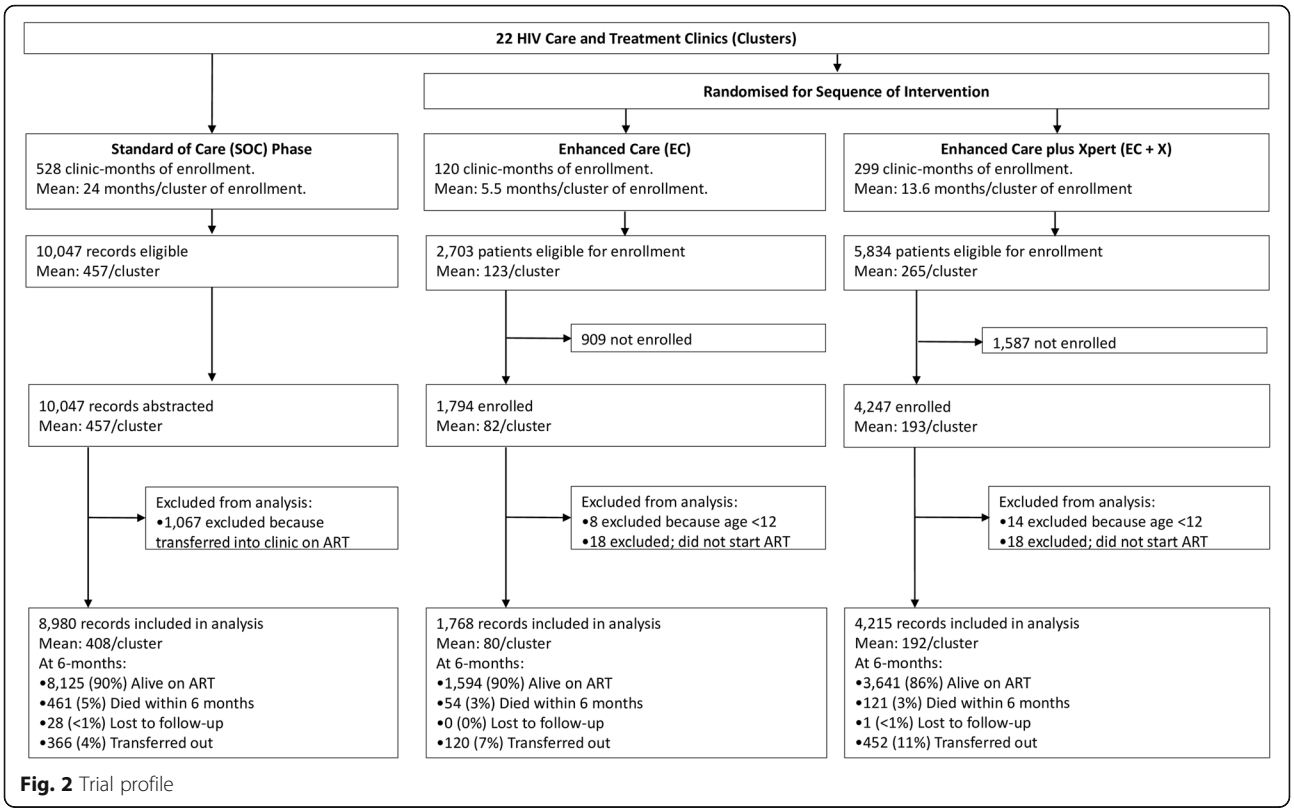


Fig. 2 Trial profile

unadjusted analysis (hazard ratio (HR) 0.58, 95% CI 0.48–0.71, $p < 0.001$) (Fig. 3, Table 2). After controlling for potential confounders, including age, sex, pregnancy status, weight, CD4 count, hemoglobin, and ART regimen, 6-month mortality remained lower in the EC+X phase compared with the SOC phase (adjusted HR, 0.77, 95% CI 0.61–0.97, $p = 0.029$).

Intervention effect size was similar across CD4 strata (see Additional file 5, a figure showing cumulative mortality incidence stratified by CD4 count at ART initiation). In addition, effect size was robust to sensitivity analyses that censored follow-up time at the time of transition between phases or assigned follow-up time to contemporary intervention phases using a time-dependent covariate (see Additional file 6, a table showing these sensitivity analyses). Effect size was robust to sensitivity analysis using an inverse probability weighting approach to account for non-enrollment in EC and EC+X phases (see Additional file 7, a table showing these sensitivity analyses).

Secondary outcomes: 12-month ART mortality in SOC versus EC+X

By 12 months after ART initiation, 551 (6.5%) of SOC versus 137 (3.7%) of EC+X phase enrollees had died. Twelve-month mortality rates were 7.3/100 person-years in the SOC versus 4.6/100 person-years in the EC+X phase. Compared with the SOC phase, 12-month mortality was lower in the EC+X phase in both unadjusted (HR 0.58, 95% CI 0.48–0.70, $p < 0.001$) and adjusted (AHR 0.76, 95% CI 0.61–0.95, $p = 0.014$) analyses (Table 2). Intervention effect size was robust to sensitivity analyses (see Additional files 6 and 7, tables showing sensitivity analyses).

Secondary outcomes: 6-month ART mortality in EC versus EC+X

By 6 months of ART follow-up among ART enrollees in the EC phase, 54 (3.2%) of enrollees had died. Six-month mortality rates were similar between the EC (6.5/100 person-years) and EC+X phases (6.3/100 person-years) in both unadjusted and adjusted pre-specified analyses (AHR 1.13, 95% CI, 0.63–2.03), where all follow-up time was assigned to the phase in which the patient started ART (Table 2). In sensitivity analyses comparing EC vs. EC+X 6-month mortality rates, the AHR was 0.90 (95% CI 0.42–1.95) when EC enrollee follow-up time was censored at the time of EC+X cross-over, and 0.79 (95% CI 0.41–1.50) when EC enrollee follow-up time in the EC+X phase was assigned to the EC+X phase using a time-dependent variable (see Additional file 6, a table showing sensitivity analyses).

Table 1 Demographic and clinical characteristics of XPRES participants at antiretroviral therapy initiation

	SOC		EC		EC+X	
	(*N* = 8980)		(*N* = 1768)		(*N* = 4215)	
	n	%/median (IQR)	*n*	%/median (IQR)	*n*	%/median (IQR)
Age (years)[a]						
n, median, (IQR)	8969	35 (30–43)	1768	34 (29–42)	4215	34 (29–41)
Gender						
Female	5624	63%	1194	68%	2797	66%
If female, pregnant?						
Yes	927	16%	271	23%	903	32%
Weight (kg)[b]						
Median (IQR)	8351	57.9 (50.5–66.6)	1765	58.6 (51.3–67.8)	4209	59.4 (52.5–68.7)
Weight (kg)						
< 45 kg	871	10%	160	9%	318	8%
45–60 kg	3971	48%	817	46%	1910	45%
> 60 kg	3509	42%	788	45%	1981	47%
Baseline CD4 (cells/μL)[c]						
Median (IQR)	8675	184 (100–241)	1765	246 (148–310)	4180	241 (132–321)
Baseline CD4 (cells/μL)						
< 50	1061	12%	132	7%	370	9%
50 to < 100	1109	13%	161	9%	371	9%
100 to < 200	2660	31%	366	21%	928	22%
200 to < 350	3456	40%	947	54%	1928	46%
350 to < 500	246	3%	93	5%	334	8%
≥ 500	143	2%	66	4%	249	6%
Baseline hemoglobin (g/dL)[d]						
Median (IQR)	7869	11.5 (10.0–13.0)	1678	11.9 (10.4–13.3)	3911	12.0 (10.6–13.3)
Hemoglobin category[e]						
Severe anemia	426	5%	68	4%	109	3%
Mild/moderate anemia	4399	56%	805	48%	1810	46%
No anemia	3044	39%	805	48%	1992	51%
TB treatment at ART initiation						
Yes	423	5%	85	5%	251	6%
Regimen[f]						
TDF/XTC/EFV or NVP	6998	78%	1615	93%	4000	96%
AZT/3TC/EFV or NVP	1045	12%	94	5%	107	3%
D4T/3TC/EFV or NVP	151	2%	2	0%	4	0%
Other	784	9%	26	1%	54	1%

Abbreviations: *SOC* standard of care phase, *EC* enhanced care phase, *EC+X* enhanced care plus Xpert phase, *IQR* interquartile range, *TDF* tenofovir, *XTC* either lamivudine or emtricitabine, *EFV* efavirenz, *NVP* nevirapine, *ddI* didanosine, *ABC* abacavir, *LPV/r* lopinavir/ritonavir, *AZT* zidovudine, *3TC* lamivudine, *D4T* stavudine

[a]11 ART enrollees in the SOC cohort had unknown age but were documented to be adult in the ART chart

[b]629 (7%), 2 (0.2%), and 6 (0.1%) had missing weights at ART initiation in the SOC, EC, and EC+X phases, respectively

[c]305 (3%), 3 (0.2%), and 35 (0.8%) had missing CD4 in the SOC, EC, and EC+X phases, respectively. For each enrollee, the CD4 count taken closest to the date of ART initiation in the 12 months before ART start was used

[d]1111 (12%), 90 (5%), and 304 (7.2%) had missing hemoglobin in the SOC, EC, and EC+X phases, respectively. For each enrollee, the hemoglobin taken closest to the date of ART initiation in the 12 months before ART start was used

[e]Anemia severity was classified according to World Health Organization criteria as follows: no anemia, hemoglobin level of ≥ 13.0 g/dL for men, ≥ 12.0 g/dL for non-pregnant females, and ≥ 11.0 g/dL for pregnant females; mild/moderate anemia, 8.0 to < 13.0 g/dL for men, 8.0 to < 12.0 g/dL for non-pregnant women, and 7.0 to < 11.0 g/dL for pregnant women; and severe anemia, < 8.0 g/dL for males and non-pregnant females and < 7.0 g/dL for pregnant women

[f]2 (0%), 31 (2%), and 50 (1%) had missing ART regimen in the SOC, EC, and EC+X phases, respectively

Table 2 Primary and secondary study outcomes—comparison of mortality rates between study phases

	ART enrollees	Deaths (*n*)[a]	Rate/100PY[b]	Crude HR[c]	(95% CI)	*p*	AHR[cd]	(95% CI)	*p*
Primary outcome: 6-month ART mortality in SOC versus EC+X phase									
SOC	8980	461	11.4	1.00	–	–	1.00	–	–
EC+X	4215	121	6.3	0.58	(0.48–0.71)	< 0.001	0.77	(0.61–0.97)	0.029
Secondary outcomes: 12-month ART mortality in SOC versus EC+X phase									
SOC	8980	551	7.3	1.00	–	–	1.00	–	–
EC+X	4215	137	4.6	0.58	(0.48–0.70)	< 0.001	0.76	(0.61–0.95)	0.014
6-month ART mortality in EC versus EC+X phase[e]									
EC	1768	54	6.5	1.00			1.00		
EC+X	4215	121	6.3	1.07	(0.62–1.84)	0.800	1.13	(0.63–2.03)	0.690

Abbreviations: *SOC* standard of care phase, *EC* enhanced care phase, *EC+X* enhanced care plus Xpert phase, *PY* person-years, *HR* hazard ratio, *AHR* adjusted hazard ratio, *CI* confidence interval, *XPRES* Xpert Package Rollout Evaluation using a Stepped-Wedge design

[a]Represents deaths observed among all ART enrollees by the time point specified

[b]Represents unadjusted 6- and 12-month ART mortality rates among all ART enrollees in each phase of the study. For mortality rates among ART enrollees included in the adjusted analyses, see Additional file 6

[c]All Cox proportional hazards regression models included a random effect for clinic

[d]Adjusted for the following characteristics at ART initiation: age, sex, pregnancy status, weight, CD4 count, hemoglobin, and ART regimen. Adjusted analysis comparing SOC versus EC+X mortality rates included 7184 SOC enrollees with 350 deaths within 6 months and 424 deaths within 12 months, and 3861 EC+X enrollees with 93 deaths within 6 months and 108 deaths within 12 months

[e]Analysis restricted to randomized stepped-wedge portion of the trial, fitting a Cox proportional hazards regression model to the data with the underlying time frame beginning August 2012 (the start of EC enrollment), and including a fixed effect for monthly changes in mortality rates during the first 6 months of ART. Adjusted analysis comparing EC versus EC+X mortality rates included 1653 EC enrollees with 43 deaths within 6 months and 3861 EC+X enrollees with 93 deaths within 6 months

TB screening and diagnosis

Among SOC, EC, and EC+X phase enrollees respectively, 359 (4%), 44 (2%), and 122 (3%) were diagnosed with TB and had started TB treatment prior to arrival at the HIV treatment clinic. Therefore, in the SOC, EC, and EC+X phases, 8621, 1724, and 4093 patients were eligible for TB symptom screening before ART initiation. Among these patients eligible for TB symptom screening before ART initiation in the SOC, EC, and EC+X phases, 1700 (20%), 1724 (100%), and 4093 (100%) were screened for at least one TB symptom and 1243 (14%), 1724 (100%), and 4093 (100%) were screened for all four TB symptoms, respectively (Fig. 4). Within the SOC phase, ART enrollees were more likely to be screened

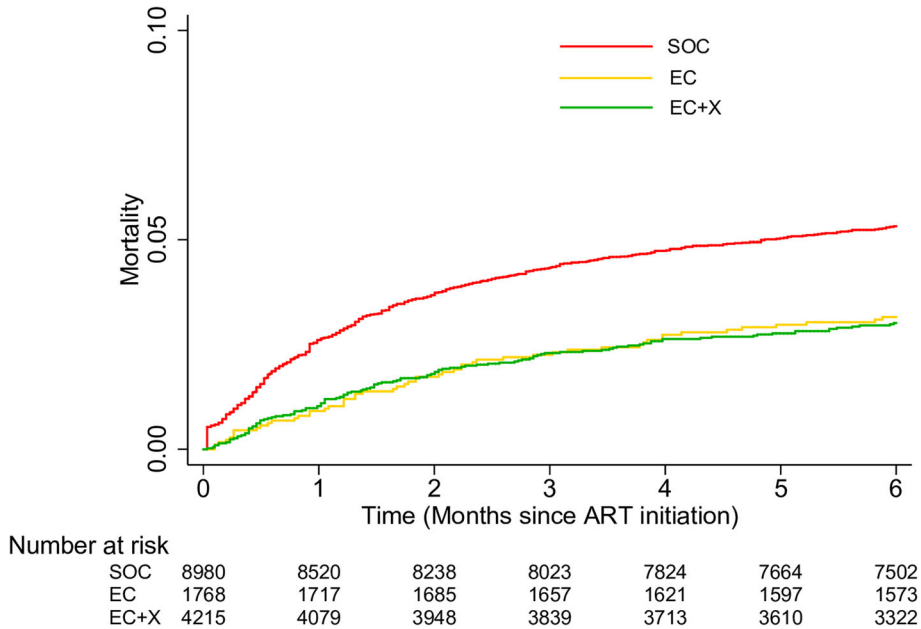


Fig. 3 Kaplan-Meier curves showing cumulative 6-month mortality among ART enrollees in SOC, EC, and EC+X phases

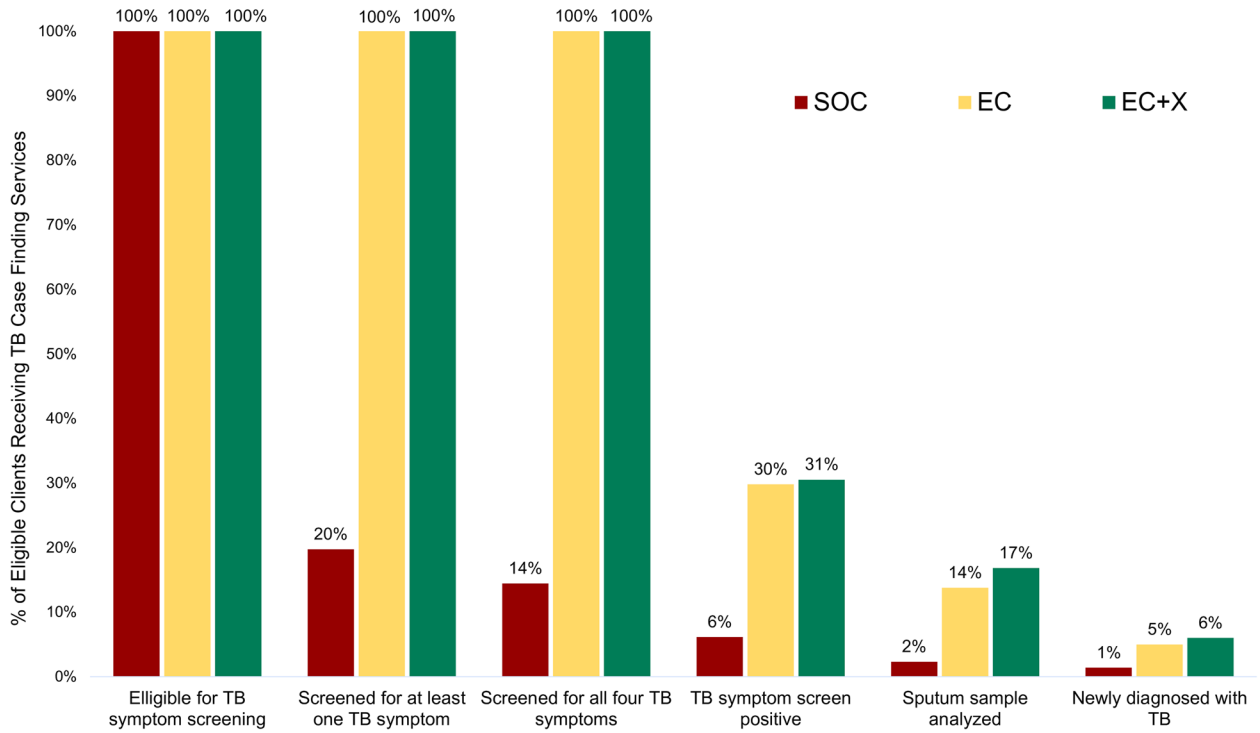


Fig. 4 Intensified TB case finding (ICF) cascade among ART enrollees in SOC, EC, and EC+X phases. Abbreviations: SOC, standard of care phase, EC, enhanced care phase, EC+X, enhanced care plus Xpert phase

for at least one TB symptom if they had lower weight and lower CD4 count at ART initiation (see Additional file 8, a table showing predictors of being screened for TB in the SOC cohort).

Among SOC, EC, and EC+X enrollees eligible for screening, 525 (6%), 514 (30%), and 1249 (31%) screened positive for at least one TB symptom and 199 (2%), 237 (14%), and 688 (17%) provided a sputum sample for TB diagnosis (Fig. 4). Ultimately, 129 (1%), 86 (5%), and 244 (6%) enrollees in the SOC, EC, and EC+X phases were newly diagnosed with TB and started TB treatment before ART initiation or during the first 6 months of ART. The number of pulmonary TB diagnoses in the SOC ($n = 123$), EC ($n = 68$), and EC+X ($n = 198$) phases that were confirmed microbiologically was 22 (18%), 35 (51%), and 129 (65%), respectively (Table 3).

Early ART LTFU

By 6 months after ART initiation, cumulative LTFU incidence, uncorrected by subsequent mortality ascertainment efforts, in the SOC, EC, and EC+X phases, was 4%, 1%, and 1%, respectively (see Additional file 9, a table summarizing these cumulative LTFU incidence percentages). Compared with 6-month LTFU rates in the SOC phase (8.3/100 person-years), rates of 6-month LTFU were lower in the EC (1.2/100 person-years) and EC+X (1.6/100 person-years) phases in both unadjusted and adjusted analyses (see Additional file 10, a table comparing LTFU rates between SOC, EC, and EC+X phases).

Discussion

In Botswana, compared with SOC, interventions to strengthen WHO-recommended TB symptom screening and ICF algorithms combined with active tracing to support retention were associated with increased TB case finding and lower early ART mortality. No additional mortality benefit of replacing sputum-smear microscopy with Xpert was observed.

Although implementation of the WHO-recommended 4-symptom TB screening rule as the first step in ICF algorithms among PLHIV starting ART has been recommended since 2011 along with TB-HIV care continuum retention interventions including active tracing [20], no study has yet reported on the potential impact on mortality of strengthening systems to implement these guidelines [7]. Although the observed reduction in all-cause mortality between SOC and subsequent EC and EC+X phases represents a pre- versus post-comparison, rather than a randomized comparison, and is therefore at risk of residual confounding, the study has a number of strengths that suggest ICF and retention interventions did independently contribute to observed mortality impact. Firstly, the reduction in all-cause mortality remained statistically significant after adjusting for key

Table 3 Methods of new TB diagnosis immediately before ART and in the first 6 months of ART in the SOC, EC, and EC+X phases of XPRES

	SOC phase		EC phase		EC+X phase	
	n	%	*n*	%	*n*	%
Pulmonary TB						
Microbiologically confirmed pulmonary TB (smear microscopy in SOC and EC, Xpert during EC+X)	22	18	23	34	113	57
Microbiologically confirmed pulmonary TB through culture[a] (missing or negative smear and Xpert)	0	0	12	18	16	8
Clinical diagnosis of pulmonary TB with negative sputum test (negative smear, Xpert, or culture documented)	6[b]	5	6[d]	9	17[f]	9
Clinical diagnosis of pulmonary TB with no documented sputum test result	95[c]	77	27[e]	40	52[g]	26
Sub-total pulmonary TB	123	100	68	100	198	100
All TB						
Pulmonary TB total	123	95	68	79	198	81
Extra-pulmonary TB total	6	5	18	21	46	19
Total	129	100	86	100	244	100

Abbreviations: *SOC* standard of care, *EC* enhanced care, *EC+X* enhanced care plus Xpert, *TB* tuberculosis, *XPRES* Xpert Package Rollout Evaluation using a Stepped-wedge design

[a]To meet other study objectives related to estimation of diagnostic accuracy of the smear microscopy-based and Xpert-based TB diagnostic algorithms, one spot sputum and the morning sputum were sent to the National TB Reference Laboratory (NTRL) for liquid culture in mycobacteria growth indicator tubes (MGIT). The liquid culture results were also returned to the clinics, although average turnaround times exceeding 49 days were expected per existing standard of care

[b]5 (83%) of 6 had documentation that x-ray findings were suggestive of pulmonary TB

[c]13 (14%) of 95 had documentation that x-ray findings were suggestive of pulmonary TB

[d]3 (50%) of 6 had documentation that x-ray findings were suggestive of pulmonary TB

[e]16 (59%) of 27 had documentation that x-ray findings were suggestive of pulmonary TB

[f]8 (47%) of 17 had documentation that x-ray findings were suggestive of pulmonary TB

[g]20 (38%) of 52 had documentation that x-ray findings were suggestive of pulmonary TB

covariates. Secondly, the improvements in TB screening, TB case finding, and uncorrected LTFU rates between SOC and subsequent EC and EC+X phases were large, providing credence that these interventions were a driver behind observed mortality reductions. Thirdly, very high ascertainment of the primary early ART mortality outcome improves ability to interpret observed mortality changes. Fourthly, the intervention effect size and statistical significance were robust to several sensitivity analyses. Therefore, these findings represent important additional evidence in support of current WHO ICF and retention guidelines, and support continued or additional investment from donors to strengthen health systems to implement these guidelines for all HIV clinic enrollees [9].

Although it was widely anticipated that introduction of the new more sensitive TB diagnostic test (Xpert) in place of sputum-smear microscopy would independently reduce mortality among PLHIV, this study and six of the seven previously reported Xpert impact trials have not observed any independent impact of Xpert versus sputum-smear microscopy on mortality [8, 21]. In the one trial that did observe Xpert impact on mortality, the mortality benefit was restricted to clients with advanced HIV disease (WHO stage III/IV) [21]. Furthermore, program data have clearly shown that leaks in the ICF cascade before a TB diagnostic test is implemented, especially failure to implement the WHO-recommended 4-symptom TB screen, may be largely responsible for unacceptably high rates of mortality due to undiagnosed TB among PLHIV engaged in care in sub-Saharan Africa [22, 23].

Per WHO guidelines, screening for the four TB symptoms (i.e., current cough, weight loss, night sweats, or fever) should occur at every clinical care encounter for PLHIV as the initial step in ICF to improve detection and treatment of HIV-associated TB [20]. The recommendation is based on a high sensitivity of the 4-symptom screening rule (89.4%) in detecting culture-positive pulmonary TB disease among ART-naïve PLHIV [24]. However, low compliance in implementing the 4-symptom TB screen at or prior to ART initiation has been consistently observed in many high burden TB-HIV countries in sub-Saharan Africa, including South Africa (59%) [23], Mozambique (61%) [25], Kenya (4%) [26], and Cote d'Ivoire (36%) [22]. Similarly, in XPRES, failure to implement TB screening before ART was the most "leaky" part of the ICF cascade in the SOC phase, with only 30% screened before ART. Improving the coverage of TB symptom screening from 30% in the SOC to 100% in the EC and EC+X phases was the main driver behind improved TB case detection from 1% in SOC to 5–6% in EC and EC+X phases and therefore appears to have been a key driver behind the declines in early ART mortality between SOC and subsequent EC and EC+X phases.

Reasons for low compliance with TB screening protocols in the SOC phase are not well understood, but

could have related to high patient load making healthcare workers more likely to omit key steps in care algorithms, inadequate training and knowledge of the guidelines, or deficiencies in monitoring and evaluation [27]. In the SOC phase, having more advanced disease at ART initiation (i.e., having a lower weight and CD4 count) was associated with higher odds of being screened for TB, suggesting that healthcare workers were triaging the clients to receive TB screening based on perception of disease stage. This finding might fit with a clinic experiencing high patient volume and HCW's rushing through patient consultations in order to complete their clinical duties within available business hours. Our intervention of providing additional nurses to implement the TB screening, additional training, and additional supervision increased the percentage of ART enrollees screened for TB from 30% to 100%.

Notably, although the percentage of enrollees screening positive for ≥ 1 TB symptom who provided ≥ 1 sputum sample increased from 38% in the SOC phase to 46% and 55% in the EC and EC+X phases, respectively, collection of sputum samples remained a challenge even in the EC phases. This low compliance with sputum collection guidelines has been observed in multiple settings [23, 27], with potential reasons being patient hesitance to provide a sputum sample for stigma-related reasons, true inability to provide a sputum sample, and HCW-related reasons such as feeling overloaded, or lack of confidence in the laboratory sample transport and diagnostic system [23]. Further research and interventions to improve this component of the cascade are needed. In addition, this finding supports calls for improved sputum-independent diagnostic tests for TB.

A key reason that prior Xpert impact trials have generally not observed independent Xpert impact on mortality is that higher rates of empiric TB treatment among clients with TB symptoms but a negative sputum-smear microscopy result replaced any potential benefit of Xpert's improved diagnostic sensitivity in detecting culture-positive TB [28, 29]. Similarly in our study, although Xpert implementation was the driver behind increased microbiological confirmation of TB diagnoses in the EC+X versus EC phase (65% vs. 51%), there was no significant difference in percentage of ART enrollees newly treated for TB (6% vs. 5%). However, as reported previously, Xpert was the driver behind reduced median time from sputum collection to TB treatment in the EC+X phase (6 days) versus the EC phase (22 days) [30]. Although no independent effect of Xpert on 6-month mortality was observed in our study, two features of the study suggest, similar to findings of a recent meta-analysis of Xpert impact trials [31], that we cannot confidently rule out the possibility of modest independent Xpert impact: (1) our study was not powered to detect a difference between EC and EC+X 6-month mortality and (2) the sensitivity analyses comparing EC vs. EC+X 6-month mortality rates generated AHRs of 0.90 (p = 0.793) and 0.79 (p = 0.472), which could possibly point to a modest Xpert impact our study was under-powered to detect.

In ART programs in resource-limited settings, observed LTFU from early ART is common, with an average of 20% LTFU by 12 months of follow-up [32, 33]. Mortality rates among LTFU ART patients are high [33]. The percentage of LTFU clients found to have died by the time of tracing ranges from 20 to 60% [13, 33]. In our study, 41% of patients LTFU in the first 6 months of ART in the SOC phase had died by 6 months of follow-up. Accumulating data show that among LTFU patients who have died by the time of tracing, mortality rates are highest shortly after the last clinic visit, the majority (> 90%) die from illness rather than other causes (e.g., trauma), and the majority had some opportunity for clinical intervention at the last visit [33]. In addition, six previous trials, which aimed to evaluate Xpert impact on patient-important outcomes, have reported that LTFU of patients with bacteriologically confirmed TB, either before or during TB treatment, almost certainly reduces the potential impact of improved TB case finding on mortality [8].

The reductions in LTFU achieved in EC and EC+X phases compared with the SOC phase are likely due to a combination of factors, including the strengthened tracing intervention, additional training and nurses, and possibly reduced incidence of missed visits due to intercurrent illness from undiagnosed TB [34]. The intensified tracing intervention might be particularly helpful in maintaining a personalized partnership with clients struggling with adherence to clinic visit schedules for a variety of reasons to ensure minimal interruption in ART pill taking [34]. These data support the underlying principle that supportive services to retain patients in HIV care are an essential component of both the ICF and HIV treatment cascade.

The absence of an interaction between CD4 count at ART initiation and intervention package effect size suggests that ICF and retention interventions could be important for all new HIV clinic enrollees, not just those with advanced disease as defined by WHO (CD4 count < 200 copies/ml) [35]. Therefore, although median CD4 count at ART initiation is increasing in many countries, including Botswana [36], with most countries having adopted WHO universal HIV treatment guidelines, these data support current WHO recommendations that high-quality implementation of ICF and retention interventions remains important for HIV clinic enrollees.

This study has a number of strengths and limitations. Strengths include the large sample size, accurate

ascertainment of the primary mortality outcome, and implementation in a real-world programmatic setting, which improves generalizability of findings. Limitations include the fact that the primary objective relies on an adjusted pre-post analysis that is subject to residual confounding, and that data from the SOC phase were collected retrospectively. In the SOC phase, TB screening or sputum sample collection may sometimes have been implemented but not documented. While retrospective data collection in the SOC phase increases the likelihood of missing covariate data, it also ensures that the type of care received by clients in the SOC phase truly represents the care provided prior to implementation of the EC and EC+X interventions. While EC and EC+X phases were of different duration, our study results show good compliance with ICF algorithm implementation and impressive active tracing impact on LTFU throughout EC and EC+X phases, indicating no discernable lag time needed for these interventions to reach maximum potential. In addition, good implementation of Xpert in the EC+X phase is evidenced by the increase in the percentage of TB cases that were microbiologically confirmed in EC+X versus EC phases, and in the shorter time from sputum collection to TB treatment in EC+X versus EC phases, with these results consistent with several prior Xpert impact trials [8]. Notably, while these data support effectiveness of the ICF and retention intervention in reducing early ART mortality, future economic evaluation would be needed to explore cost-effectiveness.

Conclusions

In summary, a health system strengthening intervention to improve compliance with WHO-recommended TB symptom screening and ICF algorithms, combined with active tracing to support retention of HIV and HIV-TB co-infected patients in care through the early period of ART, was associated with significant reductions in early ART mortality and should be considered for scale-up. In addition, similar to most other trials of Xpert impact on mortality, replacing sputum-smear microscopy with Xpert was not associated with a mortality reduction.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12916-019-1489-0.

Additional file 1. Text showing selection criteria for study clinics.

Additional file 2. Table of standard clinical follow-up of clients in SOC, EC, and EC+X phases (2010–2015).

Additional file 3. Table of indicators used to assess implementation of TB ICF and retention in the HIV care cascade.

Additional file 4. Table comparing demographic and clinical characteristics between prospective study enrollees in the EC and EC+X phases and eligible clients declining enrollment.

Additional file 5. Figure of cumulative 6-month ART mortality stratified by SOC, EC, and EC+X phases among (a) enrollees with CD4 < 200 cells/μL, (b) CD4 ≥ 200 cells/μL.

Additional file 6. Table of sensitivity analyses of primary and secondary study outcomes - comparison of mortality rates between study phases.

Additional file 7. Table of sensitivity analyses of primary and secondary study outcomes to account for non-response - comparison of mortality rates between study phases.

Additional file 8. Table showing predictors of being screened for at least one TB symptom in the standard of care phase of XPRES.

Additional file 9. Table comparing 6-month ART outcomes before versus after efforts to ascertain accurate primary mortality outcome status among clients LTFU by study phase.

Additional file 10. Table showing differences in rates of uncorrected loss to follow-up in the first 6 months of ART between SOC, EC, and EC+X phases.

Acknowledgements

The authors would like to thank all study participants, participating health facilities, and project team members for their contributions to improving TB and HIV care in Botswana. We also thank Prof. Stephen D. Lawn for his contributions as an advisor and subject matter expert prior to his death on Sept 23, 2016.

Authors' contributions

AFA, TA, RB, AD, SP, HA, JCS, and TVE were involved in the study conception and the study design. AFA, TVE, RB, TA, JCS, and AF obtained the funding. AFA, TA, AM, RB, AD, SP, CS, UM, HA, GR, PP, JCS, and AF implemented the study. AFA, SP, and KF were the study statisticians. AFA, SP, KF, ADG, CS, TA, JCS, and AF were involved in data management and planning the analysis. AFA did the analysis. All authors were involved in interpreting the data. AFA wrote the first draft. All authors read and approved the final manuscript.

Funding

This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the US Centers for Disease Control and Prevention. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to an IRB decision which was made in the interest of ensuring patient confidentiality but are available from the corresponding author on reasonable request. Per IRB guidance, the datasets will be anonymized before sharing.

Ethics approval and consent to participate

Ethical approvals for this study were obtained from the US Centers for Disease Control and Prevention (CDC) Institutional Review Board (IRB) C, the Health Research and Development Division of the Health Research and Development Committee (HRDC) in Botswana, and the University of Pennsylvania IRB No.4. All consent procedures were approved by the ethical review committees. For the SOC cohort, a waiver of informed consent for chart abstraction was granted in accordance with 45CFR 46.116 (d). Written informed consent was obtained from all EC and EC+X enrollees. XPRES is registered at ClinicalTrials.gov (trial registration no. NCT02538952). Oversight of study initiation and quarterly review of implementation was conducted by the Office of the Associate Director of Science at CDC Atlanta. Per guidance from oversight bodies, XPRES was registered at ClinicalTrials.gov following the change in the US Health and Human Services (HHS) and National Institutes of Health (NIH) accepted definition of a clinical trial on January 25, 2015 [37]. At the time of study initiation on August 1, 2012, investigators and oversight bodies did not consider XPRES met the pre-2015 HHS and NIH definition of a clinical trial that was testing the "safety and effectiveness" of an intervention, because the XPRES interventions were already considered safe

and were recommended by the Ministry of Health and World Health Organization.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Division of Global HIV & TB, Centers for Disease Control and Prevention, Atlanta, USA. [2]Center for Global Health, U.S. Centers for Disease Control and Prevention (CDC), Lilongwe, Malawi. [3]Division of TB Elimination, Centers for Disease Control and Prevention, Gaborone, Botswana. [4]Ministry of Health, Gaborone, Botswana. [5]Yale University School of Medicine, New Haven, CT, USA. [6]TB Centre, London Sch. of Hygiene & Tropical Med, London, UK. [7]School of Public Health, University of the Witwatersrand, Johannesburg, South Africa. [8]Africa Health Research Institute, School of Nursing and Public Heath, University of KwaZulu-Natal, Durban, South Africa.

Received: 18 August 2019 Accepted: 24 December 2019
Published online: 11 February 2020

References
1. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS. 2015;29(15):1987–2002.
2. Gupta A, Nadkarni G, Yang WT, Chandrasekhar A, Gupte N, Bisson GP, et al. Early mortality in adults initiating antiretroviral therapy (ART) in low- and middle-income countries (LMIC): a systematic review and meta-analysis. PLoS One. 2011;6(12):e28691.
3. Braitstein P, Brinkhof MW, Dabis F, Schechter M, Boulle A, Miotti P, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. Lancet. 2006; 367(9513):817–24.
4. Wong EB, Omar T, Setlhako GJ, Osih R, Feldman C, Murdoch DM, et al. Causes of death on antiretroviral therapy: a post-mortem study from South Africa. PLoS One. 2012;7(10):e47542.
5. World Health Organisation. Rapid Implementation of the Xpert MTB/RIF diagnostic test. http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf. Accessed 10 Aug 2019.
6. Auld AF, Agizew T, Pals S, Finlay A, Ndwapi N, Boyd R, et al. Implementation of a pragmatic, stepped-wedge cluster randomized trial to evaluate impact of Botswana's Xpert MTB/RIF diagnostic algorithm on TB diagnostic sensitivity and early antiretroviral therapy mortality. BMC Infect Dis. 2016; 16(1):606.
7. Date A, Modi S. TB screening among people living with HIV/AIDS in resource-limited settings. J Acquir Immune Defic Syndr. 2015;68(Suppl 3): S270–3.
8. Auld AF, Fielding KL, Gupta-Wright A, Lawn SD. Xpert MTB/RIF - why the lack of morbidity and mortality impact in intervention trials? Trans R Soc Trop Med Hyg. 2016;110(8):432–44.
9. Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme CC. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? Eur Respir J. 2016;48(2): 516–25.
10. Churchyard GJ, Stevens WS, Mametja LD, McCarthy KM, Chihota V, Nicol MP, et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. Lancet Glob Health. 2015;3(8):e450–7.
11. Dryden-Peterson S, Lockman S, Zash R, Lei Q, Chen JY, Souda S, et al. Initial programmatic implementation of WHO option B in Botswana associated with increased projected MTCT. J Acquir Immune Defic Syndr. 2015;68(3): 245–9.
12. Getahun H, Kittikraisak W, Heilig CM, Corbett EL, Ayles H, Cain KP, et al. Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. PLoS Med. 2011;8(1):e1000391.
13. Brinkhof MW, Pujades-Rodriguez M, Egger M. Mortality of patients lost to follow-up in antiretroviral treatment programmes in resource-limited settings: systematic review and meta-analysis. PLoS One. 2009;4(6):e5790.
14. Moulton LH, Golub JE, Durovni B, Cavalcante SC, Pacheco AG, Saraceni V, et al. Statistical design of THRio: a phased implementation clinic-randomized study of a tuberculosis preventive therapy intervention. Clin Trials. 2007;4(2):190–9.
15. Bisson GP, Gaolathe T, Gross R, Rollins C, Bellamy S, Mogorosi M, et al. Overestimates of survival after HAART: implications for global scale-up efforts. PLoS One. 2008;3(3):e1725.
16. May M, Boulle A, Phiri S, Messou E, Myer L, Wood R, et al. Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. Lancet. 2010; 376(9739):449–57.
17. Farahani M, Price N, El-Halabi S, Mlaudzi N, Keapoletswe K, Lebelonyane R, et al. Trends and determinants of survival for over 200 000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. AIDS. 2016;30(3):477–85.
18. Hemming K, Haines TP, Chilton PJ, Girling AJ, Lilford RJ. The stepped wedge cluster randomised trial: rationale, design, analysis, and reporting. BMJ. 2015; 350:h391.
19. Johnson LF, Mossong J, Dorrington RE, Schomaker M, Hoffmann CJ, Keiser O, et al. Life expectancies of South African adults starting antiretroviral treatment: collaborative analysis of cohort studies. PLoS Med. 2013;10(4): e1001418.
20. World Health Organisation. Guidelines for Intensified Tuberculosis Case-Finding and Isoniazid Preventive Therapy for People Living with HIV in Resource Constrained Settings. Available at: http://www.who.int/hiv/pub/tb/9789241500708/en/. Accessed 10 Aug 2019.
21. Ngwira LG, Corbett EL, Khundi M, Barnes GL, Nkhoma A, Murowa M, et al. Screening for tuberculosis with Xpert MTB/RIF versus fluorescent microscopy among adults newly diagnosed with HIV in rural Malawi: a cluster randomized trial (CHEPETSA). Clin Infect Dis. 2018;68(7):1176–83.
22. Auld AF, Blain M, Ekra KA, Kouakou JS, Ettiegne-Traore V, Tuho MZ, et al. Wide variations in compliance with tuberculosis screening guidelines and tuberculosis incidence between antiretroviral therapy facilities - Cote d'Ivoire. PLoS One. 2016;11(6):e0157059.
23. Chihota VN, Ginindza S, McCarthy K, Grant AD, Churchyard G, Fielding K. Missed opportunities for TB investigation in primary care clinics in South Africa: experience from the XTEND trial. PLoS One. 2015;10(9):e0138149.
24. Hamada Y, Lujan J, Schenkel K, Ford N, Getahun H. Sensitivity and specificity of WHO's recommended four-symptom screening rule for tuberculosis in people living with HIV: a systematic review and meta-analysis. Lancet HIV. 2018;5(9):e515–23.
25. Auld AF, Mbofana F, Shiraishi RW, Alfredo C, Sanchez M, Ellerbrock TV, et al. Incidence and determinants of tuberculosis among adults initiating antiretroviral therapy--Mozambique, 2004-2008. PLoS One. 2013;8(1):e54665.
26. Ndwiga C, Birungi H, Undie CC, Weyenga H, Sitienei J. Feasibility and effect of integrating tuberculosis screening and detection in postnatal care services: an operations research study. BMC Health Serv Res. 2013;13:99.
27. Christian CS, Gerdtham UG, Hompashe D, Smith A, Burger R. Measuring quality gaps in TB screening in South Africa using standardised patient analysis. International journal of environmental research and public health. 2018;15(4):729.
28. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet. 2014;383(9915):424–35.
29. Lawn SD, Nicol MP, Corbett EL. Effect of empirical treatment on outcomes of clinical trials of diagnostic assays for tuberculosis. Lancet Infect Dis. 2015; 15(1):17–8.
30. Agizew T, Nyirenda S, Mathoma A, Mathebula U, Date A, Kgwaadira B, et al. Comparison of pre- and post-Xpert tuberculosis treatment outcomes among people living with HIV in Botswana. (Abstract # PC-1124-06). 46th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease. Cape Town, 2–6 December 2015. Available at: https://theunion.org/what-we-do/journals/ijtld/body/Abstract_Book_2015-Web.pdf. Accessed 30 Nov 2019.
31. Di Tanna GL, Khaki AR, Theron G, McCarthy K, Cox H, Mupfumi L, et al. Effect of Xpert MTB/RIF on clinical outcomes in routine care settings: individual patient data meta-analysis. Lancet Glob Health. 2019;7(2):e191–9.

32. Haas AD, Zaniewski E, Anderegg N, Ford N, Fox MP, Vinikoor M, et al. Retention and mortality on antiretroviral therapy in sub-Saharan Africa: collaborative analyses of HIV treatment programmes. J Int AIDS Soc. 2018; 21(2):e25084.
33. Holmes CB, Sikazwe I, Sikombe K, Eshun-Wilson I, Czaicki N, Beres LK, et al. Estimated mortality on HIV treatment among active patients and patients lost to follow-up in 4 provinces of Zambia: findings from a multistage sampling-based survey. PLoS Med. 2018;15(1):e1002489.
34. Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. Lancet. 2015;385(9983):2173–82.
35. World Health Organisation. Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy. In: Guidelines for Managing Advanced HIV Disease and Rapid Initiation of Antiretroviral Therapy. edn. Geneva; 2017.
36. Auld AF, Shiraishi RW, Oboho I, Ross C, Bateganya M, Pelletier V, et al. Trends in prevalence of advanced HIV disease at antiretroviral therapy enrollment - 10 countries, 2004-2015. MMWR Morb Mortal Wkly Rep. 2017; 66(21):558–63.
37. NIH. Key Dates & Policy Notices - Clinical Research/Trial Policies & Effective Dates. Available at: https://grants.nih.gov/policy/clinical-trials/key-dates-and-policy-notices.htm. Accessed 10 Aug 2019.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

BMC

5.1. Published research paper supplementary material

Additional file 1 - Text: Selection criteria for study clinics

XPRES study clinics were purposively selected to be representative of ART clinics in Botswana, while also ensuring sample sizes could be reached. Clinic characteristics that were taken into account have been previously published and included:

- All 22 clinics had at least one year's experience in providing ART services.
- 21 of 22 sites had ART enrolment rates ≥8 ART patients per month (mean 23/month; range 8-46/month) according to routine program data. These enrolment rates were anticipated to meet study sample size requirements. One site had an unknown enrolment rate at study initiation (Gantsi), but enrolment rates of eight ART enrolees/month were observed during study conduct.
- The study clinic with initially unknown ART enrolment rates (Gantsi) was selected because it was thought to have a high prevalence of MDR TB among HIV clinic enrolees and MOH believed these patients would benefit from early rollout of the Xpert device.
- Accessibility to either onsite or off-site TB laboratories was representative of HIV care and treatment centres in Botswana
- All sites were implementing the microscopy-based TB diagnostic algorithm prior to study initiation.
- All sites had the ability to perform testing, or to transport specimens for, haematology, serum chemistry, and CD4 count analysis, as is standard for ART sites in Botswana.

Table 5.4. (Research paper additional file 2) Clinical follow-up of clients in SOC, EC, and EC+X phases (2010-2015)

Pre-ART, CD4 >350	**3 monthly**	Weight, CD4, TB screen
ART	**ART start**	Weight, CD4, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen, Hepatitis B screen, creatinine if TDF-based regimen
	2 weeks	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen
	1 month	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen
	3 months	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen, Viral load, creatinine if TDF-based regimen
	6months	Weight, TB screen, ALT/AST[a] if NVP-based regimen, Hb if AZT-based regimen, Viral load, CD4
	Quarterly[b]	Weight, TB screen, Viral load and CD4 6 monthly, creatinine if TDF-based regimen 6 monthly

Abbreviations: CD4, CD4 cell count; TB, tuberculosis; ALT, alanine transaminase; AST, aspartate aminotransferase; NVP, nevirapine; AZT, zidovudine; TDF, tenofovir;
[a]Routine ALT/AST not required after 6 months but may be requested by the clinician depending on the clinical situation.
[b]For those patients started on PI-based regimens, baseline and 12-monthly glucose (random or fasting) and total cholesterol/triglycerides are recommended.

Table 5.5. (Research paper additional file 3) - Table: Indicators used to assess implementation of TB ICF and retention in the HIV care cascade

Indicators used to monitor the intensified TB case finding (ICF) cascade

	Indicator	Denominator and Numerator
1	% of ART enrolees screened for ≥1 WHO-recommended TB symptom before or on the day of ART initiation	**Denominator:** The number of ART enrolees eligible for TB symptom screening (i.e., are not already diagnosed with TB). **Numerator:** The number of ART enrolees with documented screening for ≥1 of four WHO-recommended TB symptoms (cough, loss of weight, fever, night sweats) before or on the day of ART initiation
2	% of ART enrolees screened for all four WHO-recommended TB symptoms before or on the day of ART initiation	**Denominator:** Same as in #1 **Numerator:** The number of ART enrolees with documented screening for all four WHO-recommended TB symptoms (cough, loss of weight, fever, night sweats) before or on the day of ART initiation
3	% of ART enrolees screening positive for ≥1 of four WHO-recommended TB symptoms before or on the day of ART initiation	**Denominator:** Same as in #1 **Numerator:** The number of ART enrolees screening positive for ≥1 of four WHO-recommended TB symptoms before or on the day of ART initiation.
4	% of ART enrolees having a sputum sample analysed at the lab to diagnose TB	**Denominator:** Same as in #1 **Numerator:** The number of ART enrolees having a sputum sample sent for analysis on or before the date of ART initiation.
5	% of ART enrolees newly diagnosed with either extra-pulmonary or pulmonary TB	**Denominator:** Same as in #1 **Numerator:** The number of ART enrolees newly diagnosed with TB before ART initiation or during the first 6 months of ART.

Indicators used to monitor loss to follow-up from ART

	Indicator	Denominator and Numerator
1	Rate of uncorrected[a] LTFU from ART during the first 6 months of ART	**Denominator:** Person-years of follow-up starting on the day of ART initiation and ending at the event of interest, which would be the date of last attended follow-up appointment for those LTFU, date of death, date of transfer out or departure from the study, or 6 months of follow-up if still alive and on ART at 6 months after ART initiation. **Numerator:** The number of clients meeting the definition of LTFU within the first 6 months of ART (i.e., >60 days late for the next scheduled appointment).

[a]Mortality ascertainment efforts among all patients meeting the LTFU definition were implemented with subsequent correction of 6-month ART outcomes (see additional file 7).

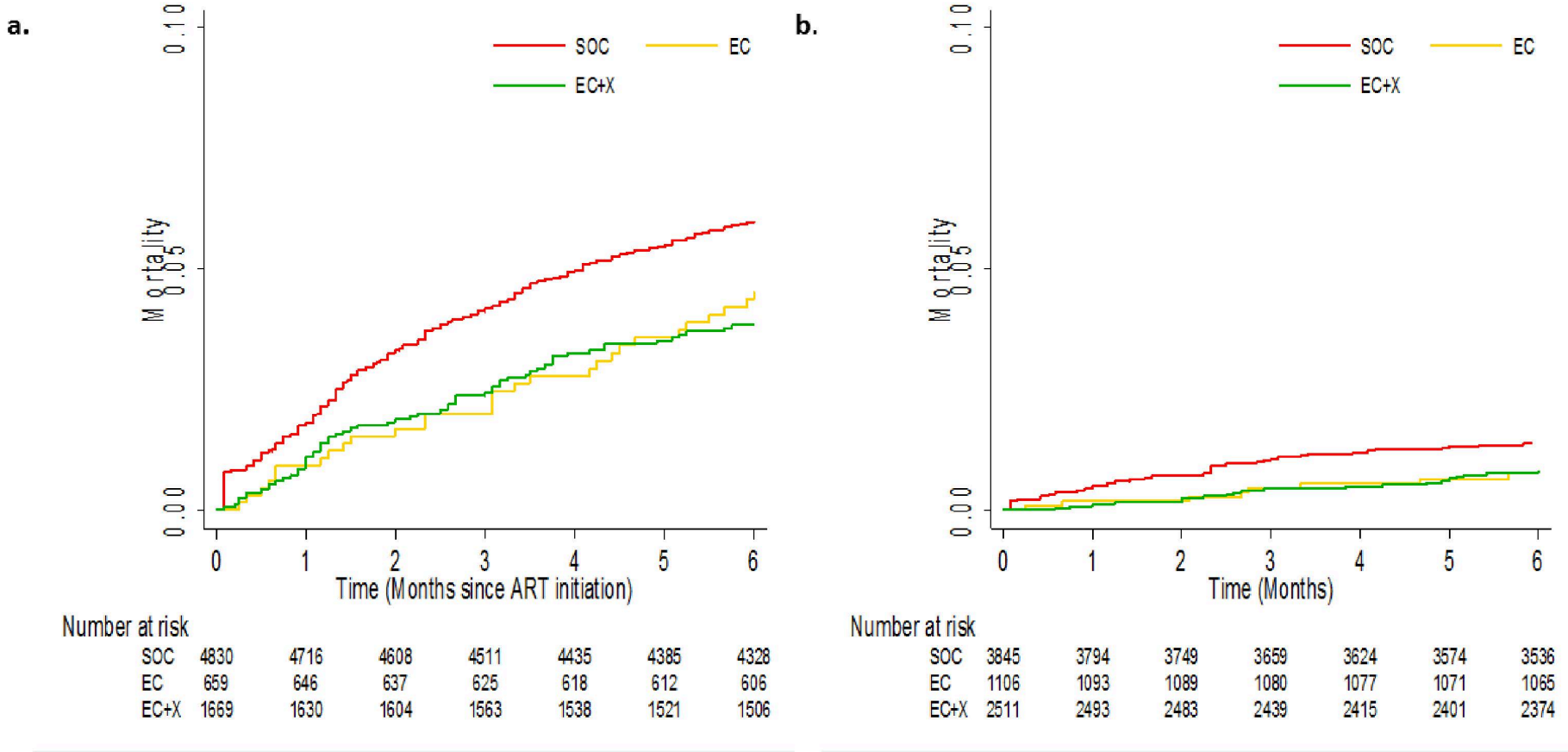
Table 5.6. (Research paper additional file 4) Comparison of demographic and clinical characteristics between prospective study enrolees in the EC and EC+X phases and eligible clients declining enrolment

	EC (N=1,768)		EC+X (N=4,215)		Declined Enrolment in Prospective Cohorts (N=2,439)[a]	
	n	%/median (IQR)	n	%/median (IQR)	n	%/median (IQR)
Age						
n, Median, (IQR)	1,768	34 (29-42)	4,215	34 (29-41)	2,439	34 (28-41)
Missing	0	0%	0	0%	0	0%
Gender						
Female	1,194	68%	2,797	66%	1,650	68%
If female, pregnant?						
Yes	271	23%	903	32%	580	35%
Weight (Kg)						
Median (IQR)	1,765	58.6 (51.3-67.8)	4,209	59.4 (52.5-68.7)	2,246	60.3 (52.8-69.5)
Missing	3	0.20%	6	0.10%	193	8%
Weight (Kg)						
<45 kg	160	9%	318	8%	177	8%
45-60 kg	817	46%	1,910	45%	931	41%
>60 kg	788	45%	1,981	47%	1,138	51%
Baseline CD4 (cells/µL)						
Median (IQR)	1765	246 (148-310)	4,180	241 (132-321)	2,367	242 (139-322)
Missing	3	0.20%	35	0.80%	72	3%
Baseline CD4 (cells/µL)						
<50	132	7%	370	9%	195	8%
50-<100	161	9%	371	9%	212	9%
100-<200	366	21%	928	22%	517	22%
200-<350	947	54%	1,928	46%	1,081	46%
350-<500	93	5%	334	8%	206	9%
≥500	66	4%	249	6%	156	7%
Baseline Haemoglobin (g/dL)						
Median (IQR)	1,678	11.9 (10.4-13.3)	3,911	12.0 (10.6-13.3)	2,169	11.9 (10.5-13.2)
Missing	90	5%	304	7.20%	270	11%
Haemoglobin category						
Severe anaemia	68	4%	109	3%	73	3%
Mild/moderate anaemia	805	48%	1,810	46%	1,017	47%
No anaemia	805	48%	1,992	51%	1,079	50%

Abbreviations: SOC, standard of care phase; EC, enhanced care phase; EC+X, enhanced care plus Xpert phase; IQR, interquartile range;

[a]Note that a total of 2,496 patients declined to enrol. However, of these patients, 57 were <12 at the time of first presentation to the clinic and were therefore ineligible for this analysis and are not included in the column of clients declining enrolment to facilitate comparisons with EC and EC+X cohorts. Of all 2,439 clients >=12 at first presentation to the study clinic, 2,430 (99.6%) were documented to have started ART by the end of the prospective cohort enrolment period (March 31, 2014). The mean percentage of patients declining enrolment by clinic was 29% (range: 6%-51%).

S5 - Figure: Cumulative 6-month ART mortality stratified by SOC, EC, and EC+X phases among (a) enrollees with CD4 <200 cells/µL, (b) CD4 ≥200 cells/µL*



*Likelihood ratio test comparing model with interaction between CD4 strata and intervention effect, and model without interaction: p=0.721

Additional file 6 - Table: Sensitivity analyses of primary and secondary study outcomes - comparison of mortality rates between study phases

		Pre-specified Multivariable Model[a]					Model 1[b] (Sensitivity Analysis)[a]					Model 2[c] (Sensitivity Analysis)[a]				
	Total ART enrollees	Total deaths incl. in adjusted analysis[d]	Rate/ 100PY among patients incl. in adjusted analysis[e]	AHR[f]	(95% CI)	p	Total deaths incl. in adjusted analysis[d]	Rate/ 100PY among patients incl. in adjusted analysis[e]	AHR[f]	(95% CI)	p	Total deaths incl. in adjusted analysis[d]	Rate/ 100PY among patients incl. in adjusted analysis[e]	AHR[f]	(95% CI)	p
Primary Outcome:																
6-month ART Mortality in SOC versus EC+X phase																
SOC	8980	350	10.8	1.00	--	--	329	12.0	1.00	--	--	329	12.0	1.00	--	--
EC+X	4215	93	5.2	0.77	(0.61-0.97)	0.029	93	5.2	0.78	(0.61-0.98)	0.037	108	4.9	0.78	(0.63-0.98)	0.033
Secondary Outcomes:																
12-month ART Mortality in SOC versus EC+X phase																
SOC	8980	424	7.0	1.00	--	--	376	8.6	1.00	--	--	376	8.6	1.00	--	--
EC+X	4215	108	3.9	0.76	(0.61-0.95)	0.014	108	3.9	0.77	(0.62-0.96)	0.021	133	3.0	0.74	(0.60-0.91)	0.005
6-month ART Mortality in EC versus EC+X phase[g]																
EC	1768	43	5.5	1.00	--	--	28	7.2	1.00	--	--	28	7.2	1.00	--	--
EC+X	4215	93	5.2	1.13	(0.63-2.03)	0.690	93	5.2	0.90	(0.42-1.95)	0.793	108	5.0	0.79	(0.41-1.50)	0.472

Abbreviations: SOC, standard of care phase; EC, enhanced care phase; EC+X, enhanced care plus Xpert phase; PY, person-years; HR, hazard ratio; AHR, adjusted hazard ratio; CI, confidence interval; XPRES, Xpert Package Rollout Evaluation using a Stepped-Wedge design

[a]All Cox proportional hazards regression models included a random effect for clinic.

[b]Model 1 sensitivity analysis censored all follow-up time for ART enrollees at the time that the new phase began. For example, if an SOC enrollee started ART in the SOC phase, but 6- or 12-month follow-up time crossed over into the EC phase, the follow-up time for that patient was censored at the start of the EC phase for that clinic.

[c]Model 2 sensitivity analysis created a new time-dependent covariate to specify exposure to the contemporary intervention phase. Therefore, all follow-up time for ART enrollees is assigned to the phase in which the follow-up time occurred. For example, if an EC enrollee started ART in the EC phase, and 6- or 12-month follow-up time crossed over into the EC+X phase, all follow-up time that occurred in the EC+X phase for that patient was assigned to the EC+X phase rather than the EC phase.

[d]Represents the total deaths included in the adjusted analysis comparing mortality rates between phases. In unadjusted analyses among all enrollees, by 6 months after ART enrollment, there were 461 deaths among SOC enrollees, 54 deaths among EC enrollees, and 121 deaths among EC+X enrollees. By 12 months after ART enrollment, there were 551 deaths among SOC, and 137 deaths among EC+X enrollees.

[e]Represents the unadjusted mortality rates among enrollees included in the complete case analysis to generate the AHRs.

[f]Adjusted for the following characteristics at ART initiation: age, sex, pregnancy status, weight, CD4 count, hemoglobin, and ART regimen. Pre-specified, complete case, adjusted analysis and sensitivity analyses comparing SOC, EC and EC+X mortality rates included 7,184 SOC, 1,653 EC, and 3,861 EC+X enrollees.

[g]Analysis restricted to randomised stepped-wedge portion of the trial, fitting a Cox proportional hazards regression model to the data with the underlying time frame beginning August 2012 (the start of EC enrollment), and including a fixed effect for monthly changes in mortality rates during the first 6 months of ART.

Additional file 7 - Table: Sensitivity analyses of primary and secondary study outcomes to account for non-response - comparison of mortality rates between study phases

		Pre-specified Multivariable Model[a]					Sensitivity Analysis using Inverse Probability Weighting to Account for non-Response[ab]					
	Total ART enrollees accepting study enrollment	Total deaths incl. in adjusted analysis[c]	Rate/100PY among patients incl. in adjusted analysis[d]	AHR[e]	(95% CI)	p	Total ART enrollees at study clinics including those not enrolled in the study[f]	Total deaths incl. in adjusted analysis[g]	Rate/100PY among patients incl. in adjusted analysis[h]	AHR	(95% CI)	p
Primary Outcome:												
6-month ART Mortality in SOC versus EC+X phase												
SOC	8,980	350	10.8	1	--	--	8,980	350	10.8	1	--	--
EC+X	4,215	93	5.2	0.77	(0.61-0.97)	0.029	5,757	122	5.2	0.75	(0.55-1.02)	0.067
Secondary Outcomes:												
12-month ART Mortality in SOC versus EC+X phase												
SOC	8,980	424	7.0	1	--	--	8,980	424	7.0	1	--	--
EC+X	4,215	108	3.9	0.76	(0.61-0.95)	0.014	5,757	143	3.9	0.75	(0.57-0.99)	0.040
6-month ART Mortality in EC versus EC+X phase[i]												
EC	1,768	43	5.5	1	--	--	2,665	60	5.4			
EC+X	4,215	93	5.2	1.13	(0.63-2.03)	0.690	5,757	122	5.2	1.12	(0.50-2.50)	0.785

Abbreviations: SOC, standard of care phase; EC, enhanced care phase; EC+X, enhanced care plus Xpert phase; PY, person-years; HR, hazard ratio; AHR, adjusted hazard ratio; CI, confidence interval; XPRES, Xpert Package Rollout Evaluation using a Stepped-Wedge design

[a]All Cox proportional hazards regression models included a random effect for clinic. All adjusted models were adjusted for the following characteristics at ART initiation: age, sex, pregnancy status, weight, CD4 count, hemoglobin, and ART regimen.

[b]Pre-specified sensitivity analysis adjusted for non-response using an inverse probability weighting approach. Inverse probability weights were calculated by applying separate adjusted logistic regression models to hospital versus clinic enrolling EC and EC+X patients, to predict the probability of being enrolled in the EC and EC+X phases. An adjusted logistic regression approach was used to estimate inverse probability weights to lower the likelihood of bias given the non-random enrollment approach.

[c]In unadjusted analysis, by 6 months after ART enrollment, there were 461 deaths among SOC enrollees, 54 deaths among EC enrollees, and 121 deaths among EC+X enrollees. By 12 months after ART enrollment, there were 551 deaths among SOC, and 137 deaths among EC+X enrollees.

[d]Represents the unadjusted mortality rates among enrollees included in the primary pre-specified complete case analysis to generate the AHRs.

[e]Pre-specified, complete case, adjusted analysis comparing SOC, EC and EC+X mortality rates included 7,184 SOC, 1,653 EC, and 3,861 EC+X enrollees.

[f]Column shows total ART enrollees in each study phase, regardless of whether they were enrolled in the study. The total number of ART enrollees included in the complete case AHR analysis after up-weighting includes 7,184 SOC, 2,375 EC, and 5,109 EC+X enrollees.

[g]Represents the number of deaths up-weighted to account for non-enrollment, that were included in the complete case AHR analysis.

[h]Represents the up-weighted unadjusted mortality rates among enrollees included in the complete case analysis to generate the AHRs.

[i]Analysis restricted to randomised stepped-wedge portion of the trial, fitting a Cox proportional hazards regression model to the data with the underlying time frame beginning August 2012 (the start of EC enrollment), and including a fixed effect for monthly changes in mortality rates during the first 6 months of ART.

Table 5.9. (Research paper additional file 8) Table of predictors of being screened for at least one TB symptom in the standard of care phase of XPRES

	Not Screened (N=6,921)		Screened (N=1,700)		OR[a]	95% CI	p-value
	n	%	n	%			
Age[b]							
Median, (IQR)	6,911	35 (30-43)	1,699	36 (31-44)	1.06	(1.00-1.13)	0.056
Gender							
Female	4,402	81%	1,056	19%	1.00		
Male	2,519	80%	644	20%	1.07	(0.91-1.25)	0.445
If female, pregnant?[c]							
No	3621	80%	914	20%	1.00		
Yes	781	85%	142	15%	0.72	(0.53-0.99)	0.041
Weight (Kg)[d]							
<45 kg	589	72%	228	28%	1.00		
45-60 kg	3,057	79%	823	21%	0.70	(0.57-0.84)	<0.001
>60 kg	2,922	83%	609	17%	0.54	(0.42-0.69)	
Baseline CD4[e]							
<50	744	76%	230	24%	1.00		
50-<200	2,879	80%	730	20%	0.82	(0.69-0.97)	
200-<350	2,754	81%	630	19%	0.74	(0.59-0.93)	<0.001
350-<500	184	79%	49	21%	0.86	(0.55-1.35)	
≥500	120	88%	17	12%	0.46	(0.25-0.84)	
Haemoglobin[f]							
severe anaemia	292	75%	96	25%	1.00		
mild/moderate anaemia	3312	79%	859	21%	0.79	(0.58-1.07)	0.285
no anaemia	2400	80%	593	20%	0.75	(0.47-1.19)	

Abbreviations: OR, Odds Ratio; CI, confidence interval; IQR, inter-quartile range; XPRES, Xpert Package Rollout Evaluation using a Stepped-wedge design

[a]All logistic regression models specified a random effect for clinic. The P-value reported is that associated with the overall model's likelihood chi-square test statistic.

[b]Odds ratio of being screened for TB associated with being 10 year's older. Likelihood ratio test for departure from linearity (p=0.574). Age was missing for 10 (0%) of those not screened and 1 (0%) of those screened

[c]Restricted to female ART patients only

[d]Weight was missing for 353 (5%) of those not screened and 40 (2%) of those screened

[e]CD4 was missing for 240 (3%) of those not screened and 44 (3%) of those screened

[f]Haemoglobin was missing for 917 (13%) of those no screened and 152 (10%) of those screened

Table 5.10. (Research paper additional file 9) Comparison of 6-month ART outcomes before versus after efforts to ascertain accurate primary mortality outcome status among clients LTFU by study phase

	Before Ascertainment of Outcomes of Clients LTFU			After Ascertainment of Outcomes of Clients LTFU		
SOC 6 month ART Outcomes						
	n	**N**	**%**	**n**	**N**	**%**
Alive	7,956	8,980	89%	8,125	8,980	90%
Dead	322	8,980	4%	461	8,980	5%
LTFU	336	8,980	4%	28	8,980	0%
Transfer Out	366	8,980	4%	366	8,980	4%
Unable to Continue	0	8,980	0%	0	8,980	0%
EC 6 month ART Outcomes						
Alive	1,585	1,768	90%	1,594	1,768	90%
Dead	53	1,768	3%	54	1,768	3%
LTFU	10	1,768	1%	0	1,768	0%
Transfer Out	76	1,768	4%	76	1,768	4%
Unable to Continue	44	1,768	2%	44	1,768	2%
EC+X 6 month ART Outcomes						
Alive	3,613	4,215	86%	3,641	4,215	86%
Dead	119	4,215	3%	121	4,215	3%
LTFU	31	4,215	1%	1	4,215	0%
Transfer Out	325	4,215	8%	325	4,215	8%
Unable to Continue	127	4,215	3%	127	4,215	3%

Abbreviations: SOC, standard of care; EC, enhanced care; EC+X, enhanced care plus Xpert; LTFU, loss to follow-up (>60 days late for last scheduled appointment).

S10 - Table: Differences in rates of uncorrected loss to follow-up in the first 6 months of ART between SOC, EC, and EC+X phases

	n	6-month Rate/ 100PY	Crude HR	(95% CI)	p	AHR[a]	(95% CI)	p
Phase of enrollment								
Standard of Care (SOC)	8,980	8.3	1.00			1.00		
Enhanced Care (EC)	1,768	1.2	0.14	(0.07-0.26)	<0.001	0.05	(0.02-0.15)	<0.001
Enhanced Care Plus Xpert (EC+X)	4,215	1.6	0.21	(0.14-0.30)	<0.001	0.18	(0.12-0.27)	<0.001

Abbreviations: ART, antiretroviral therapy; PY, person years; HR, hazard ratio; CI, confidence interval; AHR, adjusted hazard ratio

[a]Adjusted for age, sex, pregnancy status, weight at ART initiation, CD4 count at ART initiation, hemoglobin level, and ART regimen. Adjusted analysis included 7,184 SOC, 1,653 EC, and 3,861 EC+X enrollees.

Chapter 6: Results | Risk score to inform who needs intensification of ART (Research Paper 3)

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	1405072	**Title**	Dr.
First Name(s)	Francis Andrew		
Surname/Family Name	Auld		
Thesis Title	Opportunities to reduce early antiretroviral therapy mortality in sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care		
Primary Supervisor	Prof. 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?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*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?	BMC Medicine
Please list the paper's authors in the intended authorship order:	Andrew F. Auld, Katherine Fielding, Tefera Agizew, Alice Maida, Anikie Mathoma, Rosanna Boyd, Anand Date, Sherri L. Pals, George Bicego, Yuliang Liu, Ray W. Shiraishi, Peter Ehrenkranz, Christopher Serumola, Unami Mathebula,

	Heather Alexander, Salome Charalambous, Courtney Emerson, Goabaone Rankgoane-Pono, Pontsho Pono, Alyssa Finlay, James C. Shepherd, Charles Holmes, Tedd V. Ellerbrock, Alison D. Grant
Stage of publication	**Undergoing revision**

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)	The candidate conceived the idea for the paper and analysis, conducted the data analysis, and wrote the paper.

SECTION E

Student Signature	[redacted]
Date	14 July 2020

Supervisor Signature	[redacted]
Date	16 July 2020

Predicting early antiretroviral therapy mortality in sub-Saharan Africa to inform who needs intensification of care: a derivation and external validation cohort study

Andrew F. Auld[1], Katherine Fielding[2,3], Tefera Agizew[4], Alice Maida[1], Anikie Mathoma[4], Rosanna Boyd[4], Anand Date[5], Sherri L. Pals[5], George Bicego[5], Yuliang Liu[5], Ray W. Shiraishi[5], Peter Ehrenkranz[6], Christopher Serumola[4], Unami Mathebula[4], Heather Alexander[5], Salome Charalambous[7], Courtney Emerson[5], Goabaone Rankgoane-Pono[8], Pontsho Pono[8], Alyssa Finlay[4], James C. Shepherd[4,9], Charles Holmes[10], Tedd V. Ellerbrock[5], Alison D. Grant[2,3,11]

[1] Division of Global HIV & TB, Centers for Disease Control and Prevention, Lilongwe, Malawi

[2] TB Centre, London Sch. of Hygiene & Tropical Med., London, United Kingdom

[3] School of Public Health, University of the Witwatersrand, Johannesburg, South Africa

[4] Division of TB Elimination, Centers for Disease Control and Prevention, Gaborone, Botswana

[5] Division of Global HIV & TB, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.

[6] Bill and Melinda Gates Foundation, Seattle, WA, U.S.A.

[7] Aurum Institute, Johannesburg, South Africa

[8] Ministry of Health, Gaborone, Botswana

[9] Yale University School of Medicine, New Haven, CT, USA

[10] Center for Global Health Practice and Impact, Georgetown University Medical Center, Washington D.C., U.S.A.

[11] Africa Health Research Institute, School of Nursing and Public Heath, University of KwaZulu-Natal, Durban, South Africa

Abstract

Background: Clinical scores to determine early (6-month) antiretroviral therapy (ART) mortality risk have not been developed for sub-Saharan Africa (SSA), home to 70% of people living with HIV. In the absence of validated scores, WHO eligibility criteria (EC) for ART care intensification are CD4 <200/μL or WHO stage III/IV.

Methods: We used Botswana XPRES trial data for adult ART enrollees to develop CD4-independent and -dependent multivariable prognostic models for 6-month mortality. Scores were derived by rescaling coefficients. Scores were developed using the first 50% of XPRES ART enrollees and their accuracy validated internally and externally using South African TB Fast Track (TBFT) trial data. Predictive accuracy was compared between scores and WHO EC.

Results: Among 5,553 XPRES enrollees, 2,838 were included in the derivation dataset; 68% were female and 83 (3%) died by 6 months. Among 1,077 TBFT ART enrollees, 55% were female and 6% died by 6 months. Factors predictive of 6-month mortality in the derivation dataset at $p<0.01$ and selected for the CD4-independent score included: male gender (2 points), ≥1 WHO tuberculosis symptom (2 points), WHO stage III/IV (2 points), severe anemia (hemoglobin <8g/dL) (3 points), and temperature >37.5˚C (2 points). The same variables plus CD4 <200/μL (1 point) were included in the CD4-dependent score. Among XPRES enrollees, a CD4-independent score of ≥4 would provide 86% sensitivity and 66% specificity, whereas WHO EC would provide 83% sensitivity and 58% specificity. If WHO stage alone was used, sensitivity was 48% and specificity 89%. Among TBFT enrollees, the CD4-independent score of ≥4 would provide 95% sensitivity and 27% specificity, whereas WHO EC would provide 100% sensitivity but 0% specificity. Accuracy was similar between CD4-independent and -dependent scores. Categorizing CD4-independent scores into low (<4), moderate (4-6), and high-risk (≥7) gave 6-month mortality of 1%, 4%, and 17% for XPRES and 1%, 5%, and 30% for TBFT enrollees.

Conclusions: Sensitivity of the CD4-independent score was nearly twice that of WHO stage in predicting 6-month mortality and could be used in settings lacking CD4 testing to inform ART care intensification. The CD4-dependent score improved specificity versus WHO EC. Both scores should be considered for scale-up in SSA.

Background

Over the last 16 years, the scale-up of HIV treatment globally has reached over 24.5 million people living with HIV (PLHIV) with lifesaving antiretroviral therapy (ART), resulting in declines in both HIV-associated mortality and HIV incidence [1-3]. However, each year there are still about 770,000 global AIDS-related deaths, with 470,000 (61%) of these deaths occurring in sub-Saharan Africa (SSA) [1]. To reduce AIDS-related mortality, the global community is striving to reach 2030 targets of ensuring at least 90% of PLHIV are on ART [4], which will require ART enrollment for an additional 10 million of the 37.9 million PLHIV globally, about two-thirds of whom live in sub-Saharan Africa (SSA) [1]. Mortality rates during ART are highest in the first 6 months of therapy, and these early ART mortality rates continue to be highest in SSA [5, 6]. If 2030 goals of reducing AIDS-related mortality by 90% compared with 2010 are to be met, substantial progress needs to be made in addressing early ART mortality in SSA [5, 6], where 20-40% of new ART enrollees still initiate ART with relatively advanced HIV disease [7, 8].

To achieve these mortality reductions, efficient use of available resources through differentiated service delivery (DSD) models to provide tailored, patient-centered care, will be needed [9, 10]. The World Health Organization (WHO) currently recommends intensification of care for persons >5 years old starting ART with advanced HIV disease as defined by $CD4^+$ T-cell (CD4) count <200 cells/μL or WHO stage III/IV [8]. The intensification of care package, which has been shown to reduce early mortality [11], includes cotrimoxazole prophylaxis, tuberculosis (TB) screening with subsequent TB treatment or TB preventive therapy, cryptococcal antigen (CrAg) screening with pre-emptive therapy for eligible CrAg-positive people, and enhanced adherence counseling. However, the majority of health facilities providing ART in low- and middle-income countries (LMIC) lack access to rapid or point-of-care (POC) CD4 testing [8]. In these settings, up to half of adults with a CD4 count <100/μL could be categorized as WHO stage I/II, and would be missed by an advanced disease screening algorithm that relied on WHO stage alone [11]. In addition, a screening tool for advanced disease that relies only on CD4

count and WHO disease stage misses the many other demographic and clinical predictors associated with early ART mortality [9]. To date, most analyses evaluating eligibility for DSD models have focused on identifying stable patients for de-escalation of care [9]. Only one analysis from Haiti has evaluated a clinical score for determining who needs intensification of early ART care and this was not externally validated [12].

Therefore, we evaluated whether a clinical score derived from easily available covariates at ART initiation in resource-constrained clinic settings could better predict who is at risk for early (6-month) ART mortality than the current WHO advanced disease eligibility criteria. We developed clinical scores to help predict early ART mortality risk for two scenarios: (1) a scenario where on-site/rapid off-site CD4 testing is not available as is the case for the majority of ART clinics in LMIC, and (2) a scenario where on-site/rapid off-site CD4 testing is available.

Methods

We used data from the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES) trial to derive the two clinical scores to help clinicians identify those at highest risk of early ART mortality and therefore in need of ART care intensification [13]. The first clinical score assumes CD4 is unavailable at ART initiation (i.e., a CD4-independent score) and the second clinical score assumes CD4 count is available (i.e., a CD4-dependent score). We used the first 50% of XPRES cohort enrollees to derive a prediction model, and the second 50% to internally validate the model. We then used data from the TB Fast Track (TBFT) trial in South Africa (SA) to externally validate the derived clinical scores [14]. We compared screening accuracy of our derived clinical scores with existing CD4-based WHO eligibility criteria for advanced disease and ART care intensification.

XPRES study design and participants for prediction tool development

XPRES was a multi-center, stepped-wedge cluster randomized trial with a retrospective baseline component conducted at 22 health facilities, including five hospitals and 17

clinics, that were purposively selected to be representative of HIV treatment clinics in Botswana [13]. In the prospective, stepped-wedge portion of the trial, all non-incarcerated, consenting, ART-naïve, HIV-positive persons, regardless of TB treatment or symptom status, presenting to the study clinics between August 2012 and end of March 2014, were eligible for enrollment. Only adolescents and adults (aged ≥12 years old), were included in this analysis.

XPRES procedures

Per Botswana national guidelines during the time period of the study, all XPRES study participants were eligible for ART initiation if they had a CD4 count ≤350 cells/μL, were diagnosed as having WHO stage III/IV events, or were pregnant or breastfeeding [15]. All study participants received clinical care and follow-up appointments per Ministry of Health (MOH) guidelines (see Additional file 1, a table summarizing standard clinical care follow-up).

