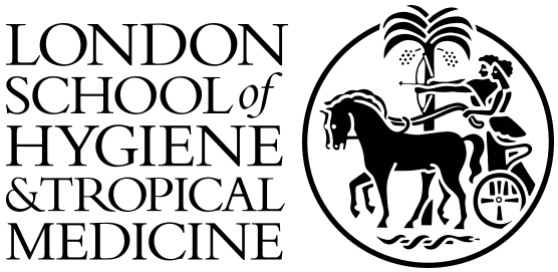

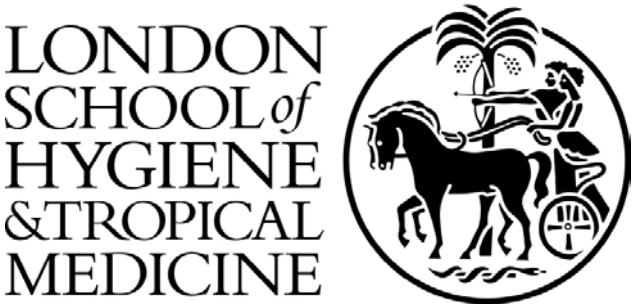

Improving outcomes for adults living with HIV admitted to hospital in the era of high antiretroviral therapy coverage

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Declaration of own work

I, Rachael Burke, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. I have read and understood the School's definition of plagiarism and cheating given in the Research Degrees Handbook.

Acknowledgements

Firstly, I want to acknowledge the CASTLE trial participants and their families. These men and women agreed to participate in research during a period of serious illness. A quarter did not survive their illness. I assume more people would have survived had the same participants presented to the London hospitals where I worked as an infectious disease registrar before moving to Malawi. This is not fair and I look forward to a day when there is more justice and equity in the world.

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Thank you to my friends and colleagues at Malawi Liverpool Wellcome. To the "Junior Researchers room" crew (particularly Ndaru Jambo), the Public Health Group (Thandie Gondwe, Luke Banda, Helena Feasey, Hannah Rickman, Linda Sande and McEwen Khundi), the Clinical Research Support Unit (Wilfred Nedi, Brian Ngwira and Markus Gmeiner), the Data Support Unit (Alfred Muyaya and the late Clemens Masesa), and the laboratory team (Brigitte Denis, Doris Shani and Joseph Bwanali). Thank you also to senior colleagues for providing encouragement and steer as I navigated clinical research in Malawi – Prof Mwandumba, Profs Gordon, Dr Jamie Rylance, Dr Nick Feasey, Dr David Lissauer, Dr Sam Lissauer, Dr Augustine Choko and Dr Marc Henrion. Thank you to colleagues at Helse Nord Tuberculosis Initiative for invaluable advice and guidance and a warm welcome into your research group, particularly Dr Marriott Nliwasa.

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Maundy Thursday, Holy Week, 2023

Abstract

Despite impressive successes with the public health approach in scaling up HIV testing and ART access in the past 20 years, advanced HIV disease remains a persistent problem. People living with HIV (PLHIV) who require admission to hospital are at extremely high risk of death. Tuberculosis (TB) remains the single biggest cause of HIV-related deaths. This thesis considers adults living with HIV admitted to hospital, with the major focus being a cluster randomised trial of enhanced TB diagnostics (CASTLE trial).

The thesis consists of the following chapters: first, a systematic review of interventions to reduce mortality among adults living with HIV admitted to hospital in low- and middle-income countries. Ten studies were identified, including two TB diagnostics intervention trials that showed mortality reductions.

Secondly, routine data from Queen Elizabeth Central Hospital in Blantyre, Malawi was used to estimate trends in incidence of adult HIV-related admission to hospital and in-hospital deaths. The population incidence of HIV-related hospital admissions declined substantially all age and sex groups from 2012 to 2019. In-hospital case-fatality for admitted PLHIV remained unchanged, at 23.5%, with no significant reduction in any age-sex group, and no association with ART use at admission.

Thirdly, a cluster-randomised trial (Computer Aided Screening for Tuberculosis in Low Resource Environments: CASTLE) using admission day as the unit of randomisation was designed and conducted. Admission days were randomly assigned to: 1) enhanced diagnostics for TB using urine lipoarabinomannan (LAM) (SILVAMP-LAM, Fujicorp, Japan and LF-LAM, Alere/Abbot, USA), digital chest X-ray with computer aided diagnosis (dCXR-CAD) using CAD4TBv6 (Delft, Netherlands) plus usual care; or 2) to usual care alone. The primary outcome was TB treatment initiation during admission. Between 2 September 2020 and 15 February 2022, 415 adults were recruited during 207 admission-days. TB treatment was initiated in 46/208 (22%) in the enhanced TB diagnostics arm and 24/207 (12%) in the usual care arm (risk ratio [RR] 1.92, 95% CI 1.20-3.08). Urine SILVAMP-LAM/LF-LAM plus dCXR-CAD diagnostics identified more hospitalised PLHIV with TB than usual care, but with no evidence of impact on survival, undiagnosed TB, or TB treatment initiation within 24 hours. Unanticipated findings in CASTLE included poor concordance between SILVAMP-LAM and LF-LAM results.