Interventions

The prospective XPRES cohort was recruited within two phases of the stepped-wedge trial. In the first phase, all prospective XPRES participants received two enhanced care interventions in addition to standard of care: (1) additional support for intensified TB case finding, and (2) intensified tracing for patients missing clinic appointments. In the second phase, the Xpert® MTB/RIF assay (Cepheid; Sunnyvale, California) (Xpert) was initiated in place of sputum smear microscopy for TB diagnosis. We have previously shown that there was no significant difference in 6-month ART mortality between the two prospective phases of XPRES [16]. Enrollment and follow-up procedures are described in a supplementary appendix (see Additional file 2, text summarizing follow-up procedures). XPRES participants were followed for 12 months, or until the end of TB treatment, whichever was later. The final follow-up visits for XPRES enrollees were in June 2015.

Development and temporal validation of the prediction model

A clinically useful prediction model should demonstrate accurate prediction of the outcome in data other than that in which the model was developed. Therefore, we split the XPRES dataset in a 1:1 ratio using the mid-point of enrollment at each of the 22 study clinics to create the derivation dataset (the first 50% of enrollees) and the temporal validation dataset (the second 50% of enrollees) [17].

Outcome

The outcome of interest for both the XPRES trial and this analysis was early (6-month) ART mortality. We implemented intensive efforts to ascertain true mortality outcomes among participants, with deaths and date of death either passively reported to the clinic by friends or relatives or actively ascertained if the client had missed an appointment or was considered LTFU (>60 days late for a scheduled appointment) [18]. Initial efforts to ascertain outcomes of clients who missed an appointment by ≥1 day included up to five phone calls to the client or contact and up to two home visits. In addition, for all clients unreachable by phone or home visit who met the LTFU definition, vital status was ascertained through national Death Registry review. By law, since 1969, all deaths need to be registered in the Death Registry, which is maintained by the Botswana Civil and National Registration Office. Available data shows Death Registry data completeness to be high [16].

Candidate predictor variables

We selected candidate predictor variables for potential inclusion in the predictive model based on prior publications, and the need for variables to be reproducible, objective, and readily available in resource-constrained clinic settings [19]. We considered variables known to be associated with mortality including age, sex (coded as male, pregnant female, and non-pregnant female [20]), education level, employment status, smoking history, prior TB treatment, number of WHO TB symptoms, weight, body mass index (BMI)

(weight/height^2), hemoglobin level, CD4 count, temperature at ART initiation in degrees Celsius, and respiratory rate at ART initiation [20-23].

Within the derivation dataset, we performed univariable analyses assessing the association of each variable with risk of mortality using logistic regression. Because follow-up of all XPRES and TBFT enrollees was complete with true ascertainment of 6-month mortality outcomes, 6-month risk was preferred to rate [16]. Continuous variables were assessed for non-linearity with log odds of death using fractional polynomials, as well as by comparing Akaike's Information Criteria and Bayesian Information Criteria between models with linear or fractional polynomial terms. Where non-linearity was observed, the appropriate fractional polynomial terms were included in the logistic regression. We also examined scatter plots of linear and transformed continuous variables and risk of mortality to assess inflexion points which might inform appropriate categorization of continuous variables.

For the multivariable analysis, a complete case analysis, whereby observations with missing data for key variables were dropped, was chosen because few data (<10%) were missing. To generate a parsimonious multivariable model, we used a stepwise backward elimination approach, starting with all candidate variables and excluding variables sequentially if $p>0.01$ using both automatic and manual approaches. We also explored how findings changed using a forward stepwise addition approach. Where two or more predictors were highly correlated, only one was selected, to simplify the prognostic model. We created two multivariable models: one in which CD4 was purposefully excluded and one in which CD4 count was included as a candidate variable to reflect situations where CD4 is either unavailable or available at the clinic. Plausible interactions between covariates (e.g., between CD4 and age) were assessed using the likelihood ratio test.

In both the derivation and temporal validation datasets, we assessed multivariable model calibration, (i.e., the agreement between probability of 6-month mortality predicted by the model and observed probability of TB within quantiles of predicted risk) graphically in a calibration plot [17], and statistically using the Hosmer-Lemeshow test. We also assessed discrimination, the ability of our model to differentiate patients who died by 6 months of ART vs. those who did not, using the area under the receiver-operating characteristic (AUROC) curve, also referred to as the C-statistic or C-index. AUROC values of 0.7 to 0.79, 0.8±0.89, and >0.9 are respectively considered acceptable, excellent and outstanding discrimination [24].

Two final multivariable models were used to generate the two clinical scores (i.e., the CD4-independent and CD4-dependent scores). For these models, continuous variables were categorized in a clinically meaningful manner based on their functional form and information from the published literature. Each beta coefficient from this logistic regression model was then rescaled to generate a clinical score by dividing each coefficient by the smallest positive model coefficient and rounding to the nearest integer. The total number of points was summed for each participant to calculate their total clinical score.

External validation of risk scores

To externally validate the clinical risk score, we used data collected independently from the TBFT trial from SA [14]. TBFT was an open-label cluster-randomized controlled trial, recruiting individuals from 24 primary health-care clinics in SA. All outpatient, HIV-positive adults (aged ≥18 years) with CD4 counts <150/µL, no TB treatment in the past 3 months, and no ART in the last 6 months were eligible. In the intervention clinics, participants were classified by a study algorithm as having high, medium, or low TB risk. High TB risk patients (i.e., those with positive lateral flow urine lipoarabinomannan assay [LF-LAM], BMI <18.5, or hemoglobin <10 g/dL) started TB treatment immediately followed by ART 2 weeks later. Medium TB risk participants (i.e., those with ≥1 WHO TB symptom only) were

recommended to have symptom-guided TB investigation. Low TB risk patients (no TB symptoms or high-risk criteria) were recommended to start ART immediately. The primary outcome was all-cause mortality at 6 months after enrollment. We restricted this analysis to intervention arm participants, for whom key variables such as temperature at enrollment were available, and to those patients who started ART, since the outcome of interest was mortality within the first 6 months of ART. The median time from trial enrollment to ART start in the intervention arm was 21 days. Participants were enrolled in TBFT between December 19, 2012, and December 18, 2014. The clinical risk score for mortality was calculated by assigning the same 'points' to variables as for the derivation cohort.

For both the XPRES cohort (combined derivation and validation datasets), and the TBFT datasets, we explored how sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and AUROC curve values varied with increasing clinical score in terms of predicting 6-month mortality and compared this screening accuracy and discrimination performance with the WHO eligibility criteria for advanced disease. Three risk groups were created to visualize increasing 6-month ART mortality risk with increasing clinical score, and the percentage of ART enrollees falling into each risk group. Kaplan-Meier (K-M) curves were used to visualize rates of early mortality within the three risk groups.

All analyses were conducted using STATA 16 (StataCorp, 2009, Stata Statistical Software, Release 16, College Station, TX). The study is reported in concordance with TRIPOD guidance for multivariable prediction models (see Additional file 3, a table with the TRIPOD checklist).

Ethics approval and consent to participate

Ethical approval for each of the source studies was obtained from the relevant ethics committees in the country of data collection and from the trial sponsors. All participants

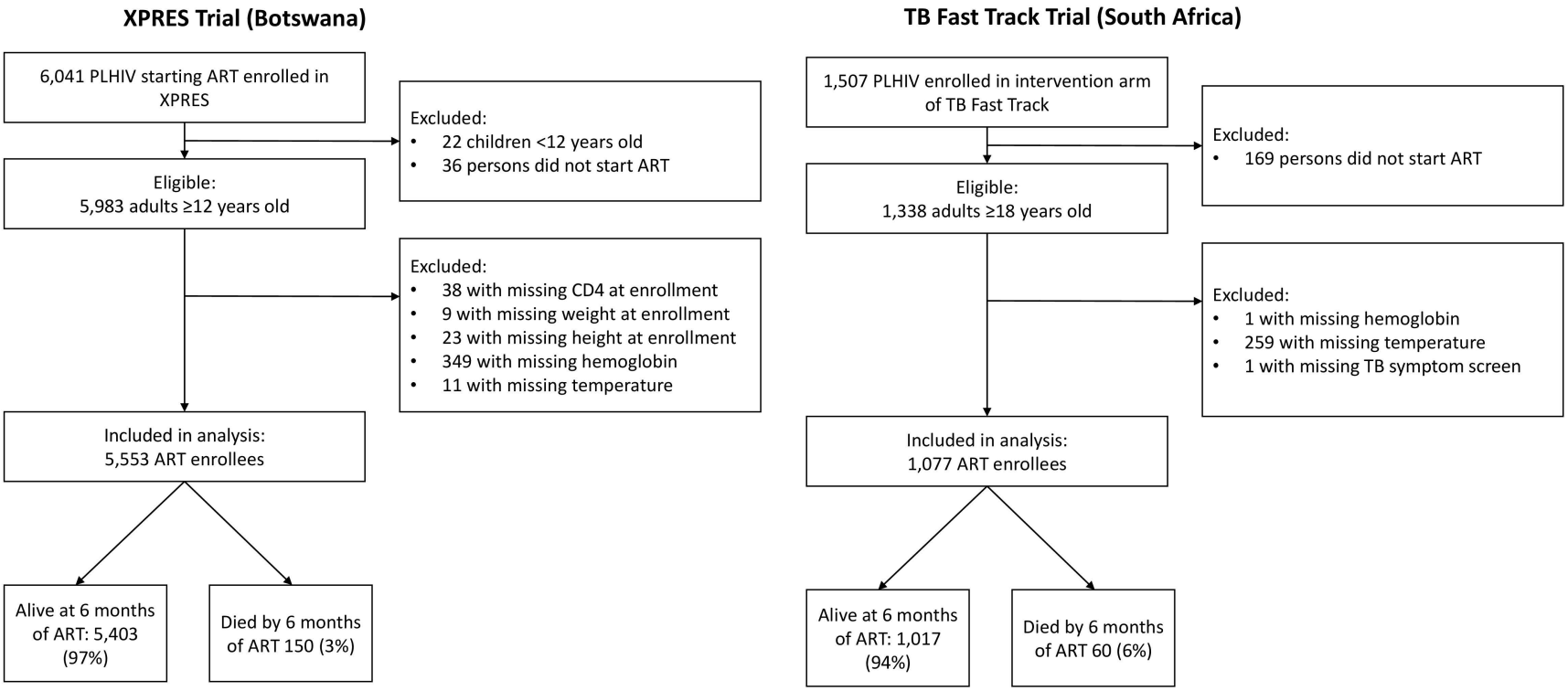
provided informed written consent, or where the enrollee could not read or write, witnessed verbal informed consent. Ethical approvals for XPRES were obtained from the U.S. Centers for Disease Control and Prevention (CDC) Institutional Review Board (IRB) C, the Health Research and Development Division of the Health Research and Development Committee (HRDC) in Botswana, and the University of Pennsylvania IRB No.4. All consent procedures were approved by the ethical review committees. Written informed consent was obtained from all prospective XPRES enrollees. XPRES is registered at ClinicalTrials.gov (trial registration no. NCT02538952). Oversight of study initiation and quarterly review of implementation was conducted by the Office of the Associate Director of Science at CDC Atlanta.

TBFT was approved by the research ethics committees of the University of the Witwatersrand and the London School of Hygiene & Tropical Medicine, and the South African Medicines Control Council. All participants provided written or witnessed verbal informed consent. This trial was registered with the ISRCTN registry, ISRCTN35344604, and the South African National Clinical Trials Register, DOH-27-0812-3902.

Results

From the XPRES cohort, 5,553 eligible ART enrollees with complete data for candidate predictors were included in the analysis (Fig 1). Overall, 150 (3%) of 5,553 ART enrollees died within 6 months of ART initiation.

Fig 1. Study profile



Internal derivation and temporal validation datasets

From the XPRES cohort, the internal derivation (N=2,838) and temporal validation (N=2,715) datasets were created (Table 1). Key characteristics including median age (34), percentage female (66-68%), median CD4 (240-245/μL), and 6-month mortality (2.5-2.9%), were similar between internal XPRES derivation and validation datasets (Table 1).

Table 1: Comparison of characteristics of antiretroviral therapy enrollees between internal derivation, internal validation, and external validation datasets

			Internal Derivation Dataset (N=2,838)		Internal Validation Dataset (N=2,715)		External Validation Dataset (TB Fast Track, SA; N=1,077)*	
Demographics			n	(median or %)	n	(median or %)	n	(median or %)
	Age, years, median (IQR)		2,838	33.8 (28.6-40.9)	2,715	34.0 (28.6-41.4)	1,077	38.0 (32.0-44.0)
	Female, n, %		1,938	68%	1,779	66%	590	55%
	If Female, Pregnant, n, %		520	27%	551	31%	0	0%
	Marital status	Married/Civil Union	300	11%	265	10%		
		Single	2,441	86%	2,346	86%		
		Widowed/Divorced	97	3%	104	4%		
	Smoking History (ever smoked), n, %		517	18%	551	20%	238	22%
	Currently Employed, n, %		1,270	45%	1,286	47%		
	Education	None	196	7%	200	7%		
		Primary	687	24%	596	22%		
		Secondary	1,734	61%	1,641	60%		
		Higher	221	8%	278	10%		
HIV/TB history								
	Previous TB treatment	Yes	277	10%	262	10%		
	WHO TB Symptoms							
	Cough	Yes	495	17%	547	20%	463	43%
	Weight loss	Yes	599	21%	555	20%	797	74%
	Fever	Yes	259	9%	245	9%	314	29%
	Night sweats	Yes	273	10%	253	9%	348	32%
	Number of WHO TB symptoms	0	1,975	70%	1,911	70%	230	21%
		1	427	15%	349	13%	285	26%
		2	202	7%	216	8%	226	21%
		3	141	5%	137	5%	178	17%
		4	93	3%	102	4%	158	15%
Clinical Characteristics								
	WHO stage III/IV, n, %		354	12%	307	11%		
	CD4	Median (IQR)	2,838	245 (143-315)	2,715	240 (134-319)	1,077	72 (36-110)
	Weight	Median (IQR)	2,838	58.8 (51.8-68.2)	2,715	59.7 (52.8-69.0)	1,077	57.9 (50.8-67.0)
	BMI	Median (IQR)	2,838	21.5 (18.9-24.9)	2,715	21.6 (19.1-25.0)	1,077	21.3 (18.8-25.0)
	Hemoglobin	Median (IQR)	2,838	11.9 (10.4-13.2)	2,715	12.0 (10.7-13.4)	1,077	11.3 (9.7-13.0)
	Temperature	Median (IQR)	2,838	36.2 (35.8-36.5)	2,715	36.2 (35.8-36.6)	1,077	36.4 (36.0-36.7)
	Respiratory rate	Median (IQR)	2,838	19 (18-20)	2,715	19 (18-20)		
Mortality within 6 months								
	Cumulative incidence		83	2.9%	67	2.5%	60	6%
	Time to death		83	50 (25-105)	67	46 (16-87)	60	55 (30-112)

Abbreviations: IQR, interquartile range; TB, tuberculosis; WHO, World Health Organization; CD4, $CD4^+$ T-cell count; TB, tuberculosis; BMI, body mass index; SA, South Africa

[a] TBFT study enrollees in the intervention arm who started ART

Development of regression model

Table 2 summarizes the results of univariable and multivariable logistic regression model development. Although age (linear continuous variable), history of smoking, weight (linear continuous variable), BMI (linear continuous variable), and respiratory rate (linear continuous variable) were associated with 6-month mortality in univariable analysis, these variables were eliminated in the stepwise backward elimination approach due to p-values in multivariable analysis >0.01.

The final multivariable Model A (which simulated the situation where CD4 is unavailable) included sex, number of WHO TB symptoms, WHO disease stage, hemoglobin concentration (continuous, linear term), and temperature (modelled as two transformed terms following output from the multivariable fractional polynomial analysis) (Table 2). In the final multivariable model B (which simulated the situation where CD4 is available), the same variables included in Model A, plus CD4 were included (Table 2).

Table 2: Univariable and multivariable logistic regression analysis in the derivation dataset (N = 2,838)

		Alive/TF by 6 months of ART (N=2,755)			Died by 6 months of ART (N=83)			Unadjusted			Model A – adjusted model excluding CD4			Model B – adjusted model including CD4		
		n	N	Median(IQR)/%	n	N	Median(IQR)/%	OR	95% CI	P	AOR	95% CI	p	AOR	95% CI	p
Demographics																
Age, years (for every 10-year increase)			2,755	34 (29-41)		83	39 (31-49)	1.44	(1.19-1.73)	<0.001						
Sex and pregnancy status	Pregnant	517	520	99%	3	520	1%	1.00	--	--	1.00	--	--	1.00	--	--
	Female non-pregnant	1,379	1,418	97%	39	1,418	3%	4.87	(1.66-14.3)	0.004	2.45	(0.76-7.88)	0.133	2.04	(0.68-6.09)	0.201
	Male	859	900	95%	41	900	5%	8.23	(2.72-24.91)	<0.001	5.47	(1.49-20.17)	0.011	4.35	(1.27-14.88)	0.019
Marital status	Married/civil union	292	300	97%	8	300	3%	1.00	--	--						
	Single	2,367	2,441	97%	74	2,441	3%	1.14	(0.65-2)	0.646						
	Widowed/Divorced	96	97	99%	1	97	1%	0.38	(0.05-3.14)	0.369						
Smoking History	Never	2,262	2,321	97%	59	2,321	3%	1.00	--	--						
	Current/ex-smoker	493	517	95%	24	517	5%	1.87	(1.11-3.15)	0.019						
Employed	Employed	1,233	1,270	97%	37	1,270	3%	1.00	--	--						
	Unemployed	1,522	1,568	97%	46	1,568	3%	1.01	(0.68-1.48)	0.971						
Education	None	188	196	96%	8	196	4%	1.00	--	--						
	Primary	664	687	97%	23	687	3%	0.81	(0.36-1.85)	0.610						
	Secondary	1,687	1,734	97%	47	1,734	3%	0.65	(0.31-1.39)	0.265						
	Higher	216	221	98%	5	221	2%	0.54	(0.23-1.26)	0.300						
HIV/TB history																
Previous TB treatment	No	2,489	2,561	97%	72	2,561	3%	1.00	--	--						
	Yes	266	277	96%	11	277	4%	1.43	(0.74-2.77)	0.290						
Number of WHO TB symptoms	0	1,955	1,975	99%	20	1,975	1%	1.00	--	--	1.00	--	--	1.00	--	--
	1	407	427	95%	20	427	5%	4.80	(2.8-8.23)	<0.001	3.39	(1.88-6.09)	<0.001	3.16	(1.84-5.43)	<0.001
	2	188	202	93%	14	202	7%	7.28	(3.58-14.8)	<0.001	4.03	(1.82-8.92)	0.001	3.64	(1.63-8.12)	0.002
	3 or 4	205	234	88%	29	234	12%	13.83	(9.04-21.13)	<0.001	5.05	(3.31-7.7)	<0.001	4.68	(2.93-7.48)	<0.001
Clinical Characteristics																
WHO Stage	I/II	2,441	2,484	98%	43	2,484	2%	1.00	--	--	1.00	--	--	1.00	--	--
	III/IV	314	354	89%	40	354	11%	7.23	(3.87-13.52)	<0.001	2.57	(1.32-4.99)	0.005	2.47	(1.24-4.89)	0.010
CD4 (per 10-cell increase)[a]			2,755	249 (149-317)		83	98 (41-218)	0.94	(0.9-0.98)	0.002				0.98	(0.95-1.01)	0.211
Weight (per 1 kg increase)			2,755	59 (52-68)		83	51 (45-60)	0.96	(0.93-0.98)	0.001						
BMI (per 1-unit increase)			2,755	21.6 (19.0-25.0)		83	19.0 (17.0-21.8)	0.86	(0.79-0.94)	0.001						
Hemoglobin (per 1g/dL increase)			2,755	11.9 (10.5-13.3)		83	9.9 (8.5-11.7)	0.69	(0.61-0.79)	<0.001	0.73	(0.65-0.81)	<0.001	0.74	(0.67-0.81)	<0.001
Temperature (per 1 ˚C increase) [b]			2,755	36.2 (35.8-36.5)		83	36.5 (36.0-37.0)	2.09	(1.47-2.96)	<0.001	1.26	(0.96-1.65)	0.092	1.25	(0.94-1.67)	0.127
Respiratory rate (per 1 breath/min increase)			2,755	18 (18-20)		83	20 (18-22)	1.02	(1.01-1.04)	0.009						

Abbreviations: ART, antiretroviral therapy; TF, transfer-out; CI, confidence interval; WHO, World Health Organization; BMI, body mass index; OR, odds ratio; AOR, adjusted odds ratio; IQR, inter-quartile range

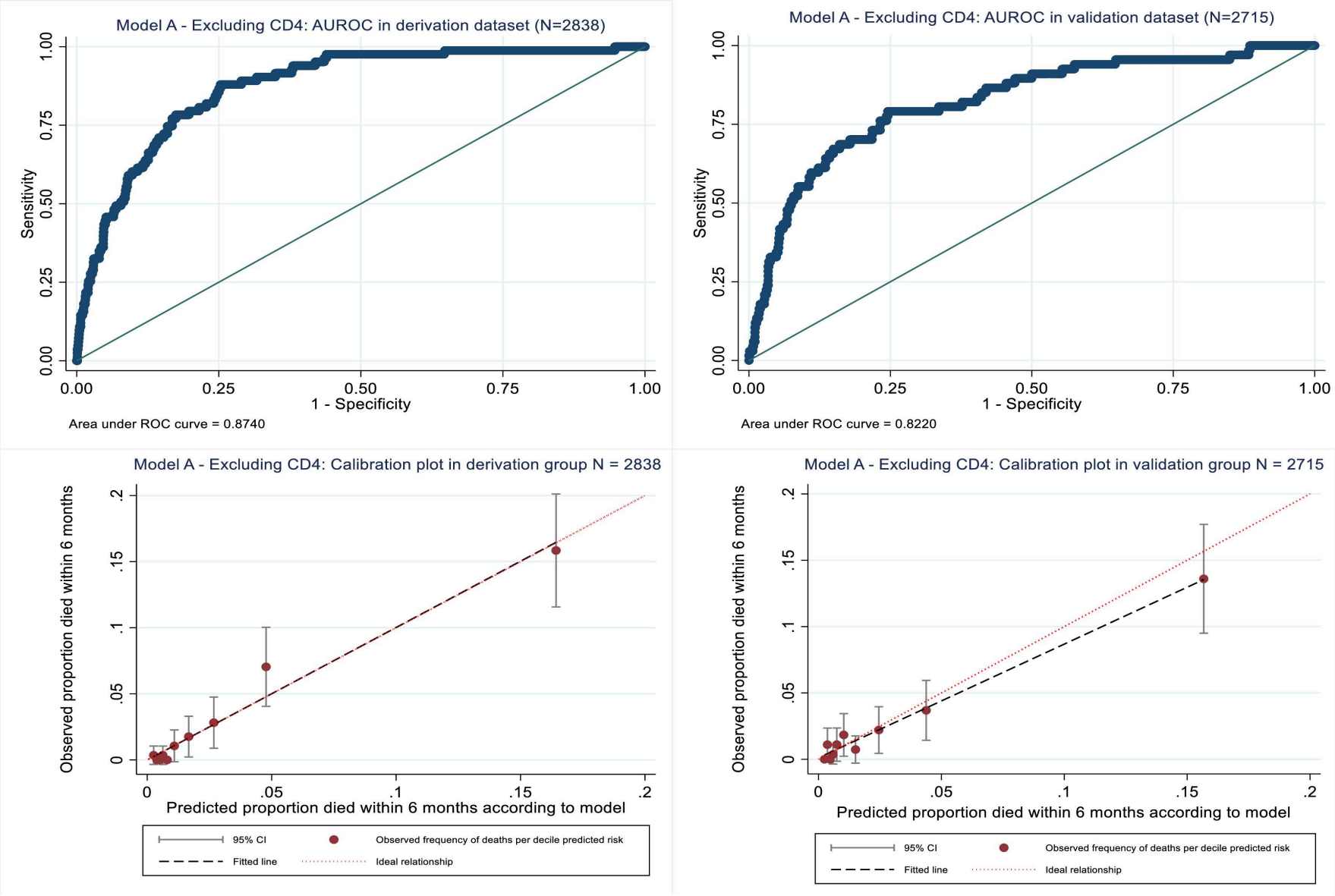
[a] Due to non-linearity in the association between CD4 and log odds of death, CD4 was modelled as two terms (term 1 = X-.2432641563 and term2 = X*ln(X)+.3438800025 if e(sample), where X = CD4/1000). Output shown is for the linear term. The p-value associated with each CD4 term was <0.001.

[b] Due to non-linearity in the association between temperature and log odds of death, temperature was modelled as two terms (term 1 = temperature^3-47148.67774 and term 2 = temperature^3*ln(temperature)-169123.2696) . Output shown is for the linear term. The p-value associated with each squared term for temperature was <0.001.

Internal validation of final regression models

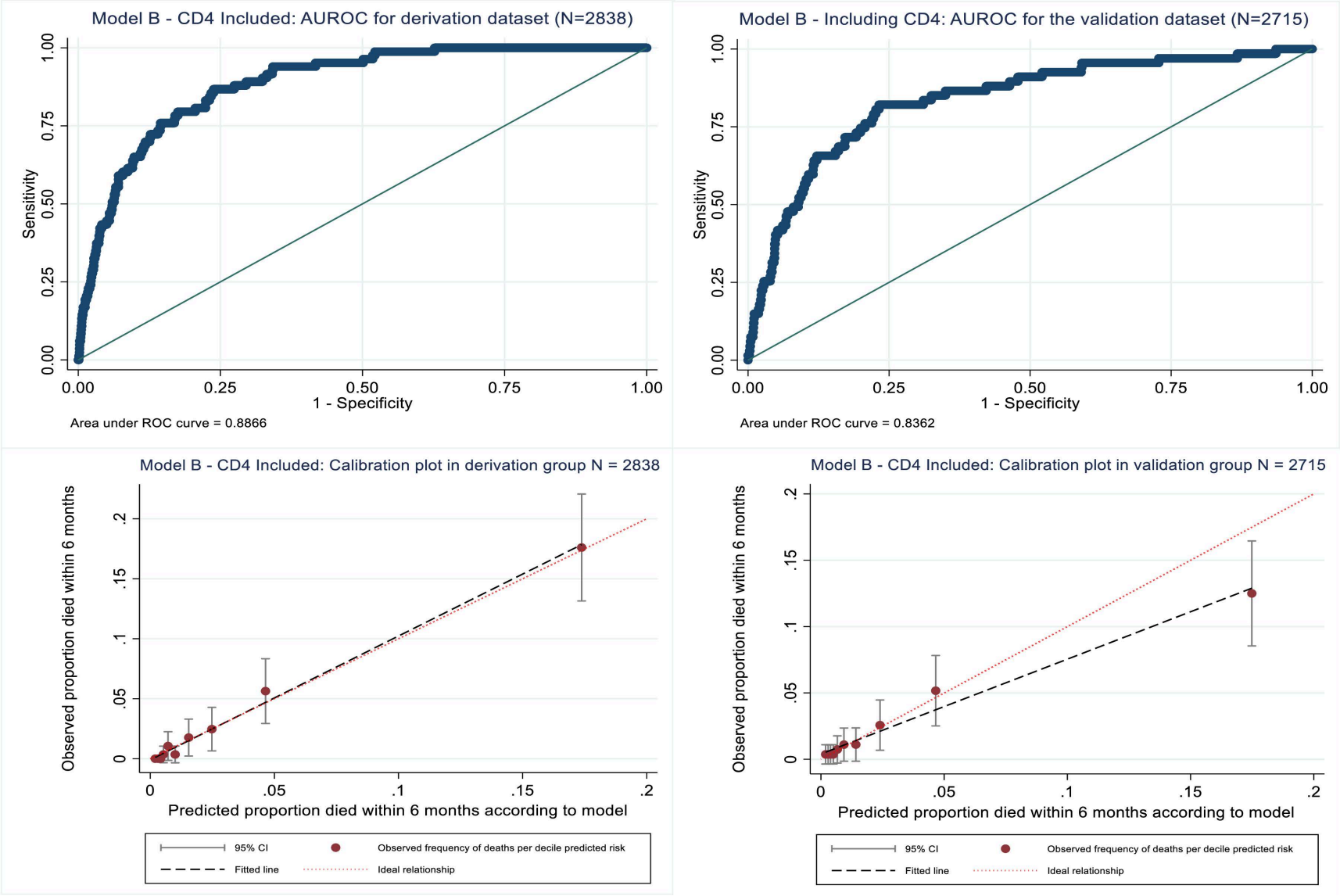
The Hosmer-Lemeshow statistics for Model A (excluding CD4) on both the derivation (p=0.381) and validation (p=0.210) datasets indicated good model fit (see Additional file 4, table showing results of Hosmer-Lemeshow tests). Similarly, the calibration curves (Fig 2) indicate adequate prediction performance for the 10 risk groups in terms of predicted number of deaths within 6 months of ART versus observed number of deaths. In addition, the AUROC curve values for the derivation (0.874) and validation (0.822) datasets indicated excellent discrimination (Fig 2).

Fig 2: Model A (excluding CD4) development and performance in the internal derivation and validation datasets respectively



The Hosmer-Lemeshow statistics for model B (including CD4) on both the derivation (p=0.735) and validation (p=0.677) datasets also indicated good model fit (see Additional file 4, table showing results of Hosmer-Lemeshow tests), with calibration curves (Fig 3) indicating adequate prediction performance for the 10 risk groups. However, in the highest risk group (risk group 10), Model B over-estimated mortality risk in the validation dataset, with 48 deaths predicted but only 34 observed (see Additional file 4, table showing results of Hosmer-Lemeshow tests). In addition, the AUROC curve values for the derivation (0.887) and validation datasets (0.836) indicated excellent discrimination (Fig 3).

Fig 3: Model B (including CD4) development and performance in the internal derivation and validation datasets



Transformation from regression model to clinical score

We used WHO advanced disease classifications for WHO stage (stage III or IV), and CD4 count (<200 cells/μL). Anemia severity in adults was classified according to WHO criteria as follows [25]: no anemia was defined as hemoglobin ≥13.0 g/dL for men, ≥12.0 g/dL for non-pregnant females, and ≥11.0 g/dL for pregnant females; mild/moderate anemia was defined as 8.0–<13.0 g/dL for men, 8.0–<12.0 g/dL for non-pregnant females, and 7.0-<11.0 g/dL for pregnant females; and severe anemia was defined as <8.0 g/dL for males and non-pregnant females and <7.0 g/dL for pregnant females. Temperature was classified as ≤37.5˚C versus >37.5˚C based on the observed distribution of mortality risk as measured temperature increased, and a common definition of a low-grade fever or higher (>37.5˚C) [26]. The multivariable model with categorization of these continuous variables in the derivation dataset is presented in Table 3.

Table 3: Multivariable model and clinical score generation from the derivation dataset (N = 2,838).

		Predictor - Model A (excluding CD4)					Predictor - Model B (including CD4)				
		AOR	95% CI	p-value	ß coefficient	Score	AOR	95% CI	p-value	ß coefficient	Score
Sex and pregnancy status	Female (pregnant)	1.00	--	--	--	0	1.00	--	--	--	0
	Female (non-pregnant)	1.94	(0.58-6.50)	0.283	0.66	1	1.71	(0.53-5.52)	0.373	0.53	1
	Male	3.54	(0.95-13.26)	0.060	1.26	2	2.93	(0.82-10.44)	0.097	1.08	2
Number of WHO TB symptoms	0	1.00	--	--	--	0	1.00	--	--	--	0
	≥1	3.65	(2.24-5.97)	<0.001	1.30	2	3.33	(2.06-5.38)	<0.001	1.20	2
WHO Stage	I/II	1.00	--	--	--	0	1.00	--	--	--	0
	III/IV	2.72	(1.42-5.20)	0.003	1.00	2	2.55	(1.32-4.92)	0.005	0.94	2
Temperature at enrollment	≤37.5°C	1.00	--	--	--	0	1.00	--	--	--	0
	>37.5°C	3.39	(1.65-6.96)	0.001	1.22	2	3.37	(1.56-7.26)	0.002	1.21	2
CD4 count	≥200/µL	--	--	--	--	N/A	1.00	--	--	--	0
	<200/µL	--	--	--	--	N/A	2.05	(1.20-3.50)	0.009	0.72	1
Anemia Status[a]	No anemia	1.00	--	--	--	0	1.00	--	--	--	0
	mild/moderate anemia	5.03	(2.57-9.87)	<0.001	1.62	2	4.58	(2.37-8.84)	<0.001	1.52	3
	Severe anemia	9.42	(3.43-25.89)	<0.001	2.24	3	8.02	(3.04-21.14)	<0.001	2.08	4

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; WHO, World Health Organization.

[a]Anemia severity was classified according to World Health Organization criteria as follows: no anemia, hemoglobin level of ≥13.0 g/dL for men, ≥12.0 g/dL for non-pregnant females, and ≥11.0 g/dL for pregnant females; mild/moderate anemia, 8.0–<13.0 g/dL for men, 8.0–<12.0 g/dL for non-pregnant women, and 7.0-<11.0 g/dL for pregnant women; and severe anemia, <8.0 g/dL for males and non-pregnant females and <7.0 g/dL for pregnant women.

Model A, categorized in this way, retained statistically excellent discrimination in both derivation (AUROC 0.867) and validation datasets (AUROC 0.818), and the Hosmer-Lemeshow statistic *p*-values were 0.269 in the derivation and 0.334 in the validation datasets indicating good calibration. Similarly, Model B AUROC statistics were 0.874 in the derivation and 0.830 in the validation datasets, with Hosmer-Lemeshow statistic *p*-values of 0.367 and 0.307 in the derivation and validation datasets respectively, indicating good model fit. The clinical scores that could be used in clinic settings to identify those at risk of early 6-month mortality, depending on availability of CD4 count, are illustrated in Fig 4.

Fig 4: CD4-independent and CD4-dependent clinical score cards

NO ← **Is CD4 Count Currently Available?** → **YES**

Risk Factor	Category	Associated points	Assigned score
Gender	Pregnant Female	0	
	Non-pregnant Female	1	
	Male	2	+
No. of WHO TB symptoms	Zero	0	
	≥1	1	+
WHO Disease stage	I or II	0	
	III or IV	2	+
Temperature (˚C)	≤37.5	0	
	>37.5	2	+
Hemoglobin Level	No anemia	0	
	Mild/Mod. anemia	2	
	Severe anemia	3	−
		Total	

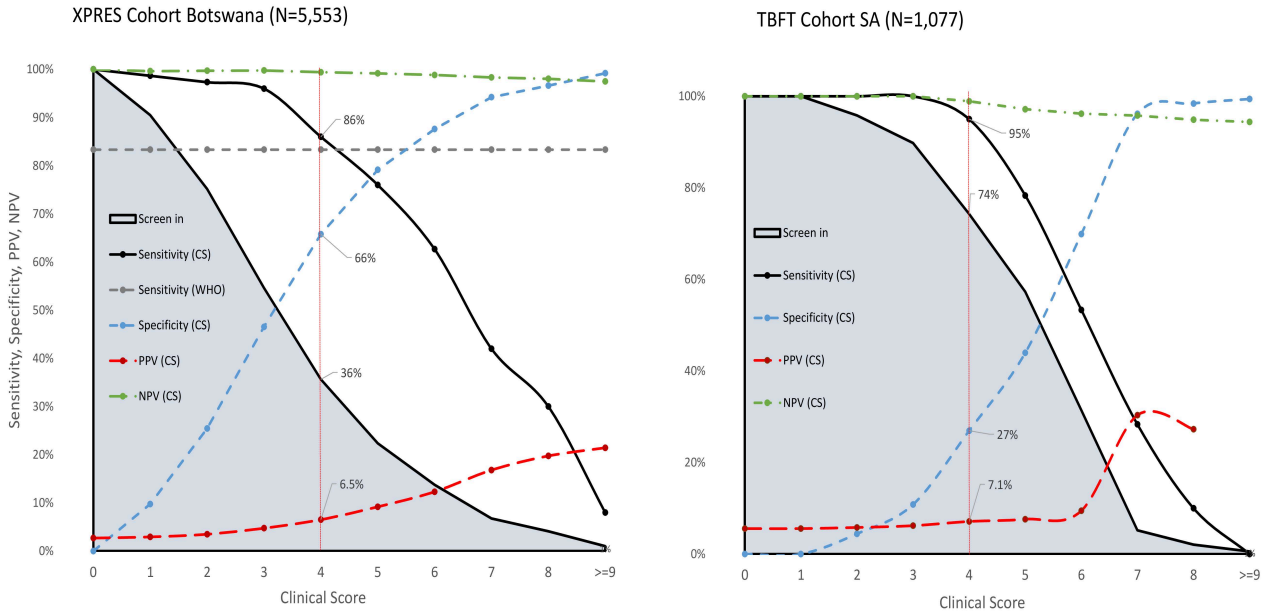
Risk Factor	Category	Associated points	Assigned score
Gender	Pregnant Female	0	
	Non-pregnant Female	1	
	Male	2	+
No. of WHO TB symptoms	Zero	0	
	≥1	2	+
WHO Disease stage	I or II	0	
	III or IV	2	+
Temperature (˚C)	≤37.5	0	
	>37.5	2	+
CD4 Count (cells/µL)	≥200	0	
	<200	1	+
Hemoglobin Level	No anemia	0	
	Mild/Mod. anemia	3	
	Severe anemia	4	−
		Total	

External validation of risk scores

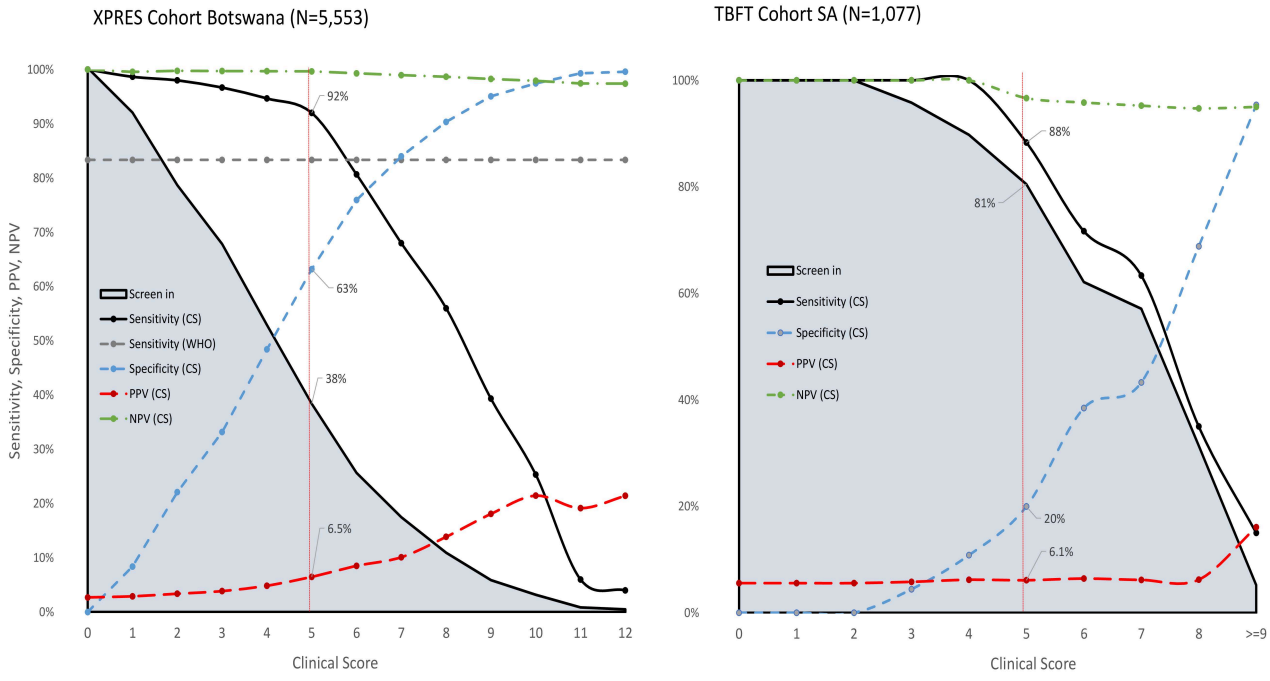
The clinical score for each predictor was generated and the possible range for the total score was 0 to 11 for Model A and 0 to 13 for Model B (see Additional file 5, tables showing performance of clinical scores). Fig 5 shows the performance of the two clinical scores at different cut-offs, in terms of sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and percentage of enrollees screened into ART care intensification. For the CD4-independent clinical score derived from Model A, (Fig 5) among XPRES enrollees, a clinical score of ≥4 would screen in 36% of ART enrollees into a care intensification pathway, providing 86% sensitivity and 66% specificity in detecting those at risk for early mortality, whereas the WHO advanced disease eligibility criteria (CD4 <200/μL or WHO stage III/IV) would screen in 44% of ART enrollees, providing 83% sensitivity and 58% specificity. Notably, if the WHO advanced disease eligibility criterion of WHO stage III/IV only was used since CD4 is unavailable, 12% of ART enrollees would be screened into an ART care intensification pathway, with only 48% sensitivity in detecting 6-month mortality and 89% specificity. Among TBFT enrollees, the clinical score of ≥4 would screen in 74% of ART enrollees, providing 95% sensitivity and 27% specificity in detecting early mortality, versus the WHO advanced disease eligibility criteria which would screen in 100% of ART enrollees, with 100% sensitivity but 0% specificity.

Fig 5: Sensitivity, Specificity, PPV, and NPV of clinical score in predicting 6-month mortality in XPRES dataset (N=5,553) and external validation TB Fast Track Dataset (N=1,077) for Models A (excluding CD4) and B (including CD4)

CD4-independent clinical score (from Model A which excludes CD4)



CD4-dependent clinical score (from Model B which includes CD4)



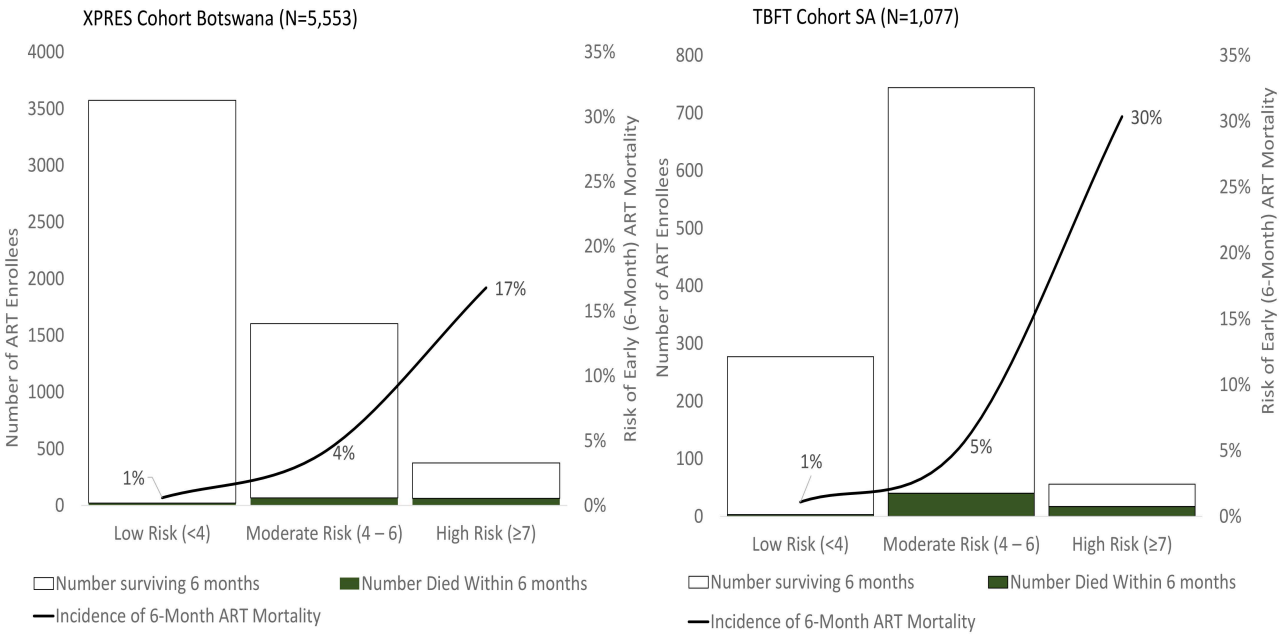
For the CD4-dependent clinical score derived from Model B, a clinical score of ≥5 would screen in 38% of ART enrollees into a care intensification pathway, providing 92% sensitivity and 63% specificity in detecting those at risk for early mortality. Among TBFT enrollees, the clinical score of ≥5 would screen in 81% of ART enrollees, providing 88% sensitivity and 20% specificity in detecting early mortality.

The AUROC for CD4-independent (0.845) and -dependent (0.852) clinical scores remained high for XPRES enrollees but was low for TB FT enrollees (0.568 for CD4-independent and 0.569 for CD4-dependent scores) (see ≤: figure of AUROC for clinical score performance).

For the CD4-independent clinical score, risk scores were grouped into low (<4), moderate (4-6), and high-risk categories (≥7) (Fig 6), with 6-month low, moderate, and high risk group incidence percentages being 1%, 4%, and 17% among XPRES enrollees and 1%, 5%, and 30% among TBFT enrollees. Similarly, for the CD4-dependent clinical score, risk scores were grouped into low (<5), moderate (5-8), and high risk categories (≥9) (Fig 6), with 6-month low, moderate, and high risk group mortality percentages being 0%, 4%, and 18% for XPRES enrollees and 3%, 5%, and 16% for TBFT enrollees. Fig 7 shows K-M failure curves of mortality over the first 6 months of ART according to the low, moderate, and high-risk groups, indicating that specific populations of moderately high- and high-risk groups, in high need of care intensification, were differentiated by the respective clinical scores.

Fig 6: Distribution of risk scores and 6-month mortality risk in the XPRES dataset (N=5,553) and external validation TB Fast Track Dataset (N=1,077) for Models A (excluding CD4) and B (including CD4)

CD4-independent clinical score (from Model A, which excludes CD4)



CD4-dependent clinical score (from Model A, which includes CD4)

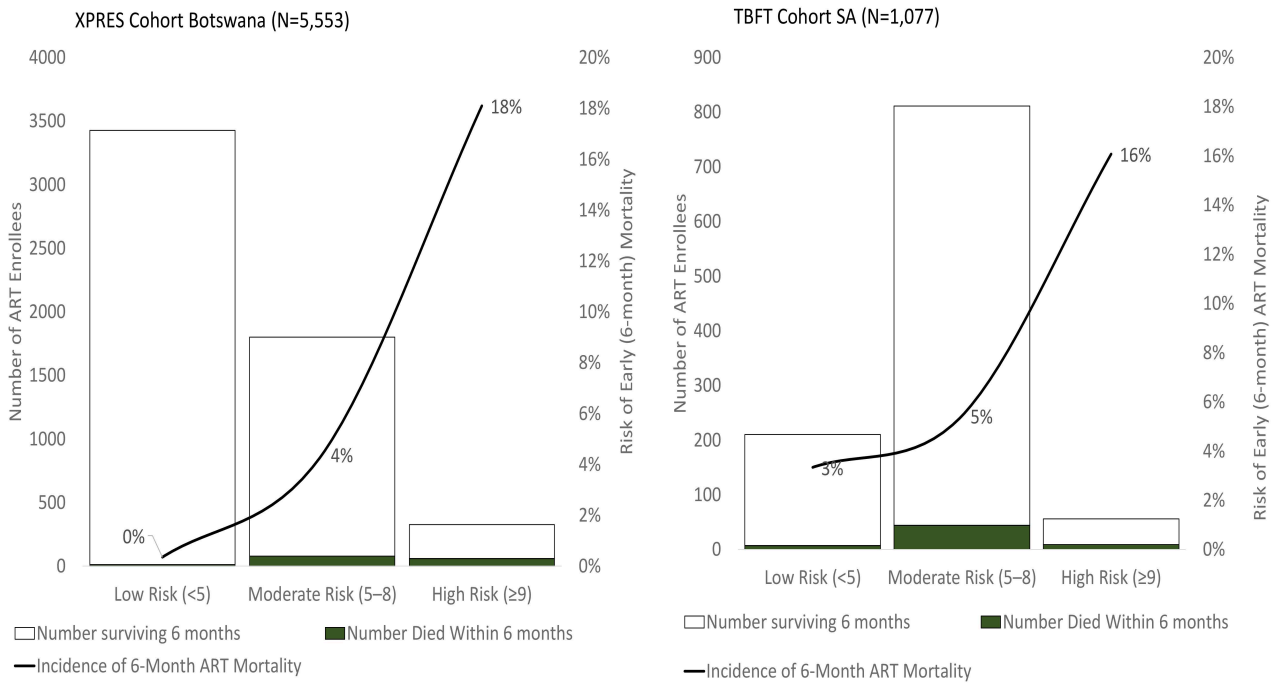
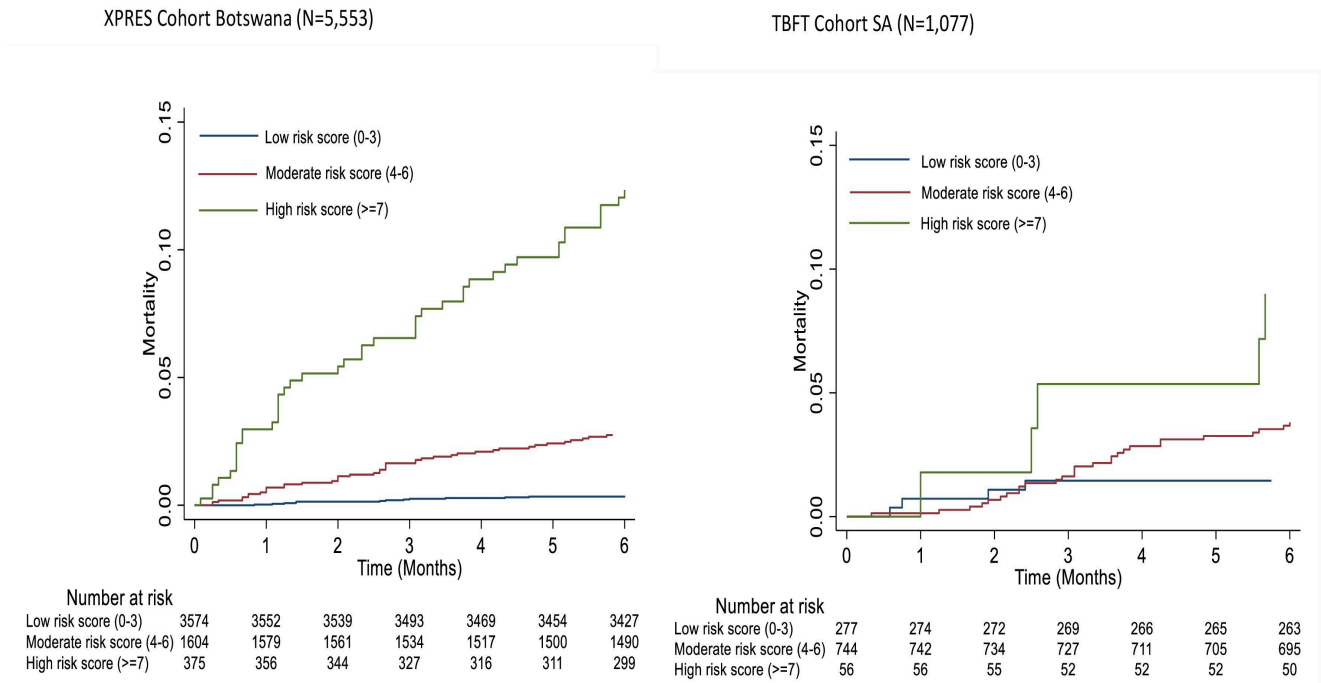
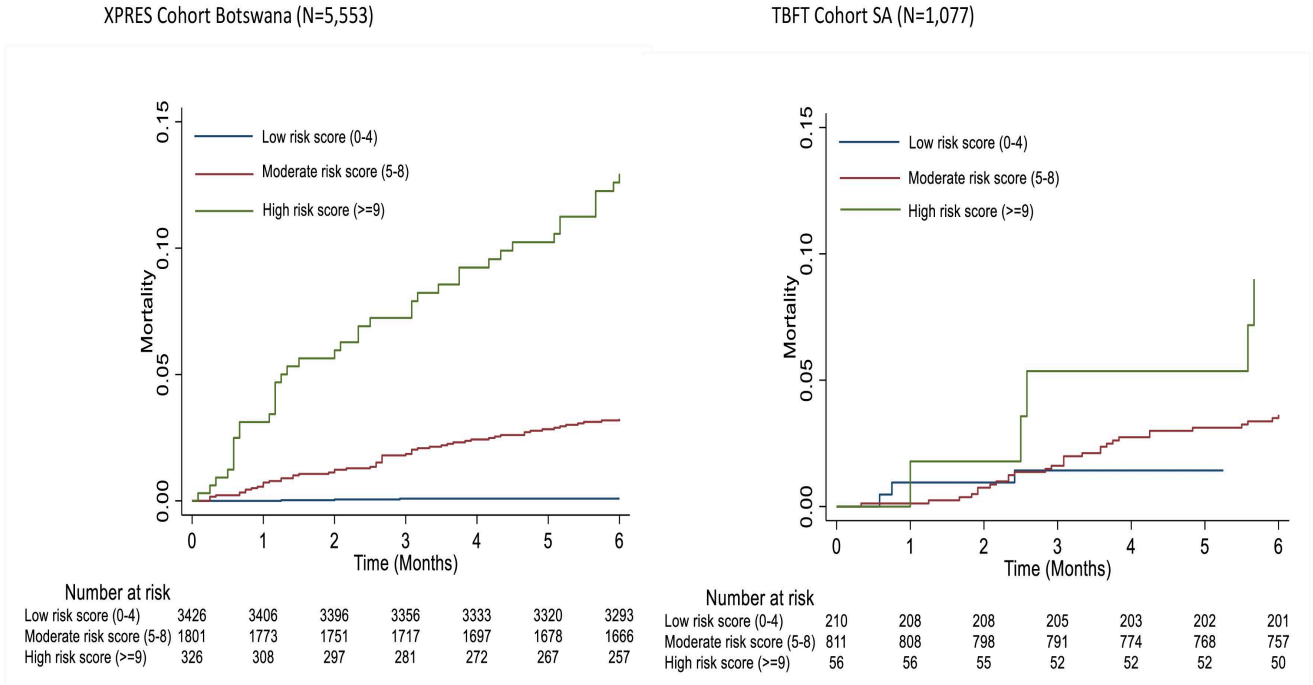


Fig 7: Survival curves stratified by risk scores in the XPRES dataset (N=5,553) and external validation TB Fast Track Dataset (N=1,077) for Models A (excluding CD4) and B (including CD4)

CD4-independent clinical score risk stratification (from Model A which excludes CD4)



CD4-dependent clinical score risk stratification (from Model B which includes CD4)



Discussion

A CD4-independent clinical score designed for settings where CD4 is unavailable at ART intitiation with a cut-off score of ≥4 was largely as sensitive in screening in persons at risk of death by 6 months as the current WHO advanced disease eligibility criteria, and nearly twice as sensitive as WHO eligibility criteria that would rely on WHO stage alone. Compared with the CD4-based WHO advanced disease eligibility criteria, the CD4-independent clinical score had higher specificity and would screen 8-26% fewer ART enrollees into intensified care pathways, suggesting the screening tool could also increase efficiency of investments in DSD models for advanced disease. Therefore, in the many settings in SSA that lack access to rapid CD4 testing, the CD4-independent clinical score should be considered for scale-up to facilitate early ART care intensification, with the potential for reductions in early ART mortality [11]. In addition, in those settings where CD4 is available, using the CD4-dependent clinical score with a cut-off score of ≥5 could increase both sensitivity and specificity over WHO advanced disease eligibility criteria, with the potential to both reduce early ART mortality and improve efficiency of DSD algorithms. To our knowledge, these are the first externally validated clinical scores for ART care intensification generated for SSA [9].

In contrast to current WHO guidelines, which recommend only the use of CD4 count and WHO HIV disease staging to identify patients at high risk for morbidity and mortality, our composite risk score provides both more comprehensive and specific information on the magnitude of risk for each patient by integrating additional objective variables into the assessment [9]. The additional variables included in our score are both clinical and demographic. The clinical variables of WHO TB symptom screen, temperature, and anemia severity are known to be associated with serious comorbidities that significantly increase early mortality risk, while the demographic variables in the scores (the gender variable of male, female non-pregnant, and female pregnant) captures important generalizable differences in early mortality risk in SSA, which are due to both psychosocial and biological factors [27, 28]. Our risk scores are careful to be simple (5 or 6 variables assessed), use

objective covariates rather than variables that are more open to interpretation, and use variables that should be available, or could easily be made available, at the POC in LMIC. The measured hemoglobin level is more available in LMIC than POC CD4, although scale-up of CD4 testing is needed and ongoing. Available POC hemoglobin measurement devices tend to be durable, easy to use, and have shown good accuracy in LMIC [29, 30], probably because to date these devices have been less expensive than currently available POC CD4 systems and are useful for non-HIV-related care (e.g., <$100/POC hemoglobin measurement device and $0.75/test [31] vs. about $7,430/POC CD4 device and about $8.70/test [32]). Both CD4 testing and hemoglobin testing are important at the point of care, and less expensive POC CD4 lateral flow assays and transcutaneous spectrophotometry solutions for hemoglobin level measurement may become available in the future [33-35].

Additional advantages of developing clinical scores with a variety of cut-offs is that it allows programme managers to choose cut-offs with associated screening accuracy characteristics, allowing programme managers to choose cut-offs based on funding availability, by trading sensitivity for improved specificity [9].

Another potential advantage of the combined clinical score over the WHO advanced disease criteria is the ability to differentiate three risk groups (low, moderate, and high), with the highest risk group having 6-month mortality rates of 16–30% versus 0–3% in the low and 4–5% in the moderate risk groups. While all patients with moderate or high scores might benefit from a standardized outpatient intensified early ART care, patients in the highest risk group might be candidates for additional interventions to help navigate the relatively complex time of early ART. During this time, clinicians need to rapidly search for, diagnose, or rule out co-morbidities, and both choose and time appropriate therapies, all within the context of ART-driven immune reconstitution [36, 37]. Our clinical score could be used to inform a clinical trial of such interventions.

Moderate to severe anemia was a stronger predictor than CD4 count and overall was the strongest predictor of early ART mortality in our cohort, similar to other studies in SSA [21, 38, 39]. Anemia is the most common hematological complication of HIV disease among PLHIV [40] and develops through several mechanisms including direct HIV infection of hematopoietic progenitor cells, dysregulated erythropoiesis through indirect effects of proinflammatory cytokines, and through anemia of chronic disorders (ACD), which is thought to be the most common pathway [41]. ACD is driven by hepatic expression of hepcidin, an acute phase reactant that causes iron to be diverted from the circulation and sequestered within cells of the reticuloendothelial system through down-regulation of ferroportin channels [42]. TB also drives ACD through this hepcidin-ferroportin-interaction [42, 43]. In turn, sequestration of iron inside macrophages and T-cells might support both intracellular mycobacterial growth [39, 42] and HIV viral replication [44], showing the potential for rapid worsening of HIV, TB, and severe hepcidin-driven anemia. Therefore, although ART is the most important treatment of HIV-associated anemia, early treatment of any associated co-infections is crucial [39]. In a separate analysis, we show that moderate to severe anemia was also predictive of active TB infection in the XPRES cohort, similar to other analyses [39]. Given the strong association between moderate to severe anemia, early mortality, and active TB, which is the most common cause of early mortality in SSA [45], the scores associated with observed moderate-severe anemia in this analysis (2–4 points) appropriately bring the total clinical score very close to the threshold for ART care intensification. Per current WHO guidelines, care intensification should include further investigations for TB, especially disseminated TB, through use of the urine TB-LAM assay and Xpert MTB/RIF [39, 43, 46, 47].

Another notable finding is that measured temperature at >37.5˚C at ART initiation was strongly predictive of early ART mortality, independent of the WHO TB symptom screen for fever or night sweats, which was also predictive of mortality. This indicates the importance of objective measures of fever in addition to patient history [23]. Notably some of the key inflammatory cytokines that drive hepcidin release and fever are the

same (e.g., interleukin (IL)-6, tumor necrosis factor which stimulates IL-6 release, interferons, and microbial-derived Toll-like receptors) are important for both pathways [42, 48]. Disseminated undiagnosed TB or TB diagnosed late is the most common infectious cause of death among PLHIV in sub-Saharan Africa, accounting for about 40% of deaths [45]. However, a recent autopsy study of causes of death among new HIV clinic enrollees in SA, found that 59% of decedents had evidence of two or more concurrent infections [49]. Most bacterial infections were due to common pathogens, such as *Klebsiella* spp., *Salmonella* spp., *H. influenzae*, and *S. aureus,* while cryptococcal infection was found in 13% [49]. Targeting an antimicrobial package of interventions to patients who screen positive for our proposed clinical scores, such as the package of interventions recommended by WHO or trialed in the REALITY trial (continuous trimethoprim–sulfamethoxazole, ≥12 weeks of isoniazid–pyridoxine (once active TB is ruled out), 12 weeks of fluconazole, 5 days of azithromycin, and a single dose of albendazole) could significantly reduce mortality for patients who screen positive [11].

The prognostic importance of male gender in predicting mortality was correlated with older age and smoking history in our model, and we chose to include the single gender variable rather than two additional variables (age ≥55 and smoking) in the CD4-dependent clinical score to make the most parsomnoius clinical score and because male gender is a more generalizable predictor of poor outcomes in SSA [28, 50, 51]. However, if ART programmes in SSA are able in the future to achieve earlier testing, ART initiation, and better adherence for male and non-pregnant female PLHIV, it is likely gender and pregnancy status could become less important predictors, while predictors like smoking and older age will become more important [50]. Although smoking is not part of the clinical score, this article provides additional evidence for the need for tobacco smoking reduction programmes for PLHIV, separate or included in early ART care intensification algorithms, to minimize not only the risk of ischemic cardiovascular diseases but also the risk of malignancies and bacterial infections, including TB [52].

Strengths of this study include the use of data from prospective cohorts nested within clinical trials, meaning there was minimal missing covariate data and strong ascertainment of the primary outcome of interest (6-month ART mortality). Additional strengths include the high screening accuracy in both the XPRES and TBFT cohorts, from two geographically separate cohorts, with very different cohort characteristics (e.g., XPRES enrollees represent general outpatient ART enrollees while TBFT enrollees had homogenously low CD4 counts (<150/µL)). Notably, while in the XPRES cohort 6% of ART enrollees were newly diagnosed and treated for TB, in the TBFT cohort 62% were treated for TB through a risk-based TB-treatment algorithm [14], suggesting that the risk score is likely to be generalizable across a wide range of new ART enrollee cohorts. Limitations include that the risk score has not yet been validated in a cohort enrolled under HIV test-and-treat guidelines, something which is planned in the near future. Other limitations include the fact that while the gender and pregnancy variable is relevant in SSA and many resource-limited settings, it is not generalizable to cohorts in resource-rich settings like the U.S. and Europe, where males often have better outcomes than female ART enrollees. Although the specificity of the clinical scores is superior to the WHO advanced disease eligibility criteria, a substantial percentage of ART enrollees (36-38% in the XPRES cohort) would be screened into receiving an advanced disease care package, which would require a monitoring system to assess implementation fidelity. In addition, these screening tools were validated in clinical trial cohorts that received relatively intensive TB screening and treatment services, and therefore those that died did so despite access these services.

Conclusions

In conclusion, where CD4 testing is not available in similar LMIC, especially in SSA, the CD4-independent risk score should be strongly considered for scale-up to facilitate early ART care intensification, with the potential for significant reductions in early ART mortality if targetted individuals are provided with evidence-based care packages [11]. For clinics where CD4 count is available, use of the CD4-dependent clinical score could improve both sensitivity and specificity over WHO advanced disease eligibility criteria, with the potential

to reduce early ART mortality and improve efficiency of DSD algorithms. Finally, further research to understand best management of ART enrollees enrolled in the highest-risk categories is warranted to further explore mortality reduction interventions. Together these actions could help drive progress to AIDS 2030 goals of zero AIDS deaths in the region of the world with the highest HIV/AIDS-associated mortality.

Acknowledgements

The authors would like to thank all study participants, participating health facilities, and project team members for their contributions to improving TB and HIV care in Botswana. We also thank Dr. Tom Spira for his contributions over many years as a mentor, advisor, subject matter expert, and for his review of this manuscript prior to his death on April 2, 2020.

Declarations

Funding

This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the United States Centers for Disease Control and Prevention (CDC). The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

References

1. UNAIDS. AIDSinfo. Available at: http://aidsinfo.unaids.org/. Accessed January 4, 2020.
2. Tanser F, Bärnighausen T, Grapsa E, Zaidi J, Newell ML. High Coverage of ART Associated with Decline in Risk of HIV Acquisition in Rural KwaZulu-Natal, South Africa. Science. 2013;339(6122);966-971.
3. Vandormael A, Akullian A, Siedner M, de Oliveira T, Barnighausen T, Tanser F. Declines in HIV incidence among men and women in a South African population-based cohort. Nat Commun. 2019;10(1):5482.
4. Stover J, Bollinger L, Izazola JA, Loures L, DeLay P, Ghys PD, et al. What Is Required to End the AIDS Epidemic as a Public Health Threat by 2030? The Cost and Impact of the Fast-Track Approach. PLoS One. 2016;11(5):e0154893.
5. Gupta A, Nadkarni G, Yang WT, Chandrasekhar A, Gupte N, Bisson GP, et al. Early Mortality in Adults Initiating Antiretroviral Therapy (ART) in Low- and Middle-Income Countries (LMIC): A Systematic Review and Meta-Analysis. PLOS One. 2011;6(12):e28691.
6. Haas AD, Zaniewski E, Anderegg N, Ford N, Fox MP, Vinikoor M, et al. Retention and mortality on antiretroviral therapy in sub-Saharan Africa: collaborative analyses of HIV treatment programmes. J Int AIDS Soc. 2018;21(2).
7. Auld AF, Shiraishi RW, Oboho I, Ross C, Bateganya M, Pelletier V, et al. Trends in Prevalence of Advanced HIV Disease at Antiretroviral Therapy Enrollment - 10 Countries, 2004-2015. MMWR Morb Mortal Wkly Rep. 2017;66(21):558-563.
8. WHO. Guidelines for Managing Advanced HIV Disease and Rapid Initiation of Antiretroviral Therapy. Available at: https://www.ncbi.nlm.nih.gov/pubmed/29341560. Accessed January 4, 2020.
9. McNairy ML, Abrams EJ, Rabkin M, El-Sadr WM. Clinical decision tools are needed to identify HIV-positive patients at high risk for poor outcomes after initiation of antiretroviral therapy. PLoS Med. 2017;14(4):e1002278.

10. WHO. HIV Treatment and Care, What's New in Service Delivery Fact Sheet. Available at: http://apps.who.int/iris/bitstream/10665/204461/1/WHO_HIV_2015.46_eng.pdf?ua=1. Accessed January 4, 2020.
11. Hakim J, Musiime V, Szubert AJ, Mallewa J, Siika A, Agutu C, et al. Enhanced Prophylaxis plus Antiretroviral Therapy for Advanced HIV Infection in Africa. N Engl J Med. 2017;377(3):233-245.
12. McNairy ML, Jannat-Khah D, Pape JW, Marcelin A, Joseph P, Mathon JE, et al. Predicting death and lost to follow-up among adults initiating antiretroviral therapy in resource-limited settings: Derivation and external validation of a risk score in Haiti. PLoS One. 2018;13(8):e0201945.
13. Auld AF, Agizew T, Pals S, Finlay A, Ndwapi N, Boyd R, et al. Implementation of a pragmatic, stepped-wedge cluster randomized trial to evaluate impact of Botswana's Xpert MTB/RIF diagnostic algorithm on TB diagnostic sensitivity and early antiretroviral therapy mortality. BMC Infect Dis. 2016;16(1):606.
14. Grant AD, Charalambous S, Tlali M, Karat AS, Dorman SE, Hoffmann CJ, et al. Algorithm-guided empirical tuberculosis treatment for people with advanced HIV (TB Fast Track): an open-label, cluster-randomised trial. The lancet HIV. 2019.
15. Dryden-Peterson S, Lockman S, Zash R, Lei Q, Chen JY, Souda S, et al. Initial programmatic implementation of WHO option B in Botswana associated with increased projected MTCT. J Acquir Immune Defic Syndr. 2015;68(3):245-249.
16. Auld AF, Agizew T, Mathoma A, Boyd R, Date A, Pals SL, et al. Effect of tuberculosis screening and retention interventions on early antiretroviral therapy mortality in Botswana: a stepped-wedge cluster randomized trial. BMC Med. 2020;18(1):19.
17. Altman DG, Vergouwe Y, Royston P, Moons KG. Prognosis and prognostic research: validating a prognostic model. BMJ. 2009;338:b605.
18. Brinkhof MW, Pujades-Rodriguez M, Egger M. Mortality of patients lost to follow-up in antiretroviral treatment programmes in resource-limited settings: systematic review and meta-analysis. PLoS One. 2009;4(6):e5790.

19. Royston P, Moons KG, Altman DG, Vergouwe Y. Prognosis and prognostic research: Developing a prognostic model. BMJ. 2009;338:b604.
20. Auld AF, Shiraishi RW, Couto A, Mbofana F, Colborn K, Alfredo C, et al. A Decade of Antiretroviral Therapy Scale-up in Mozambique: Evaluation of Outcome Trends and New Models of Service Delivery Among More Than 300,000 Patients Enrolled During 2004-2013. J Acquir Immune Defic Syndr. 2016;73(2):e11-22.
21. May M, Boulle A, Phiri S, Messou E, Myer L, Wood R, et al. Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. Lancet. 2010;376(9739):449-457.
22. Worodria W, Chang E, Andama A, Sanyu I, Byanyima P, Musisi E, et al. Predictors of Mortality Among Hospitalized Patients With Lower Respiratory Tract Infections in a High HIV Burden Setting. J Acquir Immune Defic Syndr. 2018;79(5):624-630.
23. Holmes CB, Sikazwe I, Sikombe K, Eshun-Wilson I, Czaicki N, Beres LK, et al. Estimated mortality on HIV treatment among active patients and patients lost to follow-up in 4 provinces of Zambia: Findings from a multistage sampling-based survey. PLoS Med. 2018;15(1):e1002489.
24. Moons KG, Altman DG, Vergouwe Y, Royston P. Prognosis and prognostic research: application and impact of prognostic models in clinical practice. BMJ. 2009;338:b606.
25. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity (2011). Available at: https://www.who.int/vmnis/indicators/haemoglobin/en/. Accessed January 4, 2020.
26. Del Bene VE. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Chapter 218Temperature. Available at: https://www.ncbi.nlm.nih.gov/books/NBK331/. Accessed January 4, 2020.
27. McNairy ML, Howard AA, El-Sadr WM. Antiretroviral therapy for prevention of HIV and tuberculosis: a promising intervention but not a panacea. J Acquir Immune Defic Syndr. 2013;63 Suppl 2:S200-207.

28. Auld AF, Shiraishi RW, Mbofana F, Couto A, Fetogang EB, El-Halabi S, et al. Lower Levels of Antiretroviral Therapy Enrollment Among Men with HIV Compared with Women - 12 Countries, 2002-2013. MMWR Morb Mortal Wkly Rep. 2015;64(46):1281-1286.
29. Nkrumah B, Nguah SB, Sarpong N, Dekker D, Idriss A, May J, et al. Hemoglobin estimation by the HemoCue(R) portable hemoglobin photometer in a resource poor setting. BMC Clin Pathol. 2011;11:5.
30. Sanchis-Gomar F, Cortell-Ballester J, Pareja-Galeano H, Banfi G, Lippi G. Hemoglobin point-of-care testing: the HemoCue system. J Lab Autom. 2013;18(3):198-205.
31. Medina Lara A, Mundy C, Kandulu J, Chisuwo L, Bates I. Evaluation and costs of different haemoglobin methods for use in district hospitals in Malawi. J Clin Pathol. 2005;58(1):56-60.
32. Larson B, Schnippel K, Ndibongo B, Long L, Fox MP, Rosen S. How to estimate the cost of point-of-care CD4 testing in program settings: an example using the Alere Pima Analyzer in South Africa. PLoS One. 2012;7(4):e35444.
33. Gayat E, Aulagnier J, Matthieu E, Boisson M, Fischler M. Non-invasive measurement of hemoglobin: assessment of two different point-of-care technologies. PLoS One. 2012;7(1):e30065.
34. Crowley C, Montenegro-Bethancourt G, Solomons NW, Schumann K. Validity and correspondence of non-invasively determined hemoglobin concentrations by two trans-cutaneous digital measuring devices. Asia Pac J Clin Nutr. 2012;21(2):191-200.
35. PATH. Noninvasive technology for anemia detection (2013). Seattle, WA, USA: PATH. Available at: http://www.path.org/publications/files/TS_update_anemia.pdf. Accessed January 4, 2020.
36. Mfinanga SG, Kirenga BJ, Chanda DM, Mutayoba B, Mthiyane T, Yimer G, et al. Early versus delayed initiation of highly active antiretroviral therapy for HIV-

positive adults with newly diagnosed pulmonary tuberculosis (TB-HAART): a prospective, international, randomised, placebo-controlled trial. Lancet Infect Dis. 2014;14(7):563-571.

37. Boulware DR, Meya DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. N Engl J Med. 2014;370(26):2487-2498.

38. Harris RJ, Sterne JA, Abgrall S, Dabis F, Reiss P, Saag M, et al. Prognostic importance of anaemia in HIV type-1-infected patients starting antiretroviral therapy: collaborative analysis of prospective cohort studies. Antivir Ther. 2008;13(8):959-967.

39. Kerkhoff AD, Wood R, Cobelens FG, Gupta-Wright A, Bekker LG, Lawn SD. The predictive value of current haemoglobin levels for incident tuberculosis and/or mortality during long-term antiretroviral therapy in South Africa: a cohort study. BMC Med. 2015;13:70.

40. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. Am J Med. 2004;116 Suppl 7A:27S-43S.

41. Gibellini D, Clo A, Morini S, Miserocchi A, Ponti C, Re MC. Effects of human immunodeficiency virus on the erythrocyte and megakaryocyte lineages. World J Virol. 2013;2(2):91-101.

42. Drakesmith H, Prentice AM. Hepcidin and the iron-infection axis. Science. 2012;338(6108):768-772.

43. Kerkhoff AD, Meintjes G, Burton R, Vogt M, Wood R, Lawn SD. Relationship Between Blood Concentrations of Hepcidin and Anemia Severity, Mycobacterial Burden, and Mortality Among Patients With HIV-Associated Tuberculosis. J Infect Dis. 2015.

44. Xu M, Kashanchi F, Foster A, Rotimi J, Turner W, Gordeuk VR, et al. Hepcidin induces HIV-1 transcription inhibited by ferroportin. Retrovirology. 2010;7:104.

45. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS. 2015;29(15):1987-2002.
46. Kerkhoff AD, Wood R, Lawn SD. Optimum time to start antiretroviral therapy in patients with HIV-associated tuberculosis: before or after tuberculosis diagnosis? AIDS. 2011;25(7):1003-1006.
47. WHO. Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. Available at: https://www.who.int/tb/publications/2019/LAMPolicyUpdate2019/en/. Accessed January 4, 2020.
48. Netea MG, Kullberg BJ, Van der Meer JW. Circulating cytokines as mediators of fever. Clin Infect Dis. 2000;31 Suppl 5:S178-184.
49. Karat AS, Omar T, von Gottberg A, Tlali M, Chihota VN, Churchyard GJ, et al. Autopsy Prevalence of Tuberculosis and Other Potentially Treatable Infections among Adults with Advanced HIV Enrolled in Out-Patient Care in South Africa. PLoS One. 2016;11(11):e0166158.
50. Gupta-Wright A, Corbett EL, Wilson D, van Oosterhout JJ, Dheda K, Huerga H, et al. Risk score for predicting mortality including urine lipoarabinomannan detection in hospital inpatients with HIV-associated tuberculosis in sub-Saharan Africa: Derivation and external validation cohort study. PLoS Med. 2019;16(4):e1002776.
51. Centers for Disease C, Prevention. Differences Between HIV-Infected Men and Women in Antiretroviral Therapy Outcomes - Six African Countries, 2004-2012. MMWR Morb Mortal Wkly Rep. 2013;62(47):946-952.
52. Benard A, Tessier JF, Rambeloarisoa J, Bonnet F, Fossoux H, Neau D, et al. HIV infection and tobacco smoking behaviour: prospects for prevention? ANRS CO3 Aquitaine Cohort, 2002. Int J Tuberc Lung Dis. 2006;10(4):378-383.

6.1. Research paper supplementary material

Table 6.4. (Research paper additional file 1) Table showing HIV care clinical follow-up of clients in the Botswana XPRES cohort (2010-2015)

Pre-ART, CD4 >350	**3 monthly**	Weight, CD4, TB screen
ART	**ART start**	Weight, CD4, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen, Hepatitis B screen, creatinine if TDF-based regimen
	2 weeks	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen
	1 month	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen
	3 months	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen, Viral load, creatinine if TDF-based regimen
	6months	Weight, TB screen, ALT/AST[a] if NVP-based regimen, Hb if AZT-based regimen, Viral load, CD4
	Quarterly[b]	Weight, TB screen, Viral load and CD4 6 monthly, creatinine if TDF-based regimen 6 monthly

Abbreviations: CD4, CD4 cell count; TB, tuberculosis; ALT, alanine transaminase; AST, aspartate aminotransferase; NVP, nevirapine; AZT, zidovudine; TDF, tenofovir;
[a]Routine ALT/AST not required after 6 months but may be requested by the clinician depending on the clinical situation.
[b]For those patients started on PI-based regimens, baseline and 12-monthly glucose (random or fasting) and total cholesterol/triglycerides are recommended.

Additional file 2, Text showing XPRES enrolment and follow-up procedures

At prospective cohort enrolment, research staff administered a standardized questionnaire, which captured demographic characteristics including age, sex, and pregnancy status, as well as clinical characteristics, including the WHO TB symptom screening rule for any current cough, fever, night sweats or weight loss, WHO HIV disease stage, height and weight, temperature in degrees centigrade, the most recent haemoglobin level, and the most recent CD4 count. As part of the trial intervention to strengthen ICF, all patients symptomatic for TB were encouraged to provide at least two same-day, on-the-spot (spot) sputum samples. Laboratory personnel had received refresher training on Ziehl-Neelsen staining for sputum-smear microscopy and Xpert implementation. In all phases, sputum test results were returned to the clinics within 4 days for sputum-smear microscopy and 2 days for Xpert testing. Study nurses were trained to inform patients of positive TB diagnoses the same day via phone, or if unreachable by phone, by active tracing to the household. As part of the active tracing intervention, for all patients ≥1 day late for a clinic appointment, study nurses conducted up to five telephone calls and two home visits in attempts to return these clients to care. XPRES participants were followed for 12 months, or until the end of TB treatment, whichever was later. The final follow-up visits for XPRES enrolees were in June 2015.

Additional file 3: TRIPOD Checklist: Prediction Model Development and Validation

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	4-5
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	6-7
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	6-7
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	7, 8, 12,13
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	7, 8, 12,13
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	7, 8, 12,13
	5b	D;V	Describe eligibility criteria for participants.	7, 8, 12,13
	5c	D;V	Give details of treatments received, if relevant.	7-8, 12,13
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	9
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	9-10
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	9-12
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	9-12
Sample size	8	D;V	Explain how the study size was arrived at.	6, 12
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	11
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	9-13
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	9-13
	10c	V	For validation, describe how the predictions were calculated.	9-13
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	9-13
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	9-13
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	9-13
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	12-13
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	14-15 (fig 1)
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	14-15
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	16-22
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	16-22
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	17
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	20
	15b	D	Explain how to the use the prediction model.	20-22
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	16-22
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	16-22
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	28
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	23-29
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	23-29
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	23-29
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	42
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	31

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

Table 6.6. (Research paper additional file 4) Table showing Hosmer-Lemeshow tests for calibration of final models A (CD4 excluded) and B (CD4 included)

Additional file 4a: Hosmer-Lemeshow test for calibration of final model – Model A (CD4 excluded)

Derivation Dataset					Validation Dataset				
			Death					Death	
Decile	N	Cut off*	Observed**	Predicted†	Decile	N	Cut off*	Observed**	Predicted†
1	285	0.0034	1	0.7	1	272	0.0031	0	0.7
2	283	0.0045	0	1.1	2	271	0.0042	3	1
3	284	0.0057	0	1.4	3	273	0.0053	0	1.3
4	284	0.007	1	1.8	4	270	0.0066	1	1.6
5	283	0.0091	0	2.3	5	272	0.0085	3	2
6	284	0.0131	3	3.1	6	271	0.0122	5	2.8
7	284	0.021	5	4.7	7	272	0.0189	2	4.1
8	284	0.0345	8	7.6	8	271	0.031	6	6.7
9	284	0.0689	20	13.6	9	272	0.0619	10	11.9
10	283	0.7517	45	46.6	10	271	0.5941	37	42.6
Total	2838		83	83		2715		67	75

Derivation dataset Hosmer-Lemeshow chi2(8) = 8.56, p=0.3807
Validation dataset Hosmer-Lemeshow chi2(8) = 10.91, p=0.2069
* Upper boundary of predicted risk
**Observed = observed number dying within 6 months of ART initiation
†Predicted = expected number dying within 6 months of ART initiation

Additional file 4b: Hosmer-Lemeshow test for calibration of final model – Model B (CD4 included)

Derivation Dataset					Validation Dataset				
			Death					Death	
Decile	N	Cut off*	Observed**	Predicted†	Decile	N	Cut off*	Observed**	Predicted†
1	284	0.0026	0	0.6	1	272	0.0025	1	0.5
2	284	0.0036	0	0.9	2	271	0.0034	1	0.8
3	284	0.0046	0	1.2	3	272	0.0044	1	1.1
4	284	0.0059	1	1.5	4	271	0.0057	1	1.4
5	284	0.0084	3	2	5	272	0.0078	2	1.8
6	284	0.0123	1	2.8	6	271	0.0113	3	2.5
7	284	0.0193	5	4.4	7	272	0.0177	3	3.9
8	284	0.0325	7	7.1	8	271	0.0322	7	6.5
9	284	0.0704	15	13.4	9	272	0.068	14	12.7
10	284	0.807	52	50.1	10	271	0.7138	34	47.5
	2840		84	84		2715		67	79

Derivation dataset Hosmer-Lemeshow chi2(8) 5.21, p=0.7345
Validation dataset Hosmer Lemeshow chi2(8) = 5.73, p=0.677
* Upper boundary of predicted risk
**Observed = observed number dying within 6 months of ART initiation
†Predicted = expected number dying within 6 months of ART initiation

Table 6.7. (Research paper additional file 5) Tables showing performance of clinical score in derivation and validation datasets for Models A (excluding CD4) and B (including CD4)

Additional file 5a: Performance of clinical score in derivation and validation datasets – Model A (excluding CD4)

	Derivation			Validation			TB Fast Track		
Clinical score	Total with score	Number died within 6 months	% Died	Total with score	Number died within 6 months	% Died	Total with score	Number died within 6 months	% Died
0	236	1	0%	293	1	0%	0	0	0
1	434	-	0%	415	2	0%	45	0	0%
2	573	-	0%	569	2	0%	65	0	0%
3	564	7	1%	490	8	2%	167	3	2%
4	367	11	3%	371	4	1%	183	10	5%
5	263	11	4%	214	9	4%	279	15	5%
6	203	16	11%	186	15	8%	282	15	5%
7	81	8	19%	66	10	15%	34	11	32%
8	92	20	38%	80	13	16%	16	6	38%
>=9*	25	9	27%	31	3	10%	6	0	0%
Total	2838	83		2715	67		1,077	60	

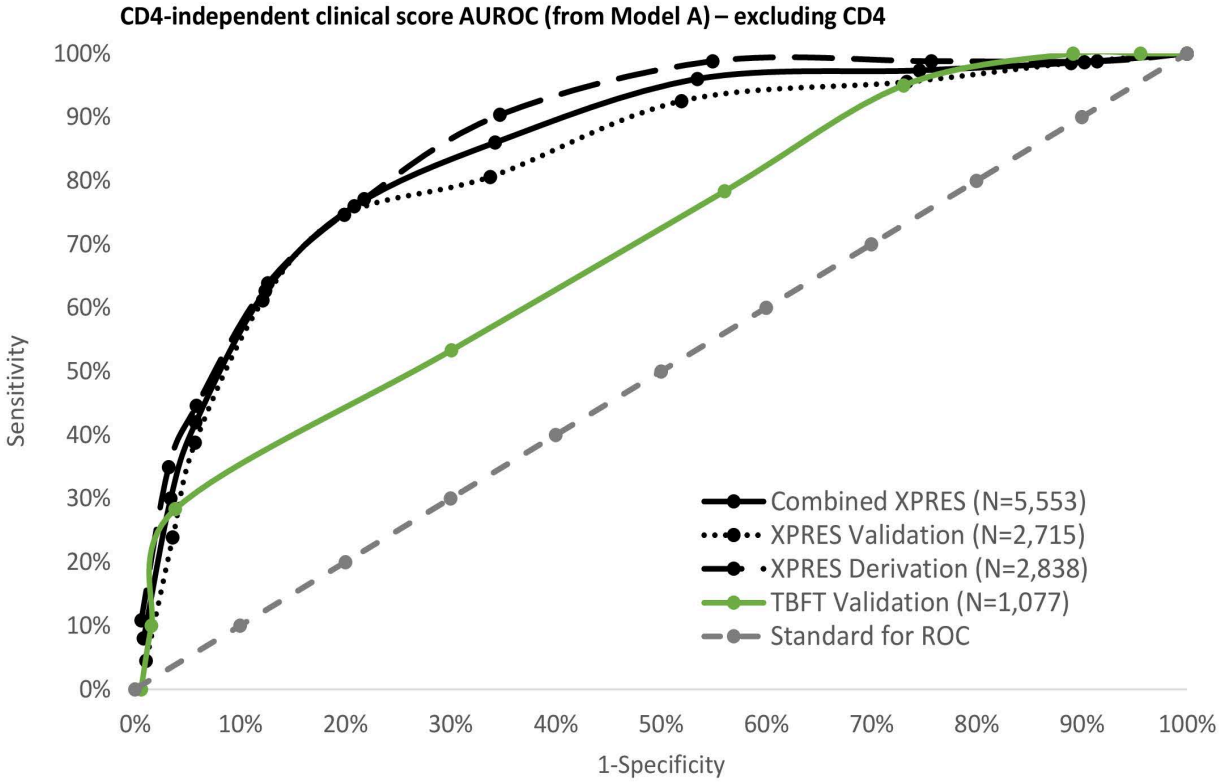
*In derivation dataset, 11, 11, and 3 patients had scores of 9, 10, and 11, with 6, 3, and 0 deaths respectively. In XPRES validation dataset, 14, 15, and 2 had scores of 9, 10, and 11 with 0, 2, and 1 deaths respectively. No scores >9 were observed in the TBFT dataset.

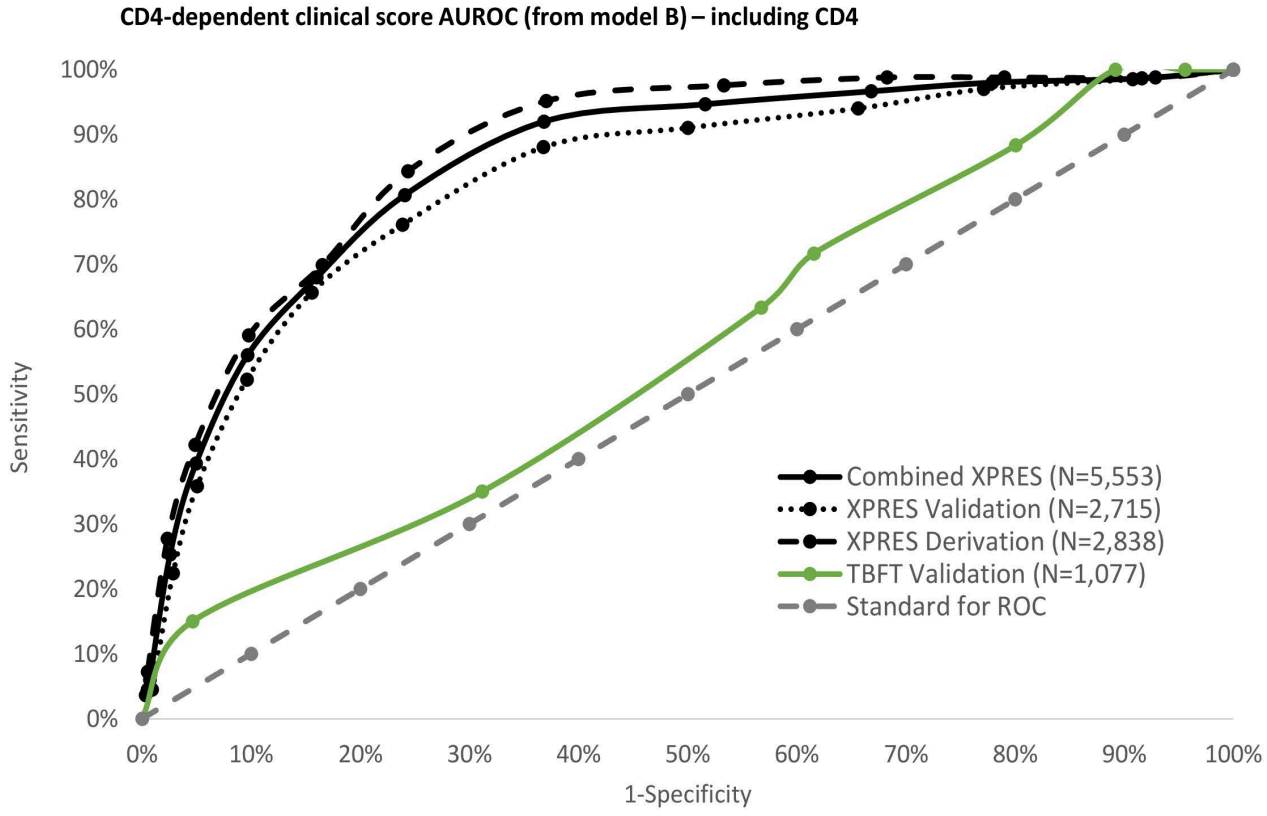
Additional file 5b: Performance of clinical score in derivation and validation datasets – Model B (including CD4)

	Derivation			Validation			TB Fast Track		
Clinical score	Total with score	Number died within 6 months	% Died	Total with score	Number died within 6 months	% Died	Total with score	Number died within 6 months	% Died
0	198	1	1%	257	1	0%	0	0	0
1	381	-	0%	361	1	0%	0	0	0
2	296	-	0%	305	2	1%	45	0	0%
3	413	1	0%	413	2	0%	65	0	0%
4	451	2	0%	351	2	1%	100	7	7%
5	358	9	3%	348	8	2%	198	10	5%
6	228	12	5%	226	7	3%	54	5	9%
7	195	9	5%	166	9	5%	277	17	6%
8	149	14	9%	131	11	8%	282	12	4%
9	82	12	15%	67	9	13%	56	9	16%
10	67	17	25%	63	12	19%			
11	8	3	38%	11	-	0%			
>=12	12	3	25%	16	3	19%			
Total	2838	83		2715	67		1,077	60	

*In derivation dataset, 9 and 3 patients had scores of 12 and 13, with 3, and 0 deaths respectively. In XPRES validation dataset, 14 and 2 had scores of 12 and 13 with 2 and 1 deaths respectively. No scores >9 were observed in the TBFT dataset.

Additional file 6: Area under the receiver operating characteristic curve for clinical score performance in combined XPRES dataset (N=5,553) and external validation TB Fast Track Dataset (N=1,077) for Models A (excluding CD4) and B (including CD4)





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	1405072	**Title**	Dr.
First Name(s)	Francis Andrew		
Surname/Family Name	Auld		
Thesis Title	Opportunities to reduce early antiretroviral therapy mortality in sub-Saharan Africa through improved tuberculosis case-finding and retention in HIV-TB care		
Primary Supervisor	Prof. 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?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*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?	PLoS Medicine
Please list the paper's authors in the intended authorship order:	Andrew F. Auld, Andrew Kerkhoff, Yasmeen Hanifa, Robin Wood, Salome Charalambous, Yuliang Liu, Tefera Agizew, Anikie Mathoma, Rosanna Boyd, Anand Date, Sherri L. Pals, Ray Shiraishi, George Bicego, Christopher Serumola,

	Unami Mathebula, Heather Alexander, Goabaone Rankgoane-Pono, Pontsho Pono, Alyssa Finlay, James C. Shepherd, Tedd V. Ellerbrock, Alison D. Grant, Katherine Fielding
Stage of publication	**Not yet submitted**

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)	The candidate conceived the idea for the paper and analysis, conducted the data analysis, and wrote the paper.

SECTION E

Student Signature	[redacted]
Date	14 July 2020

Supervisor Signature	[redacted]
Date	16 July 2020

Risk score for predicting HIV-associated tuberculosis to support case finding and preventive therapy scale-up: A derivation and external validation cohort study using regression and machine learning approaches

Andrew F. Auld[1], Andrew Kerkhoff[2], Yasmeen Hanifa[3], Robin Wood[4], Salome Charalambous[5], Yuliang Liu[1], Tefera Agizew[6], Anikie Mathoma[6, 7], Rosanna Boyd[6], Anand Date[1], Sherri L. Pals[1], Ray Shiraishi[1], George Bicego[1], Christopher Serumola[6], Unami Mathebula[6], Heather Alexander[1], Goabaone Rankgoane-Pono[8], Pontsho Pono[8], Alyssa Finlay[6], James C. Shepherd[6,9], Tedd V. Ellerbrock[1], Alison D. Grant*[3,10], Katherine Fielding*[3]

*These authors contributed equally to this work as senior authors

[1] Division of Global HIV & TB, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A

[2] Division of HIV, Infectious Diseases and Global Medicine at Zuckerberg San Francisco General Hospital and Trauma Center, Department of Medicine, University of California, San Francisco, CA, U.S.A

[3] TB Centre, London Sch. of Hygiene & Tropical Med., London, United Kingdom

[4] The Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, Cape Town, South Africa

[5] Aurum Institute, Johannesburg, South Africa

[6] Division of TB Elimination, Centers for Disease Control and Prevention, Gaborone, Botswana

[7] University of Botswana, Gaborone, Botswana

[8] Ministry of Health and Wellness, Gaborone, Botswana

[9] Yale University School of Medicine, New Haven, CT, U.S.A

[10] Africa Health Research Institute, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Abstract

Background: Among people living with HIV (PLHIV), more flexible and sensitive tuberculosis (TB) screening tools capable of detecting TB are needed to reduce morbidity and mortality from undiagnosed TB, facilitate prudent scale-up of TB preventive therapy (TPT) to over 13 million PLHIV by 2021, and allow for differentiated HIV-TB care.

Methods: We used Botswana XPRES trial data for adult HIV clinic enrolees to develop a parsimonious multivariable prognostic model for active prevalent TB using both logistic regression and random forest machine learning approaches. A clinical score (CS) was derived by rescaling final model coefficients. The CS was developed using southern Botswana XPRES data and its accuracy validated internally, using northern Botswana data, and externally using three diverse cohorts of antiretroviral therapy (ART)-naïve and ART-experienced PLHIV enrolled in XPHACTOR, TB Fast Track (TBFT), and Gugulethu studies from South Africa. Predictive accuracy of the CS was compared with the World Health Organisation (WHO) four-symptom TB screen.

Results: Among 5,418 XPRES enrolees, 2,771 were included in the derivation dataset; median CD4 was 240/µL, and 189 (7%) had undiagnosed prevalent TB. Among XPHACTOR, TBFT, and Gugulethu cohorts, median CD4 was 400/µL, 73/µL, and 167/µL, and prevalence of TB was 5%, 10%, and 18%, respectively. Factors predictive of TB in the derivation dataset and selected for the CS included: male gender (1 point), ≥1 WHO TB symptom (7 points), smoking history (1 point), temperature >37.5˚C (6 points), body mass index <18.5kg/m^2 (2 points), and severe anaemia (haemoglobin <8g/dL) (3 points). Sensitivity using the WHO four-symptom TB screen was 73%, 80%, 94% and 94% in XPRES, XPHACTOR, TBFT and Gugulethu cohorts, respectively, but increased to 88%, 87%, 97%, and 97%, when a CS of ≥2 was used. Negative predictive value (NPV) also increased 1%, 0.3%, 1.6%, and 1.7% in XPRES, XPHACTOR, TBFT, and Gugulethu cohorts, respectively when the CS of ≥2 replaced the WHO four-symptom TB screen. Categorizing risk scores

into low (<2), moderate (2-10), and high-risk categories (>10) yielded TB prevalence of 1%, 1%, 2%, and 6% in the lowest risk group and 15%, 22%, 26%, and 32% in the highest risk group for XPRES, XPHACTOR, TBFT, and Gugulethu cohorts, respectively. At CS ≥2 the number needed to screen ranged from 5.0 in Gugulethu to 11.0 in XPHACTOR.

Conclusions: The simple and feasible CS allowed improved sensitivity and NPV, which could facilitate reductions in mortality from undiagnosed TB and safer administration of TPT during proposed global scale-up efforts. Differentiation of risk by CS cut-off allows flexibility in designing differentiated HIV-TB care to maximize impact of available resources.

Introduction

Tuberculosis (TB) remains the most common cause of death among people living with HIV (PLHIV), causing 251,000 HIV-associated TB deaths in 2018, with over 95% of these deaths in low- and middle-income countries (LMIC) [1]. Among PLHIV who die from TB, TB is commonly undiagnosed at the time of death [2,3]. The World Health Organization (WHO) recommends a four-symptom TB screening rule (i.e., for cough, weight loss, night sweats or fever) to determine which PLHIV need investigation for active TB and which are eligible for immediate TB preventive therapy (TPT) [4]. The WHO four-symptom TB screening rule is recommended for LMIC regardless of expected prevalence of active TB, setting (e.g., high or low TB incidence settings), or antiretroviral therapy (ART) status (ART-naïve or ART-experienced) [4].

However, screening accuracy of the WHO four-symptom screening rule varies by population, setting, and ART status, raising the question whether a "one-size-fits-all" screening rule is appropriate. For example, a recent meta-analysis observed that while sensitivity of the WHO four-symptom TB screening rule is about 89% among ART-naïve PLHIV, it was only 51% among people on ART due to a higher prevalence of asymptomatic TB among stable ART patients [5-7]. At a time when global health donors have committed to reaching over 13 million PLHIV on ART with TPT by 2021 [8], low sensitivity of the WHO four-symptom screening rule for active TB among PLHIV on ART warrants consideration of more sensitive screening approaches [9]. Although new WHO guidelines recommend adding chest radiography (CXR) to the screening rule for PLHIV on ART to increase sensitivity and negative predictive value (NPV), this comes at the expense of specificity, carries significant additional costs and operational challenges, and might hinder rather than expedite TPT scale-up in some LMIC settings [5,10]. Asymptomatic active TB can also be present among severely immune compromised PLHIV [11], and among pre-ART patients without advanced disease in high prevalence settings [12], among whom missing asymptomatic active TB can have suboptimal health consequences for patients and

impede disease control activities [13]. Finally, the WHO four-symptom screening rule does not allow TB risk differentiation into low-, moderate-, and high-risk groups which might inform differentiated models of care.

Therefore, we aimed to develop a predictive clinical score based on variables commonly available in resource-contained clinics, to define a range of cut-offs, with associated screening sensitivity, specificity, NPV, positive predictive value (PPV), and number needed to screen (NNS) to detect one person with active TB.

Methods

We used data from the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES) trial conducted in Botswana to derive the predictive TB clinical score [14]. We split XPRES cohort data geographically into 11 southern and 11 northern clinics to serve as an internal derivation and validation datasets, respectively. We used two different but complementary modelling approaches to generate a parsimonious TB clinical risk score comprised of variables easily available in a resource-constrained clinic setting: (1) logistic regression models, and (2) random forest machine learning models. Random forest machine learning models are particularly useful for identifying important non-linear associations between predictors and outcomes because the modelling approach does not rely on assumptions of average linear or curvilinear associations [15]. Having derived the clinical score, we then used data from three other settings to validate the derived clinical score: (1) prospective cohort data for XPHACTOR study enrolees from Gauteng province, South Africa (SA), which represents a predominantly stable, long-term ART population [16]; (2) cluster-randomised trial data from the TB Fast Track (TBFT) trial from Gauteng, Limpopo, and North West Provinces in SA, which represents a population with advanced HIV disease not taking ART [17]; and (3) prospective cohort data from the Western Cape, SA, which represents an ART-naïve population in a very high TB incidence setting [18]. We compared screening accuracy of our derived clinical scores with existing WHO TB symptom screening criteria for active TB among PLHIV in each of these populations.

XPRES study design and participants for prediction tool development

XPRES was a stepped-wedge cluster-randomised trial (CRT) with a retrospective baseline component conducted at 22 health facilities, including five hospitals and 17 clinics, that were purposively selected to be representative of HIV treatment clinics in Botswana [14]. In the prospective, stepped-wedge portion of the trial, all non-incarcerated, consenting, ART-naïve, HIV-positive persons, regardless of TB treatment or symptom status, presenting to the study clinics between August 2012 and end of March 2014, were eligible for enrolment. Only adolescents and adults (aged ≥12 years old), who did not present at the study clinic with a TB diagnosis, were included in this analysis.

XPRES procedures

Per Botswana national guidelines during the time period of the study, all XPRES study participants were eligible for ART initiation if they had a CD4 count ≤350 cells/μL, were diagnosed as having WHO stage III/IV, or were pregnant or breastfeeding [19]. All study participants received clinical care and follow-up appointments per Ministry of Health and Wellness guidelines, which included WHO TB symptom screening at the first and all subsequent clinic visits (see S1, table).

Interventions

The prospective XPRES cohort was recruited within two phases of the stepped-wedge trial. In the first phase, all prospective XPRES participants received two enhanced care interventions in addition to standard of care (SOC): (1) additional support for intensified TB case finding, and (2) intensified tracing for patients missing clinic appointments. In the second phase, the Xpert MTB/RIF assay was initiated in place of sputum smear microscopy for TB diagnosis. We have previously shown that there was no significant difference in TB case finding between the first and second prospective phases of XPRES (5% vs. 6%), although the prevalence of microbiologically confirmed TB was higher in the post-Xpert study phase (51% vs. 65%) [20]. Enrolment and follow-up procedures are described in a

supplementary appendix (S2, text). XPRES participants were followed for 12 months, or until the end of TB treatment, whichever was later. The final follow-up visits for XPRES enrolees were in June 2015.

Development and internal validation of the prediction model

A clinically-useful prediction model should demonstrate accurate prediction of the outcome in data other than that in which the model was developed. Therefore, we split the XPRES dataset in an approximately 1:1 ratio into southern clinics (n=11) and northern clinics (n=11) with southern clinics serving as the derivation dataset and northern clinics as the model validation dataset [21].

Outcome

The outcome of interest for this analysis was new diagnosis of active TB (clinical or microbiologically confirmed), within 6 months of arrival at the HIV treatment clinic. In this manuscript, this outcome is referred to as undiagnosed prevalent TB at arrival at the HIV treatment clinic [16,22,23]. Active TB during this initial 6 month post-clinic enrolment period is considered prevalent rather than incident TB based on prior clinical cohort data showing that 87% of active TB cases identified in months 0-6 after HIV clinic enrolment, could have been diagnosed at the HIV clinic enrolment visit [23]. In addition, data from Zimbabwe show that the mean duration of smear-positivity prior to TB diagnosis amongst HIV-positive adults to be 18±33 weeks [22]. Precedent for this approach and definition has been previously published [16]. We implemented intensive efforts to ascertain true active TB disease among participants with TB case finding procedures previously published and provided in supplementary appendix S2 (text) [14,20].

Candidate predictor variables

We selected candidate predictor variables for potential inclusion in the predictive models based on prior publications, and the need for variables to be reproducible, objective, and readily available in resource-constrained clinic settings [24]. We considered variables

known to be associated with active TB including age, sex, marital status, education level, employment status, previous/current work as a miner, smoking history, prior TB treatment, history of a TB contact in the last 24 months, presence and number of WHO TB symptoms, body mass index (BMI) (weight/height^2), haemoglobin concentration, CD4 count, temperature at ART initiation in degrees Celsius, and respiratory rate at enrolment visit [16,25-27]. We included BMI rather than weight as a candidate variable as a better marker of nutritional status [28].

Logistic regression model approach

Within the derivation dataset, we performed univariable logistic regression analyses assessing the association of each variable with risk of prevalent active TB. Continuous variables were assessed for non-linearity with log odds of TB using multivariable fractional polynomial models, as well as by comparing Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC) between models with linear or fractional polynomial terms. Where non-linearity was observed, the appropriate fractional polynomial terms were included in the logistic regression. We also examined scatter plots of untransformed and transformed continuous variables and risk of mortality to assess inflexion points which might inform appropriate categorization of continuous variables.

For the multivariable logistic regression analysis, a complete case analysis was chosen because few data (<10%) were missing. To inform generation of a parsimonious multivariable model, we used a stepwise backward elimination approach, starting with all candidate variables and excluding variables sequentially if $p>0.01$ using both automatic and manual approaches. Prior regression derived scores used p-value cut-offs of >0.05 [16,29,30]; however, there is no accepted standard p-value cut-off for backward or forward stepwise variable elimination approaches [24]. Because we aimed to generate a parsimonious model, to increase feasibility of the practical clinical score in LMIC clinic settings, we used a >0.01 cut-off in line with recommendations from Royston *et al* [24,31]. We also explored how findings changed using a forward stepwise addition approach.

Where two or more predictors were highly correlated, only one was selected, to simplify the prognostic model. Plausible interactions between covariates (e.g., between gender and BMI [32]) were assessed using the likelihood ratio test.

Random forest model approach

We first built a random forest model with all 15 possible candidate variables that were included in the backward stepwise elimination approach. We fit the model using the randomForest R package with 1,000 trees. We used the *bestmtry* function to identify the optimum number of variables to be randomly included in each of the 1000 trees. We used this model to order the 15 variables according to importance in predicting TB as measured by both the mean decrease in accuracy and mean decrease in Gini for each variable [33]. We compared results with the logistic regression to assess if potentially important discriminatory variables had been eliminated in the backward stepwise regression. To assess any potentially important loss of discrimination through eliminating variables to create a parsimonious model, and to assess potential differences in discriminatory capacity between model approaches, we compared area under the receiver-operating characteristic (AUROC) curve values, between logistic regression and random forest models in scenarios where all 15 variables were modelled and a scenario where a parsimonious model was chosen. Information from the backward stepwise regression and random forest modelling was used to generate the final parsimonious model.

Internal validation of parsimonious model

In both the derivation and validation datasets, we assessed multivariable logistic regression model calibration graphically in a calibration plot [21], and statistically using the Hosmer-Lemeshow test. We also assessed discrimination using the AUROC values. AUROC values of 0.7 to 0.79, 0.8 to 0.89, and >0.9 are respectively considered acceptable, excellent and outstanding discrimination [34].

Clinical score generation

The final multivariable model was used to generate a practical clinical score. For these models, continuous variables were categorized in a clinically meaningful manner based on their functional form and information from the published literature. Each beta coefficient from this logistic regression model was then rescaled to generate a clinical score by dividing each coefficient by the smallest positive model coefficient and rounding to the nearest integer. The total number of points was summed for each participant to calculate their total clinical score.

External validation of risk scores

To externally validate the clinical risk score, we used data collected independently from the XPHACTOR cohort [16], TBFT trial [17], and Gugulethu cohort [18]. Characteristics of these studies as relate to setting, clinic types, eligibility criteria, dates of enrolment, ART eligibility criteria, TB symptoms screening, TB case finding approaches, and definition of active prevalent TB for this analysis, are described in Supplementary Table 3 (S3, table).

For both the XPRES cohort (combined derivation and validation datasets), and the three validation datasets, we explored how sensitivity, specificity, PPV, NPV, and AUROC curve values varied with increasing clinical score in terms of predicting active prevalent TB and compared this screening accuracy and discrimination performance with the current WHO TB symptom screening rule. Three risk groups were created to visualize increasing active prevalent TB risk with increasing clinical score, and the percentage of ART enrolees falling into each risk group. The NNS to detect one TB case was compared between WHO TB symptom screening rules and a range of clinical score cut-offs.

All logistic regression and clinical score validation analyses were conducted using STATA 16 (StataCorp, 2009, Stata Statistical Software, Release 16, College Station, TX). All Random Forest Plot analyses and analyses to assess the mean decrease in Gini associated with candidate predictor variables were done with R version 3.6.1. (R Core Team (2017). R

Foundation for Statistical Computing, Vienna, Austria). The study is reported in concordance with TRIPOD guidance for multivariable prediction models (S4, Table).

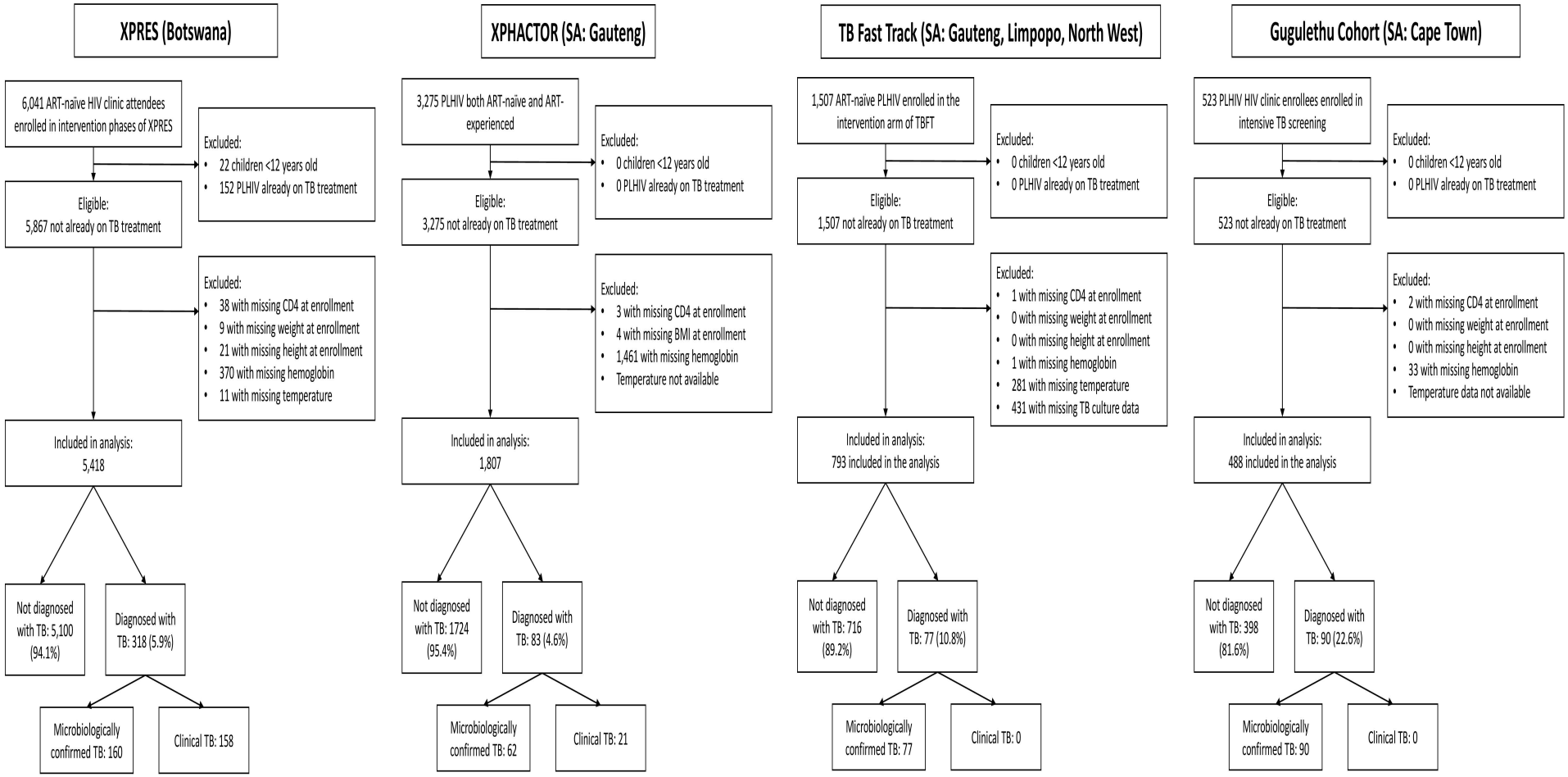
Ethical review

Ethical approval for each of the source studies was obtained from the relevant ethics committees in the country of data collection and from the trial sponsors. All participants provided informed written consent, or where the enrolee could not read or write, witnessed verbal informed consent. Ethical approvals for XPRES were obtained from the U.S. Centers for Disease Control and Prevention (CDC) Institutional Review Board (IRB) C, the Health Research and Development Division of the Health Research and Development Committee (HRDC) in Botswana, and the University of Pennsylvania IRB No.4. XPHACTOR was was approved by the ethics committees at the University of the Witwatersrand, University of Cape Town, and the London School of Hygiene & Tropical Medicine. TBFT was approved by the research ethics committees of the University of the Witwatersrand and the London School of Hygiene & Tropical Medicine, and the South African Medicines Control Council. The Gugulethu prospective cohort study was approved by the research ethics committees of the University of Cape Town, and the London School of Hygiene & Tropical Medicine.

Results

From the XPRES cohort, 5,418 eligible adult (≥12 years old) study enrolees with complete data for candidate predictors were included in the analysis (Fig 1). Overall, 318 (6%) of 5,418 enrolees had undiagnosed prevalent active TB at HIV clinic registration and study enrolment.

Fig 1. Study profile



Internal derivation and temporal validation datasets

From the XPRES cohort, the internal derivation (N=2,771) and validation (N=2,647) datasets were created (Table 1). Key characteristics including median age (34 years), percentage female (67-68%), median CD4 (240-249/μL), and prevalence of active TB at enrolment (5-7%), were similar between XPRES derivation and validation datasets (Table 1).

Table 1: Comparison of derivation and validation datasets (internal and external)*

			Derivation Dataset (Botswana southern clinics: N=2,771)		Validation Dataset (Botswana northern clinics: N=2,647)		External Validation Dataset (SA, XPHACTOR: N=1807)		External Validation Dataset (TBFT, SA: N=793)		External Validation Dataset (Gugulethu Cohort, CT, SA: N=488)	
Demographics			n	(median or %)	n	(median or %)	n	(median or %)	n	(median or %)	n	(median or %)
	Age (years) n, median (IQR)		2,771	34.3 (28.8-41.3)	2,647	33.5 (28.3-40.8)	1,807	40.0 (34.0-47.0)	793	38.0 (32.0-45.0)	488	33.6 (27.9-40.7)
	Female, n, %		1,862	67%	1,790	68%	1,290	71%	424	53%	310	64%
	If Female, Pregnant, n, %		499	27%	568	32%	0	0%	0	0%	0	0%
	Marital status, n, %	Married/Civil Union	306	11%	242	9%						
		Single	2,353	85%	2,322	88%						
		Widowed/Divorced	112	4%	83	3%						
	Smoking history (ever), n, %		466	17%	575	22%	388	21%	180	23%	185	38%
	Employed, n, %		1467	53%	1023	39%						
	Education, n, %	None	154	6%	235	9%						
		Primary	637	23%	614	23%						
		Secondary	1,689	61%	1,597	60%						
		Higher	291	11%	201	8%						
	Ever a miner, n, %		124	4%	143	5%						
HIV/TB history												
	Previous TB treatment, n, %		232	8%	169	6%						
	TB contact in last 24 months, n, %		266	10%	230	9%						
	WHO TB symptoms, n, %											
		Cough	533	19%	466	18%	364	20%	424	53%	243	50%
		Weight loss	533	19%	577	22%	243	13%	621	78%	331	68%
		Fever	262	9%	223	8%	105	6%	269	34%	139	28%
		Night sweats	257	9%	243	9%	135	7%	297	37%	199	41%
	Number of WHO TB symptoms, n, %	0	1979	71%	1837	69%	1,299	72%	133	17%	67	14%
		1	349	13%	397	15%	384	21%	173	22%	127	26%
		2	200	7%	203	8%	136	8%	191	24%	135	28%
		3	136	5%	134	5%	41	2%	145	18%	121	25%
		4	107	4%	76	3%	17	1%	151	19%	38	8%
	Duration of WHO symptoms	n, median (IQR)	792	60 (30-120)	810	60 (21-150)						
Clinical Characteristics												
	$CD4^+$ T-cell Count (cells/μL)	n, median (IQR)	2,771	240 (131-314)	2647	249 (151-321)	1807	400 (246-600)	793	73 (34-109)	488	167 (95-231)
	Weight (kg)**	n, median (IQR)	2,771	59 (52-69)	2647	60 (53-69)			793	57 (50-66)	488	64 (56-73)
	BMI (kg/m^2)	n, median (IQR)	2,771	21.8 (19.2-25.4)	2647	21.5 (18.9-24.7)	1807	25.0 (21.4-29.3)	793	20.9 (18.6-24.5)	488	23.5 (20.9-27.1)
	Haemoglobin g/dL	n, median (IQR)	2,771	11.9 (10.5-13.3)	2647	12.0 (10.7-13.4)	1,807	13.1 (11.8-14.3)	793	11.1 (9.6-12.8)	488	12.0 (10.6-13.4)
	Temperature (˚C)	n, median (IQR)	2,771	36.2 (35.8-36.7)	2647	36.1 (35.7-36.5)			793	36.3 (36.0-36.6)		
	Respiratory rate (breaths/min)	n, median (IQR)	2,771	20 (18-21)	2647	18 (17-20)						
New TB Diagnosis												
	Cumulative prevalent active TB, n, %		189	6.8%	129	4.9%	83	4.6%	77	9.7%	90	18.4%
	Cumulative incidence microbiologically confirmed TB, n, %		96	3.5%	64	2.4%	62	3.4%	77	9.7%	90	18.4%
	Time to diagnosis of prevalent TB (days)	n, median (IQR)	189	16 (7-35)	129	19 (4-48)						

Abbreviations: SA, South Africa; CT, Cape Town; WHO, World Health Organization; TB, tuberculosis; BMI, body mass index; IQR interquartile range*Where variable is blank, the data were not collected, or not provided from the source study for this analysis

**BMI was used as the covariate for nutritional status rather than weight (weight was not considered as an independent predictor)

Variable importance in logistic regression and random forest models

Table 2 summarizes the results of univariable and multivariable logistic regression model development. Although age (linear continuous variable), education level achieved, prior/current work as a miner, previous TB treatment, and respiratory rate (transformed variable), were associated with prevalent TB in univariable analysis, these variables were eliminated in the stepwise backward elimination approach due to p-values in multivariable analysis >0.01.

Rankings of variable importance as measured by size of beta coefficient in the logistic regression model and by mean decrease in Gini in the random forest model, were similar with respect to presence or absence of WHO TB symptoms, temperature, and BMI, with these variables in the top three most important predictors (Fig 2, Supplementary Appendix 5). Notably, the transformed term of BMI, which was eliminated in the backwards stepwise logistic regression at p=0.408, was considered the second most important variable according to mean decrease in Gini approach (52.766) and third most important in terms of size of the logistic regression beta coefficient (1.617). Given the importance of BMI in the variable ranking approach, importance in the published literature, and availability in resource-constrained clinics, BMI was retained in final multivariable model (Table 2). The final multivariable model included sex, smoking history, presence/absence of ≥1 WHO TB symptom, BMI (as a transformed term per the multivariable fractional polynomial (MFP) analysis), temperature (modelled as two transformed terms per the MFP analysis), and haemoglobin concentration (continuous, linear term) (Table 2).

Fig 2: Random forest model variable importance ranking by mean decrease in accuracy and mean decrease in Gini in the derivation dataset (N=2,771)

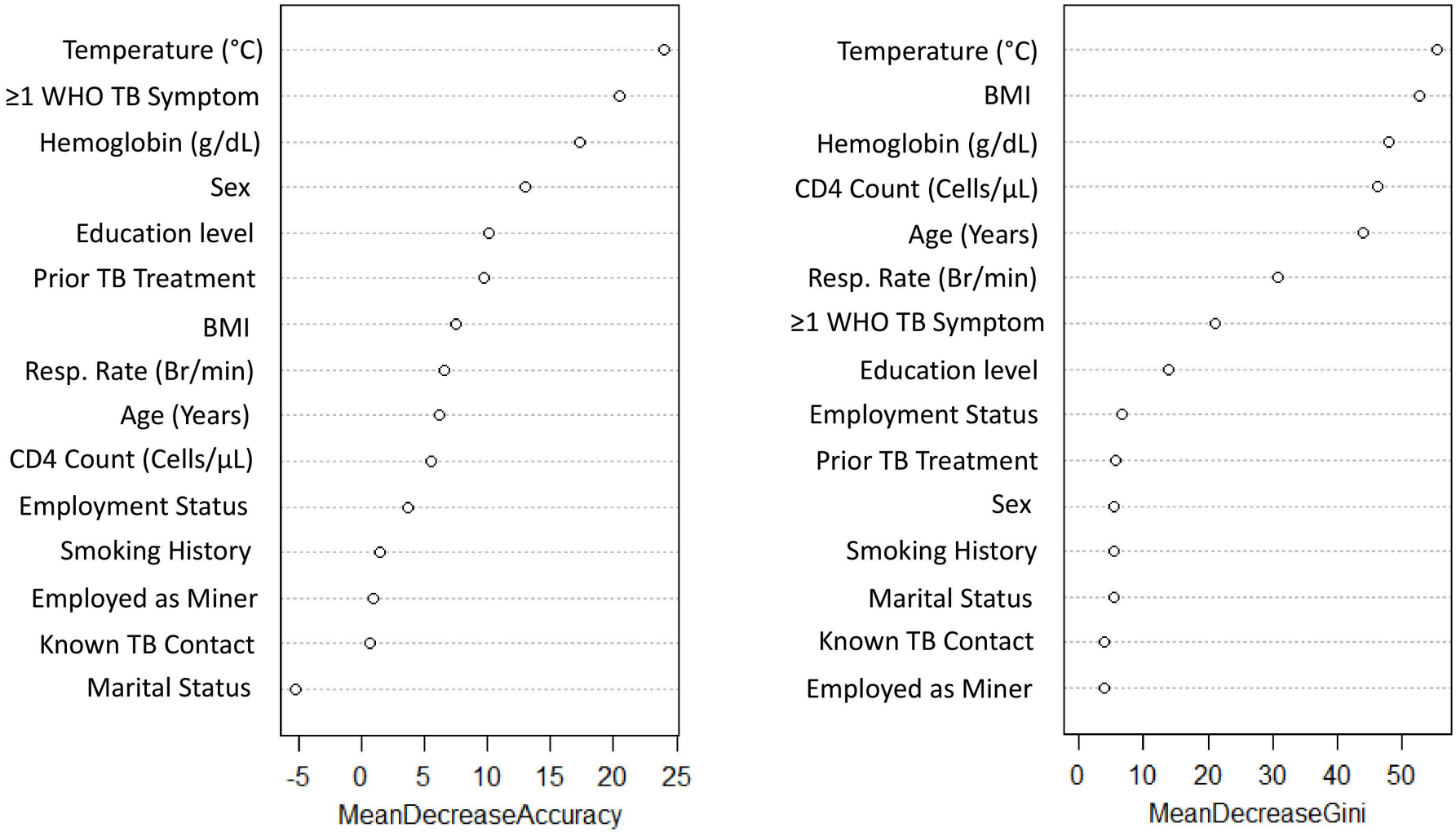


Table 2: Univariable and multivariable logistic regression analysis in the derivation dataset (N = 2,771)

		Not Diagnosed with TB (N=2,582)		Diagnosed with TB within 6 months (N=189)		Unadjusted			Final Adjusted Regression		
Demographics		n	Median(IQR)/%	n	Median(IQR)/%	OR	95% CI	p-value	AOR	95% CI	p-value
Age, years (for every 10 year increase)			34 (29-41)		38 (32-44)	1.24	(1.15-1.33)	<0.001			
Sex	Female	1,768	95%	94	5%	1.00	--	--	1.00	--	--
	Male	814	90%	95	10%	2.20	(1.63-2.95)	<0.001	1.91	(1.27-2.88)	0.002
Marital status	Married/civil union	288	94%	18	6%	1.00	--	--			
	Single	2,190	93%	163	7%	1.19	(0.71-2)	0.508			
	Widowed/Divorced	104	93%	8	7%	1.23	(0.44-3.41)	0.690			
Smoking History (ever smoked)	No	2,165	93%	154	7%	1.00	--	--	1.00	--	--
	Yes - ever smoked	417	89%	50	11%	1.82	(1.56-2.12)	<0.001	1.44	(1.12-1.85)	0.004
Employed	Employed	1,370	93%	97	7%	1.00	--	--			
	Unemployed	1,212	93%	92	7%	1.07	(0.71-1.62)	0.742			
Education	None	137	89%	17	11%	1.00	--	--			
	Primary	588	92%	49	8%	0.67	(0.38-1.18)	0.166			
	Secondary	1,576	93%	113	7%	0.58	(0.35-0.95)	0.030			
	Higher	281	97%	10	3%	0.29	(0.14-0.58)	<0.001			
Ever a miner	No	2,478	94%	169	6%	1.00	--	--			
	Yes	104	84%	20	16%	2.82	(2.04-3.9)	<0.001			
HIV/TB history											
Previous TB treatment	No	2,381	94%	158	6%	1.00	--	--			
	Yes	201	87%	31	13%	2.32	(1.43-3.78)	0.001			
Any TB contact in last 24 months	No	2,339	93%	180	7%	1.00	--	--			
	Yes	243	91%	24	9%	1.33	(0.83-2.14)	0.233			
Number of WHO symptoms	0	1,936	98%	43	2%	1.00	--	--			
	>=1	646	82%	146	18%	10.18	(6.79-15.26)	<0.001	6.91	(4.55-10.49)	<0.001
Clinical Characteristics											
CD4 (per 10-cell increase)		2,582	247 (139-316)	189	151 (57-255)	0.96	(0.94-0.97)	<0.001			
Weight (per 1 kg increase)[a]		2,582	59.4 (52.3-69.2)	189	53.7 (47.0-62.0)	0.97	(0.95-0.99)	0.001			
BMI (per 1 unit increase)[b]		2,582	21.9 (19.4-25.5)	189	19.4 (17.2-22.3)	0.90	(0.83-0.97)	0.004	0.98	(0.93-1.05)	0.612
Haemoglobin (per 1g/dL increase)		2,582	12.0 (10.6-13.3)	189	10.6 (9.2-12.3)	0.76	(0.69-0.83)	<0.001	0.78	(0.7-0.86)	<0.001
Temperature at enrolment (per 1 ˚ Celsius increase)[c]		2,582	36.2 (35.8-36.6)	189	36.4 (36.0-37.1)	2.13	(1.57-2.88)	<0.001	1.46	(1.18-1.81)	<0.001
Respiratory rate (breaths/min)[d]		2,582	20 (18-20)	189	20 (18-22)	1.03	(1.01-1.05)	0.010			

Abbreviations: TB, tuberculosis; CI, confidence interval; WHO, World Health Organization; BMI, body mass index; OR, odds ratio; AOR, adjusted odds ratio; IQR, inter-quartile range;

[a] Due to correlation with BMI, weight was not included in the stepwise backward regression.

[b] Due to non-linearity in the association between BMI and log odds TB, BMI was modelled as a transformed term from the MFP analysis (transformed BMI =X^-.5-.666749355, where: X = BMI/10). Output shown is for the single linear term to facilitate interpretation of average BMI effect (i.e., higher BMI associated with lower TB risk). In the backward stepwise regression, the p-value associated with BMI term was 0.4077 at point of elimination. Given the importance of BMI as a predictor in the Random Forest model (2nd most important predictor), ease of availability of this variable in almost all resource-limited clinics, and importance of BMI in published literature, BMI was retained in the final adjusted model.

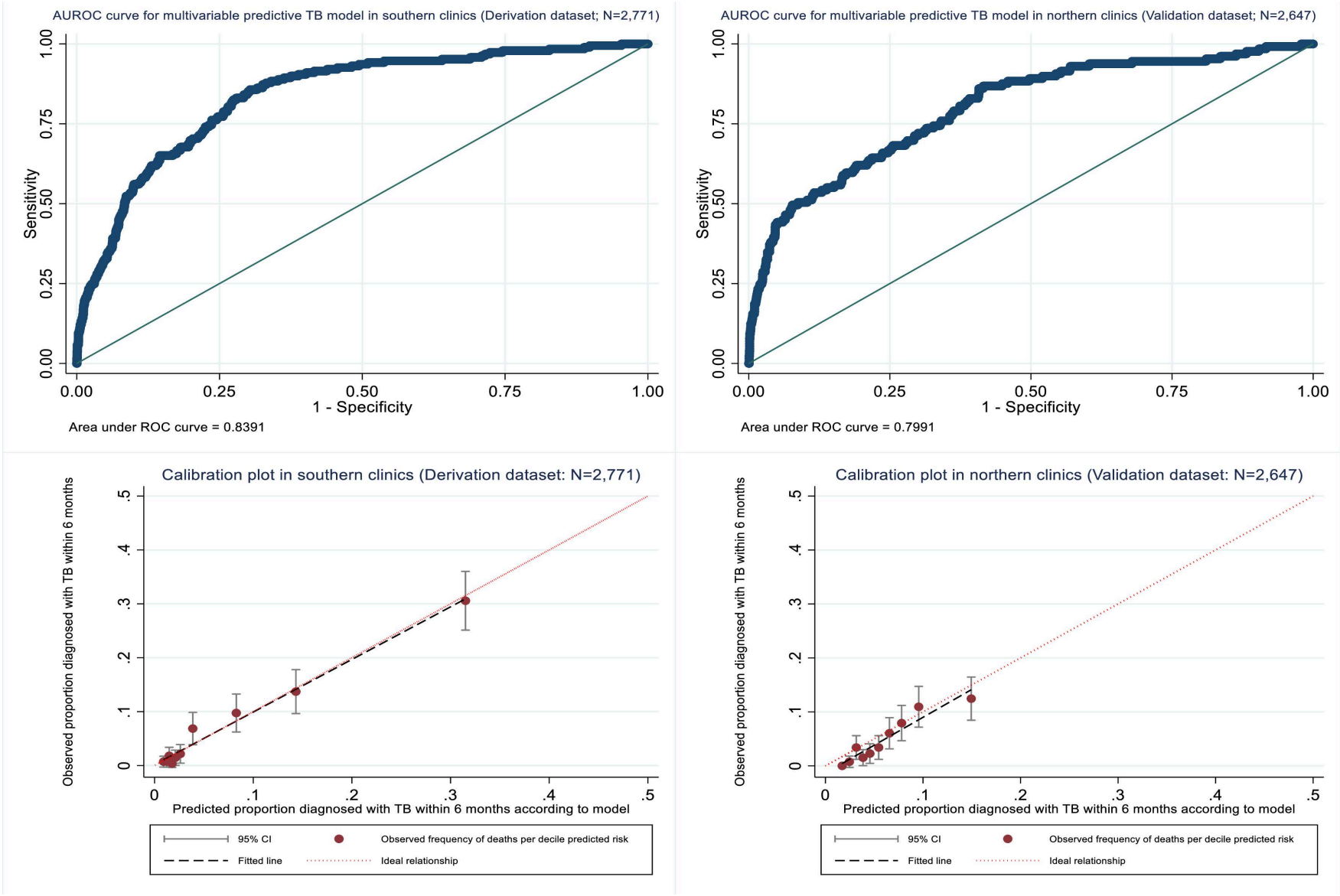
[c] Due to non-linearity in the association between temperature and log odds TB, temperature was modelled as two transformed terms (term 1 = temperature -36.12674419; term 2 = temperat^2-1305.141645). Output shown is for the single linear term to facilitate interpretation of average temperature effect (i.e., higher temperature associated with higher TB risk). In the backward stepwise regression, the p-value associated with each transformed term was 0.005 and 0.004 respectively.

[d] Due to non-linearity in the association between respiratory rate (RR) and log odds TB, RR was modelled as a transformed term from the MFP analysis (transformed term = X^-1-5.170636738, where: X = RR/100). Output shown is for the single linear term to facilitate interpretation of average RR effect (i.e., higher respiratory rate associated with higher TB risk). In the backward stepwise regression, the p-value associated with the transformed term was 0.0251 at the point of elimination from the model.

Internal validation of final multivariable regression model

For the derivation dataset, the Hosmer-Lemeshow statistic for the TB prediction model (p=0.135, see S6, table), and the calibration curve (Figure 3), indicated good model fit. Although the Hosmer-Lemeshow statistic for the internal validation dataset (p=0.0001) indicated lack of fit with over-estimation of prevalent TB, with 169 cases of prevalent TB predicted versus 129 observed, (1) the Hosmer-Lemeshow test is sensitive to sample size and our sample size is large, and (2) the calibration curve (Fig 3) indicated adequate prediction performance for the 10 risk groups. In addition, the AUROC curve values for the derivation (0.8391) and validation datasets (0.7991) indicated excellent, and borderline excellent discrimination, respectively (Fig 3).

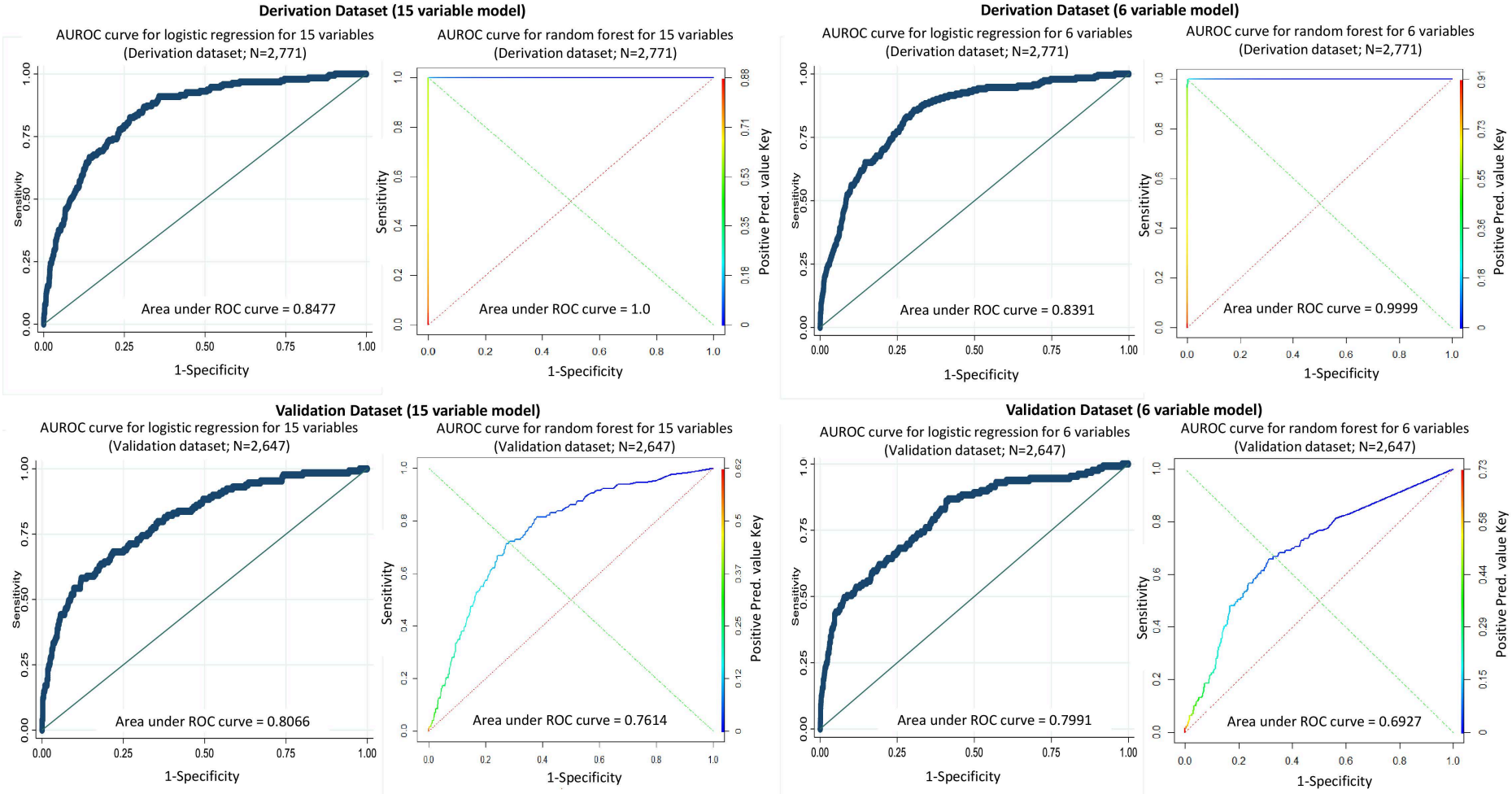
Fig 3: Logistic regression model development and performance in the internal derivation and validation datasets respectively



Comparison of regression and random forest discrimination

Comparison of discriminatory performance between 15-covariate and 6-covariate parsimonious models (Fig 4), indicated very little loss of discrimination by eliminating nine of the covariates from the predictive model, building confidence in the final multivariate model. Similarly, although the random forest approach had far superior discrimination on the derivation dataset versus the logistic regression approach, both modelling approaches had similar discrimination in the internal validation dataset (Fig 4).

Fig 4: Comparison of area under the receiver operating characteristic curves by modelling approach (logistic regression vs. random forest), covariate number (15- vs. 6-variables), and in derivation versus validation datasets



Transformation from regression model to clinical score

We used the WHO-recommended cut-offs for severe anemia in adults (<8.0 g/dL) [35] and for being underweight (BMI<18.5 kg/m^2) to categorize hemoglobin and BMI variables respectively. Temperature was classified as ≤37.5˚C versus >37.5˚C based on the observed distribution of TB prevalence risk as measured temperature increased, and a common definition of a low-grade fever or higher (>37.5˚C) (see S7, figure) [36]. The multivariable model with categorization of these continuous variables in the derivation dataset is presented in Table 4.

Table 3: Multivariable model and clinical score in the derivation dataset (N = 2,771)

Predictor		Adjusted Odds Ratio	95% CI	p-value	β coefficient	Score
WHO TB symptoms	no symptoms	1.00	--	--		
	>=1 symptom	7.00	(4.66-10.52)	<0.001	1.95	7
Sex	Female	1.00	--	--		
	Male	1.35	(0.88-2.08)	0.173	0.30	1
Smoker	Never	1.00	--	--		
	Ever smoked	1.32	(1.03-1.7)	0.030	0.28	1
Haemoglobin	>=8 g/dL	1.00	--	--		
	<8 g/dL	2.50	(1.28-4.85)	0.007	0.91	3
Temperature	<=37.5	1.00	--	--		
	>37.5	5.53	(3.5-8.72)	<0.001	1.71	6
BMI	>=18.5	1.00	--	--		
	<18.5	1.70	(1.12-2.59)	0.013	0.53	2

Abbreviations: CI, confidence interval; WHO, World Health Organization; TB, tuberculosis; BMI, body mass index

The final model, categorized in this way, retained excellent discrimination in the derivation dataset (AUROC 0.8228) and acceptable discrimination in the validation dataset (AUROC 0.7714), and the Hosmer-Lemeshow statistic *p*-values were 0.1940 in the derivation and 0.0002 in the validation datasets indicating similar goodness of fit as was observed prior to variable categorization. The clinical scores that could be used in clinic settings to identify those at risk of prevalent active TB are illustrated in Fig 5.

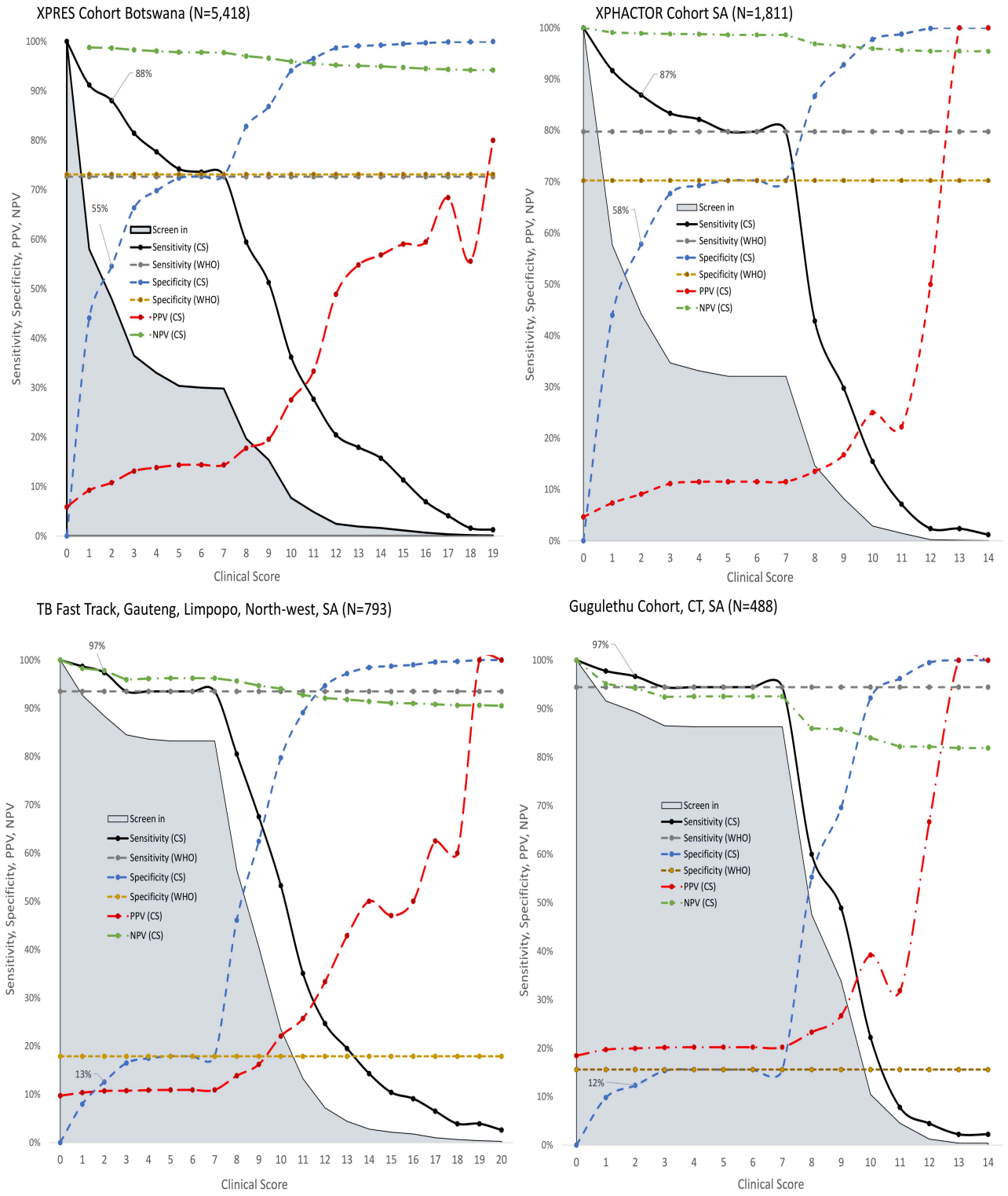
Fig 5: Clinical score for predicting tuberculosis among people living with HIV

Risk Factor	Category	Associated points	Assigned score
Gender	Female	0	
	Male	1	+
No. of WHO TB symptoms	Zero	0	
	≥1	7	+
Smoker	Never	0	
	Ever smoked	1	+
Temperature (˚C)	≤37.5	0	
	>37.5	6	+
BMI	≥18.5	0	
	<18.5	2	+
Hemoglobin Level	≥8.0	0	
	<8.0	3	=
		Total	

External validation of risk scores

The clinical score for each predictor was generated and applied to each external dataset, where the possible range for the total score was 0 to 20 (see S8, table). Fig 6 shows the performance of the clinical score at different cut-offs, in terms of sensitivity, specificity, NPV, PPV, and percentage of clinic enrolees that would be offered a TB test. Across the four datasets, a clinical score of ≥7 would give similar sensitivity and specificity to the WHO four-symptom TB screening rule. Moving the clinical score to ≥2 would give superior sensitivity versus the WHO four-symptom TB screening rule, but with some loss of specificity. For example, sensitivity in detecting prevalent active TB using the WHO four-symptom TB screening rule was 73%, 80%, 94% and 94% in XPRES, XPHACTOR, TBFT and Gugulethu cohorts, respectively, but this increased to 88%, 87%, 97%, and 97%, when a clinical score of ≥2 was used. However, specificity would decline from 73%, 70%, 18%, and 16% if the WHO four-symptom TB-screen was used to 55%, 58%, 13%, and 12% if the clinical score of ≥2 was used. Similarly, the percentage of patients offered a TB test per the WHO four-symptom TB screening rule would be 30%, 32%, 83%, and 86% in the XPRES, XPHACTOR, TBFT and Gugulethu cohorts, respectively, but this increases to 45%, 42%, 87%, and 88% if a clinical score of ≥2 is used.

Fig 6: Clinical risk scores and associated sensitivity, specificity, negative predictive value, and positive predictive value for tuberculosis across four study cohorts



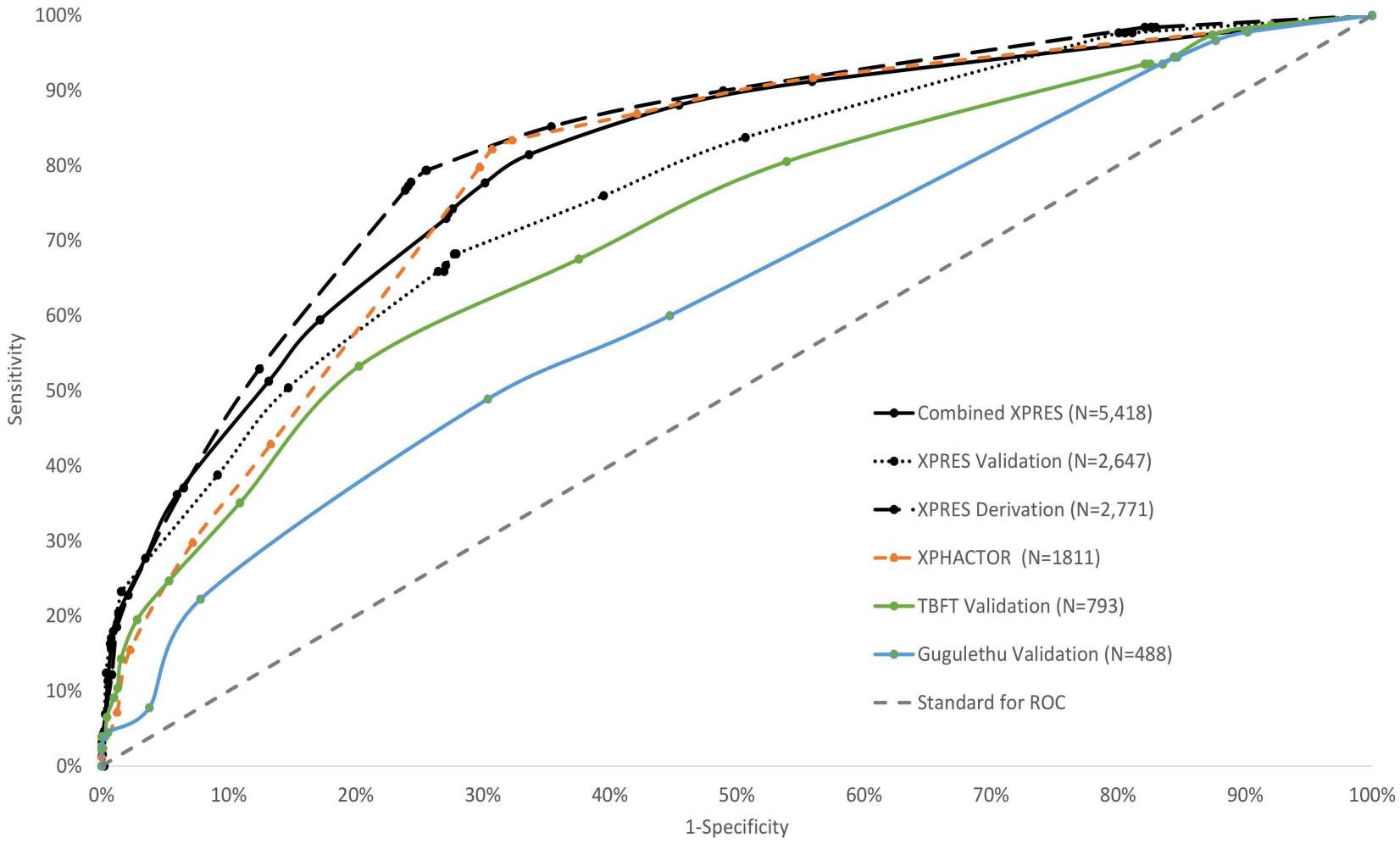
Notably, when the XPHACTOR dataset was restricted to clients on ART for >3 months, the clinical score retained high sensitivity and specificity (see S9, figure). For example, at clinical score ≥2, sensitivity was 80% and specificity 60%, versus WHO four-symptom screening criteria which provided 69% sensitivity and 72% specificity.

The NPV of the WHO 4-symptom TB screen was 97.7%. 98.6%, 96.2%, and 92.5% in the XPRES, XPHACTOR, TBFT, and Gugulethu cohorts, increasing to 98.7%, 98.9%, 97.8%, and 94.2% when the clinical score at cut-off ≥2 was used reflecting a 1%, 0.3%, 1.6%, and 1.7% increase in NPV.

When restricting the XPRES and TBFT cohorts to those who died within 6 months of clinic enrolment, the clinical score at a cut-off of ≥2 had superior sensitivity to the WHO four-symptom TB screen in predicting TB in the XPRES cohort (94% vs. 79%), and similar sensitivity in the TBFT cohort (100% vs. 100%) (see S10, figure). However, specificity of the clinical score at ≥2 was inferior to that of the WHO 4-symptom TB screen in both XPRES (16% vs. 31%) and TBFT (3% vs. 8%) cohorts.

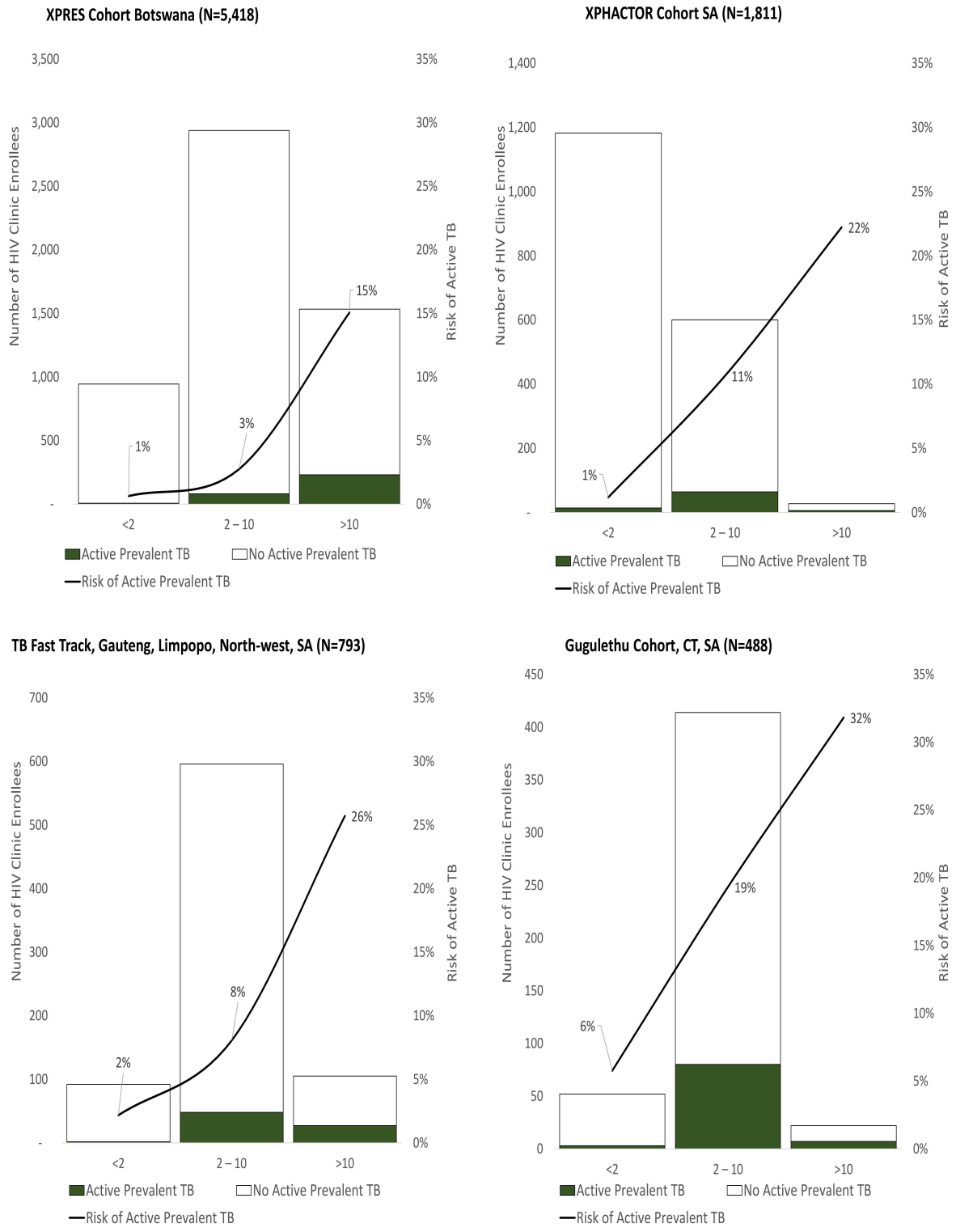
Overall, the clinical score had superior discrimination in the XPRES and XPHACTOR datasets than in the TBFT and Gugulethu cohorts (Figure 7). The XPRES and XPHACTOR cohorts were more similar with respect to median baseline CD4 count (245/µL in XPRES and 400/µL in XPHACTOR) compared with TBFT and Gugulethu cohorts (73/µL in TBFT and 167/µL in Gugulethu cohorts) (table 1). Similarly, XPRES and XPHACTOR cohorts were more similar with respect to baseline prevalence of active prevalent TB (6% in XPRES and 5% in XPHACTOR) compared with TBFT and Gugulethu cohorts (10% in TBFT and 18% in Gugulethu cohort).