The PhD highlights the ongoing high mortality of people living with HIV in hospital, the relative paucity of evidence based interventions for this population, and the persistently high death rate. I show that an enhanced TB diagnostic intervention is feasible and increases the number of people diagnosed with TB. To achieve goals to get to zero AIDS deaths, people with advanced HIV disease particularly those in hospital, deserve greater research attention.

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A note about organisation of thesis and nomenclature

This is a "papers style" thesis, consisting of three published papers, one unpublished manuscript and three narrative chapters (background, challenges faced during trial, and discussion) which are not prepared as manuscripts.

Reference lists are included within each paper / manuscript and at the end of each chapter. Pages are in numerical order throughout thesis, although pages which are part of a published manuscript may have two page numbers on them (the page in the journal, and the page in the thesis). Tables and figures in material unique to this thesis have numbers which match their section. Tables and figures in published papers or manuscript for publication are numbered according to their order within that paper / manuscript.

Where possible I have avoided using trade names or manufacturers names to refer to diagnostic tests in text. This is in light of WHO Global TB Programme decision to move away from making recommendations for individual tests, and focusing on diagnostic classes – with specific diagnostic tests within each class being evaluated through the WHO prequalification process rather than by guideline recommendation. For avoidance of doubt, "LF-LAM" refers to lateral flow lipoarabinomannan tests manufactured by Abbott (Determine LF-LAM, Abbott, USA, previously manufactured by Alere). "SILVAMP-LAM" is the name of the lipoarabinomannan test manufactured by Fujifilm (Japan). In preference to specifying a trade name, I have usually referred to sputum Nucleic Acid Amplification Tests (NAATs), meaning any WHO-approved molecular TB test, unless it is relevant what specific NAAT test was used. In practice, in CASTLE trial and in most studies referred to in this thesis, Xpert (Cephid, USA) was the specific type of TB NAAT test used – either MTB/rif or Ultra. In CASTLE the specific digital chest Xray and Computer Aided Diagnosis (dCXR-CAD) product was CAD4TB version 6 (Delft, Netherlands). Other studies have used a variety of different dCXR-CAD products and the specific product is mentioned where relevant.

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List of abbreviations and acronyms

AIDS	Acquired Immune Deficiency Syndrome
ART	Anti-retroviral therapy
BAL	Bronchoalveolar lavaga
CAD	Computer Aided Diagnosis
CAD4TB	Computer Aided Diagnosis system for chest Xrays for TB diagnosis, manufactured by Delft (Netherlands)
CI	Confidence Interval
CONSORT	Reporting Guideline for Randomised Trials
COVID-19	Coronovirus Infectious Disease 19
CrAg	Cryptococcal antigen
CRP	C-reactive protein
CRS	Clinicial Reference Standard (for TB index test evaluation)
CSF	Cerebrospinal fluid
(d)CxR	(Digital) chest X-ray
DSMC	Data and Safety Monitoring Committee
DTG	Dolutegravir, an integrase strand transfer inhibitor class of ART
HIV	Human Immunodeficiency Virus
INSTI	Integrase strand transfer inhibitor, a class of drugs used as part of ART regimens.
IPD	Individual patient data
IFU	Instructions for use
LAM	Lipoarabinomannan
LF-LAM	Lateral Flow Lipoarabinomannan, refers to urine TB diagnostic test manufactured by Alere/Abbot (USA)
M. tb	*Mycobacterium tuberculosis*
MDR	Multi-drug resistant
MGIT	Mycobacterium Growth Indictor Tubes (a form of semi-automated liquid culture for *M. tb*)
MLW	Malawi Liverpool Wellcome Trust Research Programme
MRS	Microbiological Reference Standard (for TB index test evaulation)
NAAT	Nucleic Acid Amplification Test
NPV	Negative predictor value (proportion of people with a negative index test who truly do not have disease)
PLHIV	People living with HIV
PPV	Positive predictor value (proportion of people with a positive index test who truly do have disease)
PRISMA	Reporting guideline for systematic reviews
QECH	Queen Elizabeth Central Hospital (a large hospital in Blantyre, Malawi)
Qure.AI	Refers to a Computer Aided Diagnosis software product to interpret chest X-rays, manufactuered by qXR (India).
RHZE	Rifampacin, Isoniazid, Pyrazinamide, Ethambutol (TB treatment). Also 4RHZE/2RH meaning four months of RHZE followed by two months of rifampacin and isonizaid.
SILVAMP-LAM	Urine TB diagnostic test manufactured by Fujifilm (Japan)
SPIRIT	Reporting guideline for randomised trial protocols
TB	Tuberculosis
WHO	World Health Organisation
WHO 4SS	WHO four symptom screen for TB. Consists of asking about presence of cough (any duration), fever, night sweats and weight loss. WHO 4SS is considered positive if any one or more symptom is present.
Xpert	Near point of care Nucleic acid amplification test platform manufactured by Cephid (USA)
Xpert MTB/Rif	Nucleic acid amplification test for TB manufactured by Cephid (USA)
Xpert Ultra	Nucleic acid amplification test for TB manufactured by Cephid (USA) – higher sensitivity that Xpert MTB/rif
ZCH	Zomba Central Hospital (a hospital in Southern Region, Malawi)