Fig 7: Clinical score discrimination according to area under the receiver operating characteristic curve by study cohort



Risk scores were grouped into low (<2), moderate (2-10), and high-risk categories (>10) (Fig 8). Prevalence of active TB among enrolees in low, moderate, and high risk groups was 1%, 3%, and 15% among XPRES enrolees, 1%, 11%, and 22% among XPHACTOR enrolees, 2%, 8%, and 26% for TBFT enrolees, and 6%, 19%, and 32% for Gugulethu cohorts, respectively, indicating a differentiation of prevalent TB risk by the respective clinical scores.

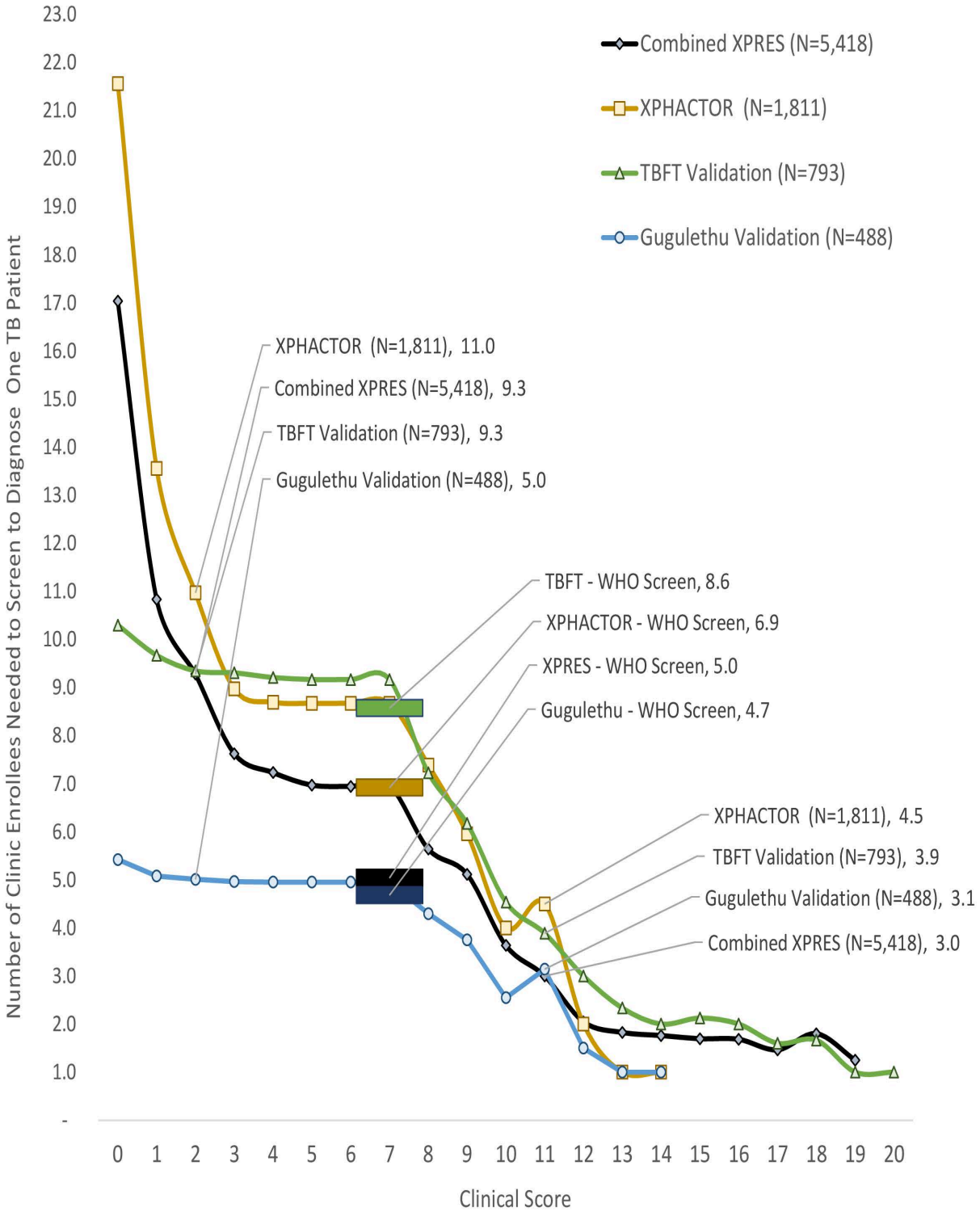
Fig 8: TB risk stratification into low, moderate, and high-risk groups by study cohort



Number needed to screen to diagnose one TB case

In the cohorts with the highest prevalence of active TB (TBFT and Gugulethu), a clinical score with cut-off of ≥2 would give a marginally higher NNS to diagnose one TB case compared with the four-symptom WHO screen (Fig 9); the NNS increased from 8.6 to 9.3 in TBFT and from 4.7 to 5.0 in Gugulethu cohorts. In contrast, in cohorts with lower prevalence of active prevalent TB (XPPRES and XPHACTOR), the NNS increased to a larger extent (from 5.0 to 9.3 in XPRES, and from 6.9 to 11.0 in XPHACTOR). The NNS in the highest risk group (clinical score >10) was uniformly low being 3.0, 4.5, 3.9, and 3.1 in XPRES, XPHACTOR, TBFT, and Gugulethu cohorts, respectively. If the NNS threshold was set at about 5.0, this would correspond to clinical scores of about ≥9-10 in XPRES, XPHACTOR, and TBFT cohorts, but ≥2 in the Gugulethu cohort.

Fig 9: Number needed to screen to detect one case of active tuberculosis by clinical score cut-off and by study cohort



Discussion

This study is the first to derive and externally validate an initial clinical score for active TB among both ART-naïve and ART-experienced PLHIV that does not rely solely on WHO TB symptom screening, and allows flexibility in choosing the desired sensitivity, specificity, NPV, PPV, and NNS across a range of cut-offs, depending on the setting, use-case scenario, and population served. In addition, the screening tool can be used to reduce the likelihood of missing asymptomatic TB, which could help reduce morbidity and mortality due to late or absent TB diagnosis in some settings, as well as reduce TPT prescription to PLHIV needing a full TB treatment course. Similarly, the screening tool's differentiation of three risk groups can be used to inform differentiated care in LMIC clinic settings, which could improve efficiency and potentially impact morbidity and mortality. Finally, the different modelling approaches provide unique insight into covariate predictor importance and practical ways machine learning can be helpful in predicting TB.

While five previous studies have generated clinical scores for TB among PLHIV, three were designed as a second step after screening positive using the WHO four-symptom TB screening rule [16,30,37], and two generated a relatively complex score (13 signs and symptoms), were focused on ART-naïve patients only in Bissau, and lack external validation [38,39]. Our tool is careful to use widely available variables in LMIC settings with external validation in three different cohorts. The only blood test needed for our score is haemoglobin concentration. Point of care (POC) haemoglobin measurement devices are widely available, durable, easy to use, have good accuracy [40,41], are useful for non-HIV-related care, and are inexpensive[42]. In addition, the importance of severe anaemia as a predictor of active TB, as well as the biological mechanism (i.e., hepcidin-driven iron sequestration in the reticuloendothelial system), has been well-described [43,44]. Another routinely available variable in LMIC clinic settings, measured temperature at >37.5˚C, was also independently predictive of TB, indicating the importance of objective measures of fever in addition to patient history [45].

A key advantage of the clinical score over the WHO four-symptom screening tool is that the score can be used by programme managers to choose the desired cut-off with associated sensitivity, specificity, NPV, PPV, and NNS. For example, among people starting or re-starting ART, among whom mortality risk from undiagnosed disseminated TB remains relatively high, a more sensitive screening tool could help reduce morbidity and mortality [2,46]. Notably, in the XPRES cohort, sensitivity of detecting TB at the initial HIV clinic visit among those who died within 6 months of clinic enrolment increased from 79% with the WHO four-symptom rule to 94% with our clinical score at cut-off ≥2, suggesting the potential for improved early case finding with possible morbidity and mortality reductions [13,20].

In addition, with support from global health donors, many countries are embarking on ambitious TPT scale-up for PLHIV, with the majority of targeted TPT recipients being long-term stable ART patients [8]. Following the 2018 United Nations high-level meeting on TB, the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) committed to reaching >13 million PLHIV with TPT by 2021 [8]. Although increases in NPV by using a clinical score cut-off of ≥2 instead of the WHO four-symptom TB screen are modest, ranging from 0.3% to 1.7%, use of the clinical score cut-off of ≥2 during the proposed TPT scale-up for PLHIV could potentially avoid 39,000 to 221,000 PLHIV with active TB being prescribed TPT. Missed active TB increases morbidity and mortality risk for the patient, but also increases risk of isoniazid-resistant TB, which is associated with worse treatment outcomes and may be transmitted to others [9,47]. Our simple screening rule approach to increasing sensitivity and NPV would be much less expensive and logistically challenging than the current WHO recommendation to consider adding CXR to the WHO symptom screen [10,48]. In addition, the NPV increase associated with adding CXR to the WHO screening rule of 0.9% is similar to the NPV increase gained by our much simpler and less costly clinical score at cut-off ≥2 (0.3-1.7%) [5].

Another potential advantage of the clinical score is that the cut-off can be tailored to the use-case scenario [49]. As described above, for clients at ART enrolment, re-enrolment, or being assessed for TPT eligibility, ruling out active TB is a high priority and therefore high sensitivity and NPV are desired and a cut-off of ≥2 could be chosen. However, for stable patients on long-term ART who have completed a course of TPT, a screening rule cut-off with higher specificity and therefore higher PPV could be chosen to lower the NNS and improve efficiency and cost-effectiveness [16,50,51].

The clinical score could also facilitate differentiated TB care based on TB risk [52]. Firstly, the clinical score is relatively simple and could be used by community healthcare workers in the community [52], with community-based care models for HIV and TB increasingly important to decongest health facilities during the COVID-19 pandemic [53]. Secondly, the score could facilitate identifying which new or long-term ART patients should be prioritized for dedicated adherence and retention resources to ensure completion of the TB diagnostic and treatment cascade, with loss to follow-up from HIV-TB care a common problem in LMIC [20,54,55]. Similarly, prioritization of limited on-site GeneXpert diagnostics can be informed by the clinical score to increase cost-effectiveness of POC Xpert use [56]. Finally, diagnostic and therapeutic algorithms could be stratfied by risk groups, with more aggressive TB case finding and treatment approaches appropriate for highest risk groups (e.g., sputum culture, urinary diagnostics, abdominal sonography, or empiric TB treatment) [17].

A strength of the analysis is the dual modelling approach of logistic regression and use of random forest machine learning to build confidence in the final practical clinical score for use in LMIC clinic settings. A key strength of the random forest approach, is that, similar to other machine learning approaches, it is better able to capture non-linear relationships between predictors and outcomes compared with well-established generalised linear regression models [15], because random forest models are not dependent on making assumptions of average linear or curvilinear associations between covariates and

outcomes. Among machine learning models, random forest models are particularly strong at predicting categorical outcomes like our TB outcome [57]. For example, despite using the fractional polynomial transformed BMI variable in the logistic regression backward stepwise elimination approach, it was eliminated from the parsimonious model at $p>0.01$. In contrast, the importance of BMI in discriminating prevalent active TB using the mean decrease in Gini analysis indicated the importance of BMI in its ability to accurately split groups of patients into those who have or do not have prevalent active TB across the 1,000 decision trees examined in our random forest model. The high ranking of BMI according to mean decrease in Gini indicates the significant decrease in average, weighted decision tree node purity that occurred when BMI was removed from the possible list of predictor variables [57].

Our analysis also indicates some of the weaknesses of machine-learning approaches. Firstly, although the random forest model is superior to single decision trees in reducing the likelihood of over-fitting to the training data [57], we observed extremely high discrimination of the random forest model on the training data and a significant drop in discrimination on the validation data. This highlights the importance of a stringent validation approach [15]. Notably the most widely available training resources and publications use a *random* 75%:25% split to create training and validation datasets for random forest models [33], but we purposefully split the dataset into northern and southern clinics in Botswana with a 50%:50% split, which is in line with more stringent validation approaches that help better assess generalizability of predictive models [15,21]. This joint approach of using multiple modelling approaches, using the strength of one approach to examine and account for weaknesses of another, represents a new and useful contribution to the TB screening literature in line with emerging expert guidance [58,59].

Additional strengths of this study include the use of data from high quality prospective cohorts, meaning there was minimal missing covariate data and strong ascertainment of the primary outcome of interest (prevalent active TB). Additional strengths include the

high screening accuracy in the three external validation cohorts, XPHACTOR, TBFT, and Gugulethu cohorts, representing three geographically separate cohorts, with very different cohort characteristics, in very different settings. Limitations include that the risk score has been validated in cohorts in SSA and may not be generalizable to cohorts in resource-rich settings like the U.S. and Europe. Another limitation is that the approaches to TB case finding were different across the four cohorts and that for the XPRES and XPHACTOR cohorts a clinical definition of TB was included in the TB outcome definition, whereas for TBFT and Gugulethu cohorts, results of enrollment sputum collection for TB culture and Xpert were used to define the TB outcome. However, model results did not change significantly when we restricted the TB outcome in XPRES and XPHACTOR datasets to microbiologically confirmed TB [16].

Another limitation is that in XPRES, sputum samples for microbiological diagnosis were only obtained from symptomatic XPRES enrolees at enrolment and during follow-up. Although the intensive TB symptom and ICF cascades during repeat visits during six months of follow-up in XPRES make it less likely that active TB disease was missed over the course of 6 months of follow-up [23], persistantly asymptomatic TB disease would have been missed. In addition, in the XPHACTOR dataset, 45% of study participants were excluded from the validation dataset due to missing haemoglobin, and in the TBFT dataset 29% were excluded because they could not produce sputum for culture at trial enrolment. Comparisons of available patient characteristics between persons excluded versus included in the validation datasets did not indicate notable differences, but additional validation exercises in contemporary cohorts with complete covariates are warranted to further build confidence in the risk score.

In conclusion, this new, simple TB screening clinical score for PLHIV, which is appropriate for both ART-naïve and ART-experienced PLHIV, and which incorporates but does not rely on the WHO four-symptom screening rule, is a timely addition to practical tools available for clinicians and programme managers in LMIC. The clinical score improves on the WHO

four-symptom screening rule's capacity to detect asymptomatic TB, carrying potential associated morbidity, mortality, and TB transmission reduction benefits. The clinical score provides improved sensitivity and NPV over the WHO four-symptom TB screen, which is needed ahead of intensive global TPT scale-up efforts. Finally, the range of clinical scores allows clinicians and programme managers to differentiate patient care and choose cut-offs based on the use-case scenario and availability of resources to improve precision and quality of patient-centered care.

Acknowledgements

The authors would like to thank all study participants, participating health facilities, and project team members for their contributions to improving TB and HIV care in Botswana. We also thank Prof. Stephen D. Lawn for his contributions as a mentor, advisor, and subject matter expert prior to his death on Sept 23, 2016.

Funding: This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the US Centers for Disease Control and Prevention. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The findings and conclusions in this report are those of the authors and do
not necessarily represent the official position of the US Centers for Disease Control and Prevention.

References

1. World Health Organization. TB and HIV, and other comorbidities - 2018 report. Available at: https://www.who.int/tb/areas-of-work/tb-hiv/en/. Accessed June 3, 2020. 2. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS. 2015;29(15):1987-2002. PubMed PMID: 26266773.

3. Gupta A, Nadkarni G, Yang WT, Chandrasekhar A, Gupte N, Bisson GP, et al. Early Mortality in Adults Initiating Antiretroviral Therapy (ART) in Low- and Middle-Income Countries (LMIC): A Systematic Review and Meta-Analysis. PLOS One. 2011;6(12):e28691. PubMed PMID: 22220193.

4. World Health Organization. Latent TB Infection : Updated and consolidated guidelines for programmatic management. Available at: http://apps.who.int/iris/bitstream/handle/10665/260233/9789241550239-eng.pdf?sequence=1. Accessed August 30, 2018.

5. Hamada Y, Lujan J, Schenkel K, Ford N, Getahun H. Sensitivity and specificity of WHO's recommended four-symptom screening rule for tuberculosis in people living with HIV: a systematic review and meta-analysis. The lancet HIV. 2018;5(9):e515-e23. PubMed PMID: 30139576.

6. Ahmad Khan F, Verkuijl S, Parrish A, Chikwava F, Ntumy R, El-Sadr W, et al. Performance of symptom-based tuberculosis screening among people living with HIV: not as great as hoped. AIDS. 2014;28(10):1463-72. PubMed PMID: 24681417.

7. Rangaka MX, Wilkinson RJ, Glynn JR, Boulle A, van Cutsem G, Goliath R, et al. Effect of antiretroviral therapy on the diagnostic accuracy of symptom screening for intensified tuberculosis case finding in a South African HIV clinic. Clin Infect Dis. 2012;55(12):1698-706. PubMed PMID: 22955441.

8. Melgar M, Nichols C, Cavanaugh JS, Kirking HL, Surie D, Date A, et al. Tuberculosis Preventive Treatment Scale-Up Among Antiretroviral Therapy Patients - 16 Countries

Supported by the U.S. President's Emergency Plan for AIDS Relief, 2017-2019. MMWR Morb Mortal Wkly Rep. 2020;69(12):329-34. PubMed PMID: 32214084.

9. Harries AD, Schwoebel V, Monedero-Recuero I, Aung TK, Chadha S, Chiang CY, et al. Challenges and opportunities to prevent tuberculosis in people living with HIV in low-income countries. Int J Tuberc Lung Dis. 2019;23(2):241-51. PubMed PMID: 30808459.

10. Pathmanathan I, Ahmedov S, Pevzner E, Anyalechi G, Modi S, Kirking H, et al. TB preventive therapy for people living with HIV: key considerations for scale-up in resource-limited settings. Int J Tuberc Lung Dis. 2018;22(6):596-605. PubMed PMID: 29862942.

11. Lawn SD, Wilkinson RJ, Lipman MC, Wood R. Immune reconstitution and "unmasking" of tuberculosis during antiretroviral therapy. Am J Respir Crit Care Med. 2008;177(7):680-5. PubMed PMID: 18202347.

12. Oni T, Burke R, Tsekela R, Bangani N, Seldon R, Gideon HP, et al. High prevalence of subclinical tuberculosis in HIV-1-infected persons without advanced immunodeficiency: implications for TB screening. Thorax. 2011;66(8):669-73. PubMed PMID: 21632522.

13. Lawn SD, Harries AD, Wood R. Strategies to reduce early morbidity and mortality in adults receiving antiretroviral therapy in resource-limited settings. Curr Opin HIV AIDS. 2010;5(1):18-26. PubMed PMID: 20046144.

14. Auld AF, Agizew T, Pals S, Finlay A, Ndwapi N, Boyd R, et al. Implementation of a pragmatic, stepped-wedge cluster randomized trial to evaluate impact of Botswana's Xpert MTB/RIF diagnostic algorithm on TB diagnostic sensitivity and early antiretroviral therapy mortality. BMC Infect Dis. 2016;16(1):606. PubMed PMID: 27782821.

15. Obermeyer Z, Emanuel EJ. Predicting the Future - Big Data, Machine Learning, and Clinical Medicine. N Engl J Med. 2016;375(13):1216-9. PubMed PMID: 27682033.

16. Hanifa Y, Fielding KL, Chihota VN, Adonis L, Charalambous S, Foster N, et al. A clinical scoring system to prioritise investigation for tuberculosis among adults attending HIV clinics in South Africa. PLoS One. 2017;12(8):e0181519. PubMed PMID: 28771504.

17. Grant AD, Charalambous S, Tlali M, Karat AS, Dorman SE, Hoffmann CJ, et al. Algorithm-guided empirical tuberculosis treatment for people with advanced HIV (TB Fast

Track): an open-label, cluster-randomised trial. The lancet HIV. 2019. PubMed PMID: 31727580.

18. Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. Lancet Infect Dis. 2012;12(3):201-9. PubMed PMID: 22015305.

19. Dryden-Peterson S, Lockman S, Zash R, Lei Q, Chen JY, Souda S, et al. Initial programmatic implementation of WHO option B in Botswana associated with increased projected MTCT. J Acquir Immune Defic Syndr. 2015;68(3):245-9. PubMed PMID: 25501611.

20. Auld AF, Agizew T, Mathoma A, Boyd R, Date A, Pals SL, et al. Effect of tuberculosis screening and retention interventions on early antiretroviral therapy mortality in Botswana: a stepped-wedge cluster randomized trial. BMC Med. 2020;18(1):19. PubMed PMID: 32041583.

21. Altman DG, Vergouwe Y, Royston P, Moons KG. Prognosis and prognostic research: validating a prognostic model. BMJ. 2009;338:b605. PubMed PMID: 19477892.

22. Corbett EL, Bandason T, Cheung YB, Makamure B, Dauya E, Munyati SS, et al. Prevalent infectious tuberculosis in Harare, Zimbabwe: burden, risk factors and implications for control. Int J Tuberc Lung Dis. 2009;13(10):1231-7. PubMed PMID: 19793427.

23. Lawn SD, Kranzer K, Edwards DJ, McNally M, Bekker LG, Wood R. Tuberculosis during the first year of antiretroviral therapy in a South African cohort using an intensive pretreatment screening strategy. AIDS. 2010;24(9):1323-8. PubMed PMID: 20386425.

24. Royston P, Moons KG, Altman DG, Vergouwe Y. Prognosis and prognostic research: Developing a prognostic model. BMJ. 2009;338:b604. PubMed PMID: 19336487.

25. Kerkhoff AD, Wood R, Vogt M, Lawn SD. Predictive value of anemia for tuberculosis in HIV-infected patients in Sub-Saharan Africa: an indication for routine microbiological investigation using new rapid assays. J Acquir Immune Defic Syndr. 2014;66(1):33-40. PubMed PMID: 24346639.

26. Auld AF, Mbofana F, Shiraishi RW, Alfredo C, Sanchez M, Ellerbrock TV, et al. Incidence and determinants of tuberculosis among adults initiating antiretroviral therapy--Mozambique, 2004-2008. PLoS One. 2013;8(1):e54665. PubMed PMID: 23349948.

27. Benard A, Tessier JF, Rambeloarisoa J, Bonnet F, Fossoux H, Neau D, et al. HIV infection and tobacco smoking behaviour: prospects for prevention? ANRS CO3 Aquitaine Cohort, 2002. Int J Tuberc Lung Dis. 2006;10(4):378-83. PubMed PMID: 16602400.

28. WHO. Global nutrition monitoring framework: operational guidance for tracking progress in meeting targets for 2025. Available at: https://www.who.int/publications/i/item/9789241513609. Accessed July 19, 2020. 2017.

29. Nanta S, Kantipong P, Pathipvanich P, Ruengorn C, Tawichasri C, Patumanond J. Screening scheme development for active TB prediction among HIV-infected patients. Southeast Asian J Trop Med Public Health. 2011;42(4):867-75. PubMed PMID: 22299469.

30. Balcha TT, Skogmar S, Sturegard E, Schon T, Winqvist N, Reepalu A, et al. A Clinical Scoring Algorithm for Determination of the Risk of Tuberculosis in HIV-Infected Adults: A Cohort Study Performed at Ethiopian Health Centers. Open forum infectious diseases. 2014;1(3):ofu095. PubMed PMID: 25734163.

31. Chowdhury MZI, Turin TC. Variable selection strategies and its importance in clinical prediction modelling. Fam Med Community Health. 2020;8(1):e000262. PubMed PMID: 32148735.

32. Mupfumi L, Moyo S, Molebatsi K, Thami PK, Anderson M, Mogashoa T, et al. Immunological non-response and low hemoglobin levels are predictors of incident tuberculosis among HIV-infected individuals on Truvada-based therapy in Botswana. PLoS One. 2018;13(1):e0192030. PubMed PMID: 29385208.

33. Weng SF, Reps J, Kai J, Garibaldi JM, Qureshi N. Can machine-learning improve cardiovascular risk prediction using routine clinical data? PLoS One. 2017;12(4):e0174944. PubMed PMID: 28376093.

34. Moons KG, Altman DG, Vergouwe Y, Royston P. Prognosis and prognostic research: application and impact of prognostic models in clinical practice. BMJ. 2009;338:b606. PubMed PMID: 19502216.

35. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Available at: https://www.who.int/vmnis/indicators/haemoglobin/en/. Accessed June 1, 2020.
36. Del Bene VE. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Chapter 218Temperature. Available at: https://www.ncbi.nlm.nih.gov/books/NBK331/. Accessed December 31, 2019.
37. Boyles TH, Nduna M, Pitsi T, Scott L, Fox MP, Maartens G. A Clinical Prediction Score Including Trial of Antibiotics and C-Reactive Protein to Improve the Diagnosis of Tuberculosis in Ambulatory People With HIV. Open forum infectious diseases. 2020;7(2):ofz543. PubMed PMID: 32042850.
38. Aunsborg JW, Honge BL, Jespersen S, Rudolf F, Medina C, Correira FG, et al. A clinical score has utility in tuberculosis case-finding among patients with HIV: A feasibility study from Bissau. Int J Infect Dis. 2020;92S:S78-S84. PubMed PMID: 32171950.
39. Rudolf F, Haraldsdottir TL, Mendes MS, Wagner AJ, Gomes VF, Aaby P, et al. Can tuberculosis case finding among health-care seeking adults be improved? Observations from Bissau. Int J Tuberc Lung Dis. 2014;18(3):277-85. PubMed PMID: 24670561.
40. Nkrumah B, Nguah SB, Sarpong N, Dekker D, Idriss A, May J, et al. Hemoglobin estimation by the HemoCue(R) portable hemoglobin photometer in a resource poor setting. BMC Clin Pathol. 2011;11:5. PubMed PMID: 21510885.
41. Sanchis-Gomar F, Cortell-Ballester J, Pareja-Galeano H, Banfi G, Lippi G. Hemoglobin point-of-care testing: the HemoCue system. J Lab Autom. 2013;18(3):198-205. PubMed PMID: 22961038.
42. Medina Lara A, Mundy C, Kandulu J, Chisuwo L, Bates I. Evaluation and costs of different haemoglobin methods for use in district hospitals in Malawi. J Clin Pathol. 2005;58(1):56-60. PubMed PMID: 15623483.
43. Kerkhoff AD, Meintjes G, Burton R, Vogt M, Wood R, Lawn SD. Relationship Between Blood Concentrations of Hepcidin and Anemia Severity, Mycobacterial Burden, and Mortality Among Patients With HIV-Associated Tuberculosis. J Infect Dis. 2015. PubMed PMID: 26136467.

44. Drakesmith H, Prentice AM. Hepcidin and the iron-infection axis. Science. 2012;338(6108):768-72. PubMed PMID: 23139325.

45. Holmes CB, Sikazwe I, Sikombe K, Eshun-Wilson I, Czaicki N, Beres LK, et al. Estimated mortality on HIV treatment among active patients and patients lost to follow-up in 4 provinces of Zambia: Findings from a multistage sampling-based survey. PLoS Med. 2018;15(1):e1002489. PubMed PMID: 29329301.

46. Karat AS, Omar T, von Gottberg A, Tlali M, Chihota VN, Churchyard GJ, et al. Autopsy Prevalence of Tuberculosis and Other Potentially Treatable Infections among Adults with Advanced HIV Enrolled in Out-Patient Care in South Africa. PLoS One. 2016;11(11):e0166158. PubMed PMID: 27829072.

47. van der Heijden YF, Karim F, Mufamadi G, Zako L, Chinappa T, Shepherd BE, et al. Isoniazid-monoresistant tuberculosis is associated with poor treatment outcomes in Durban, South Africa. Int J Tuberc Lung Dis. 2017;21(6):670-6. PubMed PMID: 28482962.

48. Samandari T, Bishai D, Luteijn M, Mosimaneotsile B, Motsamai O, Postma M, et al. Costs and consequences of additional chest x-ray in a tuberculosis prevention program in Botswana. Am J Respir Crit Care Med. 2011;183(8):1103-11. PubMed PMID: 21148723.

49. McNairy ML, Abrams EJ, Rabkin M, El-Sadr WM. Clinical decision tools are needed to identify HIV-positive patients at high risk for poor outcomes after initiation of antiretroviral therapy. PLoS Med. 2017;14(4):e1002278. PubMed PMID: 28419097.

50. Shapiro AE, Chakravorty R, Akande T, Lonnroth K, Golub JE. A systematic review of the number needed to screen to detect a case of active tuberculosis in different risk groups. Available at: https://www.who.int/tb/Review3NNS_case_active_TB_riskgroups.pdf?ua=1. Accessed June 10, 2020.

51. Khoury MJ, Iademarco MF, Riley WT. Precision Public Health for the Era of Precision Medicine. American journal of preventive medicine. 2016;50(3):398-401. PubMed PMID: 26547538.

52. Pathmanathan I, Pevzner E, Cavanaugh J, Nelson L. Addressing tuberculosis in differentiated care provision for people living with HIV. Bull World Health Organ. 2017;95(1):3. PubMed PMID: 28053356.

53. Shoptaw S, Goodman-Meza D, Landovitz RJ. Collective Call to Action for HIV/AIDS Community-Based Collaborative Science in the Era of COVID-19. AIDS Behav. 2020. PubMed PMID: 32300993.

54. Auld AF, Fielding KL, Gupta-Wright A, Lawn SD. Xpert MTB/RIF - why the lack of morbidity and mortality impact in intervention trials? Trans R Soc Trop Med Hyg. 2016;110(8):432-44. PubMed PMID: 27638038.

55. Date A, Modi S. TB screening among people living with HIV/AIDS in resource-limited settings. J Acquir Immune Defic Syndr. 2015;68 Suppl 3:S270-3. PubMed PMID: 25768866.

56. Pooran A, Theron G, Zijenah L, Chanda D, Clowes P, Mwenge L, et al. Point of care Xpert MTB/RIF versus smear microscopy for tuberculosis diagnosis in southern African primary care clinics: a multicentre economic evaluation. The Lancet Global health. 2019;7(6):e798-e807. PubMed PMID: 31097281.

53. Firmin S. Seeing the Forest for the Trees: An Introduction to Random Forest. Available at: https://towardsdatascience.com/seeing-the-forest-for-the-trees-an-introduction-to-random-forest-41a24fc842ac. Accessed June 3, 2020.

58. Chen JH, Asch SM. Machine Learning and Prediction in Medicine - Beyond the Peak of Inflated Expectations. N Engl J Med. 2017;376(26):2507-9. PubMed PMID: 28657867.

59. Rajkomar A, Dean J, Kohane I. Machine Learning in Medicine. N Engl J Med. 2019;380(14):1347-58. PubMed PMID: 30943338.

7.1. Research paper supplementary material

Table 7.4. (Research paper supplementary appendix 1 (S1)) - Table of HIV care clinical follow-up for clients in the Botswana XPRES cohort (2010-2015)

Pre-ART, CD4 >350	**3 monthly**	Weight, CD4, TB screen
ART	**ART start**	Weight, CD4, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen, Hepatitis B screen, creatinine if TDF-based regimen
	2 weeks	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen
	1 month	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen
	3 months	Weight, TB screen, ALT/AST if NVP-based regimen, Hb if AZT-based regimen, Viral load, creatinine if TDF-based regimen
	6months	Weight, TB screen, ALT/AST[a] if NVP-based regimen, Hb if AZT-based regimen, Viral load, CD4
	Quarterly[b]	Weight, TB screen, Viral load and CD4 6 monthly, creatinine if TDF-based regimen 6 monthly

Abbreviations: CD4, CD4 cell count; TB, tuberculosis; ALT, alanine transaminase; AST, aspartate aminotransferase; NVP, nevirapine; AZT, zidovudine; TDF, tenofovir;
[a]Routine ALT/AST not required after 6 months but may be requested by the clinician depending on the clinical situation.
[b]For those patients started on PI-based regimens, baseline and 12-monthly glucose (random or fasting) and total cholesterol/triglycerides are recommended.

Supplementary Appendix 2 (S2): XPRES Enrolment, TB Case Finding, and Follow-up Procedures

At prospective cohort enrolment, research staff administered a standardized questionnaire, which captured demographic characteristics including age, sex, and pregnancy status, as well as clinical characteristics, including the WHO TB symptom screening rule for any current cough, fever, night sweats or weight loss, WHO HIV disease stage, height and weight, temperature in degrees centigrade, the most recent haemoglobin level, and the most recent CD4 count.

As part of the trial intervention to strengthen ICF, all patients symptomatic for TB were encouraged to provide at least two same-day, on-the-spot (spot) sputum samples. In addition, if feasible for the patient, a morning sputum the day after screening positive for TB symptoms was recommended along with a 3rd spot sputum upon arrival at the clinic. A previously published job-aid was used by study nurses to inform the patient how to collect quality sputum samples. Laboratory personnel at the 13 laboratories serving the 22 clusters received refresher training on Ziehl-Neelsen staining for sputum-smear microscopy and Xpert implementation. When all four sputum samples were available, the morning sputum sample and second spot sputum sample for all symptomatic clients were sent to the national TB reference laboratory (NTRL) for culture using mycobacteria growth indicator tubes (MGIT).

In all phases, sputum test results were returned to the clinics within 4 days for sputum-smear microscopy and 2 days for Xpert testing. Study nurses were trained to inform patients of positive TB diagnoses the same day via phone, or if unreachable by phone, by active tracing to the household. As part of the active tracing intervention, for all patients ≥1 day late for a clinic appointment, study nurses conducted up to five telephone calls and two home visits in attempts to return these clients to care. XPRES participants were followed for 12 months, or until the end of TB treatment, whichever was later. The final follow-up visits for XPRES enrolees were in June 2015.

Supplementary appendix 3 (S3): Table Comparing XPRES and External Validation Datasets

Study Characteristic	Internal Derivation and Validation Dataset (Botswana XPRES: N=5,418)	External Validation Dataset (SA, XPHACTOR: N=1,807)	External Validation Dataset (TBFT, SA: N=793)	External Validation Dataset (Gugulethu Cohort, CT, SA: N=488)
Setting	Botswana	Gauteng Province, SA	Gauteng, Limpopo, and North West Provinces, SA	Western Cape, SA
Clinic Types	5 District Hospitals and 17 Primary Health Care Clinics, purposively selected to be nationally representative	2 hospital-based and 2 community health centre (CHC) clinics	24 primary health-care clinics; only the 12 randomly selected intervention clinics included in this analysis	Community-based HIV treatment clinic in Gugulethu township
Study Design	Stepped-wedge trial	Prospective Cohort	Cluster Randomized Trial	Cross-sectional screening at Cohort enrollment
Study Dates	Enrollment: August 2012 – March 2014. Last follow-up: June 2015	Enrollment: Sept 2012–March 2014. Last follow-up: 2015	Enrollment: December 2012 – December 2014 Last follow-up: May 2015	Enrollment: March 2010-April 2011 Last follow-up: N/A
Eligibility Criteria for Study and analysis	• ≥12 years old • New HIV clinic enrollee, ART-naïve • Not incarcerated • Not already diagnosed with TB and on TB treatment • Any CD4 count	• ≥18 years old • Newly HIV diagnosed (HTC group), or in Pre-ART care, or on ART • No TB treatment in previous 3 months • Any CD4 count	• ≥18 years old • No ART in the previous 6 months • No TB treatment in previous 3 months • CD4 count ≤150/μL • No chronic liver disease, alcohol intake < 28 units/week for men, <21 units/week for women • No signs/symptoms necessitating urgent referral to secondary care • No intent to leave clinic catchment area within 6 months	• ≥18 years old • ART-naïve • No current TB diagnosis • Any CD4 count
ART eligibility	• CD4 ≤350, stage III/IV, PBF women	• CD4 ≤200, stage IV, or CD4 ≤350 with TB or PBF women	• CD4 ≤200, stage IV, or CD4 ≤350 with TB or PBF women	• CD4 ≤200, stage IV, or CD4 ≤350 with TB or PBF women
TB Symptom Screening	• TB symptom screen at all visits*	• TB symptom screen at all visits*	• TB symptom screen at all visits*	• TB symptom screen at all visits*
TB Diagnosis Ascertainment as relevant to this analysis	• 2-4 Sputum samples collected from symptomatic enrollees • Smear-microscopy and Xpert as initial test depending on phase. • MGIT culture for all sputum samples • Ultrasound for abdominal TB • Chest x-ray per national guidelines	• ≥1 Spot sputum sample for all enrollees regardless of symptoms at enrollment • Spot sputum samples collected at subsequent visits if "high risk"** • Xpert for all sputum samples • Chest x-ray per national guidelines	• 1 Spot sputum sample for all enrollees regardless of symptoms at enrollment in the intervention arm. • Smear and MGIT culture on all enrollment samples.	• 1 Spot sputum sample, and 1 induced sputum for all enrollees regardless of symptoms at enrollment in the intervention arm. • Smear, Xpert, and MGIT culture on all enrollment samples.
TB outcome for this analysis	• Clinical or microbiologically confirmed TB within 6 months of enrollment visit.	• Clinical or microbiologically confirmed TB within 6 months of enrollment visit.	• Microbiologically confirmed TB based on sputum sample collected at enrolment for smear and culture.	• Microbiologically confirmed TB based on sputum sample collected at enrollment and +ve via Xpert, smear, or culture.
Study-specific Primary Outcome	• All cause mortality • TB case ascertainment a secondary outcome	• TB case ascertainment	• All cause mortality • TB case ascertainment a secondary outcome	• TB case ascertainment

*XPRES, XPHACTOR visits: initial visit, monthly for 3 months, then quarterly. TBFT per trial protocol. Gugulethu cohort per national guidelines.

** Spot samples were 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/m2, CD4 <100x106/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 x106/l at enrolment and (iii) all in HTC group at enrolment, the latter two categories (who were recruited for XPHACTOR sub-studies) 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.

Supplementary App. 4 (S4): TRIPOD Checklist: Prediction Model Development and Validation

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	3-4
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	5-6
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	5-6
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	6, 12
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	6, 12
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	6, 12
	5b	D;V	Describe eligibility criteria for participants.	7, 12
	5c	D;V	Give details of treatments received, if relevant.	7-8, 12
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	8
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	9-10
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	9-11
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	9-11
Sample size	8	D;V	Explain how the study size was arrived at.	6, 12
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	10
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	9-11
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	9-11
	10c	V	For validation, describe how the predictions were calculated.	9-12
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	9-12
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	9-12
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	9-12
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	12-13
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	13
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	13-14
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	16-22
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	16-22
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	17
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	20
	15b	D	Explain how to the use the prediction model.	20-22
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	16-22
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	16-22
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	28
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	23-28
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	23-28
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	23-28
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	39
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	37

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

Supplementary Appendix 5 (S5): Importance of Predictor in Logistic Regression Versus Random Forest Model

	Logistic Regression (N=2,771; 15 variables)		Random Forest Model (N=2,771; 15 variables)			
	Beta Coefficient	Rank	Mean Decrease in Gini	Rank	Mean Decrease in Accuracy	Rank
Temperature (first* transformed term) (degrees Celsius)	-8.028029	1	55.481	1	24.066	1
Number of WHO TB symptoms (>=1)	1.727382	2	20.984	7	20.516	2
BMI	1.616838	3	52.766	2	7.534	7
Smoking History (ever smoked)	0.4315334	4	5.361	12	1.422	12
Prior TB	0.4303915	5	5.781	10	9.683	6
Miner (Ever)	0.4226396	6	3.829	15	0.960	13
TB contact	0.4225646	7	3.931	14	0.704	14
Sex (Male)	0.4217461	8	5.477	11	12.982	4
Respiratory rate (transformed term)	-0.2790341	9	30.736	6	6.618	8
Hemoglobin at Enrollment	-0.2294897	10	48.020	3	17.273	3
Marital Status	0.2206944	11	5.357	13	-5.169	15
Education	-0.0648862	12	13.955	8	10.188	5
Age in years (linear)	0.004779	13	44.020	5	6.138	9
Employment Status	0.0019766	14	6.564	9	3.696	11
CD4 at Enrollment	-0.0009955	15	46.287	4	5.541	10

*Beta coefficient for second transformed term for temperature was 0.1157134

Table 7.8. (Research paper supplementary appendix 6 (S6)) - Hosmer-Lemeshow test for calibration of final tuberculosis prediction model

Derivation Dataset					Validation Dataset				
			Prevalent TB					Prevalent TB	
Decile	N	Cut off*	Observed**	Predicted†	Decile	N	Cut off*	Observed**	Predicted†
1	278	0.0111	2	2.6	1	265	0.0113	3	2.5
2	277	0.0137	2	3.4	2	265	0.0138	4	3.3
3	277	0.0162	5	4.1	3	265	0.016	0	3.9
4	277	0.0191	1	4.9	4	264	0.0189	2	4.6
5	277	0.0231	4	5.8	5	265	0.0232	6	5.5
6	277	0.0299	6	7.2	6	265	0.031	10	7
7	277	0.0551	19	10.8	7	264	0.063	15	11.4
8	277	0.1099	27	23	8	265	0.1063	12	22.7
9	277	0.1881	38	39.8	9	265	0.1684	13	35.2
10	277	0.8484	85	87.4	10	264	0.8629	64	72.8
Total	2771		189	189		2647		129	169

Derivation dataset Hosmer-Lemeshow chi2(8) = 12.39, p=0.1348

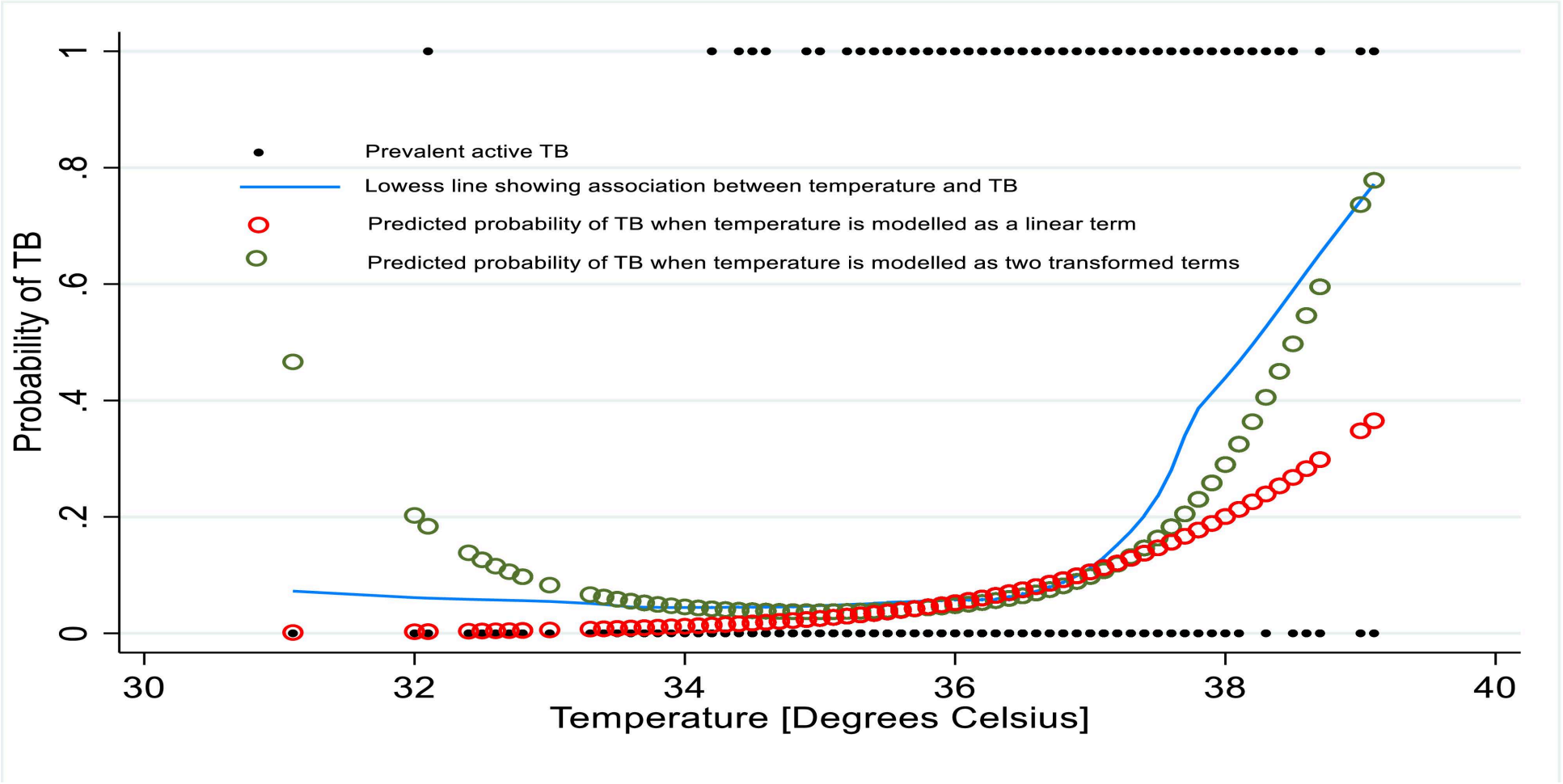
Validation dataset Hosmer-Lemeshow chi2(8) = 31.39, p=0.0001

* Upper boundary of predicted risk

**Observed = observed number diagnosed with active TB within 6 months of clinic enrolment

†Predicted = expected number diagnosed with active TB within 6 months of clinic enrolment

Supplementary appendix 7 (S7): Figure of the association between temperature modelled as a transformed versus linear variable and prevalent active TB

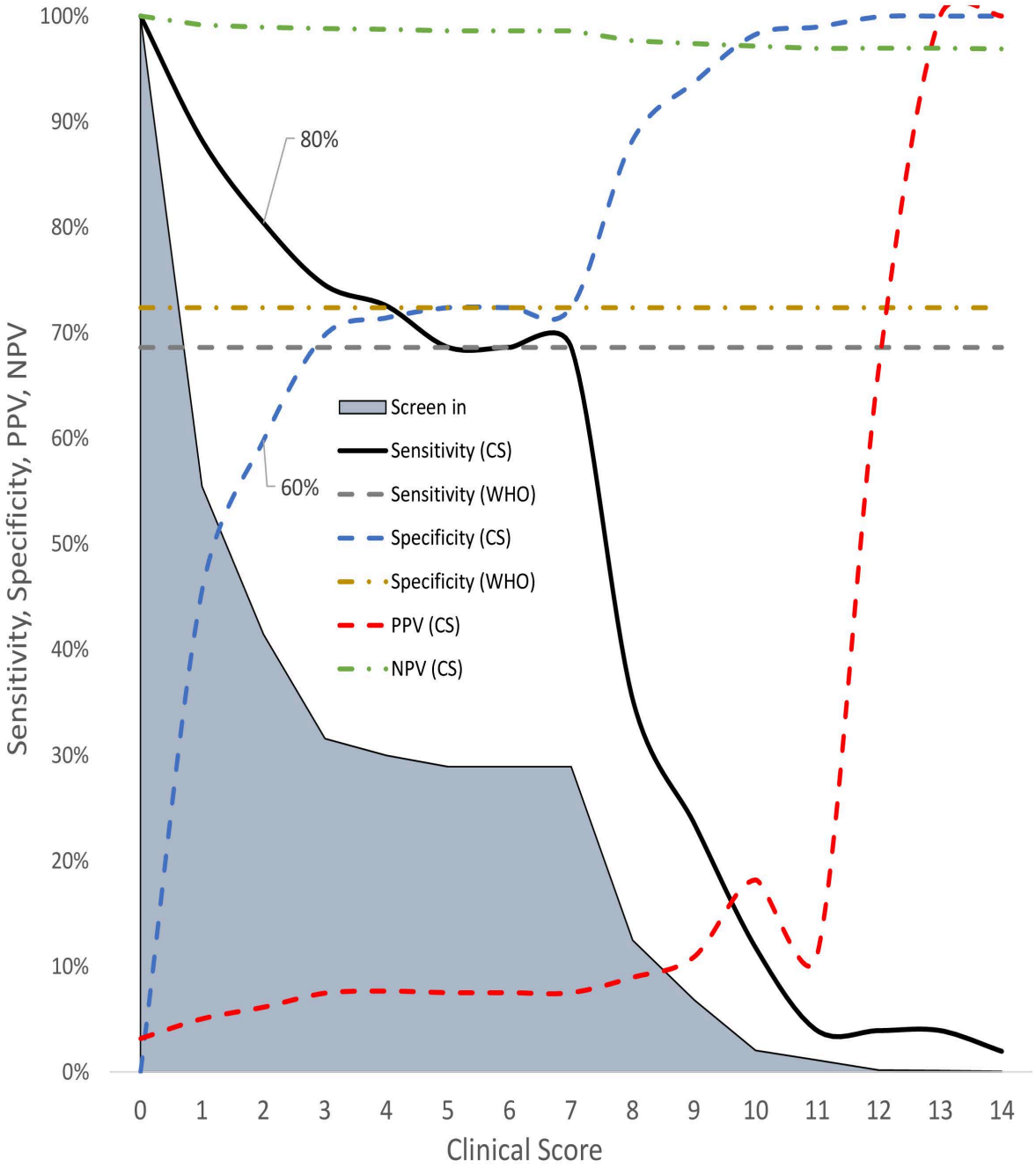


Supplementary Appendix 8: Performance of the TB prediction clinical score in derivation and validation datasets

	XPRES Cohort (N=5,418)			External Validation Dataset (SA, XPHACTOR: N=1807)			External Validation Dataset (TBFT, SA: N=793)			External Validation Dataset (Gugulethu Cohort, CT, SA: N=488)		
Clinical score	Total with score	Number diagnosed with prevalent TB	% Diagnosed	Total with score	Number diagnosed with prevalent TB	% Diagnosed	Total with score	Number diagnosed with prevalent TB	% Diagnosed	Total with score	Number diagnosed with prevalent TB	% Diagnosed
0	2,276	28	1%	767	7	1%	58	1	2%	41	2	5%
1	544	10	2%	243	4	2%	34	1	3%	11	1	9%
2	623	21	3%	173	3	2%	31	3	10%	14	2	14%
3	189	12	6%	28	1	4%	7	0	0%	1	0	0%
4	141	11	8%	19	2	11%	3	0	0%	0	0	0%
5	20	2	10%	0	0	0%	0	0	0%	0	0	0%
6	10	2	20%	0	0	0%	0	0	0%	0	0	0%
7	549	43	8%	315	31	10%	212	10	5%	189	31	16%
8	232	26	11%	117	11	9%	127	10	8%	67	10	15%
9	416	48	12%	97	12	12%	135	11	8%	114	24	21%
10	154	27	18%	25	7	28%	81	14	17%	29	13	45%
11	131	23	18%	23	4	17%	48	8	17%	16	3	19%
12	29	8	28%	2	0	0%	22	4	18%	4	2	50%
13	16	7	44%	1	1	100%	13	4	31%	0	0	-
14	27	14	52%	1	1	100%	5	3	60%	2	2	100%
15	24	14	58%				3	1	33%			
16	18	9	50%				6	2	33%			
17	10	8	80%				3	2	67%			
18	4	1	25%				2	-	0%			
19	5	4	80%				1	1	100%			
20							2	2	100%			
Total	**5,418**	**318**		**1,811**	**84**		**793**	**77**		**488**	**90**	

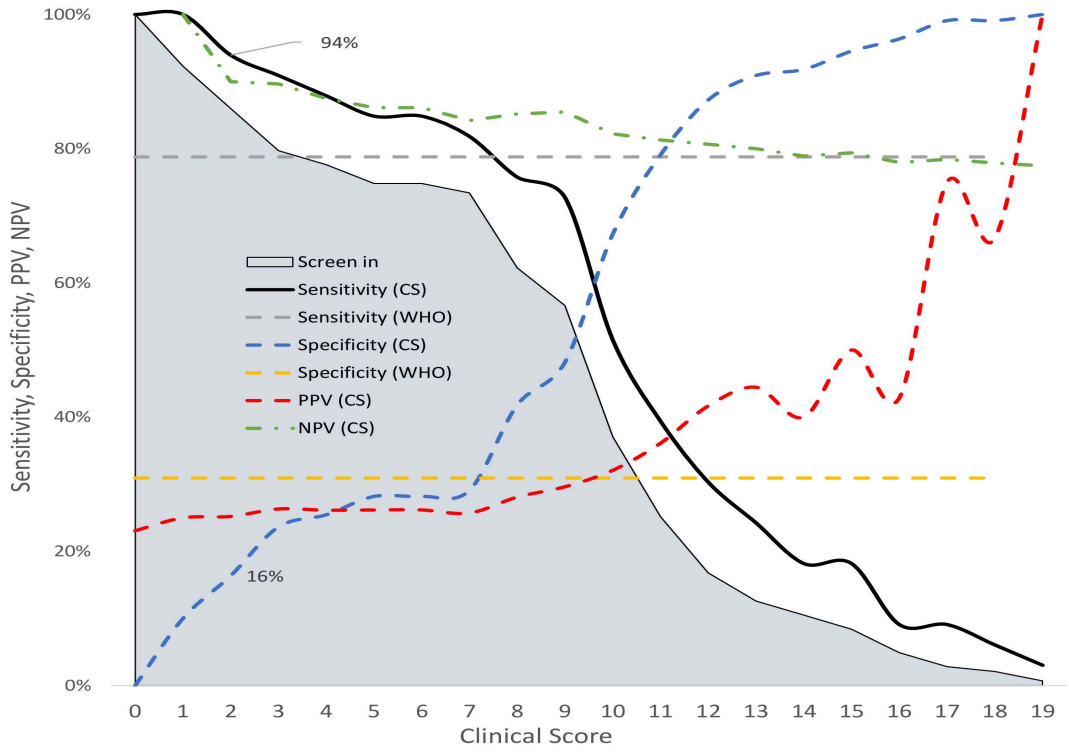
Abbreviations: 6m, 6 months; TB, tuberculosis; XPRES, Xpert Package Rollout Evaluation using a Stepped-wedge design trial; XPHACTOR, ªXpert for people attending HIV/AIDS care: test or review? trial; TBFT, TB Fast Track Trial; SA, South Africa; CT, Cape Town

Supplementary Appendix 9: Figure of sensitivity, specificity, negative predictive value, positive predictive value, of TB clinical score versus WHO 4-symptom TB screening rule among ART-experienced clinic attendees (XPHACTOR, n=1612)

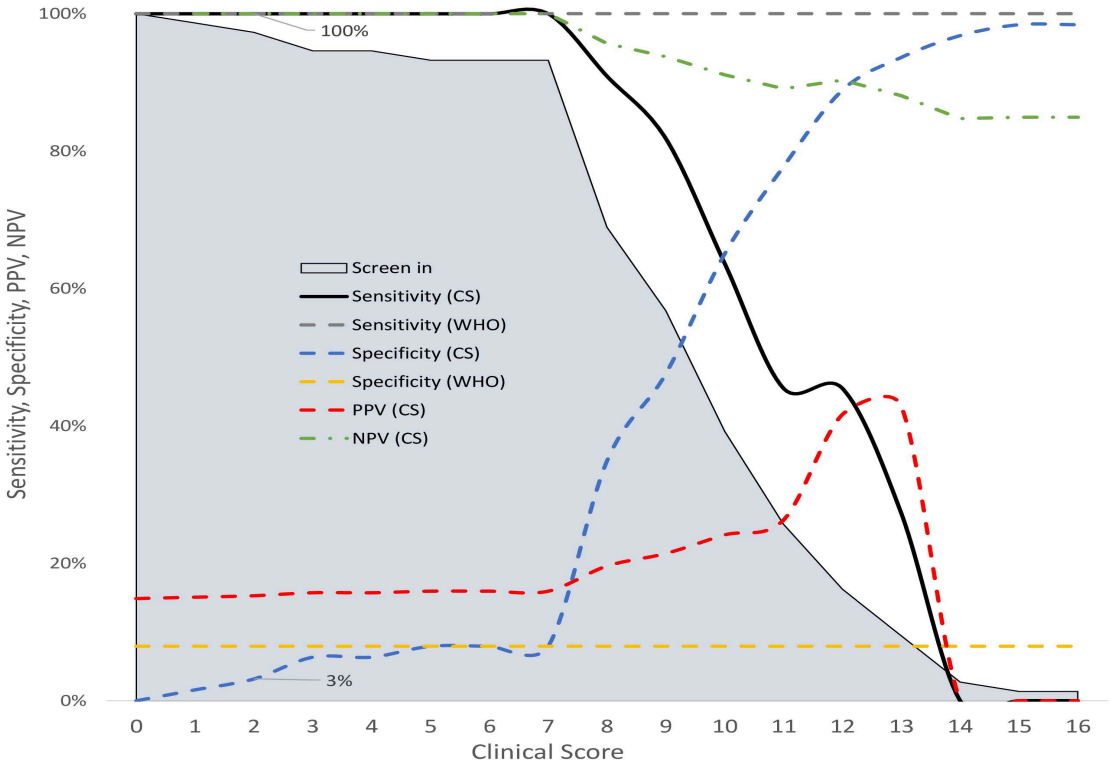


Supplementary appendix 10 (S10): Sensitivity, specificity, PPV, and NPV of clinical score in predicting prevalent TB among those who died within 6 months of clinic enrollment

XPRES Cohort Botswana (N=143)



TB Fast Track, Gauteng, Limpopo, North-west, SA (N=74)



Chapter 8. Discussion, Recommendations, and Conclusions

This thesis aimed to explore opportunities for reducing rates of early mortality on ART, as well as mortality in general, among PLHIV in sub-Saharan Africa. I endeavoured to do this through a systematic review of existing literature of Xpert impact trials, review of early ART care intensification algorithms and TB screening rules among PLHIV, as well as by designing and analysing results from the XPRES stepped-wedge trial, and using XPRES trial data to develop and externally validate new early ART care intensification scores and a TB screening score suitable for sub-Saharan Africa. This chapter provides a summary of the key results from the above work in section 8.1., shows how the key findings contribute to the existing scientific literature with associated implications of the new evidence in section 8.2., examines the limitations and strengths of the methods used in section 8.3., provides a short reflective commentary and some practical lessons learned in section 8.4., and ends with a summary set of recommendations and conclusions in sections 8.5. and 8.6., respectively.

8.1. Summary of key results

Systematic review of Xpert impact trials

Our systematic review of Xpert impact trials conducted between January 2005 and December 2015 was the first systematic review of Xpert impact trials and explored potential reasons for lack of observed impact of Xpert versus smear microscopy on patient morbidity and mortality outcomes.[146] Key reasons for lack of observed impact related to high rates of empiric TB treatment in microscopy arms and enrolment of study populations not comprised exclusively of those most likely to benefit from Xpert. In addition, a high frequency of health system weaknesses resulting in losses from TB-HIV care cascades in both microscopy and Xpert arms were observed which might have blunted potential impact of the improved diagnostic tool.

XPRES trial primary and secondary outcome analyses

XPRES was designed to address gaps in scientific literature that other Xpert impact trials could not or had not addressed.[18] Specifically, XPRES was designed to address health system weaknesses resulting in "leaky" TB case finding and TB-HIV care cascades at the design stage and ensure these weaknesses were addressed in addition to rollout of the new diagnostic device to achieve maximum impact on HIV-TB mortality.[18] Through the XPRES three-phase design, which compared historical standard of care, with enhanced care (EC), and EC plus Xpert (EC+X) phases, XPRES showed that interventions to strengthen implementation of WHO-recommended four-symptom TB screening and ICF algorithms combined with active tracing to support retention were key drivers behind increased TB case finding and lower early ART mortality. Similar to other Xpert impact trials, no independent mortality benefit of replacing sputum-smear microscopy with Xpert was observed.[161]

Risk score to inform risk of early mortality on ART

To assess further opportunities for reducing early ART mortality, the thesis reviewed how current definitions for WHO advanced HIV disease eligibility criteria (i.e., CD4 <200/μL or WHO stage III/IV) were derived and then used to inform intensification of care algorithms, as well as the limitations associated with this approach. The literature review also examined published clinical scores for early ART care intensification, most of which were prepared for resource-rich settings. I then used XPRES trial data to derive, and TB Fast Track trial data to externally validate, a clinical score potentially generalizable to sub-Saharan Africa for who needs intensification of early ART care. Key findings were that both the CD4-independent and -dependent early ART care intensification scores have potential advantages over the current WHO standard of care, which recommends ART care intensification for those with advanced HIV disease. Key advantages of the clinical scores over WHO advanced disease eligibility criteria include: (1) superior screening accuracy in predicting risk for early ART mortality, (2) independence from the requirement for availability of rapid on-site or near-site CD4 count testing, which is not yet widely

available in sub-Saharan HIV clinics, and (3) additional ability to inform differentiated care algorithms by categorizing patients into low-, moderate-, and high-risk groups.

Risk score to predict active TB among PLHIV

Similarly, building on the main XPRES trial findings, which highlight the importance of implementing a sensitive TB screening tool versus addition of a sensitive TB test (Xpert) to case finding algorithms, I conducted a literature review of meta-analyses supporting WHO recommendations for the WHO four-symptom TB screen, and emerging limitations of this TB symptom screen, especially its inability to detect asymptomatic TB. I then used XPRES trial data to derive, and XPHACTOR, TB Fast Track, and Gugulethu study data to externally validate, a flexible, initial TB clinical score for ART-naïve and ART-experienced PLHIV in sub-Saharan Africa that is capable of screening in asymptomatic TB, and can be used to prioritize either increased sensitivity and NPV or increased specificity and PPV depending on the setting and use-case scenario. In addition, by stratifying risk into low-, moderate- and high-risk groups, the new TB clinical score also provides opportunity to design differentiated models of care to maximize impact of available resources.

The section below describes how these findings complement existing literature in seven important ways with new insights and implications.

8.2. New evidence and insights from the literature review, XPRES trial, and risk score analyses

Firstly, the Xpert systematic review and XPRES trial results add to the existing published literature in at least three areas: (1) the importance of health system strengthening interventions to address “leaky” ICF and HIV-TB retention cascades to reduce all-cause early ART mortality and a public health intervention package for how to approach this; (2) the need for pragmatic trial designs for future novel TB diagnostic trials in LMIC, especially the importance of assessing at the design stage in what ways the standard of care trial arm needs to represent true standard of care rather than an “enhanced” version of existing care; and (3) the relative importance of simple sensitive screening tools that

prompt clinicians to consider risk of early death and risk of TB and prompt empirical action where needed, in addition to the need for better and more accessible diagnostic tests.

The risk score analyses to generate the externally validated early ART mortality risk score for sub-Saharan Africa, and the externally validated active TB risk score for both ART-naïve and ART-experienced PLHIV, flowed directly from insights gleaned from the Xpert impact systematic review and XPRES trial on the relative importance of sensitive screening tools, and represent the fourth and fifth contributions to the literature discussed below.

Sixth, a hybrid modelling approach that harnessed the strengths of both traditional generalized linear regression models and new machine learning approaches was used to generate a TB risk score among PLHIV, with possible implications for future prognostic research in the field of TB.

Finally, the seventh contribution of the thesis is that lessons learned from the literature reviews including the Xpert systematic review, the XPRES trial, and the risk score analyses highlight the importance of considering the operational capacity of HIV and TB clinics in sub-Saharan Africa, where the majority of clinics are under-resourced and operate within weak health systems. Within these under-resourced clinic environments, interventions to (1) strengthen the health system to do the basics right, and (2) provide simple, feasible screening tools and care algorithms, can potentially have a bigger impact on mortality than introducing new diagnostic tools, which are often not tailor-made for LMIC settings. Simple innovations to screening tools, through improving the clinician's understanding of holistic risk, and informing differentiated early ART and TB management algorithms, can maximize impact of available resources through a more precise and feasible public health approach.

8.2.1. The importance of health system strengthening interventions to address "leaky" ICF and HIV-TB retention cascades

Although implementation of the WHO-recommended four-symptom TB screening rule as the first step in ICF algorithms among PLHIV starting ART has been recommended since 2011 along with TB-HIV care continuum retention interventions,[162] and although large numbers of studies report on the challenge of "leaky" TB-HIV care continua,[163] no study has yet reported on the potential impact on mortality and other patient-relevant outcomes of strengthening systems to implement existing WHO guidelines for TB screening, ICF and TB-HIV care retention.[66] Below I summarize the extent of the problem of leaky TB-HIV care cascades, potential reasons for these weaknesses in the health system, and then how XPRES provides evidence on how to feasibly address these challenges with ultimate improvements in TB case finding and reduced mortality.

Increasing awareness of the "leaky" TB-HIV cascade problem

A key theme of the Xpert impact systematic review was the high frequency with which health system weaknesses affected both the SOC and intervention arms and patient outcomes in all eight trials. Common indicators of health system weaknesses included (1) the high frequency with which Xpert impact trial enrolees were unaware of their HIV status (with ≥15% of enrolees unaware of their status in four of five trials reporting this variable),[69,71,164,165] and (2) low ART coverage among known HIV-positive persons in all three trials reporting this variable (26%–31%).[68,69,165] Notably, sub-optimal HIV management (i.e., unknown HIV status, HIV-positive and not on ART, or HIV-positive with unknown ART status) were predictive of poor final outcomes in four trials.[68,69,73,165] Another health system-related weakness was high LTFU before TB treatment in three trials[68-70] and high LTFU following TB treatment initiation in four trials.[68,71-73]

While several meta-analyses have been published describing the problem of patient loss at various steps of the TB diagnostic and care cascade, most meta-analyses have focused on overall TB diagnostic cascades regardless of HIV status,[166,167] some have focused on individual steps within the cascade (e.g., pre-treatment loss to follow-up among

microbiologically confirmed TB cases),[166] and some have focused on specific types of TB (e.g., multi-drug resistant TB).[168] However, a systematic review and meta-analysis aiming to quantify the relative drop-off at each step in the ICF and TB treatment cascade among PLHIV in LMIC has not yet been reported.

A recent attempt to create a TB screening, diagnostic, and treatment cascade among PLHIV using data from South Africa's electronic tuberculosis register reported that only 52% of estimated total PLHIV with active TB successfully completed TB treatment with losses from the cascade estimated as follows: 3% of PLHIV never visiting a health facility, 15% not receiving a TB diagnosis despite visiting a facility, 11% not starting TB treatment despite having a TB diagnosis, and 19% not completing TB treatment despite starting therapy.[163] Notably, this analysis back-calculated the number of PLHIV accessing a diagnostic test as equal to bacteriologically confirmed TB cases via microscopy or Xpert divided by the respective TB test sensitivity. Therefore, in this analysis the 15% loss among TB-HIV co-infected patients at the diagnostic step was attributed to persistent use of less sensitive TB diagnostic tests alone (i.e., use of smear microscopy rather than Xpert),[163] which is unlikely to reflect the reality of LMIC clinics where TB symptom screening among PLHIV is poorly performed.

Sub-optimal implementation of the TB symptom screening step

A wealth of published literature shows that implementing the TB symptom screening step in the ICF cascade is commonly omitted in many high burden TB-HIV countries in sub-Saharan Africa. Low completion of the four-symptom TB screening step has been observed in South Africa through an ancillary study nested within the XTEND trial where only an estimated 59% of symptomatic persons attending the XTEND primary healthcare clinics during 2012–2013 reported being asked about any TB symptom or reporting their own TB symptoms voluntarily to the attending healthcare worker.[63] Similarly, as part of nationally representative adult ART programme evaluations in Mozambique (30 clinics) and Cote d'Ivoire (35 clinics) capturing data on ART enrolees during 2004–2007, TB symptoms were only asked about and documented in 61%[62] and 36%[98] of ART enrolees,

respectively, despite national guidelines recommending TB symptom screening at each clinic visit. In a recent report of 90,454 PLHIV attending HIV clinics in western Kenya between 2015 and 2016, 44% of PLHIV were screened for TB symptoms in <90% of their clinical encounters.[169] Similarly, in a recent study from South Africa which aimed to quantify the percentage of TB cases missed by primary healthcare clinics, authors estimated that 63–79% of TB patients with TB symptoms were missed, with 39% of TB cases missed because they were never screened for TB symptoms.[170] In a 2012 survey of 47 clinics in 26 countries, only 38% of sites reported they were using symptom-based screening to identify PLHIV in need of TB diagnostic tests.[171]

In XPRES, failure to implement TB screening before ART initiation was the most "leaky" part of the ICF cascade in the standard of care phase, with only 30% screened before ART. Improving the coverage of TB symptom screening from 30% in the SOC to 100% in the EC and EC+X phases was the main driver behind improved TB case detection from 1% in SOC to 5-6% in EC and EC+X phases, and therefore appears to have been a key driver behind the declines in early ART mortality between SOC and subsequent EC and EC+X phases.

Sub-optimal sputum sample collection from symptomatic patients

Another leaky part of the TB screening, case finding, and treatment cascade from the SOC phase of XPRES and highlighted in the literature is the persistently low percentage of persons with TB symptoms that provide sputum for diagnosis, either because symptomatic persons are not asked for sputum samples or because they are unable to produce sputum.[63,64,170] For example, through a secondary analysis of the XTEND trial, only 23% of persons with TB symptoms attending the XTEND primary healthcare clinics reported being asked to provide a sputum sample.[63] In addition, in a South African study which aimed to quantify the percentage of TB cases missed by primary healthcare clinics, authors estimated that 38–48% of microbiologically-confirmed TB patients were missed because the symptomatic patients were not asked to provide sputum samples during the clinic visit.[170] In addition, a certain percentage of symptomatic PLHIV will be unable to produce sputum spontaneously and sputum induction equipment and personnel trained

in their use are not widely available in LMIC clinic settings.[169] For example, in XPRES, although the percentage of enrolees screening positive for at least one TB symptom who provided one or more sputum samples increased from 38% in the SOC phase to 46% and 55% in the EC and EC+X phases, respectively, collection of sputum samples remained a challenge even in the EC phases mainly because symptomatic patients indicated they were unable to produce sputum.

Pre-treatment loss and poor completion of TB treatment among PLHIV

The meta-analysis of pre-treatment loss to follow-up among microbiologically confirmed TB patients reported that 18% of patients with a TB diagnosis are lost before treatment initiation in Africa.[166] Similarly, high LTFU before TB treatment was reported in three Xpert impact trials.[68-70] In the South African TB diagnosis and treatment cascade analysis using the electronic TB registers, authors estimated that 19% of TB-HIV patients do not complete TB treatment.[163] Similarly, four Xpert impact trials reported high rates of failing to complete TB treatment among patients diagnosed and starting treatment.[68,71-73]

Reasons for poor completion of the TB screening and treatment cascade

Potential reasons for poor completion of the TB investigation and care cascade can be classified as healthcare worker-related, patient-related, and public health management-related. In terms of healthcare worker-related reasons, lack of healthcare worker knowledge and training in TB case finding algorithms,[172,173] high workload and lack of motivation,[173,174] lack of confidence in the laboratory sample transport and diagnostic system,[175] and lack of supervision or mentorship, have been commonly cited reasons.[63] In terms of patient-related barriers to completing the TB diagnostic and treatment cascades, stigma,[65] long wait times,[173] logistical barriers such as cost of transport and family care responsibilities,[176] and poor treatment of patients by healthcare workers,[163,177,178] are commonly reported reasons. At the public health system level, weaker district management teams with irregular monitoring and support for healthcare workers and health facility management,[179] as well as vertical rather than integrated TB-HIV care

models,[180] have been associated with lower completion of TB case finding and care cascades.

High rates of loss to follow-up during early ART

In addition to poor implementation of the TB screening, diagnosis, and treatment cascade, there are many meta-analyses documenting drop-offs in the HIV diagnosis, treatment initiation, retention, and viral suppression cascade.[51,181,182] Recent data from the Joint United Nations Programme on HIV/AIDS (UNAIDS) show that the drop between those aware of their status and those on ART (i.e., between the first and second 90) accounts for the largest drop-off in the 90-90-90 cascades in Africa (e.g. southern and eastern Africa 90-90-90 estimates are currently 85-79-87, indicating 85% of PLHIV are aware of their status, 79% of those diagnosed are on ART, and 87% of those on ART are virally suppressed). In addition, most recent studies show that LTFU after ART initiation rather than before ART initiation is increasingly the driver for drop-offs in the second 90 with patients cycling in and out of ART.[11] Notably, LTFU rates during ART are highest in the first few months after ART initiation, with an average of 20% LTFU by 12 months of follow-up.[12,183] In addition, mortality rates among patients lost from early ART care are high with most patients dying soon after the missed appointment.[12] The percentage of LTFU clients found to have died by the time of tracing ranges from 20-60%.[12,20] Similarly in XPRES, 41% of patients LTFU in the first 6 months of ART in the SOC phase had died by 6 months of follow-up.[161]

Few trial interventions shown to comprehensively address leaky TB-HIV care cascades

Although the problems of the leaky TB screening and HIV retention cascades are well documented, and reasons for poor completion of the TB-HIV care cascades have been widely investigated, few trials have evaluated the impact of strengthening both TB screening and retention on patient-important outcomes like morbidity and mortality.

XPRES suggests that strengthened TB symptom screening could be associated with improved outcomes

Firstly, although the WHO four-symptom screen has been recommended by WHO since 2011, impact of implementing the four-symptom TB screen on patient-relevant outcomes has not yet been reported. Although the XPRES trial has several limitations, including the pre-post design for the primary mortality outcome, XPRES showed that strengthening implementation of the WHO four-symptom TB screen appeared to drive increased TB case finding with associated reductions in early ART mortality.[44,60,61,88]

As described in Chapter 5, and in the limitations section (Section 8.3.1.), the pre-post design is not an experimental design and therefore provides weaker evidence than a true experimental design such as a parallel group CRT.[144] For example, Sanson-Fisher *et al*, describe the evidence generated from a pre-post study design as providing only moderate evidence that a change has occurred, weak evidence that any change observed was due to the intervention, and moderate evidence that the degree of change is significant.[144] In contrast, evidence from a parallel group CRT would be classified as "strong" for all three categories noted above.[144] Pre-post study designs are weaker than parallel group randomised trial designs because pre-post designs are inherently at risk for residual confounding. Therefore, in XPRES, the difference in 6-month ART mortality rates between SOC and EC+X phases, might be explained by unmeasured confounders. Two potential unmeasured confounders include WHO stage and background trends of other factors over time. WHO stage was not included in the adjusted analysis per the pre-specified analysis plan, because 61% of patients in the SOC cohort had missing WHO stage data. Other variables including CD4 count, weight, and haemoglobin concentration at ART initiation, variables which were ≥90% complete in the SOC cohort and EC+X cohort, were used to control for disease stage at ART initiation, but these variables might not have fully controlled for disease stage.[32] In addition, background trends in factors such as declining national TB incidence over time (as shown in Chapter 3) might have contributed to declining risk of PLHIV mortality over time resulting in residual confounding affecting the pre-post analysis.

In addition, the 95% confidence interval associated with the adjusted hazard ratio (0.77) comparing SOC versus EC+X 6-month ART mortality rates was 0.61–0.97. Therefore, if an important unmeasured confounder exists (e.g., WHO stage or declining trends in TB risk over time), and if it were possible to add the unmeasured confounder into the adjusted analysis, it is possible that the new adjusted hazard ratio would be closer to 1.0 and the new 95% confidence interval would include 1.0.

XPRES did observe large changes in the percentage of new ART enrolees screened with the WHO four-symptom TB screen between SOC and EC+X phases (30% versus 100%). However, as described in Section 8.3.1., it is still possible that clinicians in the SOC phase did the TB screening but did not document the screening, which would introduce measurement error. In addition, the change in new TB case finding between phases, from 1% in the SOC to 6% in the EC+X phase, was relatively large, but this change might partly be explained by clinicians failing to document TB cases in the SOC cohort.

In addition, the findings from the XPRES trial pertain to new, non-incarcerated, adult (>12 years old) ART enrolees during 2010-2015 at the 22 study facilities, which were purposively selected to be representative of HIV treatment health facilities in Botswana (see section 5.1.). During this time, adult PLHIV were only eligible for ART if the CD4 count was ≤ 350 cells/μL, or WHO stage was III or IV, or the client was pregnant or breastfeeding. If a client did not meet these criteria, the client often was managed in pre-ART care at separate smaller health facilities referred to as health posts, with quarterly CD4 count monitoring. When the CD4 count fell below 350 cells/μL, or WHO stage III/IV was reached, or the client became pregnant, the client was referred to an HIV treatment clinic. Therefore, all enrolees in the SOC, EC, and EC+X cohorts represent PLHIV who had survived from the time of diagnosis until the time of ART eligibility. Although each study population (SOC, EC, and EC+X) would have been equally affected by any survivor bias, meaning comparisons between phases in the primary outcome paper (Chapter 5) remain internally valid to the population studied, care should be taken to generalize findings to

PLHIV populations at the time of HIV diagnosis. Currently, the standard of care is that all PLHIV are eligible for ART, with many newly diagnosed PLHIV starting ART on or near to the date of HIV diagnosis.

Therefore, additional contemporary evaluations of the intervention in the XPRES cohort, using a stronger study design (e.g., parallel group randomised CRT), are ideally needed to confirm the findings from the XPRES trial. However, investigators designing a parallel group CRT to evaluate effectiveness of the same interventions thought to be effective in XPRES (i.e., improved TB screening, and strengthened active tracing), would need to carefully address (1) the issue of equipoise (since TB screening and active tracing are already recommended by WHO and most ministries of health in sub-Saharan Africa), and (2) the Hawthorne effect (i.e., the possibility that the standard of care arm would experience a change in TB screening and retention practices due to health worker, and possibly study enrolee, awareness of the study hypothesis).

Need for deployment of new diagnostics, such as Xpert, as part of a holistic package

As Xpert has been rolled out globally, reaching over 122 countries and with more than 16 million tests performed since 2011, and given the lack of observed impact of Xpert versus smear microscopy in terms of reducing morbidity and mortality in trials to date, there have been several papers calling for Xpert to be rolled out alongside health system strengthening interventions.[163,184] For example, in a paper by Albert *et al* in 2016, authors explored practical lessons learned from Xpert rollout.[184] Authors argued that Xpert rollout and impact had been hampered by lack of a "complete solution package (notably comprehensive training, quality assurance, and implementation plans)" and insufficient focus on "effective linkage to care of diagnosed patients" with Xpert impact "blunted by weak health systems".[184] However, Sun *et al* used a modelling analysis to explore any differential impact weak health systems might have on Xpert- compared with microscopy-based diagnostic cascades.[185] In this analysis, similar to our Xpert impact trial review,[146] Sun *et al* showed that the biggest driver in minimizing impact of Xpert versus smear microscopy was the rates of empiric TB treatment, with higher rates of empiric TB

treatment eliminating potential impact of the improved diagnostic tool (Xpert).[185] From this analysis by Sun *et al*, any differential impact of strengthened health systems to correct "leaky" TB-HIV cascades that would result in increased Xpert versus microscopy impact on patient-important outcomes appeared to be minimal.

However, most authors agree that health system strengthening interventions that address the drop-offs throughout the TB screening, diagnosis, and treatment cascade, regardless of TB diagnostic used, should be prioritised by TB programme managers and global oversight bodies like WHO even as new diagnostics like Xpert are rolled out.[163,186,187]

Therefore, the XPRES trial results fill an important research gap by providing proof of concept data that strengthening facility-based TB symptom screening and diagnostic cascades prior to rollout of novel diagnostic devices like Xpert, can have significant independent benefit for patients served, in addition to any additional synergistic effect a strengthened cascade might afford the new diagnostic.[184,185]

XPRES adds useful evidence for active tracing to support retention during early ART

WHO guidelines for management of advanced HIV disease cite the REMSTART trial as the key evidence that adherence interventions need to be part of the package of care for PLHIV starting ART with advanced HIV disease.[57] REMSTART was an individually randomised trial among PLHIV with CD4 <200/µL comparing standard clinic-based care versus standard of care plus an intervention package. Prior to enrolment in both the standard of care and intervention arms, all participants were screened for TB with Xpert testing of sputum samples. In the intervention arm (referred to as standard care plus community support), PLHIV received two additional interventions: (1) weekly home visits for the first 4 weeks from lay counsellors who had received a two-week training and used a checklist to document each home-based care interaction. During the weekly visit, the lay counsellors delivered adherence support, monitored for signs and symptoms of disease progression or toxicity, and referred the client to the clinic if needed; and (2) screening for serum cryptococcal antigen combined with antifungal therapy for patients

testing antigen positive. In addition, a second Xpert test was provided to intervention arm enrollees at 6 weeks after ART initiation in Tanzania clinic enrollees among those not diagnosed with TB at enrollment.

At 12 months of follow-up all-cause mortality was 13% in the intervention arm and 18% in the standard of care group, with the 28% relative reduction in all-cause 12-month mortality statistically significant (p=0.004). Authors attributed about half of the mortality reduction to antifungal treatment for those with positive cryptococcal antigenemia and half of the mortality reduction to the home-based adherence counselling.[57]

XPRES adds important evidence to that contributed by the REMSTART trial. While the REMSTART community-based adherence and retention intervention was well-costed and considered relatively inexpensive ($42.60/participant in Tanzania and $45.77/participant in Zambia), these costs which are equivalent to about 56% of a person-year's supply of the current WHO-recommended first-line ART regimen for adults (about $75/person/year) are not insignificant,[188] pre-emptive home visits are not possible for all new ART enrolees, and pre-emptive home visits are not widely implemented due to the complexity of engaging new cadres, community-facility linkages, transport to people's homes, and resource limitations.[189] WHO also recommends rapid tracing for patients who miss appointments, initially by phone or through home visit if not reachable by phone, which is a retention intervention more widely feasible in sub-Saharan Africa.[44,74,75] XPRES adds evidence in support of the active tracing intervention since prior to this research, no trial had yet included active tracing as part of an intervention package to reduce early ART mortality.[76]

In addition, the most recent meta-analysis of supportive interventions to improve retention on ART in LMIC included evidence from seven trials including REMSTART.[189] Among the other six trials, two required directly observed daily medication taking at home,[190,191] one required lay counsellor support through home visits,[192,193] one required community adherence clubs,[194] and two required either daily[195] or weekly[196] visits by the patient to the health facility for directly observed therapy and clinical check-ups.

Therefore, the evidence provided by XPRES showing effectiveness of the retention intervention, comprised of facility-based, nurse-led counselling and telephonic and home tracing if a patient missed an appointment, with associated 82-95% reductions in LTFU rates, is an important addition to the available supportive retention interventions for PLHIV starting ART, with increasing relevance as phone coverage increases.

Why the XPRES health system strengthening intervention appears to have worked

Although the observed reduction in all-cause mortality between SOC and subsequent EC and EC+X phases of XPRES represents a pre- versus post-comparison, rather than a randomised comparison, and is therefore at risk of residual confounding, the study has strengths that suggest ICF and retention interventions did independently contribute to observed mortality impact. These strengths include the multivariable analysis to control for known confounders, the large improvements in TB screening, TB case finding, and uncorrected LTFU rates between SOC and subsequent EC and EC+X phases which provide credence these interventions were drivers behind observed mortality reductions, the high ascertainment of the primary early ART mortality outcome, and robustness of pre-specified primary outcome analyses to several sensitivity analyses.

As described in Chapter 5, the health system strengthening intervention in XPRES had four over-arching components: (1) additional human resources (study nurses) to support implementation, (2) additional training for clinic and laboratory personnel, (3) use of checklists and job aids to standardize implementation, and (4) regular supervisory visits to track adherence to ICF and tracing checklists. These four components directly address many of the underlying health facility-related and district health system-level reasons for leaks in the TB case finding and TB-HIV care cascades as described earlier (Table 8.1).

Table 8.1. XPRES health system strengthening components that addressed the underlying causes of missed steps in the TB and HIV care cascades

	Reason for poor completion of recommended TB and HIV cascade of care	Intervention package component number and description
Healthcare worker-related	High workload causing healthcare workers to miss components of care implementation[173,174]	HSS component #1: One additional nurse per site to focus on TB case finding and TB-HIV retention cascade. Average nurse salary about $11,400/year.[197]
	Lack of healthcare worker knowledge and training in TB case finding algorithms[172,173]	HSS component #2: Additional training at study start on TB case-finding algorithms.
	Lack of confidence in the laboratory sample transport and diagnostic system[175]	HSS component #2 and #3: HSS component #3 was the provision of job aids and checklists as well as clarifying (1) who was responsible for monitoring completion of each part of the cascade and (2) expected turnaround times for sample transport and analysis for smear microscopy and Xpert diagnostic tests.
	Rudeness/lack of support for patients at the clinic[163,177,178]	All HSS components #1-4. HSS component #4 was that each study nurse was directly supervised by a study nurse supervisor who regularly reviewed data including TB diagnostic cascade and HIV care cascade completion indictors.
	Lack of supervision or mentorship[63]	HSS component #4 as described above.
Public health system level	Irregular monitoring and support for healthcare workers and health facility management[179]	HSS component #4 as described above.
	Non-integration of TB and HIV care systems	TB screening, diagnosis, referral for TB treatment and monitoring during ART and TB treatment were the responsibility of the same nurse based at the HIV study clinic. Although the TB clinic was often not co-located, one healthcare provider was monitoring completion of both cascades.

Abbreviations: HSS, health system strengthening

Notably, our study did not specifically address patient-specific barriers to completion of TB and HIV care cascades (e.g., by providing money for transport or other incentives), but by introducing new healthcare workers, conducting new training, providing job aids and check lists for healthcare workers, and conducting supportive supervision for the new

nurses at the sites, and given the overall 82-95% reduction in LTFU rates that occurred between SOC and EC phases of XPRES, it seems likely that patients enrolled in XPRES EC phases found the care provided patient-centred and patient-friendly, although XPRES did not collect quantitative or qualitative data on patient perceptions of care provided.

Important considerations before scale-up of public health interventions

Multiple factors need to be considered prior to scale-up of any public health intervention.[10] At the planning or policy-making stage, rigorous evaluation of the scientific evidence supporting effectiveness of the public health intervention, its cost and cost-effectiveness, feasibility, and acceptability are key considerations.[10] As described in this section, and in the limitations section (Section 8.3.1.), XPRES does not provide definitive evidence that scaling up health system strengthening interventions to improve implementation of TB screening and active tracing to support retention will with certainty reduce early ART mortality in sub-Saharan Africa. However, as described above, there is a wealth of literature beyond XPRES that supports the need for health system strengthening to appropriately address "leaky" ICF and HIV-TB retention cascades, and both ICF[58] and active tracing to support retention[44] are already recommended by WHO guidelines. As stated in Chapter 5, a limitation of the XPRES trial analysis is that it was not paired with a formal cost-effectiveness analysis, although a retrospective cost-effectiveness analysis has been proposed. As described above, the health system strengthening interventions were feasible in the Botswana health system within the XPRES research context, and acceptable to healthcare workers at the XPRES trial health facilities. However, Botswana is classified as an upper middle-income country, whereas the majority of countries in sub-Saharan Africa are middle- or lower-income countries.[133,134] Any decision to scale up a public health intervention should be accompanied by a detailed strategic plan, documentation of financial considerations, monitoring plans, additional evaluations, and the future vision for sustainability.[10] Therefore, the data in the XPRES trial in Chapter 5 and the related information presented in this thesis could be used to inform discussions about scale-up of health system strengthening interventions to improve TB screening and retention, but

should be considered along with other data relating to effectiveness, cost-effectiveness, feasibility and acceptability of the interventions.[10]

8.2.2. The importance of considering the need for truly pragmatic designs for future novel TB diagnostic trials in LMIC

Increasingly trialists are asked to consider at the design stage the extent that a proposed intervention trial needs to be pragmatic (i.e., undertaken in the "real world" with the intent of assessing whether the intervention will be effective in the setting it is designed for) or explanatory (i.e., undertaken in an idealised setting, to give insight into efficacy in a carefully controlled research environment).[71,147,198,199] A recent systematic review of Xpert impact trials (currently under review and published as a pre-print)[148] argues that a key reason Xpert impact trials have not observed impact on mortality is because the standard of care arm represented an enhanced care arm (similar to the EC phase in XPRES) rather than a true SOC arm (such as the SOC phase in XPRES). Here we explore this perspective, assess how XPRES uniquely provides both pragmatic and explanatory trial components and insights, and discusses implications for future novel TB diagnostic and universal treatment trials.

Which Xpert impact trials were truly pragmatic?

The first step in considering whether a trial should be pragmatic or explanatory, even before scoring the nine components of the Pragmatic-Explanatory Continuum Indicator Summary 2 (PRECIS-2) scoring tool (see table 8.2. below), is to carefully consider the aim of the trial. For example, a truly pragmatic Xpert impact trial would be appropriate for a study question that aims to assess whether Xpert replacing sputum microscopy in an unchanged health system, which is largely a weak health system in LMIC, has independent impact on mortality. While this was the key question for the seven previous Xpert impact trials that assessed Xpert impact on mortality outcomes,[68,69,71,72,82,165,200] the XTEND trial was the closest to implementing a truly pragmatic design and therefore answering the question raised. Firstly, unlike five[68,72,82,165,201] of the other six studies, XTEND was careful not to include additional TB diagnostic tests such as chest radiography or culture on top of

microscopy that went beyond standard care in the standard of care arm. Secondly, XTEND was designed to minimise study-related changes to routine TB-HIV care in standard of care and intervention arms aside from Xpert introduction in the intervention arm, with primary mortality outcomes ascertained at 6 months through reports from participant-nominated contacts, clinic staff, and national vital statistic database review, whereas some form of system strengthening was present in both standard of care and intervention arms in all other six trials.[148]

Notably, although the trial considered the "most pragmatic" from the recent Xpert impact trial review published as a pre-print by Ochodo *et al,*[148] is the stepped-wedge trial by Trajman *et al*, from Brazil, in our own Xpert impact trial review, we did not consider that this trial truly assessed impact on all-cause or even TB-attributed mortality among any easily describable group of study enrolees for four reasons. Firstly, Trajman *et al* acknowledge that rates of loss to follow-up from both trial arms of patients starting TB treatment were very high (16% LTFU in both trial phases), and there was no mortality ascertainment among those LTFU through national mortality registry review. Mortality among patients lost to follow-up from TB treatment are high,[202] and therefore there is almost certainly outcome miss-classification in this trial. Secondly, when authors compare the composite outcome (death, LTFU, transfer, or suspicion of TB drug resistance) between study phases, these percentages were not different between phases (29.6% in intervention vs. 31.7% in control phase). Thirdly, the trial-specified study population was patients being investigated for TB (presumptive TB patients) whereas the mortality outcome reported is for those who started TB treatment with no way of assessing potential selection bias introduced by this restricted outcome analysis. Fourthly, the assignment of a death as due to TB among TB patients, which authors reported as being different between arms (2.3% vs. 3.8%) was based on clinician-reported cause of death data on death certificates, the completeness and accuracy of which is not discussed and known to be sub-optimal in most LMIC settings.[35] However, the trial does have a pragmatic design, and indicates the challenges of using data from pure pragmatic designs to understand trial outcomes and their associated policy implications.

Contribution of XPRES trial design to Xpert impact trial literature

XPRES had both pragmatic and explanatory components to its design. In our published protocol, we proposed that XPRES was primarily a pragmatic trial because the primary XPRES study question is answered by a pragmatic study design. Firstly, the SOC phase represents a true standard of care (i.e., historical data untouched by study-related procedures or interventions that might have strengthened standards of care) which is the pre-requisite for any truly pragmatic trial.[147] Secondly, XPRES specifies that the intervention is a package that includes both health system strengthening and Xpert replacing smear microscopy. By specifying that health system strengthening is part of the intervention package, XPRES addresses the importance of considering pragmatism in trial design directly. For example, if the SOC versus EC+X comparison was characterized as a pragmatic approach to understanding purely the impact of Xpert replacing sputum smear microscopy on standard of care (i.e., the same question that XTEND quite successfully addressed as described above), XPRES would represent a very poorly designed pragmatic trial to answer this question, because significant health system strengthening interventions were invested to support rollout of the Xpert device. Instead at the design stage, and inherent to the name of the XPRES trial, the intervention was described as a combination of health system strengthening interventions to support Xpert rollout.[18] In this respect, XPRES was similar to other published pragmatic trials, which frequently include complex interventions, sometimes consisting of several interacting components and often involving the skills and experience of one or more health care professionals to deliver the intervention.[198]

Four components of the PRECIS-2 score evaluate whether health system strengthening, which is separate from the specified trial intervention, have pushed the trial towards the explanatory side of the pragmatic trial continuum. Each of the nine PRECIS-2 components are given a score between 1 and 5, with scores closer to 1/5 indicating the trial feature is more explanatory and scores closer to 5/5 indicating the trial feature is more pragmatic. These four components are: component 4, which examines the degree to which the

intervention package requires additional resources to implement; component 5, which examines how flexible the investigators aim to be in allowing study clinics to determine the operationalization of the intervention; component 6, which evaluates what measures are in place to ensure adherence to intervention algorithms; and component 7, how closely participants are followed up. For the XPRES primary study question (comparing all-cause 6-month ART mortality between SOC and EC+X enrolees), because there was limited health system strengthening outside the pre-specified intervention package for comparison with true SOC, and after evaluating eligibility criteria, recruitment, setting, primary outcome, and primary analysis approaches, I believe that the XPRES design should be considered largely pragmatic (Table 8.2.).[203]

Table 8.2. Evaluating XPRES on the pragmatic vs. explanatory trial continuum

PRECIS-2 Criteria	Primary XPRES study question - Comparison of all-cause 6-month ART mortality between SOC and EC+X ART enrolees (non-randomised)	Secondary XPRES study question - Comparison of all-cause 6-month ART mortality between EC and EC+X ART enrolees
Aim	To answer the question whether the package of health system strengthening interventions to support TB screening and TB-HIV care retention combined with Xpert replacing sputum smear microscopy can impact all-cause 6-month mortality compared with true SOC.	To assess within a strengthened health system whether Xpert is superior to smear microscopy in terms of reducing 6-month all-cause mortality.
1. Eligibility	**Score: 4** Rationale: The study population eligibility criteria very closely reflected the patient population attending the study clinics. Only those new HIV clinic enrolees who were incarcerated were excluded from the study (<0.5%). As described in the limitations section, some study enrolees were referred to the XPRES study clinics after a period of pre-ART care and represent a partially pre-screened population with some patients already taking TB treatment upon arrival at the study clinics.	
2.Recruitment	**Score: 4** Rationale: All new HIV clinic enrolees in the retrospective SOC phase were included in the SOC cohort. In the EC phases, all new HIV clinic enrolees were eligible but about 30% left the clinic before they could be offered enrolment. In comparing EC and EC+X enrolees versus those not enrolled in EC phases despite being eligible, no differences were observed (see primary outcome manuscript supplementary appendix no. 4).[161] When inverse probability weights were used to account for non-enrolment in sensitivity analyses of the primary and secondary outcome, no changes were observed compared with the primary outcome analysis (see primary outcome manuscript appendix no. 7).[161]	
3.Setting	**Score: 4** Rationale: The study was done in Botswana HIV treatment clinics that were purposively chosen to be representative of HIV care and treatment clinics in Botswana (see manuscript appendix no. 1).[161] However, Botswana is considered a higher middle-income setting, unlike most other countries in Africa which are low- or middle-income.	
4.Organization	**Score: 5** Rationale: A highly pragmatic trial would aim to slot the intervention into usual care. Because the SOC arm represents true SOC, and because the intervention is a package of health system strengthening interventions combined with Xpert rollout, results represent impact of inserting the intervention package (TB screening, retention and Xpert) on top of SOC compared with true SOC.	**Score 1:** Rationale: Because both phases (EC and EC+X) were quite strictly controlled to ascertain impact of Xpert within a strengthened health system, the organization score reflects this weighting towards the "explanatory" end of the continuum.

5. Flexibility (delivery)	**Score: 3** Rationale: A perfectly pragmatic trial would allow health clinics to design the "how" of TB screening, retention, and Xpert implementation without investigator influence. In the EC+X phase, because the intervention package was designed by investigators with central Ministry of Health leadership, some of whom worked in the clinics, and in accordance with national guidelines, a score of 3 on the "pragmatism scale" is proposed.	**Score 1:** Rationale: Because both phases (EC and EC+X) were quite rigidly controlled to ascertain impact of Xpert within a strengthened health system, the flexibility score reflects this weighting towards "explanatory".
6.Flexibility (adherence)	**Score: 4** Rationale: Although the intervention implementation was closely monitored to assess adherence to implementation, this approach to monitoring was part of the intervention package.	**Score 1:** Rationale: Because in both phases (EC and EC+X) adherence to procedures was quite rigidly controlled to ascertain impact of Xpert within a strengthened health system, the flexibility score reflects this weighting towards "explanatory".
7.Follow-up	**Score: 4** Rationale: Although mortality outcomes were rigorously ascertained, in the SOC phase this was done retrospectively, with no chance that telephonic outreach or home visits could have averted 6-month ART mortality. In the intervention arm, the tracing was part of the intervention package.	**Score 1:** Rationale: Because in both phases (EC and EC+X) active tracing was implemented within a strengthened health system, the follow-up score reflects this weighting towards "explanatory", with the goal of explaining Xpert impact in a strengthened health system.
8.Primary outcome	**Score: 5** Rationale: Because the mortality outcome is directly relevant to the patient's perspective, the outcome is weighted towards pragmatic.	
9.Primary analysis	**Score: 5** Because the analysis was intention-to-treat, the primary analysis score is weighted towards pragmatic.	
Total	**38/45**	**28/45**

Abbreviations: PRECIS-2, Pragmatic-Explanatory Continuum Indicator Summary 2; ART, antiretroviral therapy; SOC, standard of care; EC, enhanced care; EC+X, enhanced care plus Xpert.

As described in Table 8.2 above, the nested stepped-wedge trial design is the explanatory component of the XPRES trial. The purpose of the nested stepped-wedge trial was to assess, as a secondary study question, whether, within a strengthened health system, Xpert has additional impact on mortality over smear microscopy.

Contributions from XTEND, XPRES, and other Xpert impact trials

Based on our Xpert impact literature review,[146] and after reviewing information in the most recent critique of Xpert impact trial designs by Ochodo *et al*,[148] XTEND is arguably the best pragmatic trial to answer the question of whether in a standard LMIC health system, replacing sputum smear microscopy with Xpert can independently drive mortality reductions among persons being investigated for TB. Simultaneously, XPRES, along with other Xpert impact trials, helps to answer the question of whether Xpert introduced into a stronger health system can independently drive mortality reductions. So far the answer to both study questions is that Xpert appears to have no easily detectable impact on mortality compared with smear microscopy, although there may be a modest impact more feasibly detected in large meta-analyses among PLHIV,[204] or in sub-groups of PLHIV with advanced HIV disease.[200]

How feasible are the XPRES health system strengthening interventions?

The XPRES health system strengthening interventions to improve TB screening and case finding and ensure retention in TB-HIV care were associated with reductions in early ART mortality. As noted above, we believe these findings are generalizable to other LMIC settings. However, one question raised by this trial finding is whether it is feasible to implement the health systems strengthening package in LMIC, including SSA, to achieve the mortality reductions we observed in XPRES. Although additional targeted investments are needed, XPRES showed that scale-up of the health system strengthening interventions was feasible, and well-supported by existing healthcare personnel and management, national guidelines, and WHO guidelines.[161] In addition, several recent papers agree that health system strengthening to address the leaky TB case finding and TB-HIV care

retention cascades in LMIC is an important contemporary priority.[16,163,184,186] Similarly, many large global funding bodies are investing in this area with an increasingly large percentage of annual investments directed to health system strengthening interventions that have proven sustainable impact.[205,206] In addition, findings from XPRES, which showed feasibility and impact of cascade strengthening interventions on TB case finding, retention, and mortality outcomes, have been used to inform national investment profiles in countries in sub-Saharan Africa since the primary trial findings were released in 2018.[207] In retrospect, an economic analysis of the XPRES intervention would have been a valuable addition to the study, and a retrospective economic analysis has been proposed to fill this gap.

8.2.3. The importance of implementing sensitive screening tools versus need for new sensitive diagnostic tests

XPRES, as well as the seven other Xpert impact trials among presumptive TB patients or PLHIV that have evaluated impact of Xpert on all-cause mortality,[68,69,71,72,82,165,200] showed that if clinicians implemented ICF and retention cascades in trial arms using microscopy, mortality outcomes were not different to well-implemented screening and retention cascades using Xpert. A key observation was that across the seven prior Xpert impact trials evaluating mortality impact, TB treatment initiation rates were similar between arms or phases in six trials and only significantly higher in the Xpert arm in one trial.[71] While participants in the Xpert arms had higher rates of microbiologically confirmed TB, higher rates of empiric TB treatment in microscopy arms balanced overall TB treatment initiation rates between arms in most trials. Our systematic review and other papers showed that empiric TB treatment of culture-positive smear-negative TB patients in the microscopy arms largely removed any potential for observed Xpert impact.[151,208] For example, in the TB-NEAT study,[68] of the 68% of patients with smear-negative tuberculosis in the microscopy arm that were later correctly detected by Xpert, 93% were treated empirically anyway. To some extent this highlights the relative importance of a sensitive TB screening

rule to prompt clinicians to "think TB" versus the need for a sensitive TB diagnostic test.[151] This concept was the reason behind development of the new clinical TB screening score and is discussed in depth in section 8.2.5. below.

Screening tools for detecting early ART mortality risk

In a similar vein, the importance of a screening tool to prompt a clinician's assessment of overall mortality risk prior to starting ART is important especially given the findings from the REALITY trial which shows that supportive and prophylactic packages of care can reduce early ART mortality.[53] However, as noted by the authors of the REALITY trial, and as acknowledged by the WHO advanced HIV disease guidelines, up to 50% of those with a CD4 count <100 cells/µL could be classified as having WHO stage I or II and therefore missed by an advanced HIV disease eligibility guideline relying on WHO stage alone.[44,53] However, most PLHIV starting ART in LMIC do not have access to rapid on-site or near-site CD4 testing that would facilitate implementation of the WHO advanced disease management guidelines.[102] Although there are calls to action to increase access to rapid CD4 testing across LMIC clinics, in most cases this scale-up needs to await release of inexpensive CD4 tests (e.g., lateral flow assays), and significant investments in scaling up these tests.[102] In the meantime, many PLHIV continue to start ART or re-initiate ART with advanced disease that is missed because of lack of access to CD4 testing. This insight was the foundation for the early ART care intensification analysis presented in the thesis.

8.2.4. New tools to inform who needs intensification of early ART care

Our CD4-independent and -dependent clinical scores, which to our knowledge are the first externally validated clinical scores to inform early ART care intensification developed for sub-Saharan Africa, have some advantages over the WHO advanced HIV disease eligibility criteria. These advantages include: (1) the CD4-independent score was nearly twice as sensitive as WHO stage used alone to detect risk of early ART mortality and could be useful for the majority of HIV clinics in sub-Saharan Africa where access to rapid on-site or

near-site CD4 testing capacity is very limited, (2) both the CD4-independent and -dependent scores improved specificity compared with the full WHO advanced HIV disease eligibility criteria (CD4 <200/μL or WHO stage III/IV), indicating the opportunity to increase efficiency of advanced HIV disease differentiated care models, (3) both scores provide flexibility for programme managers to choose cut-offs that might increase feasibility and affordability of differentiated care algorithms to maximize impact of available resources, and (4) by creating three risk groups, further differentiation of models of care is possible.[84]

Compared with other clinical scores

Our clinical scores are more appropriate for sub-Saharan Africa and other LMIC settings than the scores generated for resource-rich settings (VACS, VACS 2.0, and EuroSIDA) for several reasons, which are discussed in more depth in the literature review section of Chapter 2.3.4., and only briefly summarised here. Firstly, our score uses fewer, less complex, and more easily available scoring variables, with five routinely available variables needed for the CD4-independent and six variables needed for the CD4-dependent risk scores compared with 11 variables needed for VACS, 15 variables for VACS 2.0, and 10 variables for EuroSIDA. Secondly, the VACS, VACS 2.0, and EuroSIDA scores were generated among cohorts of PLHIV with very different demographic, clinical, and contextual factors compared to cohorts in LMIC. Thirdly, the VACS, VACS 2.0, and EuroSIDA scores have never been validated in sub-Saharan Africa or other LMIC settings as valid predictors of early ART mortality.

In addition, our scores are arguable better suited for sub-Saharan Africa, and possibly other LMIC clinic settings, than the Haiti score, which was generated using methodology with several important limitations as described in Chapter 2.3.4, and which has not been externally validated in sub-Saharan Africa or other LMIC. Firstly, while the Haiti score was generated from six non-representative clinics in Haiti and validated in one non-representative Haitian clinic with a small sample size, our scores were generated from 22 XPRES trial clinics, purposively selected to be representative of HIV care and treatment

clinics in Botswana and were externally validated in a trial dataset from South Africa (TB Fast Track). Secondly, while over 35% of study participants in the Haiti study had missing covariate data, only 7% of XPRES prospective trial participants were excluded from the analysis because of one or more missing covariates. Thirdly, in the Haiti study, the primary outcome of ART mortality was not well-ascertained, with 1-year vital status data in the derivation and validation datasets missing for 21.6% and 45.3%, respectively. In contrast, in XPRES the primary mortality outcome was almost perfectly ascertained with <1% of prospective trial participants lost to follow-up.

Next steps to validate use in contemporary routine programme LMIC clinic settings

Ideally at least two next steps are needed to further strengthen the evidence base for rollout of the CD4-dependent and CD4-independent scores: (1) in the short term, further external validation of the early ART care intensification scores in a currently available dataset of adult PLHIV starting ART in sub-Saharan Africa under test-and-treat (i.e., universal ART eligibility) guidelines is needed and ongoing, and (2) a study to assess impact of the scores and associated algorithms of care on early ART mortality is needed.

The first step of external validation in a currently available dataset is needed to address outstanding questions of how well the score discriminates risk in the current general ART enrolee population in sub-Saharan Africa. Notably, the TB Fast Track trial external validation dataset consisted of persons with homogenously advanced HIV disease and low CD4 count (CD4 <150/μL). The fact that the CD4-dependent and -independent scores and proposed cut-offs provided reasonable sensitivity in detecting early ART mortality risk compared to WHO advanced HIV disease eligibility criteria (i.e., 88-95% vs. 100%), and significantly superior specificity (20-27% vs 0%), indicates the ability of the score to differentiate risk even within a very homogenous population, which is a tougher test of discriminatory capacity compared with ability to discriminate risk in a more representative and heterogenous ART enrolee population. Therefore, we would expect, but need to confirm, that the CD4-dependent and -independent scores will have better screening

accuracy in a contemporary dataset than was observed through external validation efforts in the TB Fast Track trial dataset.

Once validated again on a regionally or nationally representative dataset, the CD4-independent score might be considered ready for use in some settings of sub-Saharan Africa lacking access to CD4 count testing, because of the very low sensitivity associated with trying to use WHO stage alone to discriminate early ART mortality risk (48% sensitivity among XPRES ART enrolees in our analysis). However, ideally impact and feasibility of both the CD4-independent and CD4-dependent screening tools would be prospectively evaluated as part of a trial to inform subsequent programmatic rollout.

Trials to improve early ART mortality risk assessments and differentiated care are still needed in sub-Saharan Africa despite rollout of new treat-all ART guidelines since 2015, because the percentage of PLHIV starting ART with advanced HIV disease remains persistently high in the region at about 15-30%, partly because many clients continue to cycle in and out of ART care.[11,38]

To inform policy makers trying to choose between use of the CD4-independent or -dependent scores rather than WHO advanced HIV disease care intensification criteria (CD4 <200/μL or WHO stage III/IV), a range of trials could be designed depending on the most pressing priorities.

In those settings lacking CD4 testing access, where the CD4-independent score might replace the use of WHO stage alone in determining early ART mortality risk and access to an advanced HIV disease care package such as that recommended by WHO (i.e., cotrimoxazole prophylaxis, TB screening with subsequent TB treatment or TB preventive therapy, cryptococcal antigen (CrAg) screening with pre-emptive therapy for eligible CrAg-positive people, and enhanced adherence counselling),[44,53] a pragmatic parallel-group

cluster-randomised trial could evaluate the potential impact of the CD4-independent score on early ART mortality.

In addition, both the CD4-independent and CD4-dependent clinical scores could inform new differentiated care packages, and this combination of clinical score plus new differentiated care might be superior and cost-effective compared with WHO ART care intensification criteria and packages for the following reasons. Firstly, a score-informed care package might be superior to current WHO advanced disease eligibility and care guidelines by (a) improving access to early ART care packages through improved sensitivity of composite scores, and (b) use of additional interventions for the highest risk patients determined by the clinical score approach.[84] The score-informed care package would have a good chance of remaining cost-effective while achieving additional impact on early ART mortality compared with standard of care, because of improved specificity of the scores compared with WHO advanced disease eligibility criteria.

8.2.5. New tools and approaches to TB screening among PLHIV

While the WHO four-symptom screening score is simple, widely known, recommended by WHO since 2011, and if implemented correctly can drive increases in case finding and associated mortality reductions among PLHIV starting ART, as was shown by the XPRES trial,[161] our TB screening score has some advantages that mean it should be considered for scale-up in sub-Saharan Africa. Below comparative advantages of our clinical TB screening score over (a) the WHO four-symptom TB screening rule, (b) the WHO-recommended approach to supplement WHO four-symptom TB screening with chest radiography where possible, and (c) other TB screening clinical scores are discussed. Drawing lessons learned from XPRES, suggested next steps for scale-up so that the TB screening clinical score is actually implemented are proposed. In addition, additional research that might support thoughtful scale-up plans to facilitate reaching ambitious TB preventive therapy goals

among PLHIV by 2021[92] and overall TB case finding, treatment, and prevention goals by 2030, is proposed.[88]

Comparative advantage of TB clinical score over WHO four-symptom TB screen

As described in the most recent meta-analysis of accuracy of the WHO four-symptom screening rule by Hamada *et al*, and as described in the TB screening rule literature review section of this thesis (Chapter 2.4.1.), an inherent weakness of the WHO four-symptom TB screening rule is its inability to detect asymptomatic TB. While this appears to have been a relatively small limitation at the time the screening rule was developed in 2011, when a much smaller percentage of PLHIV were taking ART, in 2020, when the majority of PLHIV in care are taking ART, this is a more important limitation.[61] As described in the literature review, asymptomatic active TB as a proportion of total active TB cases is relatively more common among PLHIV stable on ART than ART-naïve PLHIV for at least two important reasons: (1) PLHIV who are stable on ART are often pre-screened, which progressively reduces the relative prevalence of untreated symptomatic versus untreated asymptomatic TB,[60] and (2) PLHIV stable on ART are better able to control TB disease and are more likely to have indolent disease, possibly with intermittent symptoms.[61,90,91]

In the Hamada *et al* meta-analysis, among PLHIV on ART, sensitivity of the WHO four-symptom screening rule was only 51% compared with 89% among PLHIV who had not yet started ART.[61] At a time when global health donors have committed to reaching over 13 million PLHIV on ART with TPT by 2021,[92] low sensitivity of the WHO four-symptom screening rule for active TB among PLHIV on ART is driving consideration of more sensitive screening approaches.[16] In addition, asymptomatic active TB can be present among PLHIV with advanced HIV disease,[38,94] and among pre-ART patients without advanced disease in high prevalence settings,[96] among whom missing asymptomatic active TB can have suboptimal health consequences for patients.[24]

Key advantages of our TB clinical score over the WHO four-symptom screening rule include that it can be used to prioritise sensitivity and NPV by choosing cut-offs that prioritise these features. Scenarios where high sensitivity and NPV should be prioritised to "rule out" active TB disease include: (1) prior to TB preventive therapy prescription and (2) at HIV care or ART enrolment in sub-Saharan Africa when prevalence of active TB is high and intensified TB case finding and treatment can reduce morbidity and mortality.[16] As reported in Chapter 7, choosing a clinical score cut-off of ≥2 increased the sensitivity by 3-15% and NPV by 0.3-1.7% compared with the WHO four-symptom screening rule across the four study populations (XPRES, XPHACTOR, TBFT, and Gugulethu cohorts). Notably, when the XPHACTOR dataset was restricted to clients on ART for >3 months, the improvement in clinical score sensitivity at cut-off ≥2 (80%) versus WHO four symptom screening rule sensitivity (69%) was more pronounced than in the unrestricted mixed ART-naïve and ART-experienced XPHACTOR dataset (87% screening sensitivity at cut-off ≥2 versus 80% with WHO 4-symptom screen), indicating the potential usefulness of the TB clinical score to increase sensitivity among PLHIV on ART, in situations where sensitivity in detecting TB disease needs to be prioritized. In addition, in the XPRES cohort, among HIV-TB patients who died by 6 months after enrolment, screening sensitivity of the TB score was 94% compared with 79% using the WHO four-symptom screen, indicating the potential utility of the clinical score in diagnosing asymptomatic TB earlier before ART initiation, with associated reductions in morbidity and possibly mortality.[209]

At the same time, among PLHIV stable on ART who have received a course of TB preventive therapy, the clinical score can be used to prioritize specificity and PPV, reducing the NNS to a level that remains cost-effective in a patient population at low risk of incident TB disease and death from incident TB disease.[54,89]

Although increases in NPV by using a clinical score cut-off of ≥2 instead of the WHO four-symptom TB screen are modest, ranging from 0.3% to 1.7% in our analysis, use of the clinical score cut-off of ≥2 during the proposed near-term TPT scale-up for 13 million

PLHIV[92] could potentially avoid 39,000 to 221,000 PLHIV with active TB being prescribed TPT. Recent 2018 WHO TB screening and TB preventive therapy guidelines for PLHIV,[88] and most global experts,[16] acknowledge the need for increased sensitivity and NPV in a TB screening tool prior to TB preventive therapy prescription due to the risk of fuelling emergence and possible spread of drug-resistant TB, and missed active TB which has associated morbidity and mortality implications.[16]

Comparative advantage of TB clinical score over WHO four-symptom TB screen plus chest radiography

To achieve, the needed increase in sensitivity and NPV of the TB screening rule, WHO advises use of chest radiography where available to supplement the four-symptom TB screen, especially among PLHIV on ART, based on data from the Hamada *et al* meta-analysis.[88] In the Hamada *et al* meta-analysis, among PLHIV on ART, adding chest radiography to the four-symptom TB screening rule increased sensitivity in detecting microbiologically confirmed TB from 51.0% to 84.6%, with an associated increase in NPV of 0.2% (at TB prevalence of 1% which was about the TB prevalence among ART-experienced PLHIV in the meta-analysis) but with a major loss of specificity from 70.7% to 29.8%.[61] Because of the marginal impact, the WHO guideline committee recommends that chest radiography should only be added as an additional investigation if it does not pose a barrier to the provision of preventive treatment for people living with HIV.[88]

As described in our manuscript, compared with the WHO four-symptom TB screen, our clinical TB score at ≥2 improved sensitivity among PLHIV on ART (from 69% to 80%) and NPV (by 0.3% at 3% TB prevalence), with some loss in specificity (from 72% with the WHO four-symptom screen to 60% with our TB clinical score cut-off). Given the similar improvements in sensitivity and NPV achieved with our TB clinical score compared with WHO's recommended approach of adding chest radiography to the four-symptom TB screen, and given the relative simplicity of our TB clinical score, our TB clinical scoring

approach is arguably much more feasible and less costly and could better facilitate global TB preventive therapy scale-up and End TB goals.

Significant challenges facing widespread use of chest radiography for TB screening in LMIC

Although, with the advent of filmless or digital radiography and the validation of computer assisted diagnosis (CAD) software that can perform as well as trained clinicians in detecting both symptomatic and asymptomatic pulmonary TB disease,[210,211] and although increasing costing data suggesting that a chest radiograph, interpreted through CAD software, could be affordable (e.g., $5-10/PLHIV screened) once infrastructure is in place and human resources trained,[211,212] there remain many significant barriers to widespread rollout of chest radiography for TB screening in the near-term (e.g., to meet 2021 TB preventive therapy scale-up targets),[92] and even over the next 10 years in the lead up to End TB 2030 goals.

These barriers to scale-up of radiography for TB screening purpose are reported in a recent WHO situational assessment and include: (1) intra-reader and inter-reader variability, (2) no abnormalities are definitive of TB and therefore specificity is low, (3) a universally accepted reporting system is lacking, (4) patients are exposed to ionizing radiation although this is decreasing as advances are made, (5) special equipment with constant source of electricity needed, (6) trained personnel are needed to operate the machine, (7) chest radiography is usually not available outside district referral hospitals in sub-Saharan Africa and most LMIC, and (8) out-of-pocket expenses for patients are often high.[213]

While barriers 1-3 are increasingly ameliorated by digital radiography advances and use of CAD, barriers 4-8 make widespread radiography rollout in LMIC settings, especially in sub-Saharan Africa, a notable challenge. Each barrier 4-8 is discussed below, with a brief concluding paragraph of how our TB clinical score approach is substantially more feasible.

Firstly, although the ionizing radiation associated with one chest radiograph is low (0.1 millisieverts (mSv)), which is equivalent to 10% of the annual accepted dose of ionizing radiation for the general public, and therefore the risk of poor outcomes such as cancer from repeat exposure to the ionizing radiation associated with repeat chest radiographs is extremely small, the WHO review reports that when a large number of individuals are exposed to repeat chest radiography, "the associated risks may still constitute a public health issue.[213] The WHO report also notes that children and pregnant women are especially vulnerable to ionizing radiation, with special considerations needed for these populations.[213] Notably, pregnancy was common at ART initiation in the XPRES cohort (16-32% across the XPRES phases) and continues to be relatively common in most countries of sub-Saharan Africa.[21,161]

Secondly, equipment maintenance in most sub-Saharan health facilities is a challenge since most health systems in sub-Saharan Africa do not receive the needed government health system spending due to competing priorities.[214] A recent review of government health sector spending found that most African governments have deprioritized health in government budgets.[214] Similarly, many countries in sub-Saharan Africa lack stable electricity supplies. For example, in Malawi, only 59% of HIV clinics are connected to the electricity grid and unscheduled power outages are a daily problem affecting almost all health facilities.[215] Similarly, most countries in sub-Saharan Africa have severe shortages of skilled healthcare workers.[216,217] Given the limited infrastructure and human resources for health most PLHIV would have to travel to the nearest location with radiography services with significant out-of-pocket expenses, increasing the risk of dropping out of the TB-HIV care cascades.[163] While mobile radiography units can bring chest radiography services closer to PLHIV receiving routine care, an upfront investment would be needed to buy the purpose-built mobile units, a recurring investment would be needed to maintain them, and a large number of mobile units would be needed because many health centres in high TB-HIV burden locations hold daily HIV clinics.[207] Therefore, adding radiography to

TB screening algorithms could increase the barrier to scale-up of TB preventive therapy and access to TB diagnostics and treatment.[16] While some authors have suggested that chest radiography might facilitate immediate empiric TB treatment in the absence of a microbiological diagnosis, similarly, the TB clinical score could be used to define cut-offs above which the PPV is sufficiently high that immediate empiric TB treatment might be warranted.[117]

Therefore, our TB clinical score, that incorporates six simple score components, is careful to use variables easily available already in a LMIC clinic, requires limited training to complete the score, includes objective data points that should limit inter-operator variability, and which carries the same screening accuracy improvements of adding more expensive radiography to WHO four-symptom TB screening and also allows opportunities for differentiated TB-HIV care algorithms, represents a more feasible approach to improving TB case finding and facilitating TB preventive therapy scale-up among PLHIV.

Comparative advantage of TB clinical score over other clinical scores

As described in the literature review, six studies and six TB clinical scores for PLHIV were reviewed, but all had limitations.[118,120-123,218] Firstly, only three TB clinical scores, the Thailand score,[119] Vietnam score,[120] and TBScore from Guinea Bissau,[122] represented a clinical score used as the first step of TB screening (as opposed to the second step after the WHO four-symptom screen completion among those who initially screened TB symptom positive). Therefore, only these three scores could potentially serve to increase TB screening sensitivity and NPV compared with the WHO four-symptom screening rule. In addition, none of these three clinical scores (and none of the six TB clinical scores reviewed) had been externally validated. Of the three first step scores, several other limitations limit their use compared with our TB clinical score. Additional weaknesses of the Thailand score include the potentially biased derivation study population (since all those in the study were already suspected of having TB as described in the Chapter 2 literature review section) and dependence on CD4.[218] Additional limitations of the

Vietnam score include use of CD4 and chest radiograph as potential variables in the combination score and inability to generate categories of TB risk that could inform differentiated care.[120] Additional limitations of the TBScore study from Bissau include use of a complex 13-level score, and inclusion of more subjective variables (auscultation and anaemic eyes) in the score.[122]

Summary contribution to the literature

Therefore, our study is the first to derive and externally validate a clinical score for active TB among both ART-naïve and ART-experienced PLHIV that does not rely solely on WHO TB symptom screening, and allows flexibility in choosing the desired sensitivity, specificity, NPV, PPV, and NNS across a range of cut-offs, depending on the setting, use-case scenario, and population served. Similarly, the screening tool's differentiation of three risk groups can be used to inform differentiated care in LMIC clinic settings, which could improve efficiency and potentially impact morbidity and mortality. Given the potential advantages of our TB clinical score over the WHO four-symptom screening approach with or without chest radiography and over other published TB clinical scores, our clinical score should be evaluated further in multiple settings to generate more data about its potential utility. Some potential evaluation approaches are described in the paragraph below.

Next steps to build the TB clinical score evidence base

Given the potential advantages of the TB clinical score over existing WHO recommendations for TB screening approaches, the screening tool should be further evaluated in the near term to establish its potential utility to facilitate TB case finding and TB preventive therapy scale-up. Even if further evaluations demonstrate that there are important advantages of our TB clinical score over current WHO-recommended approaches, basic health system strengthening (i.e., ensuring healthcare workers have time to complete the score, training, checklists, job aids, and mentoring and supervision) would all be needed to ensure the clinical score is actually implemented as well as associated algorithms.[161] One additional evidence-based approach that could support

implementation of the score would be to incorporate the score in national electronic medical records (EMR) capturing data for PLHIV in care. Even in the most resource-limited settings, sustainable EMR solutions with power requirements that can be met by widely available battery and simple inexpensive solar solutions are increasingly available.[207] For example, in Malawi, incorporating routine WHO four-symptom TB screening into the point-of-care, touchscreen EMR that supports care for 70% of PLHIV across the country helped increase completion of the TB screening step.[219] However, routine monitoring and quality checks will be needed to ensure the score is being actually completed, rather than rushed through or left blank as was observed in a large Kenyan programme supported by EMR.[169]

Additional research to validate the screening accuracy of the score prospectively and evaluate effectiveness is needed. For example, a parallel-group, pragmatic cluster-randomised trial could compare standard of care (i.e., use of the WHO four-symptom screening rule and case finding algorithms) with our TB clinical score and differentiated TB management algorithms, in terms of impact on patient-important outcomes like morbidity and mortality. If paired with a costing study, this evaluation could inform understanding of cost-effectiveness. Given the continued challenge of obtaining sputum samples from 40–50% of patients who screen positive for TB, and given the Xpert trial evidence of the importance of empiric TB treatment, such a trial could help evaluate empiric TB treatment approaches that are differentiated by TB risk.[117] Differentiated TB care algorithms informed by the TB clinical score could help clinicians by: (1) helping to standardize when to use TB diagnostics (such as on-site or off-site Xpert use),[220] (2) prompting empiric TB treatment in patient groups where PPV of active TB is sufficiently high and the patient is unable to produce sputum or delays in accessing a TB test are considered unacceptably long,[221] and (3) by facilitating optimal completion of empiric TB treatment guidelines such as prompting further investigation for non-TB causes of illness if there is no response to empiric TB therapy.[222,223]

8.2.6. Machine learning as an important tool in prognostic research if understand strengths and weaknesses

A recent Institute of Medicine report concluded that a diagnostic error will be made in the care of nearly every patient in the course of his or her lifetime,[224] and receiving the right diagnosis is critical to receiving appropriate care.[128] Because of its potential to improve diagnostic decision-making in general, use of machine learning technology in medicine continues to be a point of great optimism and significant research.[128,225] Given the recent speculation that artificial intelligence can transform the way medicine is practiced, some recent authors have cautioned the medical community to avoid the "hype" of inflated expectations, focus thoughtfully on the real strengths that machine learning can bring to a particular medical challenge in a particular setting.[226] In this section, I describe how use of machine learning in generating the TB clinical score conservatively maximized the strengths of machine learning compared with traditional generalised linear regression models, while also controlling for its weaknesses, and represents a novel contribution to the TB screening literature. In addition, a short paragraph on the broader implications of machine learning in TB diagnostic and treatment research is provided to further contextualize our contribution.

Contribution of our machine learning approach to TB screening literature

Few studies have attempted to use machine learning algorithms to generate TB screening approaches and none, based on a literature review, use machine learning to generate a simple clinical score for use among PLHIV in LMIC clinic settings. One recent analysis by Melendez *et al*, combined 14 clinical features with a chest radiograph computer-assisted diagnosis score of >60 to generate a predictive model for patients suspected of having TB regardless of HIV status to predict culture-confirmed TB, but this was a second step screening approach among symptomatic patients (98.5% had cough), not focused on TB screening among PLHIV, and was reliant on availability of a computer-run algorithm and digital radiography.[227] Three other studies have used classification and regression tree

analyses, which are a type of supervised machine learning, to generate decision trees for TB investigation in emergency departments,[228] in-patients,[229] and among smear-negative persons suspected of having TB.[230] However, none of these decision trees were specific for PLHIV, one included the need for chest radiography as part of the algorithm,[229] one required pre-screening with radiography before entering the decision tree algorithm,[230] and one included the need for point of care ultrasound.[228] No study had yet used a random forest machine learning approach to investigate importance of predictors for a simple clinical TB screen among PLHIV in LMIC, and the approach presented in this thesis contributes to the growing body of literature examining the role of machine learning in global health.[231]

Maximizing strengths and controlling for weaknesses of machine learning

As described in Chapter 7, a key strength of our approach was to use random forest machine learning to assess variable importance in discriminating TB risk because of the unique ability of machine learning approaches to detect potentially important non-linear relationships between covariates and outcomes.[127] Among machine learning models, random forest models are particularly strong at predicting categorical outcomes like our TB outcome.[158] As was described in the manuscript, BMI was identified as an important predictor via the random forest variable importance analysis. Although BMI as a multifractional polynomial transformed continuous term was eliminated from the backwards stepwise regression, BMI was retained in the final model because of the importance of BMI in discriminating prevalent active TB using the mean decrease in Gini analysis. This analysis indicated the importance of BMI in its ability to accurately split groups of patients into those who have or do not have prevalent active TB across the 1,000 decision trees examined in our random forest model. The high ranking of BMI according to mean decrease in Gini indicates the significant decrease in average, weighted decision tree node purity that occurred when BMI was removed from the possible list of predictor variables.[158]

Our approach also used rigorous traditional generalised linear regression modelling approaches and stringent external validation approaches to account for a common problem faced by machine learning prediction models, namely over-fitting on the training dataset with limited external generalizability.[128,225,226] Firstly, the generalised linear regression approach, while is less likely to generate prognostic models that fit perfectly to the training dataset due to relative dependence on assumptions of average linear or transformed linear associations between covariates and outcome, is also more likely to generate prognostic models with improved external generalizability.[225] The opposite characteristics were evident with the machine learning approach where we observed extremely high discrimination of the random forest model on the training data and a significant drop in discrimination on the validation data.

Secondly, our novel approach highlights the importance of a stringent validation approach for any predictive model, but especially those derived using machine learning.[127] At the analytic design stage, we purposefully split the dataset into northern and southern clinics in Botswana with a 50%:50% split, which is in line with more stringent validation approaches that help better assess generalizability of predictive models.[124,127] In reviewing the machine learning literature, most widely available training resources and publications use a *random* 75%:25% split to create training and validation datasets for random forest models,[232] and hopefully our analysis and approach increases awareness of the need for more stringent validation approaches as described by Altman *et al*.[124]

Other and future uses of machine learning in TB diagnostic and treatment research

By far the most common use to date of machine learning in TB diagnostics or treatment research, has been in developing chest radiograph interpretation algorithms.[233] In a recent systematic review of machine learning approaches, 53 papers that used machine learning to develop or validate chest radiograph interpretation algorithms were included,

with 40 papers focused on algorithm development and 13 on external validation of the algorithms.[233] Secondly, a systematic review of machine learning for infectious disease clinical decision support included nine studies that developed automated, computer-driven algorithms to inform clinician decision-making about TB diagnosis and treatment, but none of these algorithms were documented to have been used or validated in real-world clinic settings.[234] Of all 60 machine-learning decision-support tools for infectious disease care identified in the systematic review, only three had documentation of use and evaluation in the real-world clinical setting.[234] Other uses of machine learning for TB diagnosis and treatment include in drug discovery,[235] predicting phenotypic drug resistance from genotypes,[236] and evaluation of antigens in serum as predictors of active TB in attempts to develop non sputum-based diagnostic tools.[237]

Summary of current usefulness of machine learning in TB diagnostics

While the long-term future might hold promise for machine learning diagnostic support for TB and new treatment identification, in the short term, utilizing the strengths of machine learning to help with simple prognostic scores, immediately applicable in LMIC settings, may be the most practical way to use the power of machine learning to improve patient-centred care in the reality of LMIC clinic settings.

8.2.7. Simple screening tools needed for precision public health plus strong health systems that implement them

Three over-arching principles that emerge from this thesis include (1) the need for development and validation of new and improved screening tools to detect which patients are at risk of outcomes such as early ART mortality and active TB disease, (2) the largely untapped potential for stratified risk scores to inform risk-appropriate differentiated service delivery models, and (3) the need to strengthen health systems to implement the full screening, diagnosis, and care algorithms. While all three principles are basic and

fundamental to public health approaches for care delivery in resource-constrained settings, this thesis showed many low-hanging fruits for improvement in these areas.

Firstly, the thesis shows that both WHO eligibility criteria for early ART care intensification and the four-symptom TB screening rule can be improved by thoughtful development of clinical scores using easily available variables in LMIC clinic settings. Notably, clinical scoring systems have not yet been carefully considered in the WHO guideline development process for either early ART care intensification or recent WHO TB screening guidelines.[44,88] The clinical screening tools developed in this thesis and the data provided suggest such tools should be considered in future WHO guideline development processes.

Secondly, opportunities to tailor simple care algorithms to stratified risk categories identified by clinical scores have not yet been explored widely in the area of early ART care intensification or differentiated TB-HIV care. For example, only one algorithm-guided TB case finding and treatment approach suitable for LMIC clinic settings has been evaluated by Grant *et al*,[117] compared with eight clinical trials examining potential impact of the Xpert diagnostic device.[146,161,200] In the case of advanced HIV disease guidelines, no approach to stratify risk groups into low-, moderate-, and high-risk, with associated risk-appropriate care packages has yet been evaluated. Differentiating care according to holistic risk categories has the opportunity to increase impact of the limited available resources through a precise public health approach.[238,239]

Thirdly, investments in health system strengthening to implement recommended TB screening approaches and retaining clients in TB-HIV care was shown to have significant impact on patient-important outcomes.[161] This thesis provides further insight that "leaky" TB screening and TB-HIV care cascades might be the driving force behind unacceptably high rates of HIV mortality due to undiagnosed TB or TB diagnosed late, while also providing evidence of the effectiveness of health system strengthening interventions to

improve completion of these TB screening and TB-HIV care cascades in sub-Saharan Africa.[161]

8.3. Limitations and strengths

In addition to the limitations and strengths described in the manuscripts (Chapters 5, 6, and 7), the following section describes efforts at thoroughly evaluating the potential for biases and residual confounding, which can affect internal validity, and the generalizability of study findings (i.e., external validity).

8.3.1. Evaluation of thesis limitations

Healthcare access or referral filter bias, a type of selection bias

The internal validity of a trial can be affected by several biases, which should be considered at the design stage.[240] At the time XPRES enrolled patients, national guidelines recommended ART initiation for PLHIV at CD4 count <350/μL, WHO stage III/IV, or for pregnant women regardless of CD4 count or WHO stage.[152] Some of the HIV clinic enrolees at the five large district hospitals included in XPRES, and to a lesser extent at the remaining 17 XPRES primary healthcare clinics, were referred for HIV treatment initiation after a period of CD4 count monitoring during pre-ART care at smaller health posts, with referral for ART initiation when the eligibility threshold was reached.

This means that a section of the XPRES study enrolee population represents a population that should have been pre-screened for TB. In some papers this is referred to as healthcare access bias or referral filter bias;[240] in the case of XPRES it raises the need to consider how XPRES HIV clinic enrolees might be different to all PLHIV at the point of HIV diagnosis and how findings might be different if the study population had not been pre-screened at all. For example, in XPRES 359 (4%), 44 (2%), and 122 (3%) of SOC, EC, and EC+X enrolees respectively were diagnosed with TB and had started TB treatment prior to

arrival at the study clinic. Ultimately, an additional 129 (1%), 86 (5%), and 244 (6%) enrolees in the SOC, EC, and EC+X phases were newly diagnosed with TB and started TB treatment before ART initiation or during the first 6 months of ART after study clinic enrolment.[161]

In terms of potential impact on the primary manuscript outcome findings, we might have observed an even higher impact of the TB screening and case finding intervention in a population of PLHIV that was previously completely unscreened for TB symptoms.[200] In terms of impact on the screening sensitivity of the WHO four-symptom TB screen, we might have observed a higher screening sensitivity (79%-89% according to the Getahun and Hamada meta-analyses), than the sensitivity we did observe in XPRES at the HIV clinic enrolment visit (73%).[60,61] However, our study and other studies show that TB symptom screening is generally done poorly in LMIC clinic settings.[161] In addition, if the whole population of XPRES had been thoroughly pre-screened for TB symptoms we would have expected sensitivity of the four-symptom TB screen to be even lower (41% according to the Getahun meta-analysis) than what we observed (73%).[60] Therefore, the data suggest the impact of any pre-screening might have been minimal on the key trial findings.

Survivor bias, a type of selection bias

Notably, because of the delay between HIV diagnosis and ART initiation due to ART eligibility guidelines in place at the time of study enrolment (2010–2014), our population of XPRES study enrolees represents those who had survived and been retained in the pre-ART time period after HIV diagnosis, with the need to consider the possibility of survivor bias in terms of the characteristics of patients enrolled versus characteristics of PLHIV at the point of HIV diagnosis.[240] However, each study population (SOC, EC, and EC+X) would have been equally affected by any survivor bias, which means comparisons between phases in the primary outcome paper (Chapter 5) remain internally valid to the population studied, but care should be taken to generalize findings to PLHIV populations at the time of HIV diagnosis.

Observer bias

Inherent to all Xpert impact trials is that clinics and healthcare workers where the study was being implemented were aware that Xpert is a more sensitive diagnostic test for detecting culture-positive TB than smear microscopy since this was the rationale for WHO and national guidelines for Xpert rollout.[18,77] Knowledge of this superior diagnostic accuracy may have increased the rates of empiric TB treatment in the EC phase and decreased rates of empiric TB treatment in the EC+X phase, which could be considered a type of Hawthorne effect.[151,240] However, it seems likely that any potential imbalance in likelihood of empiric TB treatment between situations where microscopy is the TB diagnostic and Xpert is the TB diagnostic would persist in the real-world,[241] and therefore the finding of no sizable impact of Xpert versus smear microscopy in reducing mortality among PLHIV is generalizable to real-world settings in sub-Saharan Africa.[146,151] In other words, if there had been feasible and ethical way to blind clinicians to which diagnostic test was being used, the study could have eliminated the potential for this particular observer bias, but then lost external validity (i.e., the ability to generalize findings to the real world). In addition, by blinding the clinician to which diagnostic was used, rates of empiric TB treatment might have been higher in both phases with clinicians erring on the side of caution and assuming that smear microscopy was the diagnostic test being used.

Contamination bias

Contamination bias occurs when the intervention affects the standard of care comparison group or standard of care affects the intervention group. In XPRES, none of the ART enrolees in the last six months of the SOC phase would have received the TB screening and retention intervention, because this was administered by the study nurse for study enrolees only. However, all healthcare providers at study clinics received a training on TB case finding at EC phase initiation and this may have benefited a few SOC enrolees who screened positive for TB symptoms during their follow-up which overlapped with the EC phase. Similarly, enrolees who started ART in the EC phase but screened positive for TB

symptoms in the EC+X phase during study follow-up, may have benefited from Xpert. Therefore, we implemented several pre-specified sensitivity analyses to assess the potential for this contamination, firstly by censoring follow-up time at the crossover in phases, and secondly by assigning follow-up time to the phase in which it occurred. For the SOC versus EC+X comparison, there was no change in effect size or direction when implementing this sensitivity analysis suggesting there was no contamination bias affecting our primary intervention effect analysis.[161]

However, for the EC vs. EC+X comparison, within the stepped-wedge portion of the trial, we did observe some changes in effect size, although 95% confidence intervals around the adjusted hazard ratios always included 1.0. For example, 6-month mortality rates were similar between the EC (6.5/100 person-years) and EC+X phases (6.3/100 person-years) in the pre-specified primary analysis where all follow-up time was assigned to the phase in which the patient started ART (AHR 1.13, 95% CI, 0.63-2.03). However, in sensitivity analyses comparing EC vs. EC+X 6-month mortality rates, the AHR was 0.90 (95% CI 0.42-1.95) when EC enrolee follow-up time was censored at the time of EC+X cross-cover, and 0.79 (95% CI 0.41-1.50) when EC enrolee follow-up time in the EC+X phase was assigned to the EC+X phase using a time-dependent variable. As stated in the discussion section of Chapter 5, this might indicate a modest Xpert impact on 6-month ART mortality that our study was not powered to detect[161] and which might be more feasibly detected in large meta-analyses.[204]

In addition, when possible, sputum samples were sent for culture in both EC and EC+X phases to help answer the second co-primary objective (not reported in this thesis), which was to compare sensitivity of the microscopy- versus Xpert-based TB diagnostic algorithms. However, only 12 (0.7%) of 1,768 EC enrolees and 16 (0.4%) of 4,215 enrolees in the EC+X phase received a TB diagnosis based on culture alone, suggesting any impact of culture on mortality outcomes between phases was very minimal.

Residual confounding

As described in the primary outcome analysis, a key limitation of the trial design is that the observed reduction in all-cause mortality between SOC and subsequent EC and EC+X phases represents a pre- versus post-comparison, rather than a randomised comparison, and is therefore at risk of residual confounding.[240] Multivariable proportional hazards regression was used to account for measured confounders, however we cannot exclude the possibility that there were unmeasured confounders affecting the pre- versus post-comparison.[240,242] Therefore, the retrospective SOC phase is both a weakness (because there was no randomised comparison and limited ability to change what covariates were collected from the retrospective phase for comparison with the prospective phase), but also a strength because it allows comparison of the intervention package with a true standard of care to allow estimation of effectiveness in the real-world.[203]

Potential impact of changing ART guidelines on generalisability

As described in the manuscript, our study enrolment occurred prior to adoption of universal test-and-treat guidelines which were adopted in Botswana in 2016 and have been implemented in most of sub-Saharan African since 2016, with gradual increases in median CD4 count at ART initiation.[161] As stated in the manuscript (Chapter 5), the absence of an interaction between CD4 count at ART initiation and intervention package effect size suggests that ICF and retention interventions are still important for all new HIV clinic enrolees, not just those with more advanced disease.[28,44] Therefore, the main trial findings still support current WHO recommendations that high quality implementation of TB screening and case finding and retention interventions remain important for all HIV clinic enrolees, even in the era of test-and-treat.[161]

Generalisability of early ART mortality and HIV-associated TB risk scores

An important limitation of the early ART mortality risk scores is that they have not yet been validated in a cohort enrolled under HIV test-and-treat guidelines, something which is planned in the near future.

Another limitation of both the early ART mortality risk scores and TB risk score is the fact that while the gender variables are relevant in sub-Saharan Africa and many resource-limited settings, the association of male gender with early ART mortality risk and TB is not generalizable to cohorts in resource-rich settings like the U.S. and Europe, where males often have better outcomes than female ART enrollees.[243]

For the early ART mortality risk scores, these screening tools were validated in trial cohorts that received relatively intensive TB screening and treatment services, and therefore those that died did so despite access to these services.[161] Evaluation of early ART risk score screening accuracy in cohorts of adult ART enrollees starting HIV treatment in the current era of "treat all" would help evaluate this limitation.

Feasibility of risk scores

Although the specificity of the early ART mortality risk scores is superior to the WHO advanced disease eligibility criteria, still a substantial percentage of ART enrollees (36-38% in the XPRES cohort) would be screened into receiving an advanced disease care package, which would require a pilot with an associated monitoring system to assess implementation fidelity and feasibility.

For the TB risk score, if the cut-off is set at ≥2, the goal is to increase sensitivity in detecting asymptomatic TB, with a resulting loss of specificity. Such a cut-off would be suitable for a population of PLHIV starting ART or immediately prior to TB preventive therapy prescription.[61] In these scenarios, the volume of patients requiring a TB test, probably with Xpert, would increase compared with a screening approach using the WHO four-symptom screening rule, and feasibility and cost-effectiveness of the approach needs

to be evaluated.[61] Arguably, by using a cut-off of >10 for those PLHIV stable on ART who have already received TB preventive therapy, this differentiated approach discussed in Chapter 5 will help improve feasibility and cost-effectiveness of the proposed TB clinical score by reducing the number of TB tests needed in this stable and low risk population compared with the WHO four-symptom TB screen, but this approach needs to be evaluated.

Outcome ascertainment error

For the early ART mortality risk score analysis, there was almost perfect ascertainment of the all-cause 6-month ART mortality outcome in both XPRES and TB Fast Track, so outcome ascertainment error would not have affected the early ART mortality risk score development.

However, within the TB risk score analyses, (1) TB case finding approaches were different across the four cohorts and (2) for the XPRES and XPHACTOR cohorts, a clinical definition of TB was included in the TB outcome definition, whereas for TBFT and Gugulethu cohorts, results of enrollment sputum collection for TB culture and Xpert were used to define the TB outcome. However, model results and risk score screening accuracy did not change significantly when we restricted the TB outcome in XPRES and XPHACTOR datasets to microbiologically confirmed TB.

Risk score effectiveness assessments needed

As described in the recommendation section below (8.3), even if screening accuracy of the risk scores is shown to be superior to the WHO-recommended standard of care, effectiveness of the risk scores and associated care algorithms on patient-important outcomes like mortality is needed to inform future scale-up.

8.3.2. Evaluation of thesis strengths

Many of the study strengths have been described in the published manuscripts and in previous sections of the thesis and these are briefly summarized below.

External validity – generalisability

As described in section 8.1.2, we believe most study features allow the study findings to be broadly generalizable to other settings in sub-Saharan Africa, with these features including: (1) purposive selection of study sites to be representative of HIV clinics in Botswana, (2) inclusive eligibility criteria for the trial with minimal exclusion criteria to ensure the study population was representative of the real-world HIV clinic patient population, (3) a census approach to trial recruitment (i.e., the aim was to enrol all eligible trial enrolees to obtain a representative study population), (4) no discernible difference between those enrolled versus not enrolled in the study, (5) no effect of inverse probability weighting approaches to account for non-enrolment on primary study outcomes, (6) the practical nature in which the intervention was designed and implemented, (7) the way outcomes were compared between intervention phases and an untouched historical standard of care phase, and (8) using an intention-to-treat analysis.

Accurate ascertainment of primary outcome, complete data, large sample size

Other study strengths include: (1) novel trial design to fill knowledge gaps not addressed by other Xpert impact trials as discussed in section 6.2.2., (2) large sample size, (3) almost perfect ascertainment of the primary outcome (early ART mortality), and (4) nearly complete data for key covariates so that adjustment could be conducted without loss of power and without introducing risk of differential measurement error.

Clinical score analyses validated externally

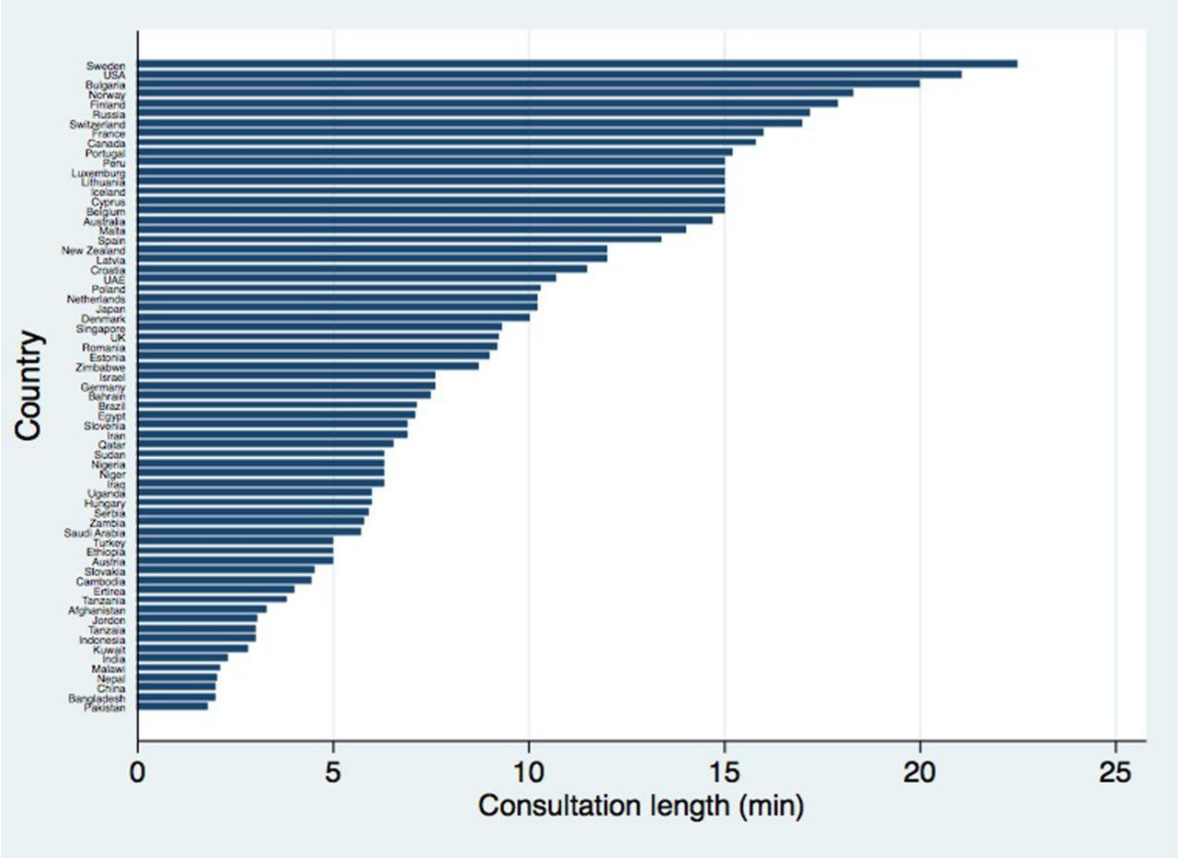
Key strengths of the clinical score analyses include many of the same strengths above as well as the extensive external validation of clinical score screening accuracy in datasets from South Africa, and use of both established and new analytic techniques to generate practical useful scores for sub-Saharan Africa.

8.4. Reflective commentary and practical lessons learned

8.4.1. Reflective commentary

Like many clinicians working in sub-Saharan Africa during the early 2000's, the experience of caring for and losing patients to preventable and treatable diseases like HIV and TB has been a motivating factor for subsequent research and public health work. Although XPRES study planning began in 2011, its intervention design was informed both by prior clinical work in South Africa during 2003–2007, and observational studies conducted in sub-Saharan Africa between 2007 and 2011.[28] By 2011, many considered Xpert to be a game changer for TB-HIV care with the estimation that hundreds of thousands of lives would be saved as Xpert was scaled up across Southern Africa.[81] Experience from clinical work helped provide insight into prevalent health system weaknesses in LMIC, with clinicians often forced to limit the length of clinical care interactions to get through the volume of patients seeking care.[244] For example, from a meta-analysis of data from 67 countries, about 50% of primary care interactions last less than 5 minutes, with national averages as low as 2 minutes/patient consultation in Malawi and 48 seconds/patient consultation in Bangladesh (Figure).[244] The length of the consultation is directly correlated with both the clinician-to-population ratios and per capita spending on health.[244]

Figure 8.1. Average length of primary care consultation by country (taken from Irving *et al*)[244]



Both the clinical insight gained from working in LMIC clinic settings, and the operational research which showed persistently incomplete performance of basic care functions like completing a four-symptom TB screen in sub-Saharan countries trying to expand TB-HIV care,[62,98,99] informed the rationale behind the health system strengthening component of the XPRES TB screening and retention package. The thesis findings have helped highlight the importance of health system strengthening, which, depending on the public health problem being addressed in LMIC, might need to be prioritised ahead of, or at least combined with, scale-up of any new technology.

Another reflection from both clinical work, prior operational research, and this thesis is the importance of prompting busy clinicians to "think TB" as part of a differential diagnosis, due to the protean manifestations of this disease in clinical practice, especially among clients living with HIV, and the importance of appropriate empiric TB treatment for

those at high risk of having active TB even if a microbiological diagnosis is not possible. This thesis provides new tools to potentially enable more accurate TB screening, and new methods to facilitate screening tool implementation in LMIC clinic settings.

8.4.2. Practical lessons learned

A key practical lesson learned in conducting this research, is the difficulty associated with conducting research using sputum-based diagnostics in LMIC clinic settings. As was described in Chapter 5, although the percentage of enrolees screening positive for at least one TB symptom who provided one or more sputum samples increased from 38% in the SOC phase to 46% and 55% in the EC and EC+X phases, respectively, collection of sputum samples remained a challenge even in the EC phases despite intensive interventions.[18] In retrospect, I might have suggested inclusion of sputum-independent TB tests, such as either testing for urinary lipoarabinomannan (LAM) (e.g., with the Alere Determine TB-LAM Antigen test) or Xpert testing of urine if I had better anticipated the challenge obtaining sputum samples from symptomatic patients. However, inclusion of this additional intervention would have made it more challenging to fully understand which part of the intervention package impacted mortality. In addition, other studies such as the STAMP trial, although conducted purely among hospitalised patients, have shown the value of these urinary diagnostics and have changed WHO guidelines.[245]

Another practical lesson learned was how challenging it was to implement a large trial in routine LMIC settings, from the conceptualization and protocol writing process,[18] through training of study nurses and health facilities involved, data entry and management tool development, monitoring implementation both in-country and remotely, preparation of quarterly progress reports for funders, tracking expenditure of available funds and applying for additional funding to complete the trial, painstaking review of central mortality registers, data management, analysis, conference presentations,[246] and

writing.[161] However, the experience also highlighted the importance of trials to solidify the evidence base for changes that need to occur to improve care for patients.

8.5. Summary recommendations

The table below provides a summary of key findings, insights and recommendations gleaned from the thesis.

Table 8.3. Summary of key recommendations in this thesis

Area	Recommendation	Chapters
HIV-TB programme implementation	1. HIV-TB programmes should prioritise health system strengthening interventions to improve completion of TB screening, case finding and TB-HIV retention cascades to reduce all-cause, early ART mortality in sub-Saharan Africa.	
	1.1. Tracing all PLHIV who missed an appointment during the first 6-12 months of ART was a feasible and effective retention intervention to reduce rates of loss to follow-up by 82-95% and should be considered for scale-up as standard of care in LMIC settings.	2.2., 5, and 8.2.1.
Needed research	1.2. Cost-effectiveness analysis of implementing the health system strengthening interventions compared with standard of care.	
Early ART care intensification at health facilities	2. HIV programmes and regulatory bodies such as the WHO should consider clinical scores as one approach to inform who needs early ART care intensification in future guideline development processes.	
	2.1. Health facilities in sub-Saharan Africa <u>lacking access</u> to rapid on-site or off-site CD4 count testing should consider initially validating and then using the CD4-independent clinical score, instead of WHO advanced HIV disease eligibility criteria, to inform who needs intensification of early ART care. Potential advantages of the clinical score include: (1) a nearly two-fold increased sensitivity in detecting early ART mortality risk compared with using WHO stage alone, (2) improved specificity compared with the full WHO advanced HIV disease eligibility criteria, and (3) improved ability to differentiate risk into low-, moderate-, and high-risk groups.	2.3., 6, and 8.2.4.
	2.2. Health facilities in sub-Saharan Africa <u>that have access</u> to rapid on-site or off-site CD4 count testing should consider initially validating and then using the CD4-dependent clinical score, instead of WHO advanced HIV disease eligibility criteria, to inform who needs intensification of early ART care. Potential advantages of the clinical score include: (1) similar or improved sensitivity in detecting early ART mortality risk, (2) improved specificity compared with the full WHO advanced HIV disease eligibility criteria, and (3) improved ability to differentiate risk into low-, moderate-, and high-risk groups.	

Needed research	2.3. External validation of the CD4-dependent and -independent clinical score in currently available datasets including PLHIV starting ART after rollout of "treat all" WHO guidelines.	
	2.4. Trial to assess early mortality impact and cost-effectiveness of using the CD4-independent and -dependent scores instead of WHO advanced HIV disease criteria to inform who needs: (a) the current standard of care for early ART care intensification (i.e., the WHO advanced HIV disease care package), and, (b) a new more differentiated package of care for moderate-, and high-risk categories.	
TB screening among PLHIV	3. HIV-TB programmes in sub-Saharan Africa should consider initially validating and then using the TB clinical score instead of the WHO four symptom TB screen to facilitate TB case finding and preventive therapy scale-up. Potential advantages of the clinical score include: (1) flexibility to choose cut-offs depending on the population and use-case scenario (e.g., prioritising increased sensitivity and NPV among new HIV clinic enrolees and among PLHIV prior to TB preventive therapy initiation and prioritising specificity and PPV among PLHIV stable on ART who have already received TB preventive therapy), (2) increased ability to detect asymptomatic TB, and (3) increased opportunity to differentiate risk into low-, moderate-, and high-risk groups.	2.4., 7., and 8.2.5
Needed research	3.1. Research to validate the screening accuracy of the score prospectively in a direct comparison with the WHO recommended four-symptom screen with and without chest radiography.	
	3.2. Trial to assess impact on patient important outcomes of using the TB clinical score instead of the WHO four-symptom screening rule to implement TB case finding, differentiated TB-HIV care for moderate-, and high-risk categories, and TB preventive therapy for those who screen negative.	

Abbreviations: TB, tuberculosis; WHO, world health organisation; PLHIV, people living with HIV; LMIC, low- and middle-income countries; ART, antiretroviral therapy; NPV, negative predictive value; PPV, positive predictive value

8.6. Conclusions

Leaks in the TB screening and TB-HIV care cascades appear to be the biggest drivers of HIV-related mortality due to undiagnosed TB or TB diagnosed late in sub-Saharan Africa. Health system strengthening to address these leaks should be prioritised by clinicians, TB-HIV programme managers, governments, and global health funders in the field of TB and HIV. In XPRES, interventions to support TB screening and active tracing for patients missing clinic appointments drove increased TB case finding and reduced loss to follow-up with associated reductions in early ART mortality.

The CD4-independent and -dependent risk scores are the first externally validated clinical scores for ART care intensification generated for sub-Saharan Africa. Sensitivity of the CD4-independent risk score with cut-off set at ≥4 was nearly twice that of WHO stage in predicting 6-month mortality and could be used in settings lacking CD4 testing to inform ART care intensification. Compared with the WHO advanced HIV disease eligibility criteria, the CD4-independent clinical score also had higher specificity and would screen 8-26% fewer ART enrollees into intensified care pathways, suggesting the screening tool could also increase efficiency of investments in differentiated service delivery models for advanced HIV disease.

In those settings where CD4 is available, using the CD4-dependent clinical score with a cut-off score of ≥5 could increase both sensitivity and specificity over WHO advanced disease eligibility criteria, with the potential to both reduce early ART mortality and improve efficiency of differentiated service delivery algorithms. Therefore, both the CD4-independent and -dependent clinical scores should be considered for scale-up to facilitate early ART care intensification in sub-Saharan Africa, with the potential for reductions in early ART mortality.

Advantages of the TB clinical score over the WHO four-symptom TB screen are that it has capacity to detect asymptomatic TB, has screening accuracy characteristics that indicate potential suitability for both ART-naïve and ART-experienced populations, allows flexibility in

choosing the desired sensitivity, specificity, NPV, PPV, and NNS across a range of cut-offs, depending on the setting, use-case scenario, and population served, and can differentiate three risk groups, which can be used to inform differentiated care in LMIC clinic settings, with the potential to improve efficiency and potentially impact morbidity and mortality.

Overall, the literature reviews including the Xpert systematic review, the XPRES trial, and the risk score analyses highlight the importance of considering the operational capacity of HIV and TB clinics in sub-Saharan Africa, where most clinics are under-resourced and operate within weak health systems. Within these under-resourced clinic environments, interventions to (1) strengthen the health system to do the basics right, and (2) provide simple, feasible, accurate screening tools, can potentially have a bigger impact on mortality than introducing new diagnostic tools, which might not be tailor-made for LMIC settings. Simple innovations to screening tools, through improving the clinician's understanding of holistic risk, and informing differentiated early ART and TB management algorithms, can maximize impact of available resources through a more precise and feasible public health approach.

9. References

1. UNAIDS. AIDSinfo. Available at: http://aidsinfo.unaids.org/. Accessed June 5, 2020.
2. Karat AS, Omar T, von Gottberg A, et al. Autopsy Prevalence of Tuberculosis and Other Potentially Treatable Infections among Adults with Advanced HIV Enrolled in Out-Patient Care in South Africa. *PLoS One* 2016; **11**(11): e0166158.
3. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. *AIDS* 2015; **29**(15): 1987-2002.
4. Group ISS, Lundgren JD, Babiker AG, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med* 2015; **373**(9): 795-807.
5. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011; **365**(6): 493-505.
6. Tanser F, Barnighausen T, Grapsa E, Zaidi J, Newell ML. High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa. *Science* 2013; **339**(6122): 966-71.
7. UNAIDS. AIDS by the numbers: 2015. Available at: http://www.unaids.org/sites/default/files/media_asset/AIDS_by_the_numbers_2015_en.pdf. Accessed February 10, 2016.
8. UNAIDS. 90-90-90: An ambitious treatment target to help end the AIDS epidemic. Available at: http://www.unaids.org/sites/default/files/media_asset/90-90-90_en_0.pdf. Accessed Apil 10, 2020.
9. UNAIDS. Fast-Track - Ending the AIDS epidemic by 2030. Available at: https://www.unaids.org/en/resources/documents/2014/JC2686_WAD2014report. Accessed January 6, 2020.
10. Bekker LG, Alleyne G, Baral S, et al. Advancing global health and strengthening the HIV response in the era of the Sustainable Development Goals: the International AIDS Society-Lancet Commission. *Lancet* 2018; **392**(10144): 312-58.

11. Osler M, Hilderbrand K, Goemaere E, et al. The Continuing Burden of Advanced HIV Disease Over 10 Years of Increasing Antiretroviral Therapy Coverage in South Africa. *Clin Infect Dis* 2018; **66**(suppl_2): S118-S25.

12. Holmes CB, Sikazwe I, Sikombe K, et al. Estimated mortality on HIV treatment among active patients and patients lost to follow-up in 4 provinces of Zambia: Findings from a multistage sampling-based survey. *PLoS Med* 2018; **15**(1): e1002489.

13. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* 2008; **22**(15): 1897-908.

14. WHO. Global Tuberculosis Report - 2019. Available at: https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf?ua=1.

15. WHO. TB and HIV, and other comorbidities. Available at: https://www.who.int/tb/areas-of-work/tb-hiv/en/. Accessed June 5, 2020.

16. Harries AD, Schwoebel V, Monedero-Recuero I, et al. Challenges and opportunities to prevent tuberculosis in people living with HIV in low-income countries. *Int J Tuberc Lung Dis* 2019; **23**(2): 241-51.

17. Brennan AT, Long L, Useem J, Garrison L, Fox MP. Mortality in the First 3 Months on Antiretroviral Therapy Among HIV-Positive Adults in Low- and Middle-income Countries: A Meta-analysis. *J Acquir Immune Defic Syndr* 2016; **73**(1): 1-10.

18. Auld AF, Agizew T, Pals S, et al. Implementation of a pragmatic, stepped-wedge cluster randomized trial to evaluate impact of Botswana's Xpert MTB/RIF diagnostic algorithm on TB diagnostic sensitivity and early antiretroviral therapy mortality. *BMC Infect Dis* 2016; **16**(1): 606.

19. Gupta A, Nadkarni G, Yang WT, et al. Early Mortality in Adults Initiating Antiretroviral Therapy (ART) in Low- and Middle-Income Countries (LMIC): A Systematic Review and Meta-Analysis. *PLOS One* 2011; **6**(12): e28691.

20. Brinkhof MW, Pujades-Rodriguez M, Egger M. Mortality of patients lost to follow-up in antiretroviral treatment programmes in resource-limited settings: systematic review and meta-analysis. *PLoS One* 2009; **4**(6): e5790.

21. Auld AF, Shiraishi R, Couto A, et al. A Decade of Antiretroviral Therapy Scale-up in Mozambique: Evaluation of outcome trends and new models of service delivery among more

than 300,000 adults starting antiretroviral therapy in Mozambique during 2004-2013. *J Acquir Immune Defic Syndr* 2016; **73**(2): e11-22.

22. Boulle A, Schomaker M, May MT, et al. Mortality in patients with HIV-1 infection starting antiretroviral therapy in South Africa, Europe, or North America: a collaborative analysis of prospective studies. *PLoS Med* 2014; **11**(9): e1001718.

23. Braitstein P, Brinkhof MW, Dabis F, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. *Lancet* 2006; **367**(9513): 817-24.

24. Lawn SD, Harries AD, Wood R. Strategies to reduce early morbidity and mortality in adults receiving antiretroviral therapy in resource-limited settings. *Curr Opin HIV AIDS* 2010; **5**(1): 18-26.

25. Wong EB, Omar T, Setlhako GJ, et al. Causes of death on antiretroviral therapy: a post-mortem study from South Africa. *PLoS One* 2012; **7**(10): e47542.

26. May M, Boulle A, Phiri S, et al. Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. *Lancet* 2010; **376**(9739): 449-57.

27. Kitahata MM, Gange SJ, Abraham AG, et al. Effect of early versus deferred antiretroviral therapy for HIV on survival. *N Engl J Med* 2009; **360**(18): 1815-26.

28. Auld AF, Shiraishi RW, Oboho I, et al. Trends in Prevalence of Advanced HIV Disease at Antiretroviral Therapy Enrollment - 10 Countries, 2004-2015. *MMWR Morb Mortal Wkly Rep* 2017; **66**(21): 558-63.

29. IeDea, Collaborations ARTC, Avila D, et al. Immunodeficiency at the start of combination antiretroviral therapy in low-, middle-, and high-income countries. *J Acquir Immune Defic Syndr* 2014; **65**(1): e8-16.

30. Cornell M, Grimsrud A, Fairall L, et al. Temporal changes in programme outcomes among adult patients initiating antiretroviral therapy across South Africa, 2002-2007. *AIDS* 2010; **24**(14): 2263-70.

31. Johnson LF, May MT, Dorrington RE, et al. Estimating the impact of antiretroviral treatment on adult mortality trends in South Africa: A mathematical modelling study. *PLoS Med* 2017; **14**(12): e1002468.

32. Farahani M, Price N, El-Halabi S, et al. Trends and determinants of survival for over 200 000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. *AIDS* 2015.

33. Farahani M, Mulinder H, Farahani A, Marlink R. Prevalence and distribution of non-AIDS causes of death among HIV-infected individuals receiving antiretroviral therapy: a systematic review and meta-analysis. *Int J STD AIDS* 2016.

34. Smith CJ, Ryom L, Weber R, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet* 2014; **384**(9939): 241-8.

35. Karat AS. An autopsy study exploring the spectrum of disease in individuals with advanced HIV in primary care clinics in South Africa. PhD (research paper style) thesis, London School of Hygiene & Tropical Medicine. DOI: https://doi.org/10.17037/PUBS.04646134. Accessed June 5, 2020.

36. IeDea, Collaborations CC. Global Trends in CD4 Cell Count at the Start of Antiretroviral Therapy: Collaborative Study of Treatment Programs. *Clin Infect Dis* 2018; **66**(6): 893-903.

37. Arinze F, Gong W, Green AF, et al. Immunodeficiency at Antiretroviral Therapy Start: Five-Year Adult Data (2012-2017) Based on Evolving National Policies in Rural Mozambique. *AIDS Res Hum Retroviruses* 2020; **36**(1): 39-47.

38. Lamp K, McGovern S, Fong Y, et al. Proportions of CD4 test results indicating advanced HIV disease remain consistently high at primary health care facilities across four high HIV burden countries. *PLoS One* 2020; **15**(1): e0226987.

39. WHO. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Available at: http://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/. Accessed October 19, 2020.

40. Rosen S, Fox MP. Retention in HIV Care between Testing and Treatment in Sub-Saharan Africa: A Systematic Review. *PLoS Med* 2011; **8**(7): e1001056.

41. Hensen B, Taoka S, Lewis JJ, Weiss HA, Hargreaves J. Systematic review of strategies to increase men's HIV-testing in sub-Saharan Africa. *AIDS* 2014; **28**(14): 2133-45.

42. Lawn SD, Harries AD, Williams BG, et al. Antiretroviral therapy and the control of HIV-associated tuberculosis. Will ART do it? *Int J Tuberc Lung Dis* 2011; **15**(5): 571-81.

43. O'Connor J, Vjecha MJ, Phillips AN, et al. Effect of immediate initiation of antiretroviral therapy on risk of severe bacterial infections in HIV-positive people with CD4 cell counts of more than 500 cells per muL: secondary outcome results from a randomised controlled trial. *The lancet HIV* 2017; **4**(3): e105-e12.

44. WHO. Guidelines for Managing Advanced HIV Disease and Rapid Initiation of Antiretroviral Therapy. Geneva; 2017.

45. Johnson LF, Rehle TM, Jooste S, Bekker LG. Rates of HIV testing and diagnosis in South Africa: successes and challenges. *AIDS* 2015; **29**(11): 1401-9.

46. Grobler A, Cawood C, Khanyile D, Puren A, Kharsany ABM. Progress of UNAIDS 90-90-90 targets in a district in KwaZulu-Natal, South Africa, with high HIV burden, in the HIPSS study: a household-based complex multilevel community survey. *The lancet HIV* 2017; **4**(11): e505-e13.

47. Iwuji CC, Orne-Gliemann J, Larmarange J, et al. Universal test and treat and the HIV epidemic in rural South Africa: a phase 4, open-label, community cluster randomised trial. *The lancet HIV* 2018; **5**(3): e116-e25.

48. Granich R, Gupta S, Hall I, Aberle-Grasse J, Hader S, Mermin J. Status and methodology of publicly available national HIV care continua and 90-90-90 targets: A systematic review. *PLoS Med* 2017; **14**(4): e1002253.

49. Auld AF, Shiraishi RW, Mbofana F, et al. Lower Levels of Antiretroviral Therapy Enrollment Among Men with HIV Compared with Women - 12 Countries, 2002-2013. *MMWR Morb Mortal Wkly Rep* 2015; **64**(46): 1281-6.

50. Auld AF, Agolory SG, Shiraishi RW, et al. Antiretroviral Therapy Enrollment Characteristics and Outcomes Among HIV-Infected Adolescents and Young Adults Compared with Older Adults - Seven African Countries, 2004-2013. *MMWR Morb Mortal Wkly Rep* 2014; **63**(47): 1097-103.

51. Green D, Tordoff DM, Kharono B, et al. Evidence of sociodemographic heterogeneity across the HIV treatment cascade and progress towards 90-90-90 in sub-Saharan Africa - a systematic review and meta-analysis. *J Int AIDS Soc* 2020; **23**(3): e25470.

52. ICAP. PHIA Project: A drop that counts. Available at: https://phia.icap.columbia.edu/resources/. Accessed July 12, 2020.

53. Hakim J, Musiime V, Szubert AJ, et al. Enhanced Prophylaxis plus Antiretroviral Therapy for Advanced HIV Infection in Africa. *N Engl J Med* 2017; **377**(3): 233-45.

54. Group TAS, Danel C, Moh R, et al. A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa. *N Engl J Med* 2015; **373**(9): 808-22.

55. Wiktor SZ, Sassan-Morokro M, Grant AD, et al. Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1-infected patients with tuberculosis in Abidjan, Cote d'Ivoire: a randomised controlled trial. *Lancet* 1999; **353**(9163): 1469-75.

56. Walker AS, Ford D, Gilks CF, et al. Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort. *Lancet* 2010; **375**(9722): 1278-86.

57. Mfinanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. *Lancet* 2015; **385**(9983): 2173-82.

58. WHO. WHO three 'I's meeting. Report of a joint WHO HIV/AIDS and TB Department Meeting; 2008. Geneva: Available at: http://www.who.int/hiv/pub/meetingreports/WHO_3Is_meeting_report.pdf. Accessed March 6, 2020.

59. WHO. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. Available at: http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf. Accessed June 5, 2020.

60. Getahun H, Kittikraisak W, Heilig CM, et al. Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. *PLoS Med* 2011; **8**(1): e1000391.
61. Hamada Y, Lujan J, Schenkel K, Ford N, Getahun H. Sensitivity and specificity of WHO's recommended four-symptom screening rule for tuberculosis in people living with HIV: a systematic review and meta-analysis. *The lancet HIV* 2018; **5**(9): e515-e23.
62. Auld AF, Mbofana F, Shiraishi RW, et al. Incidence and determinants of tuberculosis among adults initiating antiretroviral therapy--Mozambique, 2004-2008. *PLoS One* 2013; **8**(1): e54665.
63. Chihota VN, Ginindza S, McCarthy K, Grant AD, Churchyard G, Fielding K. Missed Opportunities for TB Investigation in Primary Care Clinics in South Africa: Experience from the XTEND Trial. *PLoS One* 2015; **10**(9): e0138149.
64. Christian CS, Gerdtham UG, Hompashe D, Smith A, Burger R. Measuring Quality Gaps in TB Screening in South Africa Using Standardised Patient Analysis. *International journal of environmental research and public health* 2018; **15**(4).
65. De Schacht C, Mutaquiha C, Faria F, et al. Barriers to access and adherence to tuberculosis services, as perceived by patients: A qualitative study in Mozambique. *PLoS One* 2019; **14**(7): e0219470.
66. Date A, Modi S. TB screening among people living with HIV/AIDS in resource-limited settings. *J Acquir Immune Defic Syndr* 2015; **68 Suppl 3**: S270-3.
67. Cornell M, Lessells R, Fox MP, et al. Mortality among adults transferred and lost to follow-up from antiretroviral therapy programmes in South Africa: a multicenter cohort study. *J Acquir Immune Defic Syndr* 2014; **67**(2): e67-75.
68. Theron G, Zijenah L, Chanda D, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. *Lancet* 2014; **383**(9915): 424-35.
69. Churchyard GJ, Stevens WS, Mametja LD, et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. *The Lancet Global health* 2015; **3**(8): e450-7.

70. van Kampen SC, Susanto NH, Simon S, et al. Effects of Introducing Xpert MTB/RIF on Diagnosis and Treatment of Drug-Resistant Tuberculosis Patients in Indonesia: A Pre-Post Intervention Study. *PLoS One* 2015; **10**(6): e0123536.

71. Cox HS, Mbhele S, Mohess N, et al. Impact of Xpert MTB/RIF for TB diagnosis in a primary care clinic with high TB and HIV prevalence in South Africa: a pragmatic randomised trial. *PLoS Med* 2014; **11**(11): e1001760.

72. Yoon C, Cattamanchi A, Davis JL, et al. Impact of Xpert MTB/RIF testing on tuberculosis management and outcomes in hospitalized patients in Uganda. *PLoS One* 2012; **7**(11): e48599.

73. Trajman A, Durovni B, Saraceni V, et al. Impact on Patients' Treatment Outcomes of XpertMTB/RIF Implementation for the Diagnosis of Tuberculosis: Follow-Up of a Stepped-Wedge Randomized Clinical Trial. *PLoS One* 2015; **10**(4): e0123252.

74. Fuente-Soro L, Lopez-Varela E, Augusto O, et al. Loss to follow-up and opportunities for reengagement in HIV care in rural Mozambique: A prospective cohort study. *Medicine* 2020; **99**(20): e20236.

75. Etoori D, Wringe A, Renju J, Kabudula CW, Gomez-Olive FX, Reniers G. Challenges with tracing patients on antiretroviral therapy who are late for clinic appointments in rural South Africa and recommendations for future practice. *Glob Health Action* 2020; **13**(1): 1755115.

76. Bershetyn A, Odeny TA, Lyamuya R, et al. The Causal Effect of Tracing by Peer Health Workers on Return to Clinic Among Patients Who Were Lost to Follow-up From Antiretroviral Therapy in Eastern Africa: A "Natural Experiment" Arising From Surveillance of Lost Patients. *Clin Infect Dis* 2017; **64**(11): 1547-54.

77. WHO. Rapid Implementation of the Xpert MTB/RIF diagnostic test. Available at: http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf. Accessed June 5, 2020.

78. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* 2011; **377**(9776): 1495-505.

79. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2014; **1**: CD009593.

80. Lawn SD, Meintjes G, McIlleron H, Harries AD, Wood R. Management of HIV-associated tuberculosis in resource-limited settings: a state-of-the-art review. *BMC Med* 2013; **11**: 253.

81. Menzies NA, Cohen T, Lin HH, Murray M, Salomon JA. Population health impact and cost-effectiveness of tuberculosis diagnosis with Xpert MTB/RIF: a dynamic simulation and economic evaluation. *PLoS Med* 2012; **9**(11): e1001347.

82. Mupfumi L, Makamure B, Chirehwa M, et al. Impact of Xpert MTB/RIF on Antiretroviral Therapy-Associated Tuberculosis and Mortality: A Pragmatic Randomized Controlled Trial. *Open forum infectious diseases* 2014; **1**(1): ofu038.

83. Waldrop G, Doherty M, Vitoria M, Ford N. Stable patients and patients with advanced disease: consensus definitions to support sustained scale up of antiretroviral therapy. *Trop Med Int Health* 2016; **21**(9): 1124-30.

84. McNairy ML, Abrams EJ, Rabkin M, El-Sadr WM. Clinical decision tools are needed to identify HIV-positive patients at high risk for poor outcomes after initiation of antiretroviral therapy. *PLoS Med* 2017; **14**(4): e1002278.

85. Tate JP, Sterne JAC, Justice AC, Veterans Aging Cohort S, the Antiretroviral Therapy Cohort C. Albumin, white blood cell count, and body mass index improve discrimination of mortality in HIV-positive individuals. *AIDS* 2019; **33**(5): 903-12.

86. Mocroft A, Ledergerber B, Zilmer K, et al. Short-term clinical disease progression in HIV-1-positive patients taking combination antiretroviral therapy: the EuroSIDA risk-score. *AIDS* 2007; **21**(14): 1867-75.

87. McNairy ML, Jannat-Khah D, Pape JW, et al. Predicting death and lost to follow-up among adults initiating antiretroviral therapy in resource-limited settings: Derivation and external validation of a risk score in Haiti. *PLoS One* 2018; **13**(8): e0201945.

88. WHO. Latent TB Infection : Updated and consolidated guidelines for programmatic management. Available at: http://apps.who.int/iris/bitstream/handle/10665/260233/9789241550239-eng.pdf?sequence=1. Accessed August 30, 2018.

89. Shapiro AE, Chakravorty R, Akande T, Lonnroth K, Golub JE. A systematic review of the number needed to screen to detect a case of active tuberculosis in different risk groups (2013).

Available at: https://www.who.int/tb/Review3NNS_case_active_TB_riskgroups.pdf?ua=1. Accessed June 10, 2020.

90. Ahmad Khan F, Verkuijl S, Parrish A, et al. Performance of symptom-based tuberculosis screening among people living with HIV: not as great as hoped. *AIDS* 2014; **28**(10): 1463-72.

91. Rangaka MX, Wilkinson RJ, Glynn JR, et al. Effect of antiretroviral therapy on the diagnostic accuracy of symptom screening for intensified tuberculosis case finding in a South African HIV clinic. *Clin Infect Dis* 2012; **55**(12): 1698-706.

92. Melgar M, Nichols C, Cavanaugh JS, et al. Tuberculosis Preventive Treatment Scale-Up Among Antiretroviral Therapy Patients - 16 Countries Supported by the U.S. President's Emergency Plan for AIDS Relief, 2017-2019. *MMWR Morb Mortal Wkly Rep* 2020; **69**(12): 329-34.

93. Pathmanathan I, Ahmedov S, Pevzner E, et al. TB preventive therapy for people living with HIV: key considerations for scale-up in resource-limited settings. *Int J Tuberc Lung Dis* 2018; **22**(6): 596-605.

94. Lawn SD, Wilkinson RJ, Lipman MC, Wood R. Immune reconstitution and "unmasking" of tuberculosis during antiretroviral therapy. *Am J Respir Crit Care Med* 2008; **177**(7): 680-5.

95. Lawn SD, Kranzer K, Edwards DJ, McNally M, Bekker LG, Wood R. Tuberculosis during the first year of antiretroviral therapy in a South African cohort using an intensive pretreatment screening strategy. *AIDS* 2010; **24**(9): 1323-8.

96. Oni T, Burke R, Tsekela R, et al. High prevalence of subclinical tuberculosis in HIV-1-infected persons without advanced immunodeficiency: implications for TB screening. *Thorax* 2011; **66**(8): 669-73.

97. Harries AD, Kumar AMV, Satyanarayana S, Takarinda KC, Timire C, Dlodlo RA. Treatment for latent tuberculosis infection in low- and middle-income countries: progress and challenges with implementation and scale-up. *Expert Rev Respir Med* 2020; **14**(2): 195-208.

98. Auld AF, Blain M, Ekra KA, et al. Wide Variations in Compliance with Tuberculosis Screening Guidelines and Tuberculosis Incidence between Antiretroviral Therapy Facilities - Cote d'Ivoire. *PLoS One* 2016; **11**(6): e0157059.

99. Auld AF, Tuho MZ, Ekra KA, et al. Tuberculosis in human immunodeficiency virus-infected children starting antiretroviral therapy in Cote d'Ivoire. *Int J Tuberc Lung Dis* 2014; **18**(4): 381-7.

100. Tate JP, Justice AC, Hughes MD, et al. An internationally generalizable risk index for mortality after one year of antiretroviral therapy. *AIDS* 2013; **27**(4): 563-72.

101. Diamond IR, Grant RC, Feldman BM, et al. Defining consensus: a systematic review recommends methodologic criteria for reporting of Delphi studies. *J Clin Epidemiol* 2014; **67**(4): 401-9.

102. Ehrenkranz PD, Baptiste SL, Bygrave H, et al. The missed potential of CD4 and viral load testing to improve clinical outcomes for people living with HIV in lower-resource settings. *PLoS Med* 2019; **16**(5): e1002820.

103. Justice AC, Modur SP, Tate JP, et al. Predictive accuracy of the Veterans Aging Cohort Study index for mortality with HIV infection: a North American cross cohort analysis. *J Acquir Immune Defic Syndr* 2013; **62**(2): 149-63.

104. Bebu I, Tate J, Rimland D, et al. The VACS index predicts mortality in a young, healthy HIV population starting highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2014; **65**(2): 226-30.

105. Justice AC, McGinnis KA, Skanderson M, et al. Towards a combined prognostic index for survival in HIV infection: the role of 'non-HIV' biomarkers. *HIV Med* 2010; **11**(2): 143-51.

106. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**(7): e1000097.

107. Beswick AD, Brindle P, Fahey T, Ebrahim S. A systematic review of risk scoring methods and clinical decision aids used in the primary prevention of coronary heart disease. Available at: https://www.researchgate.net/publication/259980329_A_systematic_review_of_risk_scoring_methods_and_clinical_decision_aids_used_in_the_primary_prevention_of_coronary_heart_disease. Accessed June 17, 2020.

108. Moons KG, Altman DG, Vergouwe Y, Royston P. Prognosis and prognostic research: application and impact of prognostic models in clinical practice. *BMJ* 2009; **338**: b606.

109. Bellazzi R, Zupan B. Predictive data mining in clinical medicine: current issues and guidelines. *International journal of medical informatics* 2008; **77**(2): 81-97.

110. Allison PD. Modern Methods for Missing Data. Available at: https://www.amstat.org/sections/srms/webinarfiles/ModernMethodWebinarMay2012.pdf. Accessed June 7, 2016.

111. Gerardo R, Dayana P. Development and validation of a clinical score for prognosis stratification in patients requiring antiretroviral therapy in sub-Saharan Africa: a prospective open cohort study. *Pan Afr Med J* 2011; **10**: 5.

112. Gustafson DR, Shi Q, Holman S, et al. Predicting death over 8 years in a prospective cohort of HIV-infected women: the Women's Interagency HIV Study. *BMJ open* 2017; **7**(6): e013993.

113. Brown ST, Tate JP, Kyriakides TC, et al. The VACS index accurately predicts mortality and treatment response among multi-drug resistant HIV infected patients participating in the options in management with antiretrovirals (OPTIMA) study. *PLoS One* 2014; **9**(3): e92606.

114. Letizia A, Eller MA, Polyak C, et al. Biomarkers of Inflammation Correlate With Clinical Scoring Indices in Human Immunodeficiency Virus-Infected Kenyans. *J Infect Dis* 2019; **219**(2): 284-94.

115. Nguyen HV, Tiemersma EW, Nguyen HB, et al. The second national tuberculosis prevalence survey in Vietnam. *PLoS One* 2020; **15**(4): e0232142.

116. Pathmanathan I, Pevzner E, Cavanaugh J, Nelson L. Addressing tuberculosis in differentiated care provision for people living with HIV. *Bull World Health Organ* 2017; **95**(1): 3.

117. Grant AD, Charalambous S, Tlali M, et al. Algorithm-guided empirical tuberculosis treatment for people with advanced HIV (TB Fast Track): an open-label, cluster-randomised trial. *The lancet HIV* 2019.

118. Balcha TT, Skogmar S, Sturegard E, et al. A Clinical Scoring Algorithm for Determination of the Risk of Tuberculosis in HIV-Infected Adults: A Cohort Study Performed at Ethiopian Health Centers. *Open forum infectious diseases* 2014; **1**(3): ofu095.

119. Nanta S, Kantipong P, Pathipvanich P, Ruengorn C, Tawichasri C, Patumanond J. Screening scheme development for active TB prediction among HIV-infected patients. *Southeast Asian J Trop Med Public Health* 2011; **42**(4): 867-75.

120. Nguyen DT, Hung NQ, Giang LT, et al. Improving the diagnosis of pulmonary tuberculosis in HIV-infected individuals in Ho Chi Minh City, Viet Nam. *Int J Tuberc Lung Dis* 2011; **15**(11): 1528-34, i.

121. Rudolf F, Haraldsdottir TL, Mendes MS, et al. Can tuberculosis case finding among health-care seeking adults be improved? Observations from Bissau. *Int J Tuberc Lung Dis* 2014; **18**(3): 277-85.

122. Aunsborg JW, Honge BL, Jespersen S, et al. A clinical score has utility in tuberculosis case-finding among patients with HIV: A feasibility study from Bissau. *Int J Infect Dis* 2020; **92S**: S78-S84.

123. Hanifa Y, Fielding KL, Chihota VN, et al. A clinical scoring system to prioritise investigation for tuberculosis among adults attending HIV clinics in South Africa. *PLoS One* 2017; **12**(8): e0181519.

124. Altman DG, Vergouwe Y, Royston P, Moons KG. Prognosis and prognostic research: validating a prognostic model. *BMJ* 2009; **338**: b605.

125. Ndwiga C, Birungi H, Undie CC, Weyenga H, Sitienei J. Feasibility and effect of integrating tuberculosis screening and detection in postnatal care services: an operations research study. *BMC Health Serv Res* 2013; **13**: 99.

126. Royston P, Moons KG, Altman DG, Vergouwe Y. Prognosis and prognostic research: Developing a prognostic model. *BMJ* 2009; **338**: b604.

127. Obermeyer Z, Emanuel EJ. Predicting the Future - Big Data, Machine Learning, and Clinical Medicine. *N Engl J Med* 2016; **375**(13): 1216-9.

128. Rajkomar A, Dean J, Kohane I. Machine Learning in Medicine. *N Engl J Med* 2019; **380**(14): 1347-58.

129. Shapiro AE, Hong T, Govere S, et al. C-reactive protein as a screening test for HIV-associated pulmonary tuberculosis prior to antiretroviral therapy in South Africa. *AIDS* 2018; **32**(13): 1811-20.

130. Boyles TH, Nduna M, Pitsi T, Scott L, Fox MP, Maartens G. A Clinical Prediction Score Including Trial of Antibiotics and C-Reactive Protein to Improve the Diagnosis of Tuberculosis in Ambulatory People With HIV. *Open forum infectious diseases* 2020; **7**(2): ofz543.

131. Yoon C, Semitala FC, Atuhumuza E, et al. Point-of-care C-reactive protein-based tuberculosis screening for people living with HIV: a diagnostic accuracy study. *Lancet Infect Dis* 2017; **17**(12): 1285-92.

132. Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic and prognostic value of serum C-reactive protein for screening for HIV-associated tuberculosis. *Int J Tuberc Lung Dis* 2013; **17**(5): 636-43.

133. UNDP. Human Development Report 2019. Available at: http://hdr.undp.org/sites/all/themes/hdr_theme/country-notes/BWA.pdf. Accessed June 5, 2020.

134. WorldBank. The World Bank In Botswana. Available at: https://www.worldbank.org/en/country/botswana/overview. Accessed June 5, 2020.

135. CIA. Africa: Botswana. Available at: https://www.cia.gov/library/publications/the-world-factbook/geos/print_bc.html. Accessed June 5, 2020.

136. Farahani M, Price N, El-Halabi S, et al. Trends and determinants of survival for over 200 000 patients on antiretroviral treatment in the Botswana National Program: 2002-2013. *AIDS* 2016; **30**(3): 477-85.

137. MOH. National Tuberculosis Programme Manual - Botswana. Available at: http://www.searchitech.org/itech?page=ff-08-02. Accessed June 5, 2020.

138. WHO. Tuberculosis Country Profiles. Avaiable at: https://www.who.int/tb/country/data/profiles/en/. Accessed June 5, 2020.

139. Kabongo D, Mash B. Effectiveness of home-based directly observed treatment for tuberculosis in Kweneng West subdistrict, Botswana. *African Journal of Primary Healthcare & Family Medicine* 2010; **2**(1).

140. Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. *Lancet Infect Dis* 2012; **12**(3): 201-9.

141. UNDP. South Africa Human Development Index. Available at: http://www.hdr.undp.org/en/countries/profiles/ZAF. Accessed June 5, 2020.

142. CIA. South Africa profile. Available at: https://www.cia.gov/library/publications/the-world-factbook/geos/sf.html. Accessed June 5, 2020.

143. Ansari NA, Kombe AH, Kenyon TA, et al. Pathology and causes of death in a group of 128 predominantly HIV-positive patients in Botswana, 1997-1998. *Int J Tuberc Lung Dis* 2002; **6**(1): 55-63.

144. Sanson-Fisher RW, D'Este CA, Carey ML, Noble N, Paul CL. Evaluation of systems-oriented public health interventions: alternative research designs. *Annual review of public health* 2014; **35**: 9-27.

145. Mdege ND, Man MS, Taylor Nee Brown CA, Torgerson DJ. Systematic review of stepped wedge cluster randomized trials shows that design is particularly used to evaluate interventions during routine implementation. *J Clin Epidemiol* 2011; **64**(9): 936-48.

146. Auld AF, Fielding KL, Gupta-Wright A, Lawn SD. Xpert MTB/RIF - why the lack of morbidity and mortality impact in intervention trials? *Trans R Soc Trop Med Hyg* 2016; **110**(8): 432-44.

147. Loudon K, Treweek S, Sullivan F, Donnan P, Thorpe KE, Zwarenstein M. The PRECIS-2 tool: designing trials that are fit for purpose. *BMJ* 2015; **350**: h2147.

148. Ochodo EA, Kalema N, Schumacher S, et al. Variation in the observed effect of Xpert MTB/RIF testing for tuberculosis on mortality – A systematic review and analysis of trial design considerations. Available at: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3433888. Accessed July 1, 2020.

149. Moulton LH, Golub JE, Durovni B, et al. Statistical design of THRio: a phased implementation clinic-randomized study of a tuberculosis preventive therapy intervention. *Clin Trials* 2007; **4**(2): 190-9.

150. Hussey MA, Hughes JP. Design and analysis of stepped wedge cluster randomized trials. *Contemp Clin Trials* 2007; **28**(2): 182-91.

151. Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? *Lancet Infect Dis* 2014; **14**(6): 527-32.

152. Dryden-Peterson S, Lockman S, Zash R, et al. Initial programmatic implementation of WHO option B in Botswana associated with increased projected MTCT. *J Acquir Immune Defic Syndr* 2015; **68**(3): 245-9.

153. Hayes RJ, Bennett S. Simple sample size calculation for cluster-randomized trials. *Int J Epidemiol* 1999; **28**(2): 319-26.

154. Bisson GP, Gaolathe T, Gross R, et al. Overestimates of survival after HAART: implications for global scale-up efforts. *PLoS One* 2008; **3**(3): e1725.

155. Brinkhof MW, Dabis F, Myer L, et al. Early loss of HIV-infected patients on potent antiretroviral therapy programmes in lower-income countries. *Bull World Health Organ* 2008; **86**(7): 559-67.

156. Cox JA, Lukande RL, Lucas S, Nelson AM, Van Marck E, Colebunders R. Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. *AIDS Rev* 2010; **12**(4): 183-94.

157. Agizew T, Boyd R, Ndwapi N, et al. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: Experience gained from a pragmatic, stepped-wedge trial in Botswana. *PLoS One* 2017; **12**(8): e0183237.

158. Firmin S. Seeing the Forest for the Trees: An Introduction to Random Forest. Available at: https://towardsdatascience.com/seeing-the-forest-for-the-trees-an-introduction-to-random-forest-41a24fc842ac. Accessed June 3, 2020.

159. Zhou V. Random Forests for Complete Beginners. Available at: https://victorzhou.com/blog/intro-to-random-forests/. Accessed July 10, 2020.

160. Azman AS, Lessler J, Luquero FJ, et al. Estimating cholera incidence with cross-sectional serology. *Sci Transl Med* 2019; **11**(480).

161. Auld AF, Agizew T, Mathoma A, et al. Effect of tuberculosis screening and retention interventions on early antiretroviral therapy mortality in Botswana: a stepped-wedge cluster randomized trial. *BMC Med* 2020; **18**(1): 19.

162. WHO. Guidelines for Intensified Tuberculosis Case-Finding and Isoniazid Preventive Therapy for People Living with HIV in Resource Constrained Settings. Available at: http://www.who.int/hiv/pub/tb/9789241500708/en/. Accessed March 6, 2016.

163. Naidoo P, Theron G, Rangaka MX, et al. The South African Tuberculosis Care Cascade: Estimated Losses and Methodological Challenges. *J Infect Dis* 2017; **216**(suppl_7): S702-S13.

164. Durovni B, Saraceni V, van den Hof S, et al. Impact of replacing smear microscopy with Xpert MTB/RIF for diagnosing tuberculosis in Brazil: a stepped-wedge cluster-randomized trial. *PLoS Med* 2014; **11**(12): e1001766.

165. Calligaro GL, Theron G, Khalfey H, et al. Burden of tuberculosis in intensive care units in Cape Town, South Africa, and assessment of the accuracy and effect on patient outcomes of the Xpert MTB/RIF test on tracheal aspirate samples for diagnosis of pulmonary tuberculosis: a prospective burden of disease study with a nested randomised controlled trial. *The lancet Respiratory medicine* 2015; **3**(8): 621-30.

166. MacPherson P, Houben RM, Glynn JR, Corbett EL, Kranzer K. Pre-treatment loss to follow-up in tuberculosis patients in low- and lower-middle-income countries and high-burden countries: a systematic review and meta-analysis. *Bull World Health Organ* 2014; **92**(2): 126-38.

167. Subbaraman R, Nathavitharana RR, Satyanarayana S, et al. The Tuberculosis Cascade of Care in India's Public Sector: A Systematic Review and Meta-analysis. *PLoS Med* 2016; **13**(10): e1002149.

168. Hossain ST, Isaakidis P, Sagili KD, et al. The Multi-Drug Resistant Tuberculosis Diagnosis and Treatment Cascade in Bangladesh. *PLoS One* 2015; **10**(6): e0129155.

169. Owiti P, Onyango D, Momanyi R, Harries AD. Screening and testing for tuberculosis among the HIV-infected: outcomes from a large HIV programme in western Kenya. *BMC Public Health* 2019; **19**(1): 29.

170. Kweza PF, Van Schalkwyk C, Abraham N, Uys M, Claassens MM, Medina-Marino A. Estimating the magnitude of pulmonary tuberculosis patients missed by primary health care clinics in South Africa. *Int J Tuberc Lung Dis* 2018; **22**(3): 264-72.

171. Charles MK, Lindegren ML, Wester CW, et al. Implementation of Tuberculosis Intensive Case Finding, Isoniazid Preventive Therapy, and Infection Control ("Three I's") and HIV-Tuberculosis Service Integration in Lower Income Countries. *PLoS One* 2016; **11**(4): e0153243.

172. Phetlhu DR, Bimerew M, Marie-Modeste RR, Naidoo M, Igumbor J. Nurses' Knowledge of Tuberculosis, HIV, and Integrated HIV/TB Care Policies in Rural Western Cape, South Africa. *J Assoc Nurses AIDS Care* 2018; **29**(6): 876-86.

173. Cattamanchi A, Miller CR, Tapley A, et al. Health worker perspectives on barriers to delivery of routine tuberculosis diagnostic evaluation services in Uganda: a qualitative study to guide clinic-based interventions. *BMC Health Serv Res* 2015; **15**: 10.

174. Gebreegziabher SB, Yimer SA, Bjune GA. Qualitative Assessment of Challenges in Tuberculosis Control in West Gojjam Zone, Northwest Ethiopia: Health Workers' and Tuberculosis Control Program Coordinators' Perspectives. *Tuberculosis research and treatment* 2016; **2016**: 2036234.

175. Workneh MH, Bjune GA, Yimer SA. Assessment of health system challenges and opportunities for possible integration of diabetes mellitus and tuberculosis services in South-Eastern Amhara Region, Ethiopia: a qualitative study. *BMC Health Serv Res* 2016; **16**: 135.

176. Loveday M, Padayatchi N, Voce A, Brust J, Wallengren K. The treatment journey of a patient with multidrug-resistant tuberculosis in South Africa: is it patient-centred? *Int J Tuberc Lung Dis* 2013; **17**(10 Suppl 1): 56-9.

177. Moodley N, Saimen A, Zakhura N, et al. 'They are inconveniencing us' - exploring how gaps in patient education and patient centred approaches interfere with TB treatment adherence: perspectives from patients and clinicians in the Free State Province, South Africa. *BMC Public Health* 2020; **20**(1): 454.

178. Chimbindi N, Barnighausen T, Newell ML. Patient satisfaction with HIV and TB treatment in a public programme in rural KwaZulu-Natal: evidence from patient-exit interviews. *BMC Health Serv Res* 2014; **14**: 32.

179. Loveday M, Padayatchi N, Wallengren K, et al. Association between health systems performance and treatment outcomes in patients co-infected with MDR-TB and HIV in KwaZulu-Natal, South Africa: implications for TB programmes. *PLoS One* 2014; **9**(4): e94016.

180. Schulz SA, Draper HR, Naidoo P. A comparative study of tuberculosis patients initiated on ART and receiving different models of TB-HIV care. *Int J Tuberc Lung Dis* 2013; **17**(12): 1558-63.

181. Fox MP, Rosen S. Retention of Adult Patients on Antiretroviral Therapy in Low- and Middle-Income Countries: Systematic Review and Meta-analysis 2008-2013. *J Acquir Immune Defic Syndr* 2015; **69**(1): 98-108.

182. Nosyk B, Montaner JSG, Colley G, et al. The cascade of HIV care in British Columbia, Canada, 1996-2011: a population-based retrospective cohort study. *Lancet Infect Dis* 2014; **14**(1): 40-9.

183. Haas AD, Zaniewski E, Anderegg N, et al. Retention and mortality on antiretroviral therapy in sub-Saharan Africa: collaborative analyses of HIV treatment programmes. *J Int AIDS Soc* 2018; **21**(2).

184. Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme CC. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? *The European respiratory journal* 2016; **48**(2): 516-25.

185. Sun AY, Denkinger CM, Dowdy DW. The impact of novel tests for tuberculosis depends on the diagnostic cascade. *The European respiratory journal* 2014; **44**(5): 1366-9.

186. Subbaraman R, Nathavitharana RR, Mayer KH, et al. Constructing care cascades for active tuberculosis: A strategy for program monitoring and identifying gaps in quality of care. *PLoS Med* 2019; **16**(2): e1002754.

187. Hanrahan CF, Van Rie A. A proposed novel framework for monitoring and evaluation of the cascade of HIV-associated TB care at the health facility level. *J Int AIDS Soc* 2017; **20**(1): 21375.

188. Dugdale CM, Ciaranello AL, Bekker LG, et al. Risks and Benefits of Dolutegravir- and Efavirenz-Based Strategies for South African Women With HIV of Child-Bearing Potential: A Modeling Study. *Ann Intern Med* 2019; **170**(9): 614-25.

189. Penn AW, Azman H, Horvath H, et al. Supportive interventions to improve retention on ART in people with HIV in low- and middle-income countries: A systematic review. *PLoS One* 2018; **13**(12): e0208814.

190. Munoz M, Bayona J, Sanchez E, et al. Matching social support to individual needs: a community-based intervention to improve HIV treatment adherence in a resource-poor setting. *AIDS Behav* 2011; **15**(7): 1454-64.

191. Franke MF, Kaigamba F, Socci AR, et al. Improved retention associated with community-based accompaniment for antiretroviral therapy delivery in rural Rwanda. *Clin Infect Dis* 2013; **56**(9): 1319-26.

192. Fatti G, Meintjes G, Shea J, Eley B, Grimwood A. Improved survival and antiretroviral treatment outcomes in adults receiving community-based adherence support: 5-year results from a multicentre cohort study in South Africa. *J Acquir Immune Defic Syndr* 2012; **61**(4): e50-8.

193. Grimwood A, Fatti G, Mothibi E, Malahlela M, Shea J, Eley B. Community adherence support improves programme retention in children on antiretroviral treatment: a multicentre cohort study in South Africa. *J Int AIDS Soc* 2012; **15**(2): 17381.

194. Luque-Fernandez MA, Van Cutsem G, Goemaere E, et al. Effectiveness of patient adherence groups as a model of care for stable patients on antiretroviral therapy in Khayelitsha, Cape Town, South Africa. *PLoS One* 2013; **8**(2): e56088.

195. Pearson CR, Micek MA, Simoni JM, et al. Randomized control trial of peer-delivered, modified directly observed therapy for HAART in Mozambique. *J Acquir Immune Defic Syndr* 2007; **46**(2): 238-44.

196. Braitstein P, Siika A, Hogan J, et al. A clinician-nurse model to reduce early mortality and increase clinic retention among high-risk HIV-infected patients initiating combination antiretroviral treatment. *J Int AIDS Soc* 2012; **15**(1): 7.

197. PayScale. Average Registered Nurse (RN) Salary in Botswana. Available at: https://www.payscale.com/research/BW/Job=Registered_Nurse_(RN)/Salary. Accessed July 1, 2020.

198. Simon GE, Platt R, Hernandez AF. Evidence from Pragmatic Trials during Routine Care - Slouching toward a Learning Health System. *N Engl J Med* 2020; **382**(16): 1488-91.

199. Reza TF, Nalugwa T, Farr K, et al. Study protocol: a cluster randomized trial to evaluate the effectiveness and implementation of onsite GeneXpert testing at community health centers in Uganda (XPEL-TB). *Implement Sci* 2020; **15**(1): 24.

200. Ngwira LG, Corbett EL, Khundi M, et al. Screening for tuberculosis with Xpert MTB/RIF versus fluorescent microscopy among adults newly diagnosed with HIV in rural Malawi: a cluster randomized trial (CHEPETSA). *Clin Infect Dis* 2018; **68**(7): 1176-83.

201. Westreich D, Fox MP, Van Rie A, Maskew M. Prevalent tuberculosis and mortality among HAART initiators. *AIDS* 2012; **26**(6): 770-3.

202. Squire SB, Belaye AK, Kashoti A, et al. 'Lost' smear-positive pulmonary tuberculosis cases: where are they and why did we lose them? *Int J Tuberc Lung Dis* 2005; **9**(1): 25-31.

203. Lauria ME, Fiori KP, Jones HE, et al. Assessing the Integrated Community-Based Health Systems Strengthening initiative in northern Togo: a pragmatic effectiveness-implementation study protocol. *Implement Sci* 2019; **14**(1): 92.

204. Di Tanna GL, Khaki AR, Theron G, et al. Effect of Xpert MTB/RIF on clinical outcomes in routine care settings: individual patient data meta-analysis. *The Lancet Global health* 2019; **7**(2): e191-e9.

205. IOM. Strengthening Health Systems for an Effective HIV/AIDS Response. Available at: https://www.nap.edu/read/18256/chapter/20. Accessed July 1, 2020.

206. PEPFAR. Country Operational Plan 2020 Guidance. Available at: https://www.state.gov/wp-content/uploads/2020/01/COP20-Guidance_Final-1-15-2020.pdf. Accessed July 1, 2020.

207. PEPFAR. Malawi Country Operational Plan COP 2019 Strategic Direction Summary. Available at: https://www.state.gov/wp-content/uploads/2019/09/PEPFAR-Malawi_COP19-SDS_FINAL.pdf. Accessed July 1, 2020.

208. Lawn SD, Nicol MP, Corbett EL. Effect of empirical treatment on outcomes of clinical trials of diagnostic assays for tuberculosis. *Lancet Infect Dis* 2015; **15**(1): 17-8.

209. Lung T, Marks GB, Nhung NV, et al. Household contact investigation for the detection of tuberculosis in Vietnam: economic evaluation of a cluster-randomised trial. *The Lancet Global health* 2019; **7**(3): e376-e84.

210. Philipsen RH, Sanchez CI, Maduskar P, et al. Automated chest-radiography as a triage for Xpert testing in resource-constrained settings: a prospective study of diagnostic accuracy and costs. *Scientific reports* 2015; **5**: 12215.

211. Murphy K, Habib SS, Zaidi SMA, et al. Computer aided detection of tuberculosis on chest radiographs: An evaluation of the CAD4TB v6 system. *Scientific reports* 2020; **10**(1): 5492.

212. Muyoyeta M, Kasese NC, Milimo D, et al. Digital CXR with computer aided diagnosis versus symptom screen to define presumptive tuberculosis among household contacts and impact on tuberculosis diagnosis. *BMC Infect Dis* 2017; **17**(1): 301.

213. WHO. Chest Radiography in Tuberculosis Detection. Available at: https://apps.who.int/iris/bitstream/handle/10665/252424/9789241511506-eng.pdf?sequence=1. Accessed July 2, 2020.

214. Piatti-Funfkirchen M, Lindelow M, Yoo K. What Are Governments Spending on Health in East and Southern Africa? *Health Syst Reform* 2018; **4**(4): 284-99.

215. Mweninguwe R. Coal against blackouts. Available at: https://www.dandc.eu/en/article/order-manage-its-current-energy-crisis-malawi-turns-coal. Accessed July 3, 2020.

216. Labonte R, Sanders D, Mathole T, et al. Health worker migration from South Africa: causes, consequences and policy responses. *Hum Resour Health* 2015; **13**: 92.

217. Kinfu Y, Dal Poz MR, Mercer H, Evans DB. The health worker shortage in Africa: are enough physicians and nurses being trained? *Bull World Health Organ* 2009; **87**(3): 225-30.

218. Nanta S, Kantipong P, Pathipvanich P, Ruengorn C, Tawichasri C, Patumanond J. Diagnostic value of two rapid immunochromatographic tests for suspected tuberculosis diagnosis in clinical practice. *J Med Assoc Thai* 2011; **94**(10): 1198-204.

219. Douglas GP, Gadabu OJ, Joukes S, et al. Using touchscreen electronic medical record systems to support and monitor national scale-up of antiretroviral therapy in Malawi. *PLoS Med* 2010; **7**(8).

220. Pooran A, Theron G, Zijenah L, et al. Point of care Xpert MTB/RIF versus smear microscopy for tuberculosis diagnosis in southern African primary care clinics: a multicentre economic evaluation. *The Lancet Global health* 2019; **7**(6): e798-e807.

221. Cazabon D, Alsdurf H, Satyanarayana S, et al. Quality of tuberculosis care in high burden countries: the urgent need to address gaps in the care cascade. *Int J Infect Dis* 2017; **56**: 111-6.

222. Hazard RH, Kagina P, Kitayimbwa R, et al. Effect of Empiric Anti-Mycobacterium tuberculosis Therapy on Survival Among Human Immunodeficiency Virus-Infected Adults Admitted With Sepsis to a Regional Referral Hospital in Uganda. *Open forum infectious diseases* 2019; **6**(4): ofz140.

223. Karat AS, Tlali M, Fielding KL, et al. Measuring mortality due to HIV-associated tuberculosis among adults in South Africa: Comparing verbal autopsy, minimally-invasive autopsy, and research data. *PLoS One* 2017; **12**(3): e0174097.

224. McGlynn EA, McDonald KM, Cassel CK. Measurement Is Essential for Improving Diagnosis and Reducing Diagnostic Error: A Report From the Institute of Medicine. *JAMA* 2015; **314**(23): 2501-2.

225. Kelly CJ, Karthikesalingam A, Suleyman M, Corrado G, King D. Key challenges for delivering clinical impact with artificial intelligence. *BMC Med* 2019; **17**(1): 195.

226. Chen JH, Asch SM. Machine Learning and Prediction in Medicine - Beyond the Peak of Inflated Expectations. *N Engl J Med* 2017; **376**(26): 2507-9.

227. Melendez J, Sanchez CI, Philipsen RH, et al. An automated tuberculosis screening strategy combining X-ray-based computer-aided detection and clinical information. *Scientific reports* 2016; **6**: 25265.

228. van Hoving DJ, Meintjes G, Maartens G, Kengne AP. A multi-parameter diagnostic clinical decision tree for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre (pre-print). Available at: https://wellcomeopenresearch.org/articles/5-72. Accessed July 3, 2020.

229. Aguiar FS, Almeida LL, Ruffino-Netto A, Kritski AL, Mello FC, Werneck GL. Classification and regression tree (CART) model to predict pulmonary tuberculosis in hospitalized patients. *BMC Pulm Med* 2012; **12**: 40.

230. Mello FC, Bastos LG, Soares SL, et al. Predicting smear negative pulmonary tuberculosis with classification trees and logistic regression: a cross-sectional study. *BMC Public Health* 2006; **6**: 43.

231. Schwalbe N, Wahl B. Artificial intelligence and the future of global health. *Lancet* 2020; **395**(10236): 1579-86.

232. Weng SF, Reps J, Kai J, Garibaldi JM, Qureshi N. Can machine-learning improve cardiovascular risk prediction using routine clinical data? *PLoS One* 2017; **12**(4): e0174944.

233. Harris M, Qi A, Jeagal L, et al. A systematic review of the diagnostic accuracy of artificial intelligence-based computer programs to analyze chest x-rays for pulmonary tuberculosis. *PLoS One* 2019; **14**(9): e0221339.

234. Peiffer-Smadja N, Rawson TM, Ahmad R, et al. Machine learning for clinical decision support in infectious diseases: a narrative review of current applications. *Clin Microbiol Infect* 2020; **26**(5): 584-95.

235. Lane T, Russo DP, Zorn KM, et al. Comparing and Validating Machine Learning Models for Mycobacterium tuberculosis Drug Discovery. *Mol Pharm* 2018; **15**(10): 4346-60.

236. Jamal S, Khubaib M, Gangwar R, Grover S, Grover A, Hasnain SE. Artificial Intelligence and Machine learning based prediction of resistant and susceptible mutations in Mycobacterium tuberculosis. *Scientific reports* 2020; **10**(1): 5487.

237. Agranoff D, Fernandez-Reyes D, Papadopoulos MC, et al. Identification of diagnostic markers for tuberculosis by proteomic fingerprinting of serum. *Lancet* 2006; **368**(9540): 1012-21.

238. Justman JE, Mugurungi O, El-Sadr WM. HIV Population Surveys - Bringing Precision to the Global Response. *N Engl J Med* 2018; **378**(20): 1859-61.

239. WHO. HIV Treatment and Care, What's New in Service Delivery Fact Sheet. Available at: http://apps.who.int/iris/bitstream/10665/204461/1/WHO_HIV_2015.46_eng.pdf?ua=1. Accessed June 5, 2020.

240. Delgado-Rodriguez M, Llorca J. Bias. *Journal of epidemiology and community health* 2004; **58**(8): 635-41.

241. McCarthy KM, Grant AD, Chihota V, et al. Implementation and Operational Research: What Happens After a Negative Test for Tuberculosis? Evaluating Adherence to TB Diagnostic Algorithms in South African Primary Health Clinics. *J Acquir Immune Defic Syndr* 2016; **71**(5): e119-26.

242. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med* 2017; **167**(4): 268-74.

243. Soon GG, Min M, Struble KA, et al. Meta-analysis of gender differences in efficacy outcomes for HIV-positive subjects in randomized controlled clinical trials of antiretroviral therapy (2000-2008). *AIDS Patient Care STDS* 2012; **26**(8): 444-53.

244. Irving G, Neves AL, Dambha-Miller H, et al. International variations in primary care physician consultation time: a systematic review of 67 countries. *BMJ open* 2017; **7**(10): e017902.

245. Gupta-Wright A, Corbett EL, van Oosterhout JJ, et al. Rapid urine-based screening for tuberculosis in HIV-positive patients admitted to hospital in Africa (STAMP): a pragmatic, multicentre, parallel-group, double-blind, randomised controlled trial. *Lancet* 2018; **392**(10144): 292-301.

246. Auld AF, Agizew T, Mathoma A, et al. Effect of TB screening and retention interventions on early ART mortality in Botswana. *Abstract #: 31 CROI 2018, March 4–7, 2018; Boston, Massachusetts Available at: http://wwwcroiconferenceorg/sessions/effect-tb-screening-and-retention-interventions-early-art-mortality-botswana*. Accessed June 5, 2020.

10. Appendices

10.1. Appendix 1. Published XPRES protocol

Auld et al. BMC Infectious Diseases (2016) 16:606
DOI 10.1186/s12879-016-1905-4

BMC Infectious Diseases

STUDY PROTOCOL Open Access

Implementation of a pragmatic, stepped-wedge cluster randomized trial to evaluate impact of Botswana's Xpert MTB/RIF diagnostic algorithm on TB diagnostic sensitivity and early antiretroviral therapy mortality

Andrew F. Auld[1*], Tefera Agizew[2], Sherri Pals[1], Alyssa Finlay[2,3], Ndwapi Ndwapi[4], Rosanna Boyd[2,3], Heather Alexander[1], Anikie Mathoma[2], Joyce Basotli[2], Sambayawo Gwebe-Nyirenda[2], James Shepherd[2,5], Tedd V. Ellerbrock[1] and Anand Date[1]

Abstract

Background: In 2012, as a pilot for Botswana's national Xpert MTB/RIF (Xpert) rollout plans, intensified tuberculosis (TB) case finding (ICF) activities were strengthened at 22 HIV treatment clinics prior to phased activation of 13 Xpert instruments. Together, the strengthened ICF intervention and Xpert activation are referred to as the "Xpert package".

Methods: The evaluation, called the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES), has two key objectives: (1) to compare sensitivity of microscopy-based and Xpert-based pulmonary TB diagnostic algorithms in diagnosing sputum culture-positive TB; and (2) to evaluate impact of the "Xpert package" on all-cause, 6-month, adult antiretroviral therapy (ART) mortality. A pragmatic, stepped-wedge cluster-randomized trial design was chosen. The design involves enrollment of three cohorts: (1) cohort R, a retrospective cohort of all study clinic ART enrollees in the 24 months before study initiation (July 31, 2012); (2) cohort A, a prospective cohort of all consenting patients presenting to study clinics after study initiation, who received the ICF intervention and the microscopy-based TB diagnostic algorithm; and (3) cohort B, a prospective cohort of all consenting patients presenting to study clinics after Xpert activation, who received the ICF intervention and the Xpert-based TB diagnostic algorithm. TB diagnostic sensitivity will be compared between TB culture-positive enrollees in cohorts A and B. All-cause, 6-month ART-mortality will be compared between cohorts R and B. With anticipated cohort R, A, and B sample sizes of about 10,131, 1,878, and 4,258, respectively, the study is estimated to have >80 % power to detect differences in pre-versus post-Xpert TB diagnostic sensitivity if pre-Xpert sensitivity is ≤52.5 % and post-Xpert sensitivity ≥82.5 %, and >80 % power to detect a 40 % reduction in all-cause, 6-month, ART mortality between cohorts R and B if cohort R mortality is ≥13/100 person-years.

(Continued on next page)

* Correspondence: aauld@cdc.gov
[1]Division of Global HIV & TB, Center for Global Health, United States Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, Atlanta, GA 30333, USA
Full list of author information is available at the end of the article

BioMed Central

© The Author(s). 2016 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

(Continued from previous page)

Discussion: Only one small previous trial (*N* = 424) among ART enrolees in Zimbabwe evaluated, in a secondary analysis, Xpert impact on all-cause 6-month ART mortality. No mortality impact was observed. This Botswana trial, with its larger sample size and powered specifically to detect differences in all-cause 6-month ART mortality, remains well-positioned to contribute understanding of Xpert impact.

Trial registration: Retrospectively registered at ClinicalTrials.gov: NCT02538952.

Keywords: Xpert MTB/RIF, Diagnostic accuracy, Sensitivity, Antiretroviral therapy, People living with HIV, Mortality, Stepped-wedge cluster randomized trial, Botswana

Background

In Botswana, as in the rest of sub-Saharan Africa, undiagnosed tuberculosis (TB) or TB diagnosed late in the course of disease is thought to be the most common cause of death among persons living with HIV (PLHIV), whether they are receiving antiretroviral therapy (ART) or not, with TB accounting for about 40 % of deaths according to a recent meta-analysis of pathological autopsy studies [1]. Although antiretroviral therapy (ART) reduces risk of all-cause mortality among PLHIV, early mortality in the first 3–6 months after ART initiation remains high in sub-Saharan Africa and is commonly due to undiagnosed TB or TB diagnosed late [2–4].

Reasons for failure to diagnose TB early among PLHIV can be categorized as patient-related or health facility-related. Patient-related reasons include: (1) failure to present to a health facility when symptoms arise, which may be due to poor access to health care or cultural norms that delay health-seeking behavior [5], and (2) absence of TB symptoms among late presenters with advanced immune suppression because TB symptoms are dependent on both bacillary burden and immune response [6].

Healthcare facility-related reasons for missed or late TB diagnoses among PLHIV include: (1) failure of healthcare workers (HCW) to screen for TB symptoms [7–9]), (2) failure of HCWs to request sputum or other diagnostic samples from symptomatic patients [10], (3) inability to collect high quality sputa or other appropriate diagnostic samples from symptomatic patients [11], (4) insensitive TB diagnostics, with smear microscopy alone having a sensitivity of about 45 % in diagnosing culture-positive disease among PLHIV [12], (5) inability to diagnose drug-resistant TB timeously [13], and (6) long turn-around times for some TB diagnostic tests or failure to return results to clinicians and patients [14].

In 2009, the commercial release of the Xpert MTB/RIF assay (Xpert) for the GeneXpert platform represented an important breakthrough in TB diagnostics. With features including sensitivity of about 79 % in diagnosing culture-positive TB from sputum samples among PLHIV [15], significantly superior to smear microscopy [12], ability to detect rifampicin resistance-conferring mutations, capacity to run the test on sputum samples within 100 min after brief sample processing, and minimal laboratory training requirements, Xpert significantly advanced TB diagnostic capability for clinicians managing PLHIV, especially in resource-limited settings (RLS) [16]. However, Xpert on its own, cannot solve all the facility-level challenges to diagnosing TB [17]. Strengthening of the entire TB symptom screening and diagnostic algorithm is needed for Xpert to have maximum impact on patient health outcomes in most RLS [17].

Therefore, in 2012, the Botswana Ministry of Health (MOH) and the United States Centers for Disease Control and Prevention (CDC), designed a package of intensified TB case finding (ICF) interventions to be rolled out prior to, and in coordination with, the phased activation of 13 Xpert devices in support of 22 HIV care and treatment clinics.

The package of ICF interventions included: (1) ensuring the 22 HIV care and treatment clinics adopted the World Health Organization (WHO)-recommended four-symptom TB screen for adults (>12 years old as defined by the HIV care and treatment program); (2) situating trained TB case-finding nurses in all 22 facilities to implement the screening and diagnostic algorithms; and (3) training TB case-finding nurses and other health facility personnel in both smear-microscopy-based and Xpert-based TB diagnostic algorithms for adults and children. The combination of the ICF interventions and rollout of the Xpert device is referred to as the "Xpert package" in this report.

To evaluate the accuracy of the new MOH-proposed Xpert diagnostic algorithm and also the impact of the whole Xpert package on patient outcomes, a pragmatic, stepped-wedge cluster-randomized trial (CRT), referred to as the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES), was initiated. In this paper, the protocol-specified key study objectives, design rationale, sample size, key procedures, and analytic approaches are described. In addition, the evolution of power estimates over time as real-time study enrollment numbers became available, and key amendments to study procedures, which were needed to adapt to operational challenges, are described.

Methods

Key objectives

The first objective of the evaluation is to determine whether the new MOH-recommended Xpert-based pulmonary TB diagnostic algorithm (including Xpert testing of sputum samples for all patients screening positive (i.e., presumptive TB patients) and chest x-ray for Xpert-negative presumptive TB patients) is more sensitive than the pre-Xpert smear-microscopy-based algorithm (smear microscopy and chest x-ray for smear-negative presumptive TB patients) in diagnosing culture-positive TB disease among adult PLHIV. Although it is expected that the Xpert-based TB diagnostic algorithm will be both more sensitive and more specific than the pre-Xpert algorithm, superiority of the Xpert *algorithm* has not yet been demonstrated in Botswana and thorough evaluation of the accuracy of the new diagnostic algorithm is important to guide future investments [18, 19].

The second objective is to evaluate the impact of the whole Xpert package on all-cause mortality during the first six months of ART, among adult PLHIV. With an estimated 40 % of early ART deaths due to undiagnosed TB or TB diagnosed late, the Xpert package could conceivably reduce all-cause, 6-month ART mortality by ensuring: (1) that all ART enrollees are appropriately screened for TB symptoms before and during ART, and (2) that presumptive TB patients have access to a sensitive TB test (Xpert) and early TB treatment where warranted [20]. Only one small trial ($N = 424$) in Zimbabwe has previously aimed to examine the impact of Xpert- versus microscopy-based TB diagnostic algorithms on 6-month ART mortality [21]; no difference in early ART mortality was noted between study arms, however, the small sample size and high rates of empiric TB treatment in both study arms limit study findings.

Study design rationale

A pragmatic stepped-wedge CRT design was chosen because: (1) Xpert device activation was most feasibly achieved for an entire district TB laboratory, which often served more than one health facility; this fact made an individual randomized controlled trial (RCT) design less desirable [19], (2) according to WHO guidance [22] and MOH guidelines [23], the Xpert device was expected to be beneficial for both patients and providers, and therefore it was considered ethically sub-optimal to implement a parallel group CRT, where certain district TB labs and their associated clinics were denied access to Xpert for an extended period of time [12, 24], (3) the phased rollout of Xpert provided logistical advantages, because it meant that a single site activation team, in charge of training and activation of the Xpert device, could sequentially initiate all study sites [24], (4) the need for only a single site activation team reduced projected study cost, (5) program managers and funders were interested in assessing accuracy of the Xpert diagnostic algorithm in a real-world environment rather than trying to assess accuracy in a tightly controlled research environment, with limited external validity [25], (6) in a real-world setting, the sequential rollout of an intervention allows lessons learned during earlier steps to be applied during later steps, and (7) a stepped-wedge design provides analysis options that allow for the control of trends over time [26, 27].

Study design description

Figure 1 summarizes the study design. This step-wedge design involved enrollment of three cohorts: (1) retrospective cohort (R), shaded in red in Fig. 1, (2) prospective cohort A (enrolled pre-Xpert device rollout), shaded in yellow in Fig. 1, and (3) prospective cohort B (enrolled post-Xpert device rollout), shaded in green in Fig. 1. For cohort R, all patients who initiated ART at one of the 22 HIV clinics for the first time in the 24 months before study start (i.e., before July 31, 2012), were eligible for enrollment. For cohort A, all patients who attended one of the 22 HIV clinics for the first time after study start (July 31, 2012), but before Xpert device rollout, were eligible for enrollment. For cohort B, all patients who attend one of the 22 HIV clinics for the first time after Xpert device rollout were eligible.

To answer the first primary study question, sensitivity of the pre-Xpert TB diagnostic algorithm in prospective cohort A will be compared with the post-Xpert algorithm sensitivity in prospective cohort B. Figure 2 describes the differences in TB diagnostic algorithms between cohorts A and B and how sensitivity proportions will be determined.

To meet the second key study objective (comparison of pre- versus post-Xpert package all-cause ART mortality), all-cause 6-month ART mortality rates will be compared between the retrospective cohort (cohort R) and the post-Xpert prospective cohort (cohort B). Since the cohorts being compared (cohorts R and B) do not overlap in a phased manner that would allow for controlling for secular trends according to analytic approaches recommended by Moulton et al [26] or Hussey & Hughes [27], this analysis approach is best characterized as a before and after comparison. However, secondary analyses, comparing 6-month ART mortality, and other ART outcomes, between cohorts A and B will make use of the stepped-wedge portion of the trial and analytic approaches recommended by Moulton et al [26] and Hussey & Hughes [27] to control for secular trends.

Interventions

As described above, Fig. 2 illustrates the differences between the microscopy-based algorithm used in cohort A

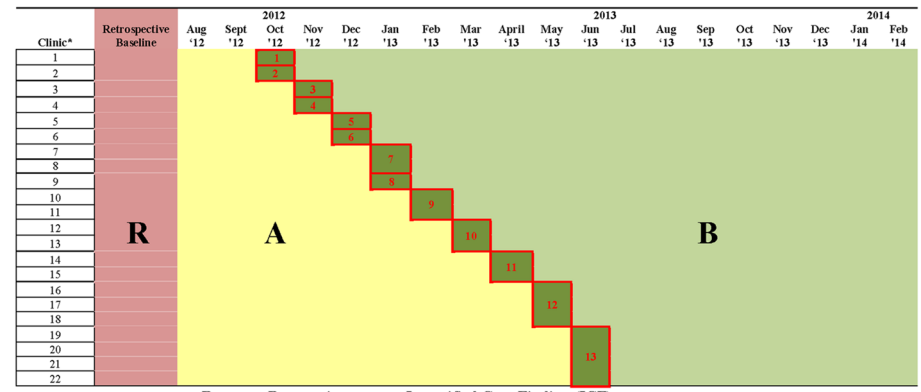


Fig. 1 Study design for the Xpert package rollout evaluation using a stepped wedge design (XPRES)

(pre-Xpert device rollout), and the Xpert-based algorithm used in cohort B (post - Xpert device rollout).

For the second key question, comparing all-cause 6-month ART mortality in cohort R with cohort B, Table 1 summarizes the differences between cohort R and B in terms of TB case finding and patient management activities. Notably, in addition to implementing ICF activities, study nurses were responsible for tracing prospectively enrolled patients (patients in cohorts A and B) who were ≥1 day late for a clinic appointment through up to five telephone calls and two home visits to return patients to HIV care. Indicators measuring compliance with all interventions, including implementation of the appropriate TB diagnostic algorithm and the tracing intervention, will help inform discussions of causal pathways during analysis of intervention impact on 6-month ART mortality.

Study population

Health facilities

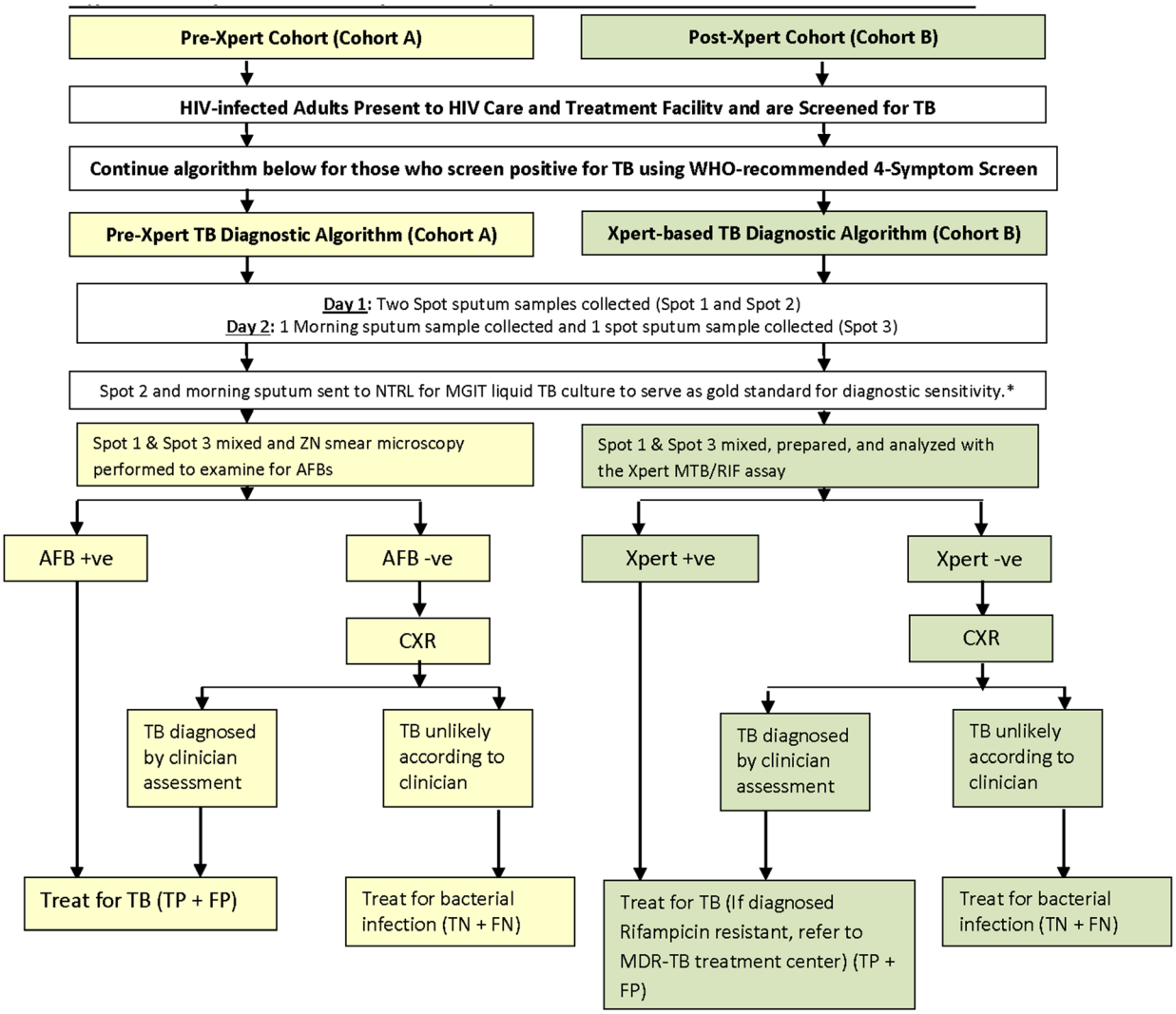
The main reason for selecting the 22 facilities in Table 2 is that they are considered by study investigators and MOH to be representative of facilities in Botswana in terms of TB case finding capacity and ART service delivery. Study facilities consist of five district hospitals and 17 primary healthcare clinics (PHCs). Other advantages of choosing these facilities are: (1) on average they had anticipated high patient enrollment rates (at 33 patients/month/clinic), which helped meet the desired study power (see below), (2) one clinic (Gantsi, in Western Botswana) is estimated to have high prevalence of multi-drug resistant TB among HIV clinic enrollees and so could benefit from early rollout of the Xpert device per WHO recommendations [22], and (3) all 22 clinics had at least one year's experience in providing ART services by the time of study start. Table 2 summarizes the study facilities chosen for XPRES.

Study patients

For the retrospective cohort, all patients starting ART in the 24 months before study start, except for prisoners, were eligible for chart abstraction to estimate all-cause ART mortality rates. For the prospective cohorts (A and B), all patients, who met consent requirements, registering for HIV care for the first time at the facility in the 19 months after study start, were eligible for enrollment, except for prisoners. Prisoners were excluded because it would be difficult to obtain comprehensive retrospective cohort data for cohort R, due to frequent unscheduled prisoner movement during incarceration, and difficult to retain prisoners in cohorts A and B for the study's duration. Children (<12 years of age) were eligible for prospective enrollment, if their assent and guardian's consent were provided, because secondary study questions aim to estimate Xpert algorithm sensitivity for children and its impact on pediatric ART outcomes.

Randomization procedures

Because some of the clinics use the same TB diagnostic facility, Xpert activation was simultaneous for these clinic consortiums (Table 2). For scheduling purposes and because clinic staff rotations occurred at the end of



Sensitivity Proportions of the Pre- and Post-Xpert TB Diagnostic Algorithms

		MGIT Culture Results*		
		TB +ve	TB -ve	
Diagnostic algorithm	TB +ve	TP	FP	TP + FP
	TB -ve	FN	TN	TN + FN
		TP + FN	FP + TN	Total (N)**
Sensitivity = TP/(TP+FN)				

*MGIT results will determine: (1) of those instances where TB treatment was started in cohorts A and B, which were true positives (TP) and which were false positives (FP), and (2) of those instances where TB treatment was not started, which were true negatives (TN) and which were false negatives (FN).

**The total (N) for cohort A, will be the total number of presumptive TB patients providing sufficient sputa for both a ZN smear microscopy test and MGIT. The total (N) for cohort B, will be the total number of presumptive TB patients providing sufficient sputa for both an Xpert MTB/RIF test and MGIT.

Abbreviations: TB, tuberculosis; Xpert, Xpert MTB/RIF Assay; AFB, Acid Fast Bacilli; ZN, Ziehl-Neelsen smear microscopy; TP, true positives; FP, false positives; FN, false negatives; TN, true negatives; N, number; MGIT, mycobacteria growth indicator tube; NTRL, National TB Reference Laboratory; -ve, negative; +ve, positive; CXR, chest X-ray.

Fig. 2 Comparison of pre-X pert and Xpert-based TB diagnostic algorithms in adults

Table 1 Comparison of TB case finding and patient management interventions for PLHIV in the retrospective and prospective cohorts

	Retrospective (R)	Prospective pre-Xpert (A)	Prospective post-Xpert (B)
TB screening algorithm for adults	1. Cough of any duration 2. Fever of any duration 3. Shortness of breath 4. Chest pain 5. Haemoptysis 6. Loss of appetite 7. Loss of weight 8. Malaise 9. Night sweats	1. Current Cough 2. Current Fever 3. Loss of weight 4. Night sweats	1. Current Cough 2. Current Fever 3. Loss of weight 4. Night sweats
Number of sputa collected from patients suspected of having TB	2 spot sputa	4 (2 spot sputa on day 1, 1 morning sputum on day 2, and one spot sputum on day 2)	4 (2 spot sputa on day 1, 1 morning sputum on day 2, and one spot sputum on day 2)
Adherence to TB screening algorithms	Estimated to be low	High	High
Specialized TB case finding nurses support TB case finding activities	No	Yes	Yes
Regular training for clinic personnel in ICF activities	No	Yes	Yes
Diagnostic algorithm in place	Microscopy + chest X-ray for smear-negative suspects	Microscopy + chest X-ray for smear-negative suspects	Xpert + chest X-ray for Xpert-negative suspects
Gold standard TB diagnostic test (MGIT) at national TB reference laboratory (NTRL)	Infrequent utilization of MGIT liquid TB culture at NTRL	MGIT liquid TB culture for all patients suspected of having TB. Prior to culture, fluorescent microscopy was conducted at NTRL.	MGIT liquid TB culture for all patients suspected of having TB. Prior to culture, fluorescent microscopy was conducted at NTRL.
TB drug resistance	Infrequent requests for TB drug resistance tests.	All positive MGIT TB cultures received: (1) LPA, (2) Phenotypic culture-based DST.	All positive MGIT TB cultures received: (1) LPA, (2) Phenotypic culture-based DST.
Patient tracing interventions in place	Irregular attempts to trace patients late for clinic appointments through telephone calls and home visits.	Tracing of patients late for clinic appointments through telephone calls and home visits.	Tracing of patients late for clinic appointments through telephone calls and home visits.

Abbreviations: *ICF* intensified TB Case Finding, *TB* tuberculosis, *MGIT* mycobacteria growth indicator tubes, *LPA* line probe assay, *DST* drug susceptibility testing

calendar months, each step in the stepped-wedge design needed to be equivalent to one calendar month. In addition, because the MOH and partners wanted the 13 Xpert devices to be operational and serving patients in nine months rather than 13 months, there was a need to initiate two Xpert devices during a single step for four of the nine steps. Taking into consideration constraints in Xpert device assignments to clinics (see Table 2 and Fig. 1), there were 9! (362,880) possible permutations of the order of Xpert rollout. The study statistician randomly selected one of these permutations [28].

Sample size and power—first key objective

Funding availability limited the prospective enrollment duration to 19 months at the 22 study facilities. MOH monitoring data reported an average of 23 new ART-eligible patients enrolling in each facility per month and investigators estimated there were 10 new ART-ineligible patients enrolled per month at each facility (i.e., potentially 33 study-eligible patients/month/clinic). To be conservative with sample size estimates, we assumed that only 70 % of study-eligible patients would agree to prospective enrollment in the study (i.e., 23 study patients/month/clinic), giving anticipated cohort A and B sample sizes of 3,266 and 6,348, respectively (N = 9,614). Since the vast majority of patients (>99 %) at these study clinics were adults (≥12 years old), for the purpose of sample size calculations, we assumed all 9,614 prospective enrollees would be adults.

Based on a published meta-analysis, we estimated that about 49 % of new adult HIV clinic enrollees would screen positive for TB [29], and that about 33 % of those screening positive would have culture-confirmed TB, giving an overall active TB prevalence among adult study enrollees of about 16.2 % [30–35]. Our literature review suggested true active TB prevalence among adult PLHIV entering HIV care ranged from 7.1 % in Ethiopia [34] to 31.5 % in South Africa [33]; since Botswana has a higher TB case notification rate (about 470/100,000 population) than Ethiopia (about 224/100,000 population) but a lower TB case notification rate than South Africa (about 860/100,000 population), our estimate of adult active TB prevalence of 16.2 % at HIV care entry in Botswana was considered reasonable [36–38]. Further literature review suggested that the pre-Xpert, microscopy-based TB diagnostic algorithm sensitivity might be as high as 62.5 %

Table 2 Selected study sites for the Xpert package rollout evaluation using a stepped-wedge design (XPRES)

District	Fixed consortiums	Clinic names	Pre-study estimates of no. new ART patients/month	Xpert location
Ngami (Maun)	fixed triplet	Letsholathebe II Memorial Hospital	36	1 × Lab Xpert
Ngami (Maun)		Boseja Clinic	28	
Ngami (Maun)		Maun Clinic	28	
Gaborone	fixed pair	Brodhurst Traditional Clinic	35	1 × Lab Xpert
Gaborone		Bontleng Clinic	45	
Francistown	fixed pair	Botswelelo Clinic	22	1 × Lab Xpert
Francistown		Area W Clinic	22	
Francistown	single facility	Nyangabgwe Referral Hospital	18	1 × Lab Xpert
Kweneng East-Molepolole	fixed quadruplet	Borakalalo Clinic	9	1 × POC Xpert
Kweneng East-Molepolole		Kgosing Clinic	8	
Kweneng East-Molepolole		Molepolole Central Clinic	8	
Kweneng East-Molepolole		Phuth-kobo Clinic	8	
Kweneng East-Mogoditsane	single facility	Nkoyaphiri Clinic	46	1 × POC Xpert
Palapye	fixed pair	Ext 3 Clinic	20	1 × POC Xpert
Palapye		Lotsane Clinic	20	
Bobirwa	single facility	Bobonong Primary Hospital	38	1 × Lab Xpert
Kanye	single facility	SDA Hospital	22	1 × Lab Xpert
Gantsi	single facility	Gantsi Clinic	unknown*	1 × Lab Xpert
Kgatleng	single facility	Deborah Memorial Hospital	26	1 × Lab Xpert
Lobatse	single facility	Athlon Clinic	18	1 × Lab Xpert
Serowe District	fixed pair	Kadimo Clinic	12	1 × POC Xpert
Serowe District		Serowe Clinic	12	
Total (22 clinics)			479	13
Average per clinic			23	

Abbreviations: *POC* point of care, *Xpert* Xpert MTB/RIF, *lab* laboratory, *ART* antiretroviral therapy
*Routine monitoring data on rate of enrollment of antiretroviral therapy patients was not available at the time of study initiation for this clinic

[34], and that Xpert algorithm sensitivity among symptomatic PLHIV could be about 82.5 % based on data from a recent multi-country Xpert accuracy study [12].

To estimate power, data were simulated according to the stepped-wedge design, using the beta-binomial model to induce the intra-cluster correlation coefficient. One thousand datasets were simulated and a mixed model appropriate for the stepped-wedge design fit to the data, as described by Hussey and Hughes [27], that included fixed effects for time and intervention condition (0 for time points before Xpert implementation and 1 afterward), and a random effect for the clinic, to take into account between-clinic variability. For protocol-specified sample sizes ($N = 9{,}614$), and assuming culture-positive TB prevalence of 16.2 % at study entry, and pre-versus post-Xpert sensitivity comparisons of 62.5 % vs. 82.5 %, we had 99.6 % power. In multiple simulations, pre-Xpert sensitivity was varied from 55 % to 62.5 % and post-Xpert sensitivity from 70 % to 82.5 % and the study had >80 % power to detect the intervention effect across all simulated scenarios.

After study initiation, monitoring data, prepared by study nurse supervisors during supervision visits, revealed that actual monthly HIV clinic enrollment numbers were lower than expected (about 21 patients/clinic/month instead of 33 patients/clinic/month). In addition, study nurses were only able to enroll about 72 % of study-eligible patients at the clinic, mostly because patients were not willing to wait while the study nurse completed enrollment of other patients, a process which took about 1 h/enrollee. Therefore, prospective enrollment occurred at about 15/month instead of the protocol-specified 23/month. In addition, the proportion of adult patients screening positive for TB at prospective cohort enrollment was lower than expected (24 % instead of 49 %), and the proportion of those screening positive, who were diagnosed with culture-positive TB, was lower than expected (17 % instead of 33 %), giving a much lower culture-positive TB prevalence at enrollment than was originally expected (about 4 % instead of 16 %).

Lower numbers of culture-positive TB cases/clinic/month (1/clinic/month instead of 4/clinic/month) resulted in inability to fit the stepped-wedge model to all simulated datasets. Therefore, the power estimation approach was simplified and Fisher's Exact Test for comparing two proportions (pre-and post-Xpert) in SAS version 9.2. software (SAS Institute Inc., Cary, NC) was used to estimate study power. Power estimates were then adjusted for the expected design effect to account for intra-cluster correlation. For design effect calculations, an intra-class correlation of 0.05 was assumed [28]. Input estimates for TB prevalence at HIV care enrollment and TB diagnostic sensitivity were varied to understand impact on study power (Fig. 3). As illustrated in Fig. 3, sample size and TB prevalence shortfalls meant we only had about 75.4 % power to detect the protocol-specified difference in sensitivity (62.5 % vs. 82.5 %) if active TB prevalence at enrollment was 4 %. However, we would have >80 % power to detect pre-versus post-Xpert TB diagnostic sensitivities at a culture-positive TB prevalence of 4 % if pre-Xpert sensitivity was ≤52.5 % and post-Xpert sensitivity ≥82.5 % (Fig. 3). Because available National TB Reference Laboratory (NTRL) monitoring data suggested pre-Xpert TB diagnostic sensitivity was ≤52.5 % and Xpert sensitivity ≥82.5 %, the study was considered still well powered to answer the first key study question at quarterly reviews conducted by the study sponsor.

Sample size and power—second key objective

To estimate power for the comparison of all-cause 6-month mortality in the retrospective cohort (cohort R) versus the post-Xpert prospective cohort (cohort B), the approach of Moulton et al, suitable for stepped-wedge trial designs, was chosen because these power estimates were more conservative than those derived from a pre-post sample size calculation [26]. Per this approach, published formulae for the comparison of two rates in an unmatched parallel group CRT [39] were adapted to the stepped-wedge design as follows:

$$Z_\beta = \sqrt{\frac{(c-1)(r_c - r_t)^2}{\left[r_0/y_c + r_1/y_t + k^2(r_0^2 + r_1^2)\right]}} - Z_{\alpha/2}$$

where Z_β is the standard normal deviate corresponding to the upper tail probability of β and β is the probability of a Type II error; c is the number of clusters (study facilities) per arm, where, since this is a stepped-wedge trial involving 22 clinics, 22/2 was used [26]; r_c is the estimated true 6-month ART mortality rate in the pre-intervention control phase (cohort R); r_t is the estimated true mortality rate in the post-intervention phase (cohort B); y_c is the average number of person-years (PYs) per clinic in the control phase, estimated as the average retrospective cohort size per clinic (552) divided by two since each patient commits 6 months of follow-up time to the analysis; y_t is average number of PYs per clinic in the intervention phase, conservatively estimated as the harmonic mean of PYs contributed by each study

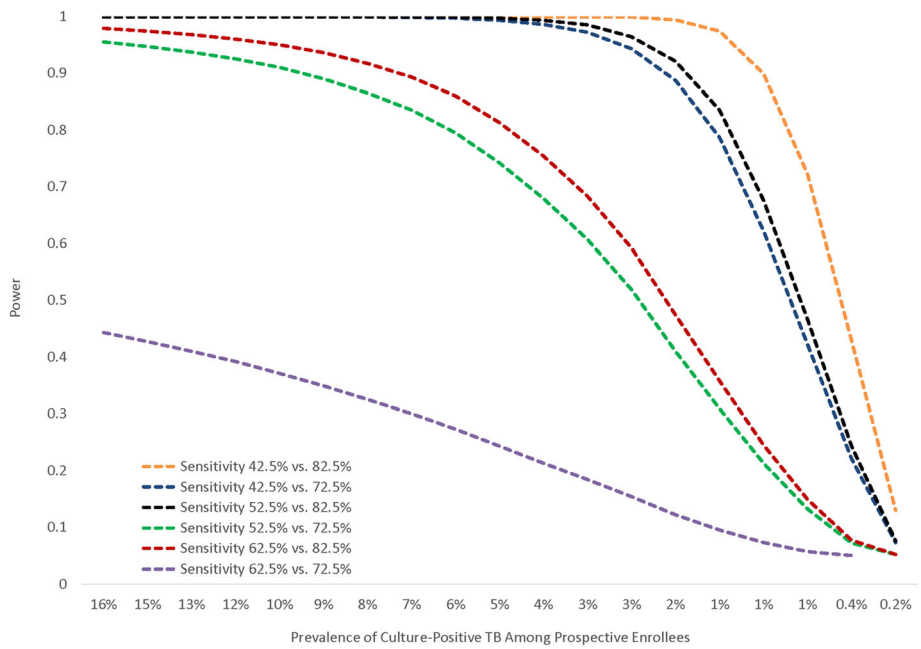


Fig. 3 Figure showing power to detect a difference in TB diagnostic algorithm sensitivity pre- versus post-Xpert over a range of culture-positive TB prevalence rates at study enrollment according to actual prospective cohort sample size ($N = 6{,}136$)

site in cohort B, again assuming 6 months of follow-up time per participant [26]; k is the estimated between-cluster coefficient of variation of the true rates in both the control and intervention phases, estimated as 0.2 [26]; $Z_{a/2}$ is the standard normal deviate corresponding to the upper tail probability of $a/2$ where a is the probability of a Type I error.

Since a log-rank test statistic for intervention effect calculated for a simulated stepped-wedge trial (Z_{SW}) will generally always be lower than the corresponding statistic (Z_E) for a parallel group trial, because allocation ratios of patients to intervention or control status for parallel group trials remain equal while for stepped-wedge trials they are usually unequal, except at the midpoint of the stepped-wedge design, the z-score in the stepped-wedge trial formula (Z_β above) was divided by a published estimate of Z_E/Z_{SW} (i.e., 1.2) prior to extrapolating the z-score to a power estimate [26]. Similarly, for Type 1 error of 5 %, instead of assuming a $Z_{\alpha/2}$ of 1.96, an inflated estimate of 2.352 was used, per published precedent [26].

Prior to study start, available data from Botswana suggested that the documented all-cause early mortality rates in the first 6 months of ART among adults were about 15 deaths per 100 PYs [40], which was similar to estimates from a meta-analyses of 18 programs in RLS with active tracing programs (14.7/100 PY) [41]. Since Botswana data, and available meta-analyses suggested about 40 % of deaths among PLHIV were due to undiagnosed TB or TB diagnosed late, and given that interrupting ART during the first 6 months of therapy by missing clinic appointments increases mortality risk [42, 43], it was considered reasonable that the Xpert package plus the tracing intervention might reduce mortality by about 40 % [2, 44]. According to protocol-specified sample sizes, the study had >80 % power to detect a difference in 6-month all-cause ART mortality between cohorts R and B of 40 % if cohort R mortality was ≥10/100 PYs (Fig. 4). According to anticipated actual sample sizes, the study has >80 % power to detect a difference in 6-month all-cause ART mortality between cohorts R and B of 40 % if cohort R mortality is ≥13/100 PYs (Fig. 4).

Study procedures related to first key objective

For prospective cohort enrollment, all new HIV clinic enrollees were informed about the study, and if interested, offered the opportunity to enroll following the Institutional Review Board (IRB)-approved consent process. An enrollment questionnaire collected important baseline demographic and clinical information, and a patient locator form was used to document telephone numbers and home addresses for patient retention activities. Adult patients screening positive for TB were asked to provide four sputa, two collected simultaneously the day of enrollment (referred to as "spot" sputa), and two the following day. Of the two sputa provided the second day, one was a morning sputum prepared by the patient soon after waking up, and the other a spot sputum provided upon arrival at the clinic. Additional file 1 shows a poster used by study nurses to inform the patient how to produce a good sputum. The poster also illustrates important infection control precautions (e.g., preparing

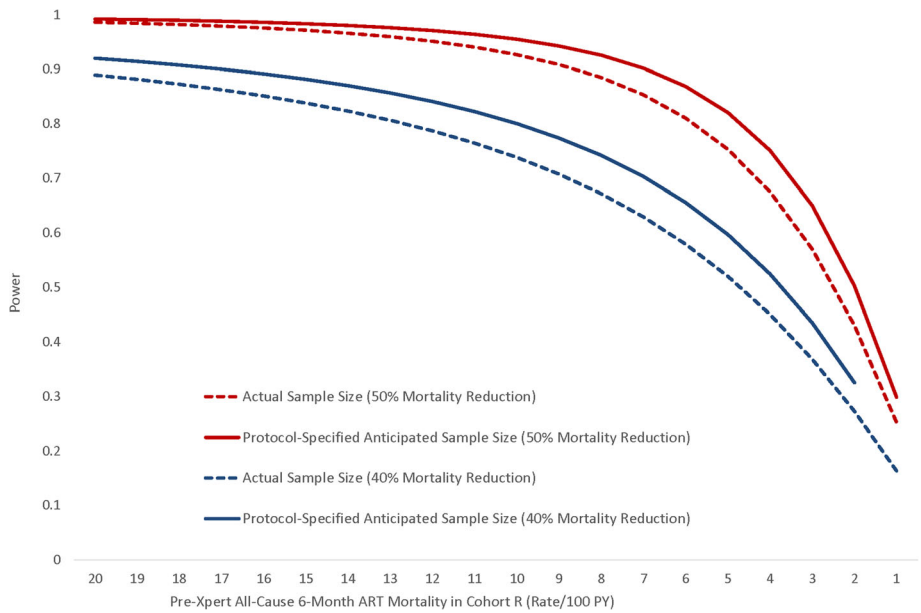


Fig. 4 Power to detect a 40 % and 50 % difference in all-cause 6-month ART mortality between pre-Xpert retrospective and post-Xpert prospective cohort enrollees over a range of pre-Xpert mortality rates

spot sputa in a well-ventilated, but still private, "cough spot" outside the clinic). If a patient screening positive for TB was unable to spontaneously produce sputa, MOH-recommended sputum-induction procedures were encouraged if there were no contra-indications [23]. For children ≤12 years old, who were unable to produce sputa spontaneously and were too young for induction (i.e., <5 years old), naso-gastric tube aspirates were recommended per MOH guidelines [23]; however, very few children <5 were expected to enroll in the study.

Spot sputa numbers one and three were sent to the on-site or peripheral district TB lab for: (1) smear microscopy, and (2) Xpert, if the Xpert device had been activated by that time. Spot sputum 2 and the morning sputum were sent to the NTRL for: (1) fluorochrome acid fast bacilli (AFB) smear microscopy on concentrated specimens, (2) liquid culture in mycobacteria growth indicator tubes, (3) confirmation of any mycobacterial growth as *Mycobacterium tuberculosis* (MTB) or non-tuberculous mycobacteria through Ziehl-Neelsen staining, blood agar plate, and immunochromatographic assays, (4) line probe assay testing for isoniazid and rifampicin resistance on MTB-positive cultures, and (5) phenotypic culture-based drug susceptibility testing on MTB-positive cultures. All test results were returned to study nurses with recommended maximum turnaround times from the time of sample collection to result return to the nurse being four days for smear microscopy at the peripheral lab, 10 days for flourochrome smear microscopy at the NTRL, two days for Xpert testing regardless of Xpert location, and 49 days for liquid culture results from the NTRL. Nurses were encouraged to inform patients of positive TB diagnoses the same day via phone, although, if the patient was unreachable by phone, the patient was informed at the next scheduled clinic appointment.

For the first 17 months of study conduct, all prospectively enrolled patients consented to 12 months of follow-up but this was shortened to 6 months of follow-up in December 2013 (17 months into study enrollment), in an attempt to reduce burden on study nurses.

Study procedures related to second key objective

Enrollment of the retrospective cohort (cohort R) was through chart abstraction of eligible patients who started ART in the 24 months before study initiation at one of the 22 study clinics. Chart abstraction procedures were similar to procedures described in previous studies [45]. Data on important demographic and clinical characteristics were abstracted to maximize opportunities to explore and control for confounding, when estimating impact of the Xpert package on all-cause mortality. Since loss to follow-up (LTFU) from ART can account for as much as 75 % of all attrition (death plus LTFU) in ART programs, and because incidence of death following LTFU ranges from 20 % to 60 % and failure to adjust mortality estimates for death among LTFU patients could bias estimates of intervention effect [40, 46], tracing patients LTFU was considered essential to answer the second key question. Tracing for mortality ascertainment purposes, was conducted through two methods in all cohorts. Firstly, up to five telephone calls and two home visits were used to determine outcomes of patients LTFU; in the retrospective cohort this occurred following documentation that the patient was >90 days late for a scheduled appointment, whereas in the prospective cohort this tracing started the day following a missed appointment. Secondly, all patients that remained LTFU following telephonic and home visit tracing activities were searched for in Botswana's national mortality database.

Since about 2,429 (28 %) of 8,565 patients eligible for the prospective cohort did not enroll due to logistical constraints (see above), a protocol amendment was approved in April 2014, that allowed retrospective chart abstraction of the missed prospective patients. By abstracting key baseline data (e.g., baseline CD4 count) and outcome data (e.g., vital status) on the 2,429 missed patients, investigators will be able to: (1) estimate if the prospective cohort is truly representative of new HIV clinic enrollees at the 22 study sites during study conduct, and (2) use appropriate methods to explore effect of non-response [47].

Analytic methods

For the first key study question, we will employ a mixed-model approach similar to that presented by Hussey and Hughes (2007) [27]. A generalized linear mixed model will be fit to the data. The dependent variable is dichotomous, indicating whether the diagnostic algorithm detected TB or not (only those with true TB detected by liquid culture will be included in the sensitivity analysis). A fixed effect for time will be included in the analysis to adjust for any time trends that might bias the pre-post comparison. A fixed effect for intervention condition (0 before Xpert implementation, 1 afterward) will also be included and is the test of the intervention effect. A random effect for clinic will be included to adjust for between-clinic variation. The intervention effect will be judged significant at $p < 0.05$ with a two-tailed test.

For the second key question, crude and multivariable Cox proportional hazards regression models, accounting for study design, will be fit to the data with a fixed effect specified for intervention status, and random effect for clinic [28]. Since there are three levels to the intervention, with cohort R receiving standard of care, cohort A receiving the ICF intervention, and cohort B receiving the ICF intervention plus the Xpert diagnostic

algorithm, intervention effect will be coded as a three-level variable to represent the three study phases. Although the protocol-specified primary question aims to compare 6-month mortality in cohort R versus cohort B, investigators will examine for any dose-response effect across cohorts R, A, and B, which could add data to inform interpretation of causal pathways [48]. Importantly, the analysis will need to control for trends over time. Several reports from RLS, including Botswana [49], have reported improvements in baseline health status at ART initiation (e.g., higher median CD4 counts) and lower incidence of 6-month ART mortality over successive annual cohorts of ART enrollees. As described earlier, because cohorts R and B do not overlap during the stepped-wedge portion of this trial, a comparison of 6-month mortality rates in cohorts R and B cannot make use of the stepped-wedge design to control for secular trends [26]. However, since most variation in 6-month ART mortality over successive annual ART cohorts is accounted for by changes in health status of ART enrollees (e.g., changes in baseline CD4 count), incorporation of these known risk factors for 6-month ART mortality into the multivariable model may fully account for secular mortality trends [7, 50].

In a secondary analysis, that excludes cohort R, we will compare 6-month ART mortality rates between cohorts A and B using analytic methods described by Moulton et al, fitting Cox proportional hazards models to the data with the underlying time frame being time since July 2012 (initiation date for the stepped-wedge component of the trial), fixed effect for intervention arm (Xpert device activation), and a random effect for clinic [26, 51]. We will also explore an alternate analytic approach, recommended by Hussey & Hughes, which utilizes a Poisson model, including fixed effect for intervention and time interval, and a random effect for cluster [27].

Ethical considerations

This research study was reviewed and approved by the CDC IRB C, the Health Research and Development Division of the Human Resource Development Council (HRDC) in Botswana, and the University of Pennsylvania IRB No.4. XPRES is registered at ClinicalTrials.gov (trial registration no. NCT02538952).

Trial status

Prospective cohort enrollment started in July, 2012 and was completed by the end of March 2014. Retrospective cohort chart abstraction was complete by December 2015. Data entry is estimated to be complete by the end of September 2016. Data analysis for the primary study questions has not yet begun. Trial data will be reported according to published guidelines for cluster-randomised trials (Additional file 2).

Discussion

The over-arching purpose of this project is to improve TB diagnostic and care services at 22 HIV care and treatment clinics through phased rollout of (1) strengthened ICF systems, and (2) 13 Xpert devices, while simultaneously answering important implementation science questions, concerning Xpert operationalization and impact.

The stepped-wedge study design was chosen for a number of reasons related to ethical, operational, and analytic needs, as described in the method's section. During the course of study implementation, the operational advantages of the phased implementation approach have been particularly notable. In our RLS of Botswana, the phased implementation approach has allowed the limited human and financial resources to be focused on smaller, more manageable pieces of the whole project, one step at a time, rather than be spread thinly across study sites, as would be required in a parallel group CRT [19]. Analytically, the stepped-wedge design allows multiple opportunities for controlling trends over time [26]. Potential disadvantages, when compared with a parallel group CRT, include: (1) moderately lower ability to assign causality to the intervention, and (2) higher sample size requirements in most circumstances, because of unequal allocation ratios for most of the duration of stepped-wedge trials [19]. The ethical, operational, and analytic advantages may help explain the increasing popularity of the stepped-wedge evaluation design, especially in RLS [24].

During trial conduct, several operational challenges were experienced, mainly related to lower than expected clinic enrolment rates, human resource constraints that reduced ability to enroll all study-eligible patients in the prospective cohort, and lower than expected prevalence of culture-positive TB at clinic enrollment. The declining HIV clinic enrolment rates probably reflect success of the HIV treatment program in reaching HIV-infected persons in prior years (i.e., during 2002–2011) [49], declining HIV incidence rates [52], and expanding numbers of alternate HIV clinics at which patients can receive care [49]. The study team probably overestimated the willingness of patients to wait at the clinic for their turn to enroll in the study. However, in response to the observation that 28 % of potentially study-eligible patients were not being enrolled in the prospective cohort, the study team wrote a protocol amendment that allowed retrospective chart abstraction for the missed prospective patients, which will allow investigators to quantify any potential selection bias incurred by non-response. The lower than expected prevalence of culture-positive TB at HIV clinic enrollment

needs further investigation once all study data are available for analysis. Fueled by the HIV epidemic, TB case notification rates in Botswana increased from about 202/100,000 population in 1990 to about 600/100,000 in 1998, plateaued at this level during 1998 through 2007, and have since declined to about 470/100,000 in recent years [36]. Increased ART coverage among HIV-infected persons might again explain declining national TB incidence and the lower-than-expected TB prevalence among HIV clinic enrollees in this study [53]. In retrospect, the protocol-specified large sample sizes and resulting high pre-study power to answer the first two primary study questions, were important precautions in place to ensure any sample size shortfalls did not result in trial futility.

Although, several Xpert impact studies have been published after this trial started, the two key study questions have not yet been answered. Firstly, data validating the Botswana Xpert diagnostic algorithm have not yet been reported, and this is an important program evaluation activity [54]. Secondly, among six trials that have compared all-cause mortality outcomes of study enrollees between microscopy and Xpert arms [17, 21, 54–57], none have observed Xpert impact on either morbidity or mortality outcomes, and only one was conducted exclusively among ART enrollees (Mupfumi et al) [21]. Certain study limitations of the trial by Mupfumi et al, including small sample size ($N = 424$) and powering the study to detect differences in a composite outcome (death or TB) between study arms, mean that XPRES, with its larger sample size ($N = 16{,}267$) and powered to detect Xpert impact on 6-month mortality rates specifically, is still positioned to provide a valuable scientific contribution. In addition, the intervention in XPRES is different from interventions employed in previous Xpert impact trials [17, 21, 54–59]—it represents a package of strengthened ICF interventions, activation of the Xpert device, and improved tracing for patients late for ART clinic appointments. In real-world settings, ICF interventions are often implemented at a sub-optimal level of quality and consistency due to health system weakness [9], and strengthening health systems to improve ICF compliance is arguably as important as the rollout of a new TB diagnostic device [17]. In addition, preventing treatment interruptions or LTFU during early ART through the tracing intervention, could contribute to reductions in all-cause, 6-month ART mortality rates [42, 43].

Additional files

Additional file 1: Study Poster of Steps to Getting a Good Sputum Sample. (PDF 804 kb)

Additional file 2: CONSORT 2010 checklist of information to include when reporting a cluster randomised trial–XPRES Trial Checklist. (PDF 392 kb)

Abbreviations

AFB: Acid fast bacilli; ART: Antiretroviral therapy; CDC: United States centers for disease control and prevention; CRT: Cluster-randomized trial; HCW: Healthcare workers; HRDC: Human resource development council; IRB: Institutional review board; LTFU: Loss to follow-up; MOH: Ministry of health; MTB: *Mycobacterium tuberculosis*; NTRL: National TB reference laboratory; PHC: Primary healthcare clinic; PLHIV: Persons living with HIV; PY: Person-years; RLS: Resource-limited settings; SAS: Statistical analysis software; TB: Tuberculosis; intensified tuberculosis case finding (ICF); WHO: World Health Organization; Xpert: Xpert MTB/RIF assay; XPRES: Xpert package rollout evaluation using a stepped-wedge design

Acknowledgements

This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the U.S. Centers for Disease Control and Prevention. We thank all study participants who made this research possible.

Availability of data and materials

The data supporting the content and discussion of the study protocol are included within the article and its additional files.

Authors' contributions

Authors AFA, SP, TA, AF, RB, HA, JB, JS, TVE, and AD conceived the study. Protocol writing was led by AFA and TA, with contributions from all authors. Design of protocol amendments were led by AFA and TA, with contributions from all authors. Power analysis and analytic approach were advised and implemented by AFA and SP. Authors TA, AF, RB, AM, JB, AFA, and SG, were primarily responsible for study implementation but all authors contributed. All authors contributed to study monitoring and supervision. All authors helped write and review the paper for intellectual content. AFA drafted the final version of the paper. All authors have approved the final manuscript submitted.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical approvals for this study were obtained from the U.S. Centers for Disease Control and Prevention (CDC) Institutional Review Board (IRB) C, the Health Research and Development Division of the Human Resource Development Council (HRDC) in Botswana, and the University of Pennsylvania IRB No.4. These are available in Additional file 2. Written informed consent was obtained from all prospectively enrolled patients. A waiver of informed consent was granted for abstraction of data from charts of retrospective cohort patients under 45CFR 46.116 (d).

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Sources of support

This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the U.S. Centers for Disease Control and Prevention.

Author details

[1]Division of Global HIV & TB, Center for Global Health, United States Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, Atlanta, GA 30333, USA. [2]Centers for Disease Control and Prevention Botswana, Plot 14818 Lebatlane Road, Gaborone, Botswana. [3]Division of TB Elimination, National Center for HIV, Hepatitis and STD Prevention, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA. [4]Ministerial Strategy Office, Ministry of Health, 24 Amos Street, Gaborone, Botswana. [5]Yale University School of Medicine, 333 Cedar St, New Haven, CT 06510, USA.

Received: 12 July 2016 Accepted: 8 October 2016
Published online: 26 October 2016

References

1. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS. 2015;29(15):1987–2002.
2. Cox JA, Lukande RL, Lucas S, Nelson AM, Van Marck E, Colebunders R. Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. AIDS Rev. 2010;12(4):183–94.
3. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. AIDS. 2008;22(15):1897–908.
4. Wong EB, Omar T, Setlhako GJ, Osih R, Feldman C, Murdoch DM, et al. Causes of death on antiretroviral therapy: a post-mortem study from South Africa. PLoS One. 2012;7(10), e47542.
5. Centers for Disease C, Prevention. Differences Between HIV-Infected Men and Women in Antiretroviral Therapy Outcomes - Six African Countries, 2004–2012. MMWR Morb Mortal Wkly Rep. 2013;62(47):946–52.
6. Lawn SD, Wilkinson RJ, Lipman MC, Wood R. Immune reconstitution and "unmasking" of tuberculosis during antiretroviral therapy. Am J Respir Crit Care Med. 2008;177(7):680–5.
7. Auld AF, Ekra KA, Shiraishi RW, Tuho MZ, Kouakou JS, Mohamed F, et al. Temporal trends in treatment outcomes for HIV-1 and HIV-2-infected adults enrolled in Cote d'Ivoire's national antiretroviral therapy program. PLoS One. 2014;9(5), e98183.
8. Auld AF, Tuho MZ, Ekra KA, Shiraishi RW, Mohamed F, Kouakou JS, et al. Temporal Trends in Mortality and Loss to Follow-up Among Children Enrolled in Cote d'Ivoire's National Antiretroviral Therapy Program. Pediatr Infect Dis J. 2014;33(11):1134–40.
9. Auld AF, Mbofana F, Shiraishi RW, Alfredo C, Sanchez M, Ellerbrock TV, et al. Incidence and determinants of tuberculosis among adults initiating antiretroviral therapy—Mozambique, 2004–2008. PLoS One. 2013;8(1), e54665.
10. Chihota VN, Ginindza S, McCarthy K, Grant AD, Churchyard G, Fielding K. Missed Opportunities for TB Investigation in Primary Care Clinics in South Africa: Experience from the XTEND Trial. PLoS One. 2015;10(9), e0138149.
11. Parsons LM, Somoskovi A, Gutierrez C, Lee E, Paramasivan CN, Abimiku A, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clin Microbiol Rev. 2011;24(2):314–50.
12. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet. 2011;377(9776):1495–505.
13. Dheda K, Gumbo T, Gandhi NR, Murray M, Theron G, Udwadia Z, et al. Global control of tuberculosis: from extensively drug-resistant to untreatable tuberculosis. Lancet Respir Med. 2014;2(4):321–38.
14. Small PM, Pai M. Tuberculosis diagnosis—time for a game change. N Engl J Med. 2010;363(11):1070–1.
15. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014;1, CD009593.
16. Lawn SD, Meintjes G, McIlleron H, Harries AD, Wood R. Management of HIV-associated tuberculosis in resource-limited settings: a state-of-the-art review. BMC Med. 2013;11:253.
17. Churchyard GJ, Stevens WS, Mametja LD, McCarthy KM, Chihota V, Nicol MP, et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. Lancet Glob Health. 2015;3(8):e450–7.
18. Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis. 2013;13(4):349–61.
19. Sanson-Fisher RW, D'Este CA, Carey ML, Noble N, Paul CL. Evaluation of systems-oriented public health interventions: alternative research designs. Annu Rev Public Health. 2014;35:9–27.
20. Lawn SD, Harries AD. Reducing tuberculosis-associated early mortality in antiretroviral treatment programmes in sub-Saharan Africa. AIDS. 2011;25(12):1554–5.
21. Mupfumi L, Makamure B, Chirehwa M, Sagonda T, Zinyowera S, Mason P, et al. Impact of Xpert MTB/RIF on Antiretroviral Therapy-Associated Tuberculosis and Mortality: A Pragmatic Randomized Controlled Trial. Open Forum Infect Dis. 2014;1(1):ofu038.
22. World Health Organization. Rapid Implementation of the Xpert MTB/RIF diagnostic test: 2011. Available at: http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf. Accessed 18 Aug 2015.
23. Ministry of Health, Republic of Botswana. National Tuberculosis Programme Manual. Seventh Edition. 2011.
24. Mdege ND, Man MS, Taylor Nee Brown CA, Torgerson DJ. Systematic review of stepped wedge cluster randomized trials shows that design is particularly used to evaluate interventions during routine implementation. J Clin Epidemiol. 2011;64(9):936–48.
25. Maclure M. Explaining pragmatic trials to pragmatic policy-makers. CMAJ. 2009;180(10):1001–3.
26. Moulton LH, Golub JE, Durovni B, Cavalcante SC, Pacheco AG, Saraceni V, et al. Statistical design of THRio: a phased implementation clinic-randomized study of a tuberculosis preventive therapy intervention. Clin Trials. 2007;4(2):190–9.
27. Hussey MA, Hughes JP. Design and analysis of stepped wedge cluster randomized trials. Contemp Clin Trials. 2007;28(2):182–91.
28. Hayes R, Moulton LH. Cluster Randomized Trials. Boca Raton: CRC Press; 2009.
29. Getahun H, Kittikraisak W, Heilig CM, Corbett EL, Ayles H, Cain KP, et al. Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. PLoS Med. 2011;8(1), e1000391.
30. Lawn SD, Brooks SV, Kranzer K, Nicol MP, Whitelaw A, Vogt M, et al. Screening for HIV-Associated Tuberculosis and Rifampicin Resistance before Antiretroviral Therapy Using the Xpert MTB/RIF Assay: A Prospective Study. PLoS Med. 2011;8(7), e1001067.
31. Bassett IV, Wang B, Chetty S, Giddy J, Losina E, Mazibuko M, et al. Intensive tuberculosis screening for HIV-infected patients starting antiretroviral therapy in Durban, South Africa. Clin Infect Dis. 2010;51(7):823–9.
32. Lawn SD, Edwards DJ, Kranzer K, Vogt M, Bekker LG, Wood R. Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy diagnostic yield and association with immune reconstitution disease. AIDS. 2009;23(14):1875–80.
33. Lawn SD, Kranzer K, Edwards DJ, McNally M, Bekker LG, Wood R. Tuberculosis during the first year of antiretroviral therapy in a South African cohort using an intensive pretreatment screening strategy. AIDS. 2010;24(9):1323–8.
34. Shah S, Demissie M, Lambert L, Ahmed J, Leulseged S, Kebede T, et al. Intensified tuberculosis case finding among HIV-Infected persons from a voluntary counseling and testing center in Addis Ababa, Ethiopia. J Acquir Immune Defic Syndr. 2009;50(5):537–45.
35. Cain KP, McCarthy KD, Heilig CM, Monkongdee P, Tasaneeyapan T, Kanara N, et al. An algorithm for tuberculosis screening and diagnosis in people with HIV. N Engl J Med. 2010;362(8):707–16.
36. World Health Organization. African Health Observatory: Better information, better action for health. Available at: http://www.aho.afro.who.int/profiles_information/index.php/Botswana:Analytical_summary_-_Tuberculosis. Accessed 18 Aug 2015.
37. Ministry of Health, Ethiopia. Incidence of Tuberculosis in Ethiopia. Available at: http://www.tradingeconomics.com/ethiopia/incidence-of-tuberculosis-per-100-000-people-wb-data.html. Accessed 18 Aug 2015.
38. World Health Organization. Global Tuberculosis Report-2014. Available at: http://www.who.int/tb/publications/global_report/gtbr14_main_text.pdf. Accessed 18 Aug 2015.
39. Hayes RJ, Bennett S. Simple sample size calculation for cluster-randomized trials. Int J Epidemiol. 1999;28(2):319–26.
40. Bisson GP, Gaolathe T, Gross R, Rollins C, Bellamy S, Mogorosi M, et al. Overestimates of survival after HAART: implications for global scale-up efforts. PLoS One. 2008;3(3), e1725.
41. Braitstein P, Brinkhof MW, Dabis F, Schechter M, Boulle A, Miotti P, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. Lancet. 2006;367(9513):817–24.
42. Brinkhof MW, Dabis F, Myer L, Bangsberg DR, Boulle A, Nash D, et al. Early loss of HIV-infected patients on potent antiretroviral therapy programmes in lower-income countries. Bull World Health Organ. 2008;86(7):559–67.
43. Cornell M, Lessells R, Fox MP, Garone DB, Giddy J, Fenner L, et al. Mortality among adults transferred and lost to follow-up from antiretroviral therapy programmes in South Africa: a multicenter cohort study. J Acquir Immune Defic Syndr. 2014;67(2):e67–75.

44. Ansari NA, Kombe AH, Kenyon TA, Hone NM, Tappero JW, Nyirenda ST, et al. Pathology and causes of death in a group of 128 predominantly HIV-positive patients in Botswana, 1997–1998. Int J Tuberc Lung Dis. 2002;6(1):55–63.
45. Auld AF, Mbofana F, Shiraishi RW, Sanchez M, Alfredo C, Nelson LJ, et al. Four-Year Treatment Outcomes of Adult Patients Enrolled in Mozambique's Rapidly Expanding Antiretroviral Therapy Program. PLoS One. 2011;6(4), e18453.
46. Geng EH, Glidden DV, Bangsberg DR, Bwana MB, Musinguzi N, Nash D, et al. A causal framework for understanding the effect of losses to follow-up on epidemiologic analyses in clinic-based cohorts: the case of HIV-infected patients on antiretroviral therapy in Africa. Am J Epidemiol. 2012;175(10):1080–7.
47. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. Stat Methods Med Res. 2013;22(3):278–95.
48. Lucas RM, McMichael AJ. Association or causation: evaluating links between "environment and disease". Bull World Health Organ. 2005;83(10):792–5.
49. Farahani M, Vable A, Lebelonyane R, Seipone K, Anderson M, Avalos A, et al. Outcomes of the Botswana national HIV/AIDS treatment programme from 2002 to 2010: a longitudinal analysis. Lancet Glob Health. 2014;2(1):e44–50.
50. Auld AF, Shiraishi R, Couto A, Mbofana F, Xavier C, Alfredo C, et al. Evaluation of outcome trends and determinants and new models of service delivery among more than 300,000 adults starting antiretroviral therapy in Mozambique during 2004–2013. JAIDS. 2016;(in press).
51. Durovni B, Saraceni V, Moulton LH, Pacheco AG, Cavalcante SC, King BS, et al. Effect of improved tuberculosis screening and isoniazid preventive therapy on incidence of tuberculosis and death in patients with HIV in clinics in Rio de Janeiro, Brazil: a stepped wedge, cluster-randomised trial. Lancet Infect Dis. 2013;13(10):852–8.
52. UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2013. Available at: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf. Accessed 14 Dec 2015.
53. Lawn SD, Harries AD, Williams BG, Chaisson RE, Losina E, De Cock KM, et al. Antiretroviral therapy and the control of HIV-associated tuberculosis. Will ART do it? Int J Tuberc Lung Dis. 2011;15(5):571–81.
54. Yoon C, Cattamanchi A, Davis JL, Worodria W, den Boon S, Kalema N, et al. Impact of Xpert MTB/RIF testing on tuberculosis management and outcomes in hospitalized patients in Uganda. PLoS One. 2012;7(11), e48599.
55. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet. 2014;383(9915):424–35.
56. Cox HS, Mbhele S, Mohess N, Whitelaw A, Muller O, Zemanay W, et al. Impact of Xpert MTB/RIF for TB diagnosis in a primary care clinic with high TB and HIV prevalence in South Africa: a pragmatic randomised trial. PLoS Med. 2014;11(11), e1001760.
57. Calligaro GL, Theron G, Khalfey H, Peter J, Meldau R, Matinyenya B, et al. Burden of tuberculosis in intensive care units in Cape Town, South Africa, and assessment of the accuracy and effect on patient outcomes of the Xpert MTB/RIF test on tracheal aspirate samples for diagnosis of pulmonary tuberculosis: a prospective burden of disease study with a nested randomised controlled trial. Lancet Respir Med. 2015;3(8):621–30.
58. Durovni B, Saraceni V, van den Hof S, Trajman A, Cordeiro-Santos M, Cavalcante S, et al. Impact of replacing smear microscopy with Xpert MTB/RIF for diagnosing tuberculosis in Brazil: a stepped-wedge cluster-randomized trial. PLoS Med. 2014;11(12), e1001766.
59. van Kampen SC, Susanto NH, Simon S, Astiti SD, Chandra R, Burhan E, et al. Effects of Introducing Xpert MTB/RIF on Diagnosis and Treatment of Drug-Resistant Tuberculosis Patients in Indonesia: A Pre-Post Intervention Study. PLoS One. 2015;10(6), e0123536.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

Copyright Clearance Center RightsLink®

Help Email Support

SPRINGER NATURE

Implementation of a pragmatic, stepped-wedge cluster randomized trial to evaluate impact of Botswana's Xpert MTB/RIF diagnostic algorithm on TB diagnostic sensitivity and early antiretroviral therapy mortality

Author: Andrew F. Auld et al

Publication: BMC Infectious Diseases

Publisher: Springer Nature

Date: Oct 26, 2016

Copyright © 2016, Springer Nature

Creative Commons

This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

You are not required to obtain permission to reuse this article.

CC0 applies for supplementary material related to this article and attribution is not required.

© 2020 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Terms and Conditions

Comments? We would like to hear from you. E-mail us at customercare@copyright.com

10.2. Appendix 2. Data collection and consent forms for XPRES

10.2.1. Information and consent form – prospective adult enrolees (>18 years old at time of Consent) in EC and EC+X phases

Instructions for study nurse only:

1. *The statement should be read to eligible enrolees aged ≥18 years old.*
2. *Only read the non-italicized parts.*
3. *Throughout the process of obtaining consent, it is important that you are patient and allow the respondent to ask questions and to consider the decision. Never rush or otherwise pressure the respondent to give consent.*
4. *Offer a copy of this consent script to all eligible patients who signed the consent.*
5. *Keep the other copy in the consent form folder, locked in a cabinet, at the clinic.*

(Flesch-Kincaid Grade Level: 7.5)

See next page for consent form

[Title of the study]: *Evaluating Performance, Impact, and Operational Challenges of GeneXpert use for TB Case Finding among HIV-infected Persons in Botswana during 2011-2013.*

[Introduction]:

Hello, my name is…….I am a trained health care provider working with the Ministry of Health of Botswana and the United States Centers for Disease Control and Prevention or CDC. I am responsible for checking whether you might have the sickness called tuberculosis. There is also a research study at this clinic and I am responsible for managing the research study. I will explain the research study, but first let me tell you about the sickness called TB.

TB is caused by a germ which is too small to see. The germ usually lives in a person's lungs. Coughing is one sign that a patient might have TB. TB is a common disease especially in people living with HIV. It is also a common cause of death amongst people with HIV. Once TB is detected, it can be treated in most patients. Treatment helps patients to feel better and live longer. However, detecting which people have TB, is difficult.

Recently, a new TB test was developed. The new test is called "Gene Xpert". Gene Xpert is a machine that can detect TB germs in a person's cough sputum. Sputum is the liquid that a person produces when they cough . The test can be done in less than 24 hours. GeneXpert is better than TB tests which rely on microscopes (microscopes are tools which help people to look at very tiny things like TB germs).

We are doing a research study to find out the best way to use GeneXpert to detect TB in people living with HIV in Botswana. We think that if we can find the best way to use GeneXpert, we can detect TB infection earlier, treat TB earlier and make people healthier.

The CDC has paid for this research study. CDC and the Ministry of Health are partners in this study.

[Reason for Asking the Patient to be Part of the Study]

We are going to ask if you want to be part of this study. The reason we are asking you, and not all patients at this clinic is that we need patients visiting the clinic for the first time to be in the study. We think about 10,000 people will be enrolled in the study in total. About 500 of these people might be children less than 18 years old.

[Procedures]

If you agree to be in the study, your part in the study will start today. We will do the following today and tomorrow.:

1. Ask you to sign this paper, showing that you agree to be part of the study.

2. Ask you some questions like “What is your age?” and “Are you married?”
3. Ask you some questions about whether you are coughing, sweating at night, losing weight, or have a fever.
4. Review your records prepared by the doctor to get information like your weight, height, and health history.
5. Ask you for contact details (telephone numbers) so that we can contact you or a friend or a family member if you miss a future clinic visit. Note that this is standard practice recommended by the Ministry of Health and is not something specific to this study.
6. Ask you for your omang number to help us with data collection from your medical records.
7. If you have one of the signs of TB, we will ask you to cough sputum into two cups for us today. If you are unable to cough, we may need to assist you by advising you how to produce sputum.
8. Once you have produced the cough sputum, we will ask you to return tomorrow to the clinic. We will give you a container to cough into when you wake up tomorrow morning and will ask you to bring the container with you to the clinic tomorrow.
9. When you arrive at the clinic tomorrow, we will ask for a fourth cup of cough sputum.
10. All sputa will go to laboratories for TB tests, including TB microscopy and culture.

If the GeneXpert test has been set up at the TB lab at the time we collect your sputum, we will test the sputum with the GeneXpert device. (Note that all patients attending the clinic will have access to the GeneXpert device, after its set up, even if they are not part of the study). Any left-over sputum at the lab will be discarded according to government guidelines. However, if TB germs are identified, these germs will be studied to identify their type and, if you agree, may be used in future studies. After tomorrow, we will contact you by telephone if we find that you have TB and will ask you to come back to the clinic as soon as possible to start TB treatment. If you do not have TB, we will ask you to visit with me (the study nurse), each time you come to the clinic to see the doctor treating you for HIV. The clinic visit schedule varies depending on whether the doctor recommends you start HIV treatment with drugs called antiretrovirals or not. If you don’t start antiretrovirals, clinic visits will be according to the schedule provided by your doctor. If you do start antiretrovirals, clinic visits will be at 2 weeks, 1 month, 2 months, 3 months and then 3 monthly thereafter.

At each clinic visit, after you have seen the doctor, you will come to see me (the study nurse) and we will:

1. Make sure you have the results, which are ready, from your previous sputum tests.
2. Explain the results to you.
3. Ask additional questions about your health.

Record additional data from you medical record.

Most of the procedures we have just described would occur even if you did not enroll in the study. However, the following procedures will occur only because you agree to be in this study: (1) interview at each clinic visit, (2) collection of data from your medical record, (3) 4 instead of 2 sputa collected if you have TB symptoms, and (4) testing of your sputum for a drug resistant form of TB.

[Time Commitment]

If you agree to the study, today we will need about an extra hour of your time, in addition to the time you would have needed to spend at the clinic if there were no study.

At each subsequent visit, we will need about half an hour to an hour of your time for the study.

Your part in the study will end 6 months from the time you agreed to be part of the study, or, if you start TB treatment during the next 6 months, and TB treatment ends more than 6 months from now, your part in the study will end at the end of TB treatment.

[Confidentiality]

As part of the study, we will collect your name and telephone number so that we can contact you if you miss an HIV clinic appointment. However, everything we write down about you will be kept confidential as far as possible. We will store the paper questionnaires in sealed brown envelopes and in locked steel cabinets here at the clinic. When transporting the paper questionnaires to Gaborone, sealed boxes will be used. The questionnaires will be stored in Gaborone in locked steel cabinets and entered into a computer protected by a password. We will not use your name in any printed reports for the study.

[Risks/Discomfort]

We do not anticipate any additional health risks due to enrolment in the study. However, there is small risk of loss of privacy. For example, if you miss a clinic visit, and we cannot reach you by phone, we would like to visit your home to find out if there is a problem preventing you attending the clinic. We will only visit your home if you have missed the clinic appointment by more than 7 days and we are unable to reach you or a friend by phone.

[Benefits for You]

There may be additional health benefits for you due to enrolment in the study because if you have symptoms of TB, as part of the study, we will test the sputum for drug resistant TB, a test not routinely offered to all patients with TB symptoms. This might help to identify and treat this form of TB early which could improve your health.

[Benefits for the Program]

By taking part in the study, you will be helping the Ministry of Health and CDC find the best ways to use GeneXpert. For example, the study may show that GeneXpert works best in district laboratories rather than in small peripheral clinics. This finding would direct future use of GeneXpert in Botswana maximizing the usefulness of GeneXpert for future patients.

[Your Rights]

You can choose not to take part in the study. If you choose not to take part in the study, we will still give you the standard of care recommended by the Ministry of Health. There will be no penalties for choosing not to

take part. You will also be able to leave the study at any time, without penalty. You may take a copy of the consent form with you. Now, or at any time in the future, you can ask any member of the study team any questions about the study.

[Contact Persons for Additional Information]

If you have any questions about this study now, please ask us now.

If you have any questions about this study in the future, please contact.

[Dr James Shepherd Principal Investigator at* 367-2430*]

If you feel that you have been harmed by this study, please contact ***[Chawangwa Lesedi Study Coordinator at 367-2534 CDC Botswana]***

If you have any concerns about your rights in this study, please contact ***[Mr. Pilate Khulumani, Health Research Development Committee - Ministry of Health at 3632775]***

[Consent]

If you agree to the following statements, please sign your name below: "I agree to be in this study. The information in this consent form has been explained to me. I have been given a chance to ask questions. I feel that all of my questions have been answered. I know that being in this study is my choice. I know that after choosing to be in this study, I may leave the study at any time. If I wish to leave this study, I will contact ***the study nurse at my clinic***. If I leave this study, I will continue to get regular medical care at this clinic or hospital.

I understand that I will receive a copy of this signed and dated consent form. By signing and dating this consent form, I have not waived any of the legal rights that I would have if I were not a participant in the study." I have read or heard this form read to me.

By signing below, I consent to join this study.

By ticking this box ☐ **I also agree** *to let the researchers use any TB germs for future studies.*

_____ _____

Name of participant Signature or thumb print of participant

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

I verify that the consent form has been read and explained accurately by a member of the study staff.

_____ _____

Name of witness Signature of witness

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

Study Staff Administering Consent _____

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

10.2.2. Information and consent form for guardians of minors (<18 years)

Instructions for study nurse only:

1. *The statement should be read to legal guardians of eligible enrolees aged <18 years old.*
2. *Only read the non-italicized parts.*
3. *Throughout the process of obtaining consent, it is important that you are patient and allow the respondent to ask questions and to consider the decision. Never rush or otherwise pressure the respondent to give consent.*
4. *Offer a copy of this consent script to all guardians whether they consent to enrolment of the child in their care or not.*
5. *Keep the other copy in the consent form folder, locked in a cabinet, at the clinic. (Flesch-Kincaid Grade Level: 7.7)*

See next page for consent form

[Title of the study]: *Evaluating Performance, Impact, and Operational Challenges of GeneXpert use for TB Case Finding among HIV-infected Persons in Botswana during 2011-2013.*

[Introduction]:

Hello, my name is…….I am a trained health care provider working with the Ministry of Health of Botswana and the United States Centers for Disease Control and Prevention or CDC. I am responsible for checking whether patients might have the sickness called tuberculosis or TB. . There is also a research study at this clinic and I am responsible for managing the research study. I will explain the study, but first let me tell you about the sickness called TB.

TB is caused by a germ which is too small to see. The germ usually lives in a person's lungs. Coughing is one sign that a patient might have TB. TB is a common disease especially in people living with HIV. It is also a common cause of death amongst people with HIV. Once TB is detected, it can be treated in most patients. Treatment helps patients to feel better and live longer. However, detecting which people have TB, is difficult.

Recently, a new TB test was developed. The new test is called "GeneXpert". GeneXpert is a machine that can detect TB germs in a person's cough sputum. Sputum is the liquid that a person produces when they cough. Young children sometimes do not cough up sputum. Instead, young children often swallow it. The GeneXpert test can be done in less than 24 hours. GeneXpert is better than TB tests which rely on microscopes (microscopes are tools which help people to look at very tiny things like TB germs).

We are doing a research study to find out the best way to use GeneXpert to detect TB in people living with HIV in Botswana. We think that if we can find the best way to use GeneXpert, we can detect TB infection earlier, treat TB earlier and make people healthier.

The CDC has paid for this research study. The CDC and the Ministry of Health are partners in this study.

[Reason for Asking the Patient to be Part of the Study]

We are going to ask if you will allow the child in your care to be part of this research study. The reason we are asking for your child to be enrolled in the study, and not all patients at this clinic, is that we need patients visiting the clinic for the first time to be in the study. We think about 10,000 people will be enrolled in the study in total. About 500 of these people might be children less than 18 years old.

[Procedures]

If you agree for the child in your care to be in the study, the child's part in the study will start today. We will do the following today and tomorrow:

1. Ask you to sign this paper, showing that you agree for the child in your care to be part of the study.
2. Ask you some questions like “What is your child’s age?”
3. Ask you some questions such as whether your child is coughing, sweating at night, losing weight, has a fever, or has reduced playfulness.
4. Review your child’s records, prepared by the doctor, to get information like your child’s weight, height, and health history.
5. Ask you for contact details (telephone numbers) so that we can contact you or a friend or a family member if your child misses a future clinic visit. Note that this is standard practice recommended by the Ministry of Health and is not something specific to this study.
6. Ask you for your child’s omang number to help us with data collection from medical records.
7. If your child has one of the signs of TB, we will ask your child to cough sputum into two cups for us today. If your child is unable to cough, we may need to advise and encourage your child to cough, or we may need to use a tube to suck swallowed sputum out of your child’s stomach.
8. Once your child has produced the cough sputum, we will ask you and your child to return tomorrow to the clinic. If your child can cough, we will give you a container for the child to cough into when your child wakes up tomorrow morning. We will then ask you to bring the container with you to the clinic tomorrow. If your child cannot cough, we will collect two sputa tomorrow when you get to the clinic.
9. If your child is <=12 years old, even if he/she has no TB symptoms, we will ask your child to cough in one sputum container today and one container tomorrow. If your child is unable to cough, we may need to advise and encourage your child to cough, but we will not use a tube to suck swallowed sputum out of your child’s stomach, if he/she has no symptoms of TB.
10. All sputa will go to laboratories for TB tests.

If the GeneXpert test has been set up at the TB lab at the time we collect your child’s sputum, we will test the sputum with the GeneXpert device. (Note that all patients attending the clinic will have access to the GeneXpert device, after its set up, even if they are not part of the study).Any left-over sputum at the lab will be discarded according to government guidelines. However, if TB germs are identified, these germs will be studied to identify their type and, if you agree, may be used in future studies. After tomorrow, we will contact you by telephone if we find that your child has TB and will ask you and your child to come back to the clinic as soon as possible to start TB treatment. If your child does not have TB, we will ask you and your child to visit with me (the study nurse), each time you and your child comes to the clinic to see the doctor treating your child for HIV. The clinic visit schedule varies depending on whether the doctor recommends your child starts HIV treatment with drugs called antiretrovirals or not. If your child does not start antiretrovirals, clinic visits will be according to the schedule provided by your doctor. If your child does start antiretrovirals, clinic visits will be at 2 weeks, 1 month, 2 months, 3 months and then 3 monthly thereafter. At each clinic visit, after you have seen the doctor, you and your child will come to see me (the study nurse) and we will:

11. Make sure you have the results, which are ready, from your child’s previous sputum tests.

12. Explain the results to you and your child.
13. Ask additional questions about your child's health.
14. Record additional data from your child's medical record.

Most of the procedures we have just described would occur even if your child did not enroll in the study. However, the following procedures will occur only because you agree for your child to be in this study: (1) interview at each clinic visit, (2) collection of data from your child's medical record, (3) 4 instead of 2 sputa collected if your child has TB symptoms, (4) testing of your child's sputum for a drug resistant form of TB.

[Time Commitment]

If you agree to the study, today we will need about an extra hour of your time, in addition to the time you and your child would have needed to spend at the clinic if there were no study.

At each subsequent visit, we will need about half an hour to an hour of your time and your child's time for the study.

Your child's part in the study will end 6 months from the time you and your child agreed to be part of the study, or, if your child starts TB treatment during the next 6 months, and TB treatment ends more than 6 months from now, your child's part in the study will end at the end of TB treatment.

[Confidentiality]

As part of the study, we will collect your name and telephone number and your child's name so that we can contact you and your child if your child misses an HIV clinic appointment. However, everything we write down about you and your child will be kept confidential as far as possible. We will store the paper questionnaires in sealed brown envelopes and in locked steel cabinets here at the clinic. When transporting the paper questionnaires to Gaborone, sealed boxes will be used. The questionnaires will be stored in Gaborone in locked steel cabinets and entered into a computer protected by a password. We will not use your name or your child's name in any printed reports for the study.

[Risks/Discomfort]

We do not anticipate any additional health risks due to enrolment in the study. However, there is a small risk of loss of privacy. For example, if your child misses a clinic visit, and we cannot reach you by phone, we would like to visit your home to find out if there is a problem preventing you and your child attending the clinic. We will only visit your home if your child has missed the clinic appointment by more than 7 days and we are unable to reach you or a friend by phone. If your child does not have TB symptoms, this does not guarantee that your child does not have TB. By collecting 2 sputa samples from your child, we will help to rule out the possibility of TB, but this may cause some discomfort, especially if sputum needs to be suctioned. Currently the Ministry of Health guidelines do not recommend collection of sputa from children if they have no TB symptoms, but we want to assess whether these guidelines are correct. By allowing your child, who does not have TB symptoms, to provide sputa, you and your child will help the Ministry of

Health to decide on the best way to find TB in children.

[Benefits for You]

There may be additional health benefits for you due to enrolment in the study because: if your child has symptoms of TB, as part of the study, we will test the sputum for drug resistant TB, a test not routinely offered to all patients with TB symptoms. This might help to identify and treat this form of TB early which could improve your child's health.

[Benefits for the Program]

By taking part in the study, your child will be helping the Ministry of Health and CDC find the best ways to diagnose TB in children using GeneXpert. For example, the study may show that GeneXpert works best in district laboratories rather than in small peripheral clinics. This finding would direct future use of GeneXpert in Botswana maximizing the usefulness of GeneXpert for future patients.

[Your Rights]

You and your child can choose not to take part in the study. If you choose for your child not to take part in the study, we will still give your child the standard of care recommended by the Ministry of Health. There will be no penalties for choosing not to take part. You will also be able to leave the study at any time, without penalty. You may take a copy of the consent form with you. Now, or at any time in the future, you can ask any member of the study team any questions about the study.

[Contact Persons for Additional Information]

If you have any questions about this study now, please ask us now.

If you have any questions about this study in the future, please contact.

[Dr James Shepherd Principal Investigator at* 367-2430*]

If you feel that you or your child has been harmed by this study, please contact ***[Chawangwa Lesedi Study Coordinator at 367-2534 CDC Botswana]***

If you have any concerns about your rights in this study, please contact ***[Mr. Pilate Khulumani, Health Research Development Committee - Ministry of Health at 3632775]***

[Consent]

If you agree to the following statements, please sign your name below: "I am the legal guardian of the child. I agree for the child in my care to be in this study. The information in this consent form has been explained to me. I have been given a chance to ask questions. I feel that all of my questions have been answered. I know that allowing my child to be in this study is my choice and my child's choice. I know that after choosing to allow my child to be in this study, my child and I may leave the study at any time. If I wish for my child and I to leave this study, I will contact ***the study nurse at my clinic***. If my child and I leave this study, we will continue to get regular medical care at this clinic or hospital. I understand that I

will receive a copy of this signed and dated consent form. By signing and dating this consent form, I have not waived any of the legal rights that I or my child would have if my child were not a participant in the study."

I have read or heard this form read to me. By signing below, I consent for my child to join this study.

By ticking this box ☐ **I also agree** *to let the researchers use any TB germs for future studies.*

_____

Name of participant (child)

_____ _____

Name of Guardian Signature or thumb print of participant

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

I verify that the consent form has been read and explained accurately by a member of the study staff.

_____ _____

Name of witness Signature of witness

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

Study Staff Administering Consent _____

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

10.2.3. Information and assent form for minors aged 13-17

Instructions for study nurse only:

1. *The statement should be read to eligible child enrolees aged 13-17 years old. The assent form should only be read if the guardian provided consent for enrolment.*
2. *Only read the non-italicized parts.*
3. *Throughout the process of obtaining assent, it is important that you are patient and allow the child to ask questions and to consider the decision. Never rush or otherwise pressure the child to give assent.*
4. *Offer a copy of this assent script to all guardians whether the child assented to enrolment or not.*
5. *Keep the other copy in the assent form folder, locked in a cabinet. (Flesch-Kincaid Grade Level: 6.5)*

See next page for consent form.

[Title of the study]: *Evaluating Performance, Impact, and Operational Challenges of GeneXpert use for TB Case Finding among HIV-infected Persons in Botswana during 2011-2013.*

[Introduction]:

Hello, my name is…….I am a trained nurse. I need to check whether you have a sickness called tuberculosis. I am also managing a research study at this clinic. I will explain the research study, but first let me tell you about the sickness called TB.

TB is caused by a germ which is too small to see. The germ usually lives in a person's lungs. Coughing is one sign that someone might have TB. TB is common. It can cause death. If we find TB we can treat it most of the time. Treatment helps patients feel better and live longer. However, finding which people have TB, is difficult.

Recently, a new TB test was developed. The new test is called "Gene Xpert". Gene Xpert is a machine. GeneXpert is better than TB tests which rely on microscopes (microscopes are tools which help people to look at very tiny things like TB germs). GeneXpert tests sputum for TB germs. Sputum is the liquid that a person produces when they cough . We are doing a research study to find out the best way to use GeneXpert to find TB. If we find the best way to use GeneXpert, we can make sick people healthier.

The CDC has paid for this research study. CDC and the Ministry of Health are partners in this study.

[Reason for Asking the Patient to be Part of the Study]

We are going to ask if you want to be part of this study. The reason we are asking you, and not all patients at this clinic is that we need patients visiting the clinic for the first time to be in the study.

[Procedures]

If you agree to be in the study, we'll start today. We will do the following today and tomorrow:

1. Ask you to sign this paper, showing that you agree to be part of the study. Your mother/father/aunt/uncle/(other____) *[choose correct word for guardian]* has already given consent for you to join the study if you agree.
2. Ask you and your mother/father/aunt/uncle/(other____) *[choose correct word for guardian]* some questions like "What is your age?"
3. Ask some questions about whether you are coughing, sweating at night, losing weight, or have a fever.
4. Review your records prepared by the doctor.
5. Ask for your mother's/father's/aunt's/uncle's/(other____) *[choose correct word for guardian]* telephone number so that we can contact you if you miss a future clinic visit.

6. Ask your mother/father/aunt/uncle for contact details (telephone numbers) so that we can contact you or a friend or a family member if you miss a future clinic visit. Note that this is standard practice recommended by the Ministry of Health and is not something specific to this study.
7. Ask you for your omang number to help us with data collection from your medical records.
8. If you have one of the signs of TB, we will ask you to cough sputum into two cups for us today.
9. Once you have produced the cough sputum, we will ask you to return tomorrow to the clinic. We need two more sputa from you tomorrow.
10. If you have no signs of TB, we will still collect one cup of sputum today and one cup tomorrow, to help rule out the possibility that you have TB. If you have no signs of TB, and cannot cough, we will encourage you to cough, but will not use a tube to suck sputum from your stomach.
11. All sputa will go to laboratories for TB tests.
12. If you are unable to cough, we may need to advise you how to cough, or use a tube to suck sputum from your stomach. These are normal clinic tests and would be needed even if you did not enroll in the study.
13. If the GeneXpert test has been set up at the TB lab at the time we collect your sputum, we will test the sputum with the GeneXpert device. (Note that all patients attending the clinic will have access to the GeneXpert device, after its set up, even if they are not part of the study).After the TB tests, we will discard any left-over sputum. However, if TB germs are identified, these germs will be studied to identify their type and, if you agree, may be used in future studies.

After tomorrow, we will contact you if you have TB. We will ask you to come back so you can start TB treatment. If you do not have TB, we will ask you to visit with me (the study nurse), each time you come to the clinic to see the doctor treating you for HIV. At each clinic visit, we will:

14. Make sure you have the results of your tests.
15. Explain the results to you.
16. Ask you questions about your health.
17. Look at your medical record.

[Time Commitment]

We need about an extra hour of your time at this clinic visit and every future clinic visit for about 1 year. The number of future clinic visits with me depends on whether the doctor recommends you start HIV treatment with drugs called antiretrovirals or not. If you don't start antiretrovirals, clinic visits will be according to the schedule provided by your doctor. If you do start antiretrovirals, clinic visits will be at 2 weeks, 1 month, 2 months, 3 months and then 3 monthly thereafter.

[Confidentiality]

As part of the study, we will collect your name and your mother's/father's/aunt's/uncle's/(other____) *[choose correct word for guardian]* name and telephone number so that we can contact you if you miss an

HIV clinic appointment. We will keep your information as secret as possible. We will not use your name in any printed reports for the study.

[Risks/Discomfort]

We do not anticipate any additional health risks due to enrollment in the study. However, there is a small risk of loss of privacy. For example, if you miss a clinic visit, and we cannot reach you by phone, we would like to visit your home to find out if there is a problem preventing you attending the clinic.

[Benefits for You]

There may be additional health benefits for you due to enrolment in the study because we will test your sputum, as part of the study, for a dangerous form of TB which is resistant to standard treatment. This is not routinely available for all persons with TB symptoms and the test could help to improve your health sooner.

[Benefits for the Program]

By taking part in the study, you will be helping the Ministry of Health and CDC find the best ways to use GeneXpert. For example, the study may show that GeneXpert works best in district laboratories rather than in small peripheral clinics. This finding would direct future use of GeneXpert in Botswana maximizing the usefulness of GeneXpert for future patients.

[Your Rights]

You can choose not to take part in the study. If you choose not to take part in the study, we will still give you the standard of care recommended by the Ministry of Health. You will also be able to leave the study at any time, without penalty. You may take a copy of the assent form with you. Now, or at any time in the future, you can ask any member of the study team any questions about the study.

[Contact Persons for Additional Information]

If you have any questions about this study now, please ask us now.

If you have any questions about this study in the future, please contact.

[Dr James Shepherd Principal Investigator at* 367-2430*]

If you feel that you have been harmed by this study, please contact ***[Chawangwa Lesedi Study Coordinator at 367-2534 CDC Botswana]***

If you have any concerns about your rights in this study, please contact ***[Mr. Pilate Khulumani, Health Research Development Committee - Ministry of Health at 3632775]***

[Assent]

If you agree to the following statements, please sign your name below: “I agree to be in this study. The information in this assent form has been explained to me. I have been given a chance to ask questions. I

feel that all of my questions have been answered. I know that being in this study is my choice. I know that after agreeing to be in this study, I may leave the study at any time. If I wish to leave this study, I will inform my mother/father/uncle/aunt [*choose correct word for guardian*] and we will contact ***the study nurse at my clinic***. If I leave this study, I will continue to get regular medical care at this clinic or hospital. I understand that I will receive a copy of this signed and dated assent form. By signing and dating this assent form, I have not waived any of the legal rights that I would have if I were not a participant in the study."

I have read or heard this form read to me. By signing below, I agree to join this study.

By ticking this box ☐ **I also agree** *to let the researchers use any TB germs for future studies.*

_____ _____

Name of participant (child) Signature or thumb print of participant

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

I verify that the assent form has been read and explained accurately by a member of the study staff.

_____ _____

Name of witness Signature of witness

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

Study Staff Administering Consent _____

Date: ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]

10.2.4. Prospective cohort enrolment form for EC and EC+X enrolees

XPRES Enrolment Questionnaire for Consenting New HIV Clinic Enrolees

Instructions for Study Nurse:

1. *Complete a separate form for each consenting patient at Prospective Study Enrolment.*

2. *Some questions require interview responses. Others require review of the patient's medical record and others require patient measurement*

A. Patient Study Identification		
1. Name of Facility	_____	
2. Name of staff completing the form:	_____	
3. Date of form initiation:	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]	
4. Time of form initiation:	☐☐ : ☐☐ [HH (24hr format) : min]	
5. Patient Clinic Registration number	_____	
6. Patient Serial Log Number	☐☐☐-☐-☐☐☐☐-☐	
7. Patient Study Identification number	☐☐☐-☐-☐☐☐☐-☐	
B. Demographics		
8. Is the patient male or female?	☐ **Male**	☐ **Female**
9. What is your age?	☐☐☐ (Years)	☐ Refused to Answer ☐ Unknown
10. What is your date of birth?	☐☐/☐☐/☐☐☐☐	☐ Unknown
[Nurse]: Either age or date of birth or both must be recorded.		
11. What is your current marital status (check one):	☐ Never married ☐ Married ☐ Divorced	☐ Widowed ☐ Refused to Answer
12. What is the highest level of school you attended: primary, secondary, higher?	☐ None ☐ Primary ☐ Secondary	☐ Higher ☐ Refused to Answer
[Nurse]: If "None", skip to question 14.		
13. How many years of schooling have you completed?	☐☐ Years	☐ Refused to Answer ☐ Unknown
14. Are you employed?	☐ **Yes**	☐ **No**
[Nurse]: If "No", skip to question 16.		
15. What type of work do you do?		

16. Which of the following options best describes your living arrangements?	☐ **Live in a house/apartment we own** ☐ **Live in a house/apartment we rent** ☐ **Am homeless** ☐ **Refused to answer**

[Nurse]: If "Am homeless", skip to question 19.

17. Please indicate which of the following is present in your home?	**Electricity**	☐**Yes**	☐**No**
	Running water from a tap	☐**Yes**	☐**No**
	Brick wall (or modern wall)	☐**Yes**	☐**No**
	Mud walls (or traditional walls e.g. reeds)	☐**Yes**	☐**No**
	A fridge	☐**Yes**	☐**No**
	A television	☐**Yes**	☐**No**
	A radio	☐**Yes**	☐**No**
	Refused to answer	☐**Yes**	☐**No**

18. How many people live in your home?	☐☐ ☐ **Refused to answer** ☐ **Unknown**
19. *[Skip this question if enrolee is <13 years old]* How many biological children do you have?	☐☐ ☐ **Refused to answer** ☐ **Unknown**
20. *[Skip this question if enrolee is ≥18 years old]* How many siblings do you have?	☐☐ ☐ **Refused to answer** ☐ **Unknown**
21. a. How many kilometres away from the clinic do you live?	☐☐☐☐ ☐ **Refused to answer** ☐ **Unknown**
21.b. How do you travel to the clinic?	☐ **By taxis** ☐ **In my own car** ☐ **In a friend's or relative's car** ☐ **By foot (walking)** ☐ **Refused to answer** ☐ **Unknown**
22. How long does it take you to reach the clinic from home?	☐☐h ☐☐mins ☐ **Refused to answer** ☐ **Unknown**
23. How much does it cost you to reach the clinic from home	☐☐☐☐Pula ☐ **Refused to answer** ☐ **Unknown**

[Nurse]: If "patient is ≤12, skip to Q 26] 24. Have you told a close family member or friend about your HIV status?	☐ **Yes**	☐ **No**

[Nurse]: If "No", skip to question 26.

25. If yes, who have you told	☐ **Husband/wife**	☐ **Boyfriend/girlfriend**
	☐ **Friend**	☐ **Family member other than spouse**
	☐ **Other**_____	☐ **Refused to answer** ☐ **Unknown**

[Nurse]: If "patient is ≥18, skip to Q 27] 26. Is the child aware of his/her HIV status?	☐ **Yes**	☐ **No**

C. HIV Clinic Visit Information

27. What date did you first test HIV-positive?	☐☐/☐☐/☐☐☐☐ ☐ Unknown

		Adults	Children (≤12)
28. WHO stage? ***1.Measure and cross check with medical record.*** ***2.Record all relevant conditions experienced in the past or presently*** ***3.WHO stage cannot improve with time (e.g.from IV to III)***	☐ **Stage I**	☐ Asymptomatic HIV infection ☐ Persistent, generalized lymphadenopathy	☐ Asymptomatic HIV infection ☐ Persistent, generalized lymphadenopathy
	☐ **Stage II**	☐ Moderate weight loss (<10% of body weight). ☐ Recurrent respiratory infections, sinusitis, tonsillitis, otitis media and pharyngitis ☐ Herpes zoster ☐ Sores/cracks around lips ☐ Recurrent mouth ulcers ☐ Itchy skin rash (papular pruritic eruptions) ☐ Itchy, scaly skin condition (seborrhoeic dermatitis) ☐ Fungal nail infections of fingers	☐ Unexplained persistent hepatosplenomegaly ☐ Papular pruritic eruptions ☐ Extensive wart virus infection ☐ Extensive molluscum contagiosum ☐ Recurrent oral ulcerations ☐ Unexplained persistent parotid enlargement ☐ Lineal gingival erythema ☐ Herpes zoster ☐ Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea, sinusitis, tonsillitis) ☐ Fungal nail infections
	☐ **Stage III**	☐ Unexplained severe weight loss (>10% of body weight) ☐ Unexplained chronic diarrhea (> 1 mo) ☐ Unexplained persistent fever (> 1 mo) ☐ Oral candidiasis ☐ Oral hairy leukoplakia ☐ Pulmonary TB (current) ☐ Severe bacterial infection (e.g., pneumonia, empyema, pyomositis, bone or joint infection, meningitis or bacteremia) ☐ Severe painful oral ulcers (i.e., acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis) ☐ Unexplained anaemia (<8 g/dl), neutropenia (<0.5 X 109 per litre) or chronic thrombocytopaenia (<50 X 109 per litre)	☐Unexplained moderate malnutrition not adequately responding to standard therapy ☐Unexplained persistent diarrhoea (14 days or more) ☐Unexplained persistent fever (above 37.5 degrees C, intermittent or constant, for longer than one month) ☐Persistent oral Candidiasis (after first 6 weeks of life) ☐Oral hairy leukoplakia ☐Acute necrotizing ulcerative gingivitis/periodontitis ☐Lymph node TB ☐Pulmonary TB ☐Severe recurrent bacterial pneumonia ☐Symptomatic lymphoid interstitial pneumonitis ☐Chronic HIV-associated lung disease including bronchiectasis ☐Unexplained anaemia (<8.0 g/dl), neutropenia (<0.5x109/L3) or chronic thrombocytopenia (<50 x 109/L3)
	☐ **Stage IV**	☐ HIV wasting syndrome (> 10% wt loss and > 1 mo diarrhea and > 1 mo fever) ☐ Pneumocystis pneumonia (P. *jiroveci)* ☐ Recurrent severe bacterial pneumonia ☐ Chronic herpes simplex (> 1 mo) ☐ Oesophageal candidiasis ☐ Extrapulmonary TB ☐ Kaposi's sarcoma ☐ CNS toxoplasmosis ☐ HIV encephalopathy ☐ Cytomegalovirus infection (retinitis	☐Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy ☐Pneumocystis pneumonia ☐Recurrent severe bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia) ☐Chronic herpes simplex infection; (orolabial or cutaneous of more than one month's duration, or visceral at any site) ☐Extrapulmonary TB ☐Kaposi sarcoma

		or infection of other organs, excluding liver, spleen and lymph nodes) ☐ Extrapulmonary cryptococcosis including meningitis ☐ Other stage 4 diagnosis. ☐ Disseminated nontuberculous mycobacteria infection ☐ Progressive multifocal leukoencephalopathy ☐ Chronic cryptosporidiosis ☐ Chronic isosporiasis ☐ Disseminated mycosis (histoplasmosis, coccidiomycosis) ☐ Recurrent septicaemia (including nontyphoidal *Salmonella*) ☐ Lymphoma (cerebral or B cell non-Hodgkin) ☐ Invasive cervical carcinoma ☐ Atypical disseminated leishmaniasis ☐ Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy	☐Oesophageal candidiasis (or candiadisis of trachea, bronchi or lungs) ☐Central nervous system toxoplasmosis (after the neonatal period) ☐HIV encephalopathy ☐Cytomegalovirus (CMV) infection; retinitis or CMV infection affecting another organ, with onset at age more than 1 month ☐Extrapulmonary cryptococcosis including meningitis ☐Disseminated endemic mycosis (extrapulmonary histoplasmosis, coccidioidomycosis, penicilliosis) ☐Chronic cryptosporidiosis (with diarrhoea) ☐Chronic isosporiasis ☐Disseminated non-tuberculous mycobacterial infection ☐Cerebral or B cell non-Hodgkin lymphoma ☐Progressive multifocal leukoencephalopathy ☐HIV-associated cardiomyopathy or nephropathy

29. Weight *[measure]*	☐☐☐.☐ kg
30. a. Height/Length *[measure]*	☐☐☐ cm
30.b.Mid upper arm circumference for children aged ≤ 5	☐☐☐ mm
30.c. Is the child assessed as having developmental delay (children ≤12 only)?	☐**Yes** ☐ **No**
31. Heart rate *[measure]*	☐☐☐ per min
32. Respiratory rate *[measure]*	☐☐☐ per min
33. Blood Pressure *[measure]*	☐☐☐/☐☐☐ in mmHg
34. Temperature *[measure]*	☐☐.☐ Degrees Celsius ☐ Oral ☐Axillary
[Nurse]: Review the patient's medical record for these values. If lab test results are not in the patient medical record, check that the tests have been done, and get the results when they return.	
35. Date of current clinic visit	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
36. a. Current CD4 count	☐☐☐☐ cells/ul ☐☐.☐☐ % (for children <5) Date blood was taken: ☐☐/☐☐/☐☐☐☐
36.b. Current Viral Load	☐☐☐☐☐☐☐ copies/ml Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**

37. Current Haemoglobin	☐☐.☐ g/dL Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
38. Current ALT	☐☐☐☐ IU/L Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
39. Current AST	☐☐☐☐ IU/L Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
40. Current Creatinine	☐☐☐☐ mg/dL ☐ **Not done** Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
41. Has the patient been started on Cotrimoxazole or Dapsone prophylaxis?	☐ **Co-trimoxazole** Start ☐☐/☐☐/☐☐☐☐ ☐ **Dapsone** Start ☐☐/☐☐/☐☐☐☐ ☐ **Neither Co-trimoxazole nor Dapsone started**
[Nurse]: If patient is male or <13 years old, skip to (Q 46). For other patients, If these data are missing from the patient's medical record, ask the patient.	
42. Is the patient pregnant?	☐ **Yes** EDD ☐☐/☐☐/☐☐☐☐ ☐ **No [If no, skip to 46]**
43. If the patient is pregnant, has she been prescribed PMTCT antiretrovirals?	☐ **No** ☐ **Yes, the following antiretrovirals:**_____ Date started: ☐☐/☐☐/☐☐☐☐
44. Has this female patient previously taken antiretrovirals for PMTCT?	☐ **No** ☐ **Yes, the following antiretrovirals:**_____ Date started: ☐☐/☐☐/☐☐☐☐ Date completed: ☐☐/☐☐/☐☐☐☐
[Nurse]: If "patient is >12, skip to Q 46] 45. Was the child exposed to PMTCT antiretrovirals after birth?	☐ **No** ☐ **Yes, the following antiretrovirals:**_____

46. Is the patient eligible for ART?	☐ **Yes**	☐ **No** ***[If "No", Skip to 50]***
47. If yes, on what criteria is the patient ART eligible? *[Can check more than 1]*	**Adult/child (≥5 years old)** ☐ WHO Clinical Stage IV other than TB ☐ WHO Clinical Stage III other than TB ☐ CD4 count <350 cells/uL ☐ Diagnosis of TB	**Children <5 years old** ☐ <2year old ☐ >2year old with Stage III other than TB ☐ >2year old with Stage IV other than TB ☐ 24-59 months with CD4%<25% or CD4<750 ☐ Diagnosis of TB
48. If ART-eligible, did the patient start ART?	☐**No** ***[Nurse]: If no, skip to Q 50]***	☐ **Yes,** Started on ☐☐/☐☐/☐☐☐☐
49. If the patient started ART, what regimen was started?	☐ **TDF/FTC/EFV** ☐ **TDF/FTC/NVP** ☐ **TDF/3TC/ EFV** ☐ **TDF/3TC/ NVP** ☐ **TDF/ABC/LPV/r** ☐ **ddI/ABC/LPV/r** ☐ **AZT/3TC/NVP** ☐ **AZT/3TC/EFV**	☐ **D4T/3TC/NVP** ☐ **D4T/3TC/EFV** ☐ **AZT/3TC/ABC** **other [specify below]** **Drug 1:**_____**Missing** ☐ **Drug 2:**_____**Missing** ☐ **Drug 3:**_____**Missing** ☐

D. Past Medical History

50. Have you been treated for TB before?	☐ **Yes, on** ☐ **occasions** ☐ **No** ***[Nurse, If "No", Skip to 52]***

51. If yes, when did TB treatment start and stop?	**Dates of start and stop of episodes of TB**	**Outcome**
	1st: Started on ☐☐/☐☐/☐☐☐☐ ☐**Pulmonary** ☐**Extra-Pulmonary**	☐ Cured ☐ Complete ☐ Failure ☐ Default ☐ Transfer out ☐ Unknown ☐ Treatment ongoing
	1st: Ended on ☐☐/☐☐/☐☐☐☐ ☐**Pulmonary** ☐**Extra-Pulmonary**	
	2nd: Started on ☐☐/☐☐/☐☐☐☐ ☐**Pulmonary** ☐**Extra-Pulmonary**	☐ Cured ☐ Complete ☐ Failure ☐ Default ☐ Transfer out ☐ Unknown ☐ Treatment ongoing
	2nd: Ended on ☐☐/☐☐/☐☐☐☐ ☐**Pulmonary** ☐**Extra-Pulmonary**	
	3rd: Started on ☐☐/☐☐/☐☐☐☐ ☐**Pulmonary** ☐**Extra-Pulmonary**	☐ Cured ☐ Complete ☐ Failure ☐ Default ☐ Transfer out ☐ Unknown ☐ Treatment ongoing
	3rd: Ended on ☐☐/☐☐/☐☐☐☐	

	☐ **Pulmonary** ☐ **Extra-Pulmonary**	
52. Has anyone living in your home received TB treatment in past 24 months?	☐ **Yes**	Started on ☐☐/☐☐/☐☐☐☐
	☐ **No** ☐ **Refused to answer** ☐ **Don't know**	
53. Have you been in contact with someone, who lives outside your home, who has received TB treatment in the past 24 months?	☐ **Yes**	Date (most recent) ☐☐/☐☐/☐☐☐☐
	☐ **No** ☐ **Refused to answer** ☐ **Don't know**	
54. In the past 24 months have you received medicines to prevent Tuberculosis (TB) called Isoniazid or INH or IPT?	☐ **Yes**	Started on ☐☐/☐☐/☐☐☐☐ Ended ☐☐/☐☐/☐☐☐☐ or, ☐ **Currently taking INH**
	☐ **No** ☐ **Refused to answer** ☐ **Don't know**	
55. In the past 6 months, has your sputum been tested for Tuberculosis?	☐ **Yes**	Date ☐☐/☐☐/☐☐☐☐
	☐ **No** ☐ **Refused to answer** ☐ **Don't know**	
56. Are you currently taking medicines besides antiretrovirals, co-trimoxazole, dapsone, and INH?	☐ **Yes** ☐ **No *[Nurse, If "No", skip to 58]***	
57. Besides antiretrovirals, co-trimoxazole, dapsone, and INH, which other medicines are you currently taking?	**Medicine 1:**_____ **Medicine 2:**_____ **Medicine 3:**_____ **Medicine 4:**_____ **Medicine 5:**_____ **Medicine 6:**_____ **Medicine 7:**_____	
E.	**TB Screening Questions**	
58. Do you currently have a cough?	☐ **Yes** ☐ **No *[If "No", skip to 61]***	
59. When did the cough start?	☐☐☐ days ago **or** ☐☐ years ago	
60. Since the cough started, have you coughed up blood?	☐ **Yes** ☐ **No**	
61. Do you have a fever?	☐ **Yes** ☐ **No *[If "No", skip to 63]***	
62. When did the fever start?	☐☐☐ days ago **or** ☐☐ years ago	
63. Do you have drenching sweats at night?	☐ **Yes** ☐ **No *[If "No", skip to 65]***	
64. When did the night sweats start?	☐☐☐ days ago **or** ☐☐ years ago	

65. Have you lost weight?	☐ **Yes**	☐ **No** ***[If "No", skip to 68]***
66. When did the weight loss start?	☐☐☐ days ago **or** ☐☐ years ago	
67. How have you noticed the weight loss?	☐ **Observed weight loss as measured by a scale** ☐ **Clothes fit less tightly** ☐ **Observed my physical appearance changing** ☐ **Others observed my physical appearance changing** ☐ **Other**_____	
[Nurse, examine the patient to answer this question] 68. Does the patient have asymmetric lymphadenopathy?	☐ **Yes**	☐ Cervical ☐ armpit ☐ groin ☐ other,_____
	☐ **No**	
F. Potential Risk Factors for Poor Outcomes		
[Nurse, skip to 79 if <10 years old] 69. Do you drink alcohol?	☐ **Yes**	☐ **No** ***[If "No", skip to 72]***
70. How often do you drink alcohol?	☐ **Every day** ☐ **5-6 times per week** ☐ **3-4 times per week** ☐ **1-2 times per week** ☐ **<1 times per week**	
71. When you drink, on average, how many units do you drink? One unit is equivalent to one beer, one glass of wine, or one shot of spirits. One whole Chibuku is equivalent to 3 units. One glass of Chibuku is one unit.	☐☐ units	
72. Have you ever smoked cigarettes?	☐ **Yes**	☐ **No** ***[If "No", skip to 76]***
73. Are you a current smoker or an ex-smoker?	☐ **Current smoker** ☐ **Ex-smoker**	
74. When you smoke/smoked, how many cigarettes do/did you smoke per day on average?	☐ **<1 per day** ☐ **1-5 per day** ☐ **6-10 per day** ☐ **11-20 per day** ☐ **21-30 per day** ☐ **>30 per day**	
75. For how long have you been smoking? *[or for how long did you smoke if an ex-smoker]*	☐☐ years	
76. Are you currently, or have you ever been, a miner?	☐ **Yes**	☐ **No** ***[If "No", skip to 79]***
77. What do/did you mine?	☐ **Diamonds** ☐ **Gold** ☐ **Coal** ☐ **Copper** ☐ **Other**_____	
78. For how long have you been/were you a miner?	☐☐ years	
79. Have you been	☐ **Yes**	On ☐☐ occasions

hospitalized in the last 24 months?	☐ **No** ***[If "No", skip to 81]***		

80. For each occasion you were hospitalized, for how long were you hospitalized?	**Duration**	**Date**	**Diagnosis**
	1st ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____
	2nd ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____
	3rd ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____
	4th ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____
	5th ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____
	6th ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____

81. Does the patient currently work in a health facility or has the patient ever worked in a health facility?	☐ **Yes, currently working in a health facility**	Describe:_____
	☐ **Yes, worked in a health facility in the past**	Describe:_____
	☐ **No**	

82. Does the patient work in a prison?	☐ **Yes**	☐ **No**
83. Does the patient work in a TB lab?	☐ **Yes**	☐ **No**
84. Has the patient been in contact with someone who is being treated or has been treated for drug resistant TB?	☐ **Yes**	☐ **No**

G. Questions Specific for Children (≤12 years old). Address interview questions to the guardian. [Skip to 96 if >12 years old]

85. What was the child's birth weight?	☐☐☐ grams	☐ **Unknown**		
86. What was the child's APGAR score?	☐☐	☐ **Unknown**		
87. Has the child been assessed as having developmental delay?*[Review the medical record for this information]*	☐ **Yes**	☐ **No**	☐ **Unknown**	
88. Did the child receive a BCG vaccination?	☐ **Yes**	☐ **No**	☐ **Unknown**	
89. Is the child's vaccination schedule up to date? *[Review Immunization Card]*	☐ **Yes**	☐ **No**		
90. Please describe the growth curve in the last **3 months.**	☐ **Child has lost weight** ☐ **Child has not gained weight** ☐ **Child has gained some weight**			
91. Does the child have any enlarged lymph nodes (>1cmx1cm)?	☐ **Yes**	☐ Cervical ☐ armpit ☐ groin ☐ other,_____		
	☐ **No**			
92. Have you noticed reduced playfulness of the child?	☐ **Yes**	☐ **No**	***[If no, skip to 94]***	
93. When did the reduced playfulness start?	☐☐☐ days ago			
94. Is the child going to	☐ **Yes**	☐ **No**	***[If no, skip to 96]***	

school?	
95. What grade at school is the child attending?	____________ ☐ **Unknown**
H. TB Diagnostic Tests Performed	
96. Did the patient screen positive for TB?	☐ **Yes** ☐ **No**

Instructions for Specimen Collection:

	Adult (>12 years old)	***Child <=12 years old***
*Screened **positive** for one or more TB symptoms*	***Collect 4 sputa***	***Collect 4 sputa***
*Screened **negative** for all TB symptoms*	***Collect 0 sputa***	***Collect 2 spot sputa***

97. How many sputum specimens were collected in total?	☐ specimens [**If zero sputa specimens go to Q.99**]			
	Date and Time Acquired by Nurse	Induced?	Gastric Aspirate?	Spot/Morning
98. For each specimen	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐**Y** ☐**N**	☐**Y** ☐**N**	☐**S** ☐**M**
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐**Y** ☐**N**	☐**Y** ☐**N**	☐**S** ☐**M**
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐**Y** ☐**N**	☐**Y** ☐**N**	☐**S** ☐**M**
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐**Y** ☐**N**	☐**Y** ☐**N**	☐**S** ☐**M**
99. Was the patient referred for X-Ray	☐ **Yes** ☐ **No** **If yes please ensure the CXR form is completed**			
100. Were any other TB diagnostic specimens taken?	☐ **Yes** ☐ **No [if no skip to Q 102]**			

	Date and Time Acquired by Nurse	Type of Specimen	Location
101. Other Specimens	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min		
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min		
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min		
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min		
102. What is the date of the next appointment after the appointment today (date of enrollment)?	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]		
103. Was the patient diagnosed as having TB	☐ **Yes** ☐ **No**	If yes, please specify: ☐ **Pulmonary TB** ☐ **Extra-Pulmonary TB** (Site of extra-pulmonary TB_ ____________)	

COMPLETENESS/QUALITY CHECK

Form has been completed by the Study Nurse and checked for quality and completeness: (Signature) _____

Date: ☐☐/☐☐/☐☐☐☐

DATA ENTRY CHECK (to be completed by data entry clerks in Gaborone)

☐ **First data-entry completed:_____ Date:** ☐☐/☐☐/☐☐☐☐

☐ **Second data-entry completed:_____Date:** ☐☐/☐☐/☐☐☐☐

10.2.5. Patient locating information – kept by study nurse for tracing purposes

Patient Locating Information	
Instructions For Study Nurse: ***1.*** *Complete a separate form for each consenting patient at Prospective Study Enrolment.* ***2.*** *Questions require interview responses.*	
A. Patient Study Identification	
1. Name of Facility	_____
2. Name of staff completing the form:	_____
3. Date of form completion:	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
4. Patient Clinic Registration number	_____
4.1. Omang Number	_____ ☐ **Missing**
4.2. If Omang missing, list Passport/ birth certificate / resident permit number	_____ ☐ **Missing** ☐**Passport** ☐**Resident permit** ☐**Birth certificate**
5. Patient Name	
6. Patient Serial Log Number	☐☐☐-☐-☐☐☐☐-☐
7. Patient Study Identification number	☐☐☐-☐-☐☐☐☐-☐
B. Locating Information	
8. Patient telephone number(s)	**1.** _____ **2.** _____
9. Patient residential address	
10. Family/friend's telephone number(s) who will know where you are if we can't reach you (must be >=18 years old)?	**1. Name:**_____**Number:**_____ **2. Name:**_____**Number:**_____
11. Family/friend's	**1. Name:**_____

residential addresses who will know where you are if we can't reach you (someone at the address must be >= 18 years old)?	**Address:**_____ **2. Name:**_____ **Address:**_____

COMPLETENESS/QUALITY CHECK

Form is complete and has been reviewed by the Study Nurse: _____

Date: ☐☐/☐☐/☐☐☐☐

DATA ENTRY CHECK

Study Nurse Completed Data Entry: _____

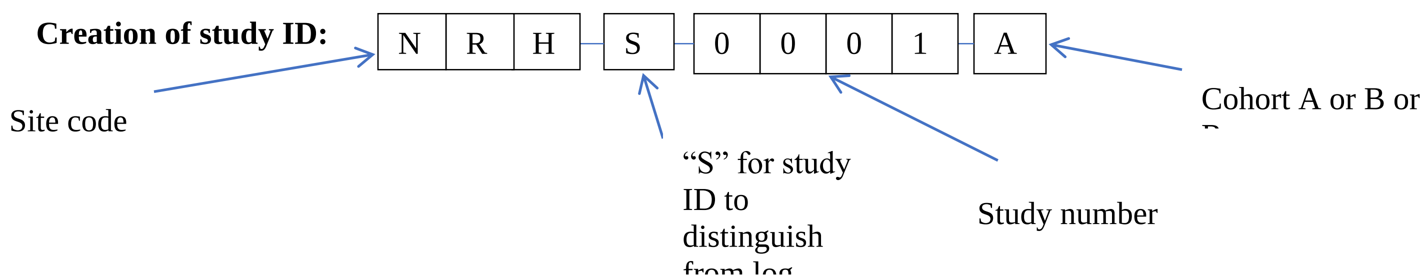
Date: ☐☐/☐☐/☐☐☐☐

10.2.6. Prospective study register to facilitate appointment tracking

PAGE 1 FOR A GROUP OF 10 PATIENTS (E.G. NRH-S-0001-A THROUGH NRH-S-0010-A)																		
							1st Visit		2nd Visit		3rd Visit		4th Visit		5th Visit		6th Visit	
Nurse Initials	Site* (e.g. NRH)	Study # (e.g. S-0001)	Cohort (A or B)	Clinic Registration #	Name (First name & surname)	Tel #	Date (dd/mm/ yyyy)	On time/ Late (OT/L)	Date (dd/mm/ yyyy)	On time/ Late (OT/L)	Date (dd/mm/ yyyy)	On time/ Late (OT/L)	Date (dd/mm/ yyyy)	On time/ Late (OT/L)	Date (dd/mm/ yyyy)	On time/ Late (OT/L)	Date (dd/mm/ yyyy)	On time/ Late (OT/L)
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
							__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	

***If a study nurse works at more than one study site, this nurse will have one register per site.**

Creation of study ID:



Study # (e.g. S-0001)	**PAGE 2 FOR A GROUP OF 10 PATIENTS**																			
	7th Visit		8th Visit		9th Visit		10th Visit		11th Visit		12th Visit		13th Visit		14th Visit		15th Visit		16th Visit	
	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)	Date (dd/mm/yyyy)	On time/Late (OT/L)
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	
	__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____		__/__/____	

10.2.7. Follow-up questionnaire for prospective EC and EC+X cohorts

XPRES Follow-up Questionnaire

Instructions for Study Nurse:

1. *Complete a separate form for each consenting patient returning for Prospective Study Follow-up.*

2. *Some questions require interview responses. Others require review of the patient's medical record and others require patient measurement*

A. Patient Study Identification

1. Name of Facility	_____
2. Name of staff completing the form:	_____
3. Date of form completion:	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
4. Time of form completion:	☐☐ : ☐☐ [HH (24hr format) : min]
5. Patient Clinic Registration number	_____
6. Patient Serial Log Number	☐☐☐-☐-☐☐☐☐☐-☐
7. Patient Study Identification number	☐☐☐-☐-☐☐☐☐☐-☐

B. Updated Demographics

[Nurse: If enrolee is <13 years old, skip to Q10] 8. Since the last clinic visit, have you told a close family member or friend about your HIV status?	☐ **Had disclosed status by time of enrolment *[Skip to Q 11]***	☐ **Yes**	☐ **No *[Skip to Q 11]***

9. If yes, who have you told	☐ **Husband/wife**	☐ **Boyfriend/girlfriend**
	☐ **Friend**	☐ **Family member other than spouse**
	☐ **Other**_____	☐ **Refused to answer** ☐ **Unknown**

[Nurse: If enrolee is ≥18 years old, skip to Q11] 10. Is the child aware of his/her HIV status?	☐ **Yes, Was aware of HIV status at time of enrolment**	☐ **Yes**	☐ **No**

C. HIV Clinic Visit Information

C.1. Outcomes

11. What was the scheduled appointment date?	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
12. What is the actual visit date?	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
13. Based on question 11 and 12, is the patient late for his/her	☐ **On time: Attended clinic on scheduled date *[Nurse: If checked skip to Q15]***
	☐ **Late for scheduled visit [*Nurse: If checked, ensure that a tracing***

appointment?	***form was completed]***
14. If the patient is late, did the patient return to the clinic due to the active tracing of the study team?	☐ **Yes – returned because of tracing interventions. Please check how patient was reached:** ☐ **Phone call** ☐ **SMS** ☐ **Home visit to patient** ☐ **Home visit to patient's friend/neighbour** ☐ **Other**_____
	☐ **Patient returned on his/her own accord (was never reached by the study team)**
14.b. What was the reason for the missed visit? (in patient's own words)	_____ _____ _____ _____
14.c. If the reason for not attending the clinic can be summarized, please check the appropriate box?	☐ **Transport problems** ☐ **Work responsibilities** ☐ **Childcare responsibilities** ☐ **Hospitalized** ☐ **Forgot about appointment** ☐ **Spouse did not allow clinic attendance** ☐ **Significant social event (e.g. funeral)** ☐ **Unsatisfied with quality of care** ☐ **Unsatisfied with long waiting times at clinic** ☐ **Other**_____
	☐ **Hospitalized** **Date of hospitalization:** ☐☐/☐☐/☐☐☐☐ **Reason:**_____

C.2. Clinic Visit Data

		Adults	Children (≤12)
15. WHO stage? ***1.Measure and cross check with medical record.*** ***2.Record all relevant conditions experienced in the <u>past or presently</u>*** ***3.WHO stage cannot***	☐ **Stage I**	☐ Asymptomatic HIV infection ☐ Persistent, generalized lymphadenopathy	☐ Asymptomatic HIV infection ☐ Persistent, generalized lymphadenopathy
	☐ **Stage II**	☐ Moderate weight loss (<10% of body weight). ☐ Recurrent respiratory infections, sinusitis, tonsillitis, otitis media and pharyngitis ☐ Herpes zoster ☐ Sores/cracks around lips ☐ Recurrent mouth ulcers ☐ Itchy skin rash (papular pruritic eruptions) ☐ Itchy, scaly skin condition (seborrhoeic dermatitis) ☐ Fungal nail infections of fingers	☐ Unexplained persistent hepatosplenomegaly ☐ Papular pruritic eruptions ☐ Extensive wart virus infection ☐ Extensive molluscum contagiosum ☐ Recurrent oral ulcerations ☐ Unexplained persistent parotid enlargement ☐ Lineal gingival erythema ☐ Herpes zoster ☐ Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea, sinusitis, tonsillitis) ☐ Fungal nail infections
	☐ **Stage III**	☐ Unexplained severe weight loss (>10% of body weight) ☐ Unexplained chronic diarrhea (> 1 mo) ☐ Unexplained persistent fever (> 1 mo) ☐ Oral candidiasis ☐ Oral hairy leukoplakia ☐ Pulmonary TB (current) ☐ Severe bacterial infection (e.g., pneumonia, empyema, pyomositis, bone or joint infection, meningitis or bacteremia) ☐ Severe painful oral ulcers (i.e., acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis) ☐ Unexplained anemia (<8 g/dl),	☐Unexplained moderate malnutrition not adequately responding to standard therapy ☐Unexplained persistent diarrhoea (14 days or more) ☐Unexplained persistent fever (above 37.5 oC, intermittent or constant, for longer than one month) ☐Persistent oral Candidiasis (after first 6 weeks of life) ☐Oral hairy leukoplakia ☐Acute necrotizing ulcerative gingivitis/periodontitis ☐Lymph node TB ☐Pulmonary TB

improve with time (e.g.from IV to III)		neutropenia (<0.5 X 109 per litre) or chronic thrombocytopaenia (<50 X 109 per litre)	☐Severe recurrent bacterial pneumonia ☐Symptomatic lymphoid interstitial pneumonitis ☐Chronic HIV-associated lung disease including bronchiectasis ☐Unexplained anaemia (<8.0 g/dl), neutropenia (<0.5x109/L3) or chronic thrombocytopenia (<50 x 109/L3)
	☐ **Stage IV**	☐ HIV wasting syndrome (> 10% wt loss and > 1 mo diarrhea and > 1 mo fever) ☐ Pneumocystis pneumonia (P. *jiroveci)* ☐ Recurrent severe bacterial pneumonia ☐ Chronic herpes simplex (> 1 mo) ☐ Oesophageal candidiasis ☐ Extrapulmonary TB ☐ Kaposi's sarcoma ☐ CNS toxoplasmosis ☐ HIV encephalopathy ☐ Cytomegalovirus infection (retinitis or infection of other organs, excluding liver, spleen and lymph nodes) ☐ Extrapulmonary cryptococcosis including meningitis ☐ Other stage 4 diagnosis. ☐ Disseminated nontuberculous mycobacteria infection ☐ Progressive multifocal leukoencephalopathy ☐ Chronic cryptosporidiosis ☐ Chronic isosporiasis ☐ Disseminated mycosis (histoplasmosis, coccidiomycosis) ☐ Recurrent septicaemia (including nontyphoidal *Salmonella*) ☐ Lymphoma (cerebral or B cell non-Hodgkin) ☐ Invasive cervical carcinoma ☐ Atypical disseminated leishmaniasis ☐ Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy	☐Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy ☐Pneumocystis pneumonia ☐Recurrent severe bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia) ☐Chronic herpes simplex infection; (orolabial or cutaneous of more than one month's duration, or visceral at any site) ☐Extrapulmonary TB ☐Kaposi sarcoma ☐Oesophageal candidiasis (or candiadisis of trachea, bronchi or lungs) ☐Central nervous system toxoplasmosis (after the neonatal period) ☐HIV encephalopathy ☐Cytomegalovirus (CMV) infection; retinitis or CMV infection affecting another organ, with onset at age more than 1 month ☐Extrapulmonary cryptococcosis including meningitis ☐Disseminated endemic mycosis (extrapulmonary histoplasmosis, coccidioidomycosis, penicilliosis) ☐Chronic cryptosporidiosis (with diarrhoea) ☐Chronic isosporiasis ☐Disseminated non-tuberculous mycobacterial infection ☐Cerebral or B cell non-Hodgkin lymphoma ☐Progressive multifocal leukoencephalopathy ☐HIV-associated cardiomyopathy or nephropathy

16. Functional Status *[observe]*	☐ **Able to work (play for children)** ☐ **Ambulatory (Mobile, for children)** ☐ **Bed ridden**
17. Weight *[measure]*	☐☐☐.☐ kg
17.1. Mid-upper arm circumference for children (≤ 5years old)	☐☐☐ mm
17.2. Is the child assessed as having developmental delay (children ≤12 only)?	☐**Yes** ☐ **No**
18. Height/Length *[measure]*	☐☐☐ cm
19. Heart rate *[measure]*	☐☐☐ per min
20. Respiratory rate *[measure]*	☐☐☐ per min
21. Blood Pressure *[measure]*	☐☐/☐☐ in mmHg
22. Temperature *[measure]*	☐☐.☐ Degrees Celsius ☐ Oral ☐Axillary

[Nurse]: Review the patient's medical record for these values. If lab test results are not in the patient medical record, check that the tests have been done, and get the results when they return.	
23. Current CD4 count	☐☐☐☐ cells/ul ☐☐.☐☐ % (for children <5) Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
23.b. Current viral load	☐☐☐☐☐☐☐ copies/ml Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
24. Current Hemoglobin	☐☐.☐ g/dL Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
25. Current ALT	☐☐☐☐ IU/L Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
26. Current AST	☐☐☐☐ IU/L Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
27. Current Creatinine	☐☐☐☐ mg/dL ☐ **Not done** Date blood was taken: ☐☐/☐☐/☐☐☐☐ ☐ **Not done**
28. Is the patient currently taking Cotrimoxazole or Dapsone?	☐ **Co-trimoxazole** Start ☐☐/☐☐/☐☐☐☐ ☐ **Dapsone** Start ☐☐/☐☐/☐☐☐☐ ☐ **Neither Co-trimoxazole nor Dapsone started**
[Nurse]: If patient is male or <13 years old, skip to Q 31. For patients ≥13, if these data are missing from the patient's medical record, ask the patient.	
29. Is the patient pregnant?	☐ **Yes** EDD ☐☐/☐☐/☐☐☐☐ ☐ **No *[If no, skip to 31]***
30. If the patient is pregnant, has she been prescribed PMTCT antiretrovirals?	☐ **No** ☐ **Yes, the patient is currently taking ART for her own health** ☐ **Yes, the patients was prescribed the following PMTCT antiretrovirals:** **Drug 1:**_____ **Drug 2:**_____ **Drug 3:**_____

	Date started: ☐☐/☐☐/☐☐☐☐				
31. Has the patient already started ART?	☐ **No** ***[If no, skip to 34]*** ☐ **Yes, Date started**: ☐☐/☐☐/☐☐☐☐				
32. Did the doctor change the patient's initial regimen?	☐ **Yes** ☐ **No**				
33. Current ART regimen for continuing patients	☐ **TDF/FTC/EFV** ☐ **TDF/FTC/NVP** ☐ **TDF/3TC/ EFV** ☐ **TDF/3TC/ NVP** ☐ **TDF/ABC/LPV/r** ☐ **ddI/ABC/LPV/r**	☐ **AZT/3TC/NVP** ☐ **AZT/3TC/EFV** ☐ **D4T/3TC/NVP** ☐ **D4T/3TC/EFV** ☐ **AZT/3TC/ABC** ☐ **other** ***[specify below]***			
	Drug 1: _ _ _ _	**Drug 2:** _ _ _ _	**Drug3:** _ _ _ _		
34. If not yet started on ART, is the patient newly eligible for ART at this visit?	☐ **Yes** ☐ **No** ***[If "No", Skip to 38]***				
35. If yes, on what criteria is the patient newly ART eligible? *[Can check more than 1]*	**Adult (≥5 years old)**		**Children <5 years old**		
	☐ WHO Clinical Stage IV other than TB ☐ WHO Clinical Stage III other than TB ☐ CD4 count <350 cells/uL ☐ Diagnosis of TB		☐ <2year old ☐ >2year old with Stage III ☐ >2year old with Stage IV ☐ 24-59 months with CD4%<25% or CD4<750		
36. If newly ART-eligible, did the patient start ART?	☐ **Yes,** Started on ☐☐/☐☐/☐☐☐☐ ☐ **No** ***[Skip to 38]***				
37. If patient started ART, what regimen was started?	☐ **TDF/FTC/EFV** ☐ **TDF/FTC/NVP** ☐ **TDF/3TC/ EFV** ☐ **TDF/3TC/ NVP** ☐ **TDF/ABC/LPV/r** ☐ **ddI/ABC/LPV/r**		☐ **AZT/3TC/NVP** ☐ **AZT/3TC/EFV** ☐ **D4T/3TC/NVP** ☐ **D4T/3TC/EFV** ☐ **AZT/3TC/ABC** ☐ **other [specify below]**		
	Drug 1: ☐ **Missing** _ _ _ _	**Drug 2:** ☐ **Missing** _ _ _ _	**Drug3:** ☐ **Missing** _ _ _ _		
D. TB Outcomes					
38. Were you tested for Tuberculosis at the last clinic visit?	☐ **Yes** ☐ **No** ***[If "No", Skip to 41]***				
39. For each specimen tested, give the result	**Specimen**	**Test**	**Result (NR=Not Ready)**	**Date Sample Taken**	**Date Nurse Received Result**
	Spot #1	☐**Microscopy**	☐ **No AFB seen** ☐ **Scanty(_AFB)** ☐ **1+** ☐ **2+** ☐ **3+**	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _

		☐Xpert	☐ MTB detected ☐ MTB not detected ☐ MTB invalid ☐ MTB indeterminate ☐ MTB error ☐ RIF Resistance Detected ☐ RIF Resistance Not Detected ☐ RIF Resistance Indeterminate	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
	Spot #2	☐ Flourochrome Microscopy	☐ No AFB seen ☐ Scanty(_AFB) ☐ 1+ ☐ 2+ ☐ 3+	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
		☐Culture	☐ MTB growth detected ☐ Mycobacterium other than TB (MOTT) detected_____ ☐ No growth detected ☐ Contamination	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
	Spot #3	☐Microscopy	☐ No AFB seen ☐ Scanty(_AFB) ☐ 1+ ☐ 2+ ☐ 3+	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
		☐Xpert	☐ MTB detected ☐ MTB not detected ☐ MTB invalid ☐ MTB indeterminate ☐ MTB error ☐ RIF Resistance Detected ☐ RIF Resistance Not Detected ☐ RIF Resistance Indeterminate	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
	Morning sputum	☐ Flourochrome Microscopy	☐ No AFB seen ☐ Scanty(_AFB) ☐ 1+ ☐ 2+ ☐ 3+	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
		☐Culture	☐ MTB growth detected ☐ Mycobacterium other than TB (MOTT) detected_____ ☐ No growth detected ☐ Contamination	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
	Other____	_____	☐ +ve ☐ NR ☐ -ve	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
	Other____	_____	☐ +ve ☐ NR ☐ -ve	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _

Note to Nurse: Please comment if TB tests were done at another clinic:_____
_____
_____
_____
_____
_____

40. Was a chest X-Ray taken?	☐ **Yes**	X-ray date ☐☐/☐☐/☐☐☐☐ X-ray result: ☐**Suspect TB** ☐ **TB not suspected**
	☐ **No**	
41. Did Patient start TB treatment?	☐ **Yes**	Start date: ☐☐/☐☐/☐☐☐☐ ☐**Pulmonary TB** ☐**Extra-pulmonary TB: (Site of extra-pulmonary TB:**_____**)**
	☐ **No**	
42. Was TB drug resistance detected?	☐ **Yes**	☐**INH** ☐**RIF** ☐**PYZ** ☐**ETH** ☐**other**_____ date: ☐☐/☐☐/☐☐☐☐
	☐ **No** ***[If "No", Skip to 44]***	
43. Which test(s) detected resistance? *[Tick all that apply]*	☐ **Xpert** ☐ **Line Probe Assay** ☐ **Drug Susceptibility test**	

44. Which TB drug regimen was started?	**Phase**	**Duration**	**Drugs**	**Drugs if Other**
	Intensive Phase:	☐ months	☐ 2HRZE ☐ HRZES/1HRZE ☐ other	☐Pyrazinamide ☐Amikacin ☐ Levofloxacin ☐ Ethionamide ☐ Cycloserine ☐ P-aminosalicylic acid (PAS) # ☐ Levofloxacin ☐ Streptomycin
	Continuation Phase:	☐ months	☐4HRE ☐5HRE ☐other	☐Pyrazinamide ☐Amikacin ☐ Levofloxacin ☐ Ethionamide ☐ Cycloserine ☐ P-aminosalicylic acid (PAS) # ☐ Levofloxacin ☐ Streptomycin

E. Other Outcomes

45. Has the patient been diagnosed as having (1) an opportunistic infection (OI) (other than TB) or, (2) cancer since the last clinic visit?	☐ **Yes**	#1:_____: ☐☐/☐☐/☐☐☐☐
		#2:_____: ☐☐/☐☐/☐☐☐☐
		#3:_____: ☐☐/☐☐/☐☐☐☐
		#4:_____: ☐☐/☐☐/☐☐☐☐
	☐ **No**	
46. H as the patient been hospitalized since the last clinic visit?	☐ **Yes**	on ☐☐ occasions
	☐ **No** *[If no, skip to 48]*	

47. For each occasion you were hospitalized, for how long were you hospitalized?	Duration	Date	Diagnosis
	1st ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____
	2nd ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____
	3rd ☐☐☐ days	☐☐/☐☐/☐☐☐☐	_____

F. TB Risk Factors

48. Has anyone living in your home received TB treatment since your last clinic visit?	☐ **Yes**	Started on ☐☐/☐☐/☐☐☐☐
	☐ **No** ☐ **Refused to answer** ☐ **Don't know**	
49. Have you been in contact with someone, living outside your home, who is receiving TB treatment, since your last clinic visit?	☐ **Yes**	Date (most recent) ☐☐/☐☐/☐☐☐☐
	☐ **No** ☐ **Refused to answer** ☐ **Don't know**	
50. Have you started medicines to prevent Tuberculosis (TB) called INH (or IPT) since the last clinic visit?	☐ **Yes**	Started on ☐☐/☐☐/☐☐☐☐ Ended ☐☐/☐☐/☐☐☐☐ or, ☐ **Currently taking INH**
	☐ **No** ☐ **Refused to answer** ☐ **Don't know**	
51. Are you taking medicines besides antiretrovirals, co-trimoxazole, dapsone, and INH?	☐ **Yes**	☐ **No** ***[If "No", skip to 53]***
52. Besides antiretrovirals, co-trimoxazole, dapsone, and INH, which other medicines are you currently taking?	**Medicine 1:**_____ **Medicine 2:**_____ **Medicine 3:**_____ **Medicine 4:**_____ **Medicine 5:**_____ **Medicine 6:**_____ **Medicine 7:**_____	

G. TB Screening Questions

53. Do you currently have a cough?	☐ **Yes**	☐ **No** ***[If "No", skip to 56]***
54. When did the cough start?	☐☐☐ days ago **or** ☐☐ years ago	
55. Since the cough started, have you coughed up blood?	☐ **Yes**	☐ **No**
56. Do you have a fever?	☐ **Yes**	☐ **No** ***[If "No", skip to 58]***
57. When did the fever start?	☐☐☐ days ago **or** ☐☐ years ago	
58. Do you have drenching sweats at night?	☐ **Yes**	☐ **No** ***[If "No", skip to 60]***
59. When did the night sweats start?	☐☐☐ days ago **or** ☐☐ years ago	

60. Have you lost weight?	☐ **Yes** ☐ **No** ***[If "No", skip to 63]***	
61. When did the weight loss start?	☐☐☐ days ago **or** ☐☐ years ago	
62. How have you noticed the weight loss?	☐ **Observed weight loss as measured by a scale** ☐ **Clothes fit less tightly** ☐ **Observed my physical appearance changing** ☐ **Others observed my physical appearance changing** ☐ **Other**_____	
63. *[Examine the patient to answer this question]* Does the patient have asymmetric lymphadenopathy?	☐ **Yes**	☐ Cervical ☐ armpit ☐ groin ☐ other,_____
	☐ **No**	
64. Has the patient been in contact with someone who is being treated or has been treated for drug resistant TB?	☐ **Yes** ☐ **No**	

Questions Specific for Children (≤12 years old). Address interview questions to the guardian. [Skip if >12 years old]

65. Is the child's vaccination schedule up to date? *[Review Immunization Card]*	☐ **Yes** ☐ **No**	
66. Please describe the growth curve in the last 3 months.	☐ **Child has lost weight** ☐ **Child has flat growth (i.e. has not gained or lost weight)** ☐ **Child has gained some weight**	
67. Does the child have any enlarged lymph nodes (>1cmx1cm)?	☐ **Yes**	☐ Cervical ☐ armpit ☐ groin ☐ other,_____
	☐ **No**	
68. Have you noticed reduced playfulness of the child?	☐ **Yes** ☐ **No** ***[If no, skip to 70]***	
69. When did the reduced playfulness start?	☐☐☐ days ago	
70. Is the child going to school?	☐ **Yes** ☐ **No** ***[If no, skip to 72]***	
71. What grade at school is the child attending?	_____ ☐ **Unknown**	

TB Diagnostic Tests Performed

72. Did the patient screen positive for TB at this clinic visit?	☐ **Yes** ☐ **No**

Instructions for Specimen Collection during follow-up:

	Adult (>12 years old)	***Child <=12 years old***
*Screened **positive** for one or more TB symptoms*	***Collect 4 sputa***	***Collect 4 sputa***
*Screened **negative** for all TB symptoms*	***Collect 0 sputa***	***Collect 0 sputa***

73. How many sputum specimens were collected in total?	☐ specimens [If zero sputa, go to Q75]

74.	Date and Time Acquired by Nurse	Induced?	Gastric Aspirate?	Spot/Morning
or each specimen	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐**Y** ☐**N**	☐**Y** ☐**N**	☐**S** ☐**M**

	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐Y ☐N	☐Y ☐N	☐S ☐M
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐Y ☐N	☐Y ☐N	☐S ☐M
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min	☐Y ☐N	☐Y ☐N	☐S ☐M
75. Was the patient referred for X-Ray		☐ **Yes** ☐ **No** **[*If yes, make sure x-ray form is completed*]**		
76. Were any other TB diagnostic specimens taken?		☐ **Yes** ☐ **No** [If No, go to Q78]		
77. Other Specimens	Date and Time Acquired by Nurse	Type of Specimen	Location	
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min			
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min			
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min			
	☐☐/☐☐/☐☐☐☐ Time: ☐☐h☐☐min			
78. What is the date of the next appointment after the appointment today (date of interview)?		☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]		
79. Was the patient diagnosed as having TB	☐ **Yes** ☐ **No**	If yes, please specify: ☐ **Pulmonary TB** ☐ **Extra-Pulmonary TB** (Site of extra-pulmonary TB_ _____)		

☐ Form is complete and has been reviewed by the Study Nurse: _____
Date: _____

☐ First data-entry completed: _____ Date: _____

☐ Second data-entry completed: _____ Date: _____

10.2.8. TB treatment chart abstraction form

TB Patient Chart Abstraction Instrument	
To be abstracted from ETR.net/Paper Chart at TB Clinic	
1. TB and HIV patient information	
1. Name of Facility	_____
2. Name of staff completing the form:	_____
3. Date of form initiation:	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
4. Patient Clinic Registration number	_____
5. Patient Serial Log Number	☐☐☐-☐-☐☐☐☐-☐
6. Patient Study Identification number	☐☐☐-☐-☐☐☐☐-☐
7.a. Was the study nurse able to access the patient's TB chart at the TB clinic?	☐Yes ☐No: Specify reason (E.g. distance >60klm to TB clinic):_____ _____
7.b. Date of TB registration	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing**
8. TB register number (assigned by district TB Coordinator):	☐**Missing**
9. ame of clinic where s/he received TB treatment:	☐TB clinic in the study clinic ☐Other TB clinic (specify) _____ ☐**Missing**
10. as patient referred for TB treatment from an HIV care and treatment facility?	☐Yes ☐No ☐**Missing**
11. f yes, what was the name of the HIV care and treatment clinic?	☐**Missing**
12. id the patient start ART at any time before or during TB treatment?	☐Yes ☐No ☐**Missing**
13. f yes, what was the date of ART start?	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing**
14. id the patient start	☐Yes ☐No ☐**Missing**

Cotrimoxazole prophylaxis at any time before or during TB treatment?	
15. f yes, what was the date of Cotrimoxazole start?	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing**
16. isease diagnosis site:	☐ Sputum-smear positive pulmonary TB ☐ Sputum-smear negative pulmonary TB ☐ Sputum-Xpert positive pulmonary TB ☐ Sputum-Xpert negative pulmonary TB ☐ Smear or Xpert not done, pulmonary TB ☐ Culture positive pulmonary TB ☐ Culture negative pulmonary TB ☐ Culture not done, pulmonary TB ☐ Extra-pulmonary TB, ☐ specify:_____ ☐**Missing**
17. reatment in Intensive Phase:	□ Category I - 2HRZE/4HRZ □ Category II - 2HRZES/1HRZE/5HRE □ Unknown □ Other (specify)_____ ☐**Missing**
18. putum results for follow-up of TB treatment (Sputum Smear Microscopy):	**Month: 0 Date:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing** **Result:** ☐ Neg. ☐ Actual ____ ☐ 1+ ☐ 2++ ☐ ≥3+++ ☐**Missing** **Month: 2 Date:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing** **Result:** ☐ Neg. ☐ Actual ____ ☐ 1+ ☐ 2++ ☐ ≥3+++ ☐**Missing** **Month: 3 Date:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing** **Result:** ☐ Neg. ☐ Actual ____ ☐ 1+ ☐ 2++ ☐ ≥3+++ ☐**Missing** **Month: 5 Date:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing** **Result:** ☐ Neg. ☐ Actual ____ ☐ 1+ ☐ 2++ ☐ ≥3+++ ☐**Missing** **Month: 6 Date:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing** **Result:** ☐ Neg. ☐ Actual ____ ☐ 1+ ☐ 2++ ☐ ≥3+++ ☐**Missing** **Month: 8 Date:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] ☐**Missing** **Result:** ☐ Neg. ☐ Actual ____ ☐ 1+ ☐ 2++ ☐ ≥3+++ ☐**Missing**
19. reatment outcome (select one):	☐ Cured ☐ **Missing** ☐ Completed Treatment ☐ Died ☐ Treatment failure

	☐ Defaulted/Lost-to-follow-up/missing ☐ Transferred out ☐ Treatment not completed before the end of the study follow-up

10.2.9. Study exit form for EC and EC+X enrolees

Study Exit Form		
Complete one of these forms for every patient enrolled in the prospective or retrospective cohorts (Cohort A or B or R), when they leave the study		
Patient Identification Information		
1. Name of Facility	_____	
2. Name of staff completing the form:	_____	
3. Date of form initiation:	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]	
4. Patient Clinic Registration number	_____	
5. Patient Serial Log Number	☐☐☐-☐-☐☐☐☐-☐	
6. Patient Study Identification number	☐☐☐-☐-☐☐☐☐-☐	
7. Date of study enrollment	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]	
Outcomes		
8. What was the patient's final outcome?	☐ Completed Follow-up. If yes: ☐End of 6 months of follow-up ☐End of TB treatment which was >12 months after enrollment	**Date follow/up completion (last study visit):** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
	☐ **Transferred to another clinic. If yes, why?:** ☐ Patient migrated from study area ☐ Patient transferred to a clinic closer to home ☐ Patient transferred to another clinic because not ART eligible ☐ Other_____	**Date of transfer:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
	☐ Patient lost to follow-up (>90 days late for their next scheduled appointment)	**Date last attended clinic:** ☐☐/☐☐/☐☐☐☐ **Date of missed visit:** ☐☐/☐☐/☐☐☐☐ **Date of study exit:** ☐☐/☐☐/☐☐☐☐
	☐ Unable to attend further clinic visits. Give	**Date of study exit:**

	reason:_____ _____ _ _____ __	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
	☐ Unwilling to attend further clinic visits (includes those who withdrew consent). Give reason:_____ _____ _____	**Date of study exit:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
	☐ Investigator decides to end study subject's participation. Specify reason: _____ _ _____ _ _____ __	**Date of study exit:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
	☐ Patient died (Complete Appendix 9.2 as well).	**Date of last clinic visit:** ☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY] **Date of death:** ☐☐ DD ☐unknown ☐☐ MM ☐unknown ☐☐☐☐ YYYY ☐unknown
9. If patient died, did patient die in hospital?	☐ Yes (if yes, enter date) ☐ No	**Date of hospital admission:** ☐☐ DD ☐unknown ☐☐ MM ☐unknown ☐☐☐☐ YYYY ☐unknown

Form has been completed by the Study Nurse and checked for quality and completeness: (Signature) _____

Date:

☐☐/☐☐/☐☐☐☐

DATA ENTRY CHECK (to be completed by data entry clerks in Gaborone)

☐ **First data-entry completed:**_____ **Date:** ☐☐/☐☐/☐☐☐☐

☐ **Second data-entry completed:**_____**Date:** ☐☐/☐☐/☐☐☐☐

10.2.10. Adult SOC (retrospective) cohort data abstraction questionnaire

Instructions For Study Nurse:

1. *Use the "Retrospective Cohort Tracking Form" to identify those ART patients selected for this part of the study.*
2. *Locate their permanent paper ART medical records at the facility and complete the relevant lines on the "Retrospective Cohort Tracking Form"*
3. *Abstract data from the medical records of patients known to have died, been transferred to another clinic, stopped ART, or been lost to follow-up first.*
4. *Flag folders of patients who are currently retained on ART to indicate their potential eligibility for an interview, and document the next appointment date on the "Retrospective Cohort Tracking Form"*
5. *Among patients who are currently retained on ART, patients who screen negative for TB will not be interviewed, and the data from their chart should be abstracted.*
6. *Among patients who are currently retained on ART, patients who screen positive for TB will be interviewed, and the data from their chart should be abstracted and included in the study database only if they give consent for this.*

A. Patient Study Identification

a) Name of Facility	_____
b) Name of staff completing the form:	_____
c) Date of form completion:	☐☐/☐☐/☐☐☐☐ [DD/MM/YYYY]
d) Time of form completion:	☐☐ : ☐☐ [HH (24hr format) : min]
e) Patient Clinic Registration number	_____
f) Patient Serial Log Number	☐☐☐-☐-☐☐☐☐-☐
g) Patient Study Identification number	☐☐☐-☐-☐☐☐☐-☐

A. DEMOGRAPHIC INFORMATION

Chart ART number	_____
1. Date of birth:	__ __/__ __/__ __ __ __ (D D / M M / Y Y Y Y) ☐ **Missing**
2. Age at ART start	_ _ **years** ☐ **Missing (cannot be missing if Q.1. is missing)**
3. Sex:	☐ **Male** ☐ **Female** **(cannot be missing)**
4. Residence at ART initiation:	**Region :**_____ ☐ **Missing** **Village/Town:**_____ ☐ **Missing**
Partner Information (Check Pre-ART Readiness Form)	
5a. Marital Status of Patient	☐ **Married** ☐ **Single** ☐ **Widowed** ☐ **Divorced** ☐ **Other**..... ☐ **Missing.**
5b. Did the patient give a phone number?	☐ **Yes** ☐ **No** ☐ **Missing**

5c. Did the patient consent to being called by phone by the clinic if needed?	☐ **Yes**	☐ **No**	☐ **Missing**
5d. Has patient disclosed status to family	☐ **Yes**	☐ **No**	☐ **Missing**
5e. Has patient disclosed status to partner	☐ **Yes**	☐ **No**	☐ **Missing**
5f. Has partner been tested for HIV	☐ **Yes**	☐ **No**	☐ **Missing**
5g. HIV status of partner	☐ **Positive**	☐ **Negative**	☐ **Missing**

Employment and Treatment Support Information

6a. Was the patient employed at ART initiation?	☐ **Yes**	Check "Yes" if there is documentation of any of: Occupation, Employer, work address, or work telephone number
	☐ **No, patient unemployed**	Check "No" if the patient is documented to be unemployed.
	☐ **Missing**	Check "Missing" if there is no documentation of whether the patient is employed or unemployed

6b. Occupation (main activity)	_____	☐ **Missing**
7. Does the patient have one or more treatment supporters?	☐ **Yes** ☐ **No**	☐ **Missing**
8. Is the patient a member of a support group	☐ **Yes** ☐ **No**	☐ **Missing**

B. HIV DIAGNOSIS

9. Date patient first tested HIV-positive?	**/ /** D D M M Y Y Y Y	☐ **Missing**

10. Was this patient transferred in?	☐ **Yes (if yes, record date of transfer-in below)** **/ /** D D M M Y Y Y Y ☐ **Missing date**	☐ **No**

11. If patient is documented to have transferred in, is there a documented date of ART initiation at a previous facility?	☐ **Yes, date of ART start at previous facility:** **/ /** D D M M Y Y Y Y	☐ **Patient transferred in but there is no date of ART start at a previous facility**	☐ **Patient is not a transfer in**

C. TIME LINE

12. Date of ART initiation at **this facility**.	**/ /** D D M M Y Y Y Y	**(cannot be missing)**
13. Date of most recent visit to **this facility**.	**/ /** D D M M Y Y Y Y	**(cannot be missing)**

D. ART INITIATION VISIT

14. WHO stage at ART initiation	☐ **I** ☐ **II** ☐ **III** ☐ **IV**	☐ **Missing**
15. Functional Status at ART initiation	☐ **A**mbulatory ☐ **W**orking ☐ **B**edridden	☐ **Missing**
16. Weight at ART initiation	**_ _ . _ kg**	☐ **Missing**
17. Was patient pregnant at time of initiation of ART?	☐ **Yes** ☐ **No** ☐ **Missing** ☐ **N/A (male)**	
18. CD4 count at ART start	**_ _ _ _ cells/mm³** ☐ **Missing**	**/ /** D D M M Y Y Y Y
19. Has the patient had previous PMTCT antiretrovirals?	☐ **Yes** ☐ **No** ☐ **Missing** ☐ **N/A (male)**	
20. Has the patient had previous ART?	☐ **Yes** ☐ **No** ☐ **Missing**	
21. Prescribed Cotrimoxazole (CTX)/Dapsone?	☐ **Yes** ☐ **No**	
22. Is there documentation the patient had TB prior to ART start?	☐ **Yes** ☐ **No**	

E. TB SCREENING AND DIAGNOSIS INFORMATION AT OR PRIOR TO ART START

23. Has the recommended TB screening form been completed?	☐ **Yes (could be part of another form)** ☐ **No (cannot find form or form is not completed)** ☐ **N/A (there is no recommended TB screening form)**
24. What is the date of the earliest (1st) TB screen	**/ /** (D D M M Y Y Y Y) ☐ **Missing**

25. Which of the following symptoms screened positive at the earliest (1st) visit?

Symptom	Yes	No	Missing
Cough >=2 weeks	☐	☐	☐
Current cough (any duration)	☐	☐	☐
Fever (any duration)	☐	☐	☐
Night Sweats(any duration)	☐	☐	☐
Loss of appetite	☐	☐	☐
Loss of Weight (any duration)	☐	☐	☐
Malaise (any duration)	☐	☐	☐
Shortness of breath (any duration)	☐	☐	☐
Any chest pain? (any duration)	☐	☐	☐
Hemoptysis (coughing blood) (any duration)	☐	☐	☐

26. What is the documented TB screening result at the earliest (1st) visit?	☐ **positive** ☐ **negative** ☐ **Missing**

27. Was patient tested for active TB?

Test	Test done?	Result?		
Sputum smear?	☐ **Yes** ☐ **No**	☐ **pos**	☐ **neg**	☐ **missing**
Sputum culture?	☐ **Yes** ☐ **No**	☐ **pos**	☐ **neg**	☐ **missing**
Chest X-ray	☐ **Yes** ☐ **No**	☐ **pos**	☐ **neg**	☐ **missing**
Other_____ (e.g. Biopsy)	☐ **Yes** ☐ **No**	☐ **pos**	☐ **neg**	☐ **missing**

28. Was the patient diagnosed as having TB?	☐ **Yes** **If Yes:** **Date of TB treatment start: __ __ /__ __ /__ __ __ __** (D D / M M / Y Y Y Y) ☐ **Pulmonary TB** ☐ **Extra-Pulmonary TB** ***[Note: Remember to complete App. 8.2 for each documented episode of TB treatment]***	☐ **No**
29. Was the patient taking TB treatment at ART start?	☐ **Yes**	☐ **No**
30. Was the patient prescribed IPT?	☐ **Yes** **/ /** (D D M M Y Y Y Y)	☐ **No**

F. ANTIRETROVIRAL THERAPY REGIMEN

31. Indicate if one of the following <u>standard regimens</u> was used:	☐ **AZT/3TC/NVP** ☐ **AZT/3TC/EFZ** ☐ **D4T/3TC/NVP** ☐ **D4T/3TC/EFZ**	☐ **TDF/3TC/NVP** ☐ **TDF/3TC/EFZ** ☐ **TDF/ABC/LPV/r** ☐ **ddI/ABC/LPV/r**	☐ **TDF/FTC/EFZ (ATRIPLA)** ☐ **TDF/FTC/NVP** ☐ **other (see below)**
32. If "other", please describe the regimen used (write 3 or 4 letter code: e.g. AZT for zidovudine, LPV/r for Lopinavir ritonavir):	**<u>Drug 1</u>:** ☐ **Missing** __ __ __ __	**<u>Drug 2</u>:** ☐ **Missing** __ __ __ __	**<u>Drug3</u>:** ☐ **Missing** __ __ __ __

NRTI	NNRTI	PI's and Boosted PI's	FDC

☐ **Zidovudine** (AZT) ☐ **Lamivudine** (3TC) ☐ **Stavudine** (d4T) ☐ **Abacavir** (ABC) ☐ **Didanosine** (Videx, ddI) ☐ **Tenofovir** (TDF)	☐ **Nevirapine** (Viramune, NVP) ☐ **Efavirenz** (EFZ)	☐ **Lopinavir (Lop)** ☐ **Nelfinavir (NFV)** ☐ **Indinavir (IND)** ☐ **Saquinavir (Saq)** ☐ **Lopinavir/ritonovir(Lop/r)** ☐ **Nelfinavir/ritonovir (Nfv/r)** ☐ **Indinavir/ritonovir (Ind/r)** ☐ **Saquinavir/ritonovir (Saq/r)**	☐ **Virolans** (d4T/3TC/Nevirapine) ☐ **Combivir** (AZT/3TC) ☐ **Triomune** (d4T/NVP/3TC) ☐ **TZV** (ABC/3TC/ZDV) ☐ **Other, specify:_____** ☐ **Missing**

G. MOST RECENT VISIT

33. Date of most recent **clinic visit**	**/ /** (D D M M Y Y Y Y) **(cannot be missing)**	
34. WHO Stage recorded	☐ **I** ☐ **II** ☐ **III** ☐ **IV** ☐ **Missing**	
35. Functional status	☐ **A**mbulatory ☐ **W**orking ☐ **B**edridden ☐ **Missing**	
36. Weight	**_ _ . _ kg** ☐ **Missing**	
37. If Female, did she ever become pregnant since ART initiation?	☐ **Yes** **/ /** (D D M M Y Y Y Y) ☐ **Missing date**	☐ **No pregnancy documented** ☐ **N/A (male)**
38. Most recent CD4 count	**_ _ _ _ cells/mm³** ☐ **Missing**	**/ /** (D D M M Y Y Y Y) **D**
39. Prescribed Cotrimoxazole or Dapsone?	☐ **Yes**	☐ **No documentation of this**
40. Date of next appointment?	**/ /** (D D M M Y Y Y Y)	☐ **Missing**

H. KEY EVENTS DURING ART

41. As of today, since ART initiation, the patient is…. *(choose one, except if patient stopped and/or restarted ART):* **Note any additional comments here:** **_____** **_____** ***[Note: Remember to complete App. 8.3 – the Exit Form - for each patient who is known to have died, stopped ART, or transferred to another clinic].*** ***[Note: Remember to complete App.9 if the patient is late for next appointment (see Q.40) or thought to be LTFU]***	☐ **Retained; patient is alive and on ART**	**Date of most recent visit (either to see clinician or pick up medication)** **/ /** (D D M M Y Y Y Y)
	☐ **Stopped ART**	**A) / /** (D D M M Y Y Y Y) **UNTIL** **B) / /** ☐ **No restart**
	☐ **Transferred out (officially documented)**	**/ /** (D D M M Y Y Y Y)
	☐ **Died**	**/ /** (D D M M Y Y Y Y)
	☐ **Been lost to follow up (Last visit >90 days ago) (LTFU)**	**/ /** (D D M M Y Y Y Y)
42. If patient died, what was the cause of death documented? ☐ **N/A, patient did not die**	☐ **Respiratory (not pulmonary TB)** ☐ **Acute diarrhea** ☐ **Chronic diarrhea** ☐ **TB (pulm + extra pulm)** ☐ **IRIS** ☐ **Other……** ☐ **Unknown**	

I. DRUG CHANGES

43. If the antiretroviral medications changed during treatment, please indicate the new drugs, the date of change and the reason for change (1= Immunologic failure, 2=Virologic failure, 3=Clinical Failure, 4 = Neuropathy, 5 = Anemia, 6 = Hepatitis, 7 = toxicity (other than neuropathy and anemia), 8 = pregnancy, 9 = presumed pregnancy , 10 = active TB, 11 = stock-out of medicine, 12 = new medication available, 13 = other, please specify_____).

☐ **No change in ARVs**

Change	Antiretrovirals	DD / M M / Y Y	Reason
1st	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing
2nd	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing
3rd	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing
4th	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing
5th	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing
6th	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing
7th	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing
8th	_ _ _ / _ _ _ / _ _ _	/ / ☐ Missing	_____(1-13) ☐ Missing

10.3. Appendix 3. IRB approvals for XPRES

10.3.1. CDC IRB C approval

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Centers for Disease Control
and Prevention (CDC)

Memorandum

Date July 19, 2012

From Betty Wong, MPH, CHES
IRB C Administrator, Human Research Protection Office

Subject Site Restricted IRB Approval of New CDC Protocol #6294, "Evaluating Performance, Impact, and Operational Challenges of GeneXpert Use for TB Case Finding among HIV-infected Persons in Botswana during 2012-2013: The Xpert Package Rollout Evaluation Study (XPRES)" (Convened)

To Tedd Ellerbrock, MD
CGH/DGHA

CDC's IRB C has reviewed the request for approval of new protocol #6294 and has approved the protocol for the maximum allowable period of one year. CDC IRB approval will expire on 07/17/2013. The study involves no greater than minimal risk to subjects.

COLLABORATOR/SITE RESTRICTION: Institutions that receive federal support who are engaged in human subjects' research are required to obtain and provide documentation of IRB approval and maintain a valid Federal wide Assurance (FWA). CDC investigators who interact with institutions that have failed to meet these requirements are collaborating with noncompliant institutions. Study activities may not begin with the collaborators listed below until documentation indicating current IRB approval has been received by CDC's Human Research Protection Office (HRPO) and the PI has been notified by HRPO that this restriction has been lifted and study activities may begin:

University of Pennsylvania

If other institutions involved in this protocol are being awarded CDC funds through the CDC Procurement and Grants Office (PGO), you are required to send a copy of this IRB approval to the CDC PGO award specialist handling the award. You are also required to verify with the award specialist that the awardee has provided PGO with the required documentation and has approval to begin or continue research involving human subjects as described in this protocol.

As a reminder, the IRB must review and approve all human subjects' research protocols at intervals appropriate to the degree of risk, but not less than once per year. There is no grace period beyond one year from the last IRB approval date. It is ultimately your responsibility to submit your research protocol for continuation review and approval by the IRB along with available IRB approvals from all collaborators. Please keep this approval in your protocol file as proof of IRB approval and as a reminder of the expiration date. **To avoid lapses in approval of your research and the possible suspension of subject enrollment and/or termination of the**

protocol, please submit your continuation request along with all completed supporting documentation at least six weeks before the protocol's expiration date of 07/17/2013.

Any problems of a serious nature must be brought to the immediate attention of the CDC IRB, and any proposed changes to the protocol should be submitted as an amendment to the protocol for CDC IRB approval before they are implemented.

If you have any questions, please contact your National Center Human Subjects' Contact or the CDC Office of Scientific Integrity (404) 639-7570 or e-mail: huma@cdc.gov.

cc:
CGH Human Subjects

10.3.2. Botswana national ethics committee approval (HRDC)

Telephone: (267) 363200
FAX (267) 353100
TELEGRAMS: RABONGAKA
TELEX: 2818 CARE BD

MINISTRY OF HEALTH
PRIVATE BAG 0038
GABORONE

REPUBLIC OF BOTSWANA

REF NO: PPME-13/18/1 Vol VII (472) 24 May 2012

Health Research and Development Division

Notification of IRB Review: New Application

Dr James Shepherd
P.O. Box 90
BOTUSA

Protocol Title: **OPERATIONAL CHALLENGES OF GENE EXPERT USE FOR TB CASE FINDING AMONG HIV-INFECTED PERSONS IN BOTSWANA DURING 2011-2013: VERSION 1.3**

HRDC Protocol Number:	HRDC **00687**
Sponsor:	PEPFAR
HRDC Review Date:	15 May 2012
HRDC Expiration Date:	14 May 2013
HRDC Review Type:	HRDC reviewed
HRDC Review Determination:	Approved
Risk Determination:	Minimal risk

Dear Dr Shepherd

Thank you for submitting a new application for the above referenced study. This approval includes the following:

1. Application Form
2. Proposal
3. Data collection tools

This permit does not however give you authority to collect data from the selected sites without prior approval from the management. Consent from the identified individuals should be obtained at all times.

1

The research should be conducted as outlined in the approved proposal. Any changes to the approved proposal must be submitted to the Health Research and Development Division in the Ministry of Health for consideration and approval.

Furthermore, you are requested to submit at least one hardcopy and an electronic copy of the report to the Health Research, Ministry of Health within 3 months of completion of the study. Copies should also be submitted to all other relevant authorities.

Continuing Review
In order to continue work on this study (including data analysis) beyond the expiry date, submit a Continuing Review Form for Approval at least three (3) months prior to the protocol's expiration date. The Continuing Review Form can be obtained from the Health Research Division Office (HRDD), Office No. 9A 10 or Ministry of Health website: www.moh.gov.bw or can be requested via e-mail from Mr. Kgomotso Motlhanka, e-mail address: kgmmotlhanka@gov.bw As a courtesy, the HRDD will send you a reminder email about eight (8) weeks before the lapse date, but failure to receive it does not affect your responsibility to submit a timely Continuing Report form.

Amendments
During the approval period, if you propose any change to the protocol such as its funding source, recruiting materials, or consent documents, you must seek HRDC approval before implementing it. Please summarize the proposed change and the rationale for it in the amendment form available from the Health Research Division Office (HRDD), Office No. 9A 11 or Ministry of Health website: www.moh.gov.bw or can be requested via e- mail from Mr. Kgomotso Motlhanka, e-mail address: kgmmotlhanka@gov.bw . In addition submit three copies of an updated version of your original protocol application showing all proposed changes in bold or "track changes".

Reporting
Other events which must be reported promptly in writing to the HRDC include:
- Suspension or termination of the protocol by you or the grantor
- Unexpected problems involving risk to subjects or others
- Adverse events, including unanticipated or anticipated but severe physical harm to subjects.

If you have any questions please do not hesitate to contact Mr. P. Khulumani at pkhulumani@gov.bw, Tel +267-3914467 or Lemphi Moremi at lamoremi@gov.bw or Tel: +267-3632466

Thank you for your cooperation and your commitment to the protection of human subjects in research.

Yours sincerely

[redacted]
P. Khulumani
For Permanent Secretary

10.3.3. University of Pennsylvania IRB approval

University of Pennsylvania
Office of Regulatory Affairs
3624 Market St., Suite 301 S
Philadelphia, PA 19104-6006
Ph: 215-573-2540/ Fax: 215-573-9438
INSTITUTIONAL REVIEW BOARD
(Federalwide Assurance # 00004028)

27-Aug-2012

Andrew Steenhoff
Attn: Rosemarie Kappes
steenhoff@email.chop.edu
rkappes@mail.med.upenn.edu

PRINCIPAL INVESTIGATOR	: Andrew Steenhoff
TITLE	: XPRES: Evaluating Performance, Impact, and Operational Challenges of GeneXpert use for TB Case Finding among HIV-infected Persons In Botswana during 2011-2013
SPONSORING AGENCY	: No Sponsor Number
PROTOCOL #	: 815871
REVIEW BOARD	: IRB #4

Dear Dr. Steenhoff:

IRB approval has been given to the above referenced protocol as of 24-Aug-2012. This study will be due for continuing review on or before 18-Jun-2013.

Approval by the IRB does not necessarily constitute authorization to initiate the conduct of a human subject research study.

Principal investigators are responsible for assuring final approval from other applicable school, department, center or institute review committee(s) or boards has been obtained. This includes, but is not limited to, the University of Pennsylvania Cancer Center Clinical Trials Scientific Review and Monitoring Committee (CTSRMC), Clinical and Translational Research Center (CTRC) review committee, CAMRIS committee, Institutional Bio-safety Committee (IBC), Environmental Health and Radiation Safety Committee (EHRS), and Standing Conflict of Interest (COI) Committee. Principal investigators are also responsible for assuring final approval has been obtained from the FDA as applicable, and a valid contract has been signed between the sponsor and the Trustees of the University of Pennsylvania. If any of these committees require changes to the IRB-approved protocol and informed consent/assent document(s), the changes must be submitted to and approved by the IRB prior to beginning the research study.

If this protocol involves cancer research with human subjects, biospecimens, or data, you may not begin the research until you have obtained approval or proof of exemption from the Cancer Center Clinical Trials Review and Monitoring Committee.

Please note that you are responsible for registering this study on ClinicalTrials.gov if this study qualifies as a clinical trial (as defined in section 50.3 of title 21, Code of Federal Regulations) and the principal investigator is designated as the responsible party (as defined in Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA)(PL110-85).

The revisions, in response to review by the convened IRB, were reviewed and approved by Dr. Emma Meagher, Executive Chair of the IRB (or her authorized designee).

The following documents were included in this review:

_HS-ERA IRB Application, submitted 08/22/12 [confirmation code: jigaiia]
_Report of CDC IRB C, CDC Protocol #6294, dated 06/21/12
_Report of CDC IRB C, CDC Protocol #6294, dated 06/04/12
_Summary of Changes, dated 08/22/12

The documents noted below were submitted with confirmation code: jeccgfa and are now approved:

-HRDC Approval letter dated 5/24/12
-Informed Consent Form – Prospective Adult HIV Clinic Enrollees (>18 years old at time of Consent) uploaded 7/25/12

-Information and Consent Form for Guardians of HIV Clinic Enrollees who are Minors (<18 years old at time of Consent) 7/25/12
-Information and Assent Form for HIV Clinic Enrollees who are Minors Aged 7-12 uploaded 7/25/12
-Information and Assent Form for HIV Clinic Enrollees who are Minors Aged 13-17 uploaded 7/25/12
-Informed Consent Form for Cross-Sectional Interview – Adult ART Enrollees (>18 years old at time of Consent) uploaded 7/25/12
-Appendix 4.3.2.: Informed Consent Form for Guardians of Minors (Currently <18 Years Old), who are Eligible for the Cross-Sectional Interview uploaded 5/25/12
-Information and Assent Form for Cross-Sectional Interview Enrollees who are Minors Aged 7-12 uploaded 7/25/12
-Information and Assent Form for Cross-Sectional Interview Enrollees who are Minors Aged 13-17
-GeneXpert Technician User Acceptance Consent form and Survey uploaded 7/25/12
-Information and Consent Form for "Cause of death interview" for the Relative/friend/guardian/acquaintance of the deceased – (interviewee must be >18 years old at time of Consent)
-Full protocol dated 3/30/12 Version: 3.0 Date: 16 July 2012
-Cover letter to the IRB dated 7/25/12

The documents noted below, submitted with confirmation code: jbbdcab, were reviewed by the convened IRB on 19-Jun-2012, and are now approved:

-Cover letter to the IRB dated 5/25/12
-List of Other Investigators /Co-Principal Investigators uploaded 5/25/12
-TB Treatment Record Abstraction Form uploaded 5/25/12
-Study Exit Form uploaded 5/25/12
-Specimen Collection Form uploaded 5/25/12
-Pediatric Retrospective Data Abstraction Questionnaire (<12 Years old at ART Start) uploaded 5/25/12
-Adult Retrospective Data Abstraction Questionnaire (>12 Years old at ART Start) uploaded 5/25/12
-Questionnaire for Patients Late for Clinic Appointments uploaded 5/25/12
-Chest X-ray Interpretation and Results Form uploaded 5/25/12
-Questionnaire to Ascertain Cause of Death uploaded 5/25/12
-Xpert MTB/RIF User Acceptance uploaded 5/25/12
-Questionnaire for Health Care Provider at the Clinic uploaded 5/25/12
-Prospective Study Enrollment Questionnaire uploaded 5/25/12
-Interview for Patients Enrolled Retrospectively uploaded 5/25/12
-Follow-up Questionnaire for Patients Enrolled in Prospective Cohorts uploaded 5/25/12
- Interview for Health Care Worker at Each Participating Study Site Information Sheet: Botswana Xpert Rollout Evaluation Study (XPRES) uploaded 5/25/12
-Quality assurance monitoring document uploaded 5/25/12
-Analysis plan document uploaded 5/25/12
-Study Procedures document uploaded 5/25/12
-Vulnerable populations Supplemental form: Children document uploaded 5/23/12
-Vulnerable populations Supplemental form: Pregnant Women document uploaded 5/23/12

Please Note: The IRB reviewed and approved a waiver or alteration of the required elements of consent for the retrospective portion of the study under § 46.116(d) as it was determined that (1) the study is no greater than minimal risk, (2) conducting the study would be impracticable without the waiver, (3) waiving does not adversely affect subjects and, if applicable, (4) pertinent information will be provided to the subjects later.

Please Note: The IRB reviewed and approved the Subpart B review as per Federal Regulations 45 CFR 46.204 regarding pregnant women and fetuses and all criteria have been met. Additionally, the study was reviewed for subjects undergoing minimal risk procedures under 21 CFR 50.51 (45 CFR. 46.404) regarding research involving children and all criteria have been met.

Please Note: The IRB reviewed and approved the Subpart D review as per Federal Regulations 45 CFR 46.404 (FDA 50.51), as the research was determined to be no greater than minimal risk. The IRB determined that permission of one parent is sufficient and that adequate provisions are made for soliciting permission. The IRB has determined that assent must be obtained from subjects and provisions for obtaining assent are appropriate.

When enrolling subjects at a site covered by the University of Pennsylvania's IRB, a copy of the IRB approved informed consent form with the IRB approved from/to stamp must be used unless a waiver of written documentation of consent has been granted.

The IRB has received a HIPAA Authorization Form which will be used for all study subjects, which is presumed to be accurate. Disclosure of any protected health information outside the constraints of the authorization is prohibited. It is mandatory that you obtain a new authorization or submit a waiver request to change the current terms of the disclosure authorization in any way.

If you have any questions about the information in this letter, please contact the Regulatory Affairs administrative staff. Contact information is available at our website: http://www.upenn.edu/regulatoryaffairs.

Thank you for your cooperation.

Sincerely,

Jenna Dragani

Digitally signed by Jenna Dragani
DN: cn=Jenna Dragani, o=ORA, ou=IRB, email=jdragani@upenn.edu, c=US
Reason: I attest to the accuracy and integrity of this document
Date: 2012.08.27 15:44:39 -04'00'

IRB Administrator

10.3.4. London School of Hygiene & Tropical Medicine ethics approval

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

Dr Francis Auld
LSHTM

21 September 2016

Dear Francis,

Study Title: Evaluating Performance, Impact, and Operational Challenges of GeneXpert Use for TB Case Finding among HIV-infected Persons In Botswana: The Xpert Package Rollout Evaluation Study (XPRES)

LSHTM ethics ref: 11779

Thank you for your application for the above research, which has now been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Local Approval	HRDC Approval - New Protocol Approval letter - 2012	24/05/2012	1
Local Approval	CDC IRB Approval - protocol 6294 - New Protocol Approval - 2012	19/07/2012	1
Local Approval	UPenn IRB Approval - New Protocol Approval - 2012	27/08/2012	1
Protocol / Proposal	XPRES_(6294)_Protocol_CLEAN_version_of_Protocol_v5_12Dec2013	12/12/2013	5
Protocol / Proposal	XPRES_(6294)_Appendices_All_V5_Dec12_2013	12/12/2013	5
Investigator CV	CV_A.F.Auld_M.D_M.Sc_Feb2016_final	29/02/2016	1
Investigator CV	Curriculum Vitae -- Tedd V. Ellerbrock, MD -- April 2016	30/04/2016	1
Investigator CV	CV Dr Agizew T 08 Jul 2016	08/07/2016	1
Investigator CV	CV_Finlay-Vickers	26/07/2016	1
Investigator CV	GrantCVshort_investigator_dec15	26/07/2016	1
Investigator CV	Lawn_Biosketch_NewFormat_DRAFT_09.16.15	26/07/2016	1
Investigator CV	CV_katherine_Fielding_PhD_LSHTM	26/07/2016	1

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]
Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/