Chapter 1: Introduction

1.1 Aims

The broad aim of this research is to better understand and to reduce mortality among adults living with HIV admitted to hospital. Because tuberculosis is the most common cause of hospital admission and death, the focus of the research is on improving tuberculosis diagnosis in this group of people. The research was conducted in Malawi, which is a high HIV prevalence, high TB burden country, where much HIV care is delivered through a public health approach [1,2] and population coverage of ART is high among people living with HIV.[3] The findings are likely to be generally applicable to Southern Africa, which is the region where most advanced HIV disease and most HIV related deaths occur.[4]

1.2 Objectives

The specific objectives were to:

- Systematically review and synthesise the literature about interventions to reduce all-cause mortality among adults living with HIV admitted to hospital in low- and middle- income countries.
- Assess and quantify trends in HIV related hospital admission and in-hospital deaths between 2012 and 2019 in Blantyre City. This time period is concurrent with substantial ART scale up in Blantyre.
- Design, implement and conduct a cluster randomised trial of enhanced tuberculosis diagnostics (using digital chest X-ray with computer aided diagnosis and urine LF-LAM and SILVAMP-LAM testing) among adults living with HIV admitted to hospital.
- Analyse, present, and discuss the results of the cluster randomised trial of enhanced tuberculosis diagnostics.

1.3 Structure of thesis

This thesis consists of eight chapters.

Chapter 1: This introduction chapter sets out the aims, objectives and my role in the research.

Chapter 2 gives scientific background firstly to advanced HIV disease, HIV-related deaths and HIV related hospital admissions, with a focus on Southern Africa. Secondly chapter two provides the scientific background about TB diagnostics in people living with HIV. This material is specific to the thesis, and is not published or prepared for publication elsewhere.

Chapter 3 is systematic review of interventions to reduce all-cause mortality among adults living with HIV admitted to hospital in low- and middle- income countries. This review is of interventions that are broadly applicable to all or most of the population of HIV positive adults admitted to hospital. Studies which only include people who already have an aetiological diagnosis in addition to HIV at the time of recruitment into the study (for example, studies of treatments for cryptococcal meningitis) are not included. This is a published article in PLoS Global Public Health, reproduced under the terms of the CC-BY 4.0 license. Chapter 3 also contains an introduction and appendices to the review.

> Burke R.M., Twabi H.H., Johnston C., Nliwasa M., Gupta-Wright A., Fielding K., Ford, N., MacPherson, P., Corbett, E. L. (2023) Interventions to reduce deaths in people living with HIV admitted to hospital in low- and middle-income countries: A systematic review. PLOS Global Public Health 3(2): e0001557.

Chapter 4 is the author accepted manuscript of a paper where I analyse change in incidence of HIV-related adult hospital admissions and in-hospital deaths in Queen Elizabeth Central Hospital, Blantyre between 2012 and 2019, together with an introductory "linking" section. The paper is published in AIDS, and the material reproduced for this thesis under a CC-BY license applied by authors ("rights retention" route for Plan S Green Open Access).

> Burke, R. M., Henrion, M. Y. R., Mallewa, J., Masamba, L., Kalua, T., Khundi, M., Gupta-Wright, A., Rylance, J., Gordon, S. B., Masesa, C., Corbett, E. L., Mwandumba, H. C., & Macpherson, P. (2021). Incidence of HIV-positive admission and inpatient mortality in Malawi (2012-2019). *AIDS (London, England)*, *35*(13), 2191–2199.

Chapter 5 is the protocol paper, summarising the design and protocol for a cluster randomised trial of the impact of dCXR-CAD plus SILVAMP-LAM plus LF-LAM plus usual care, compared to usual care alone for increasing TB treatment initiation among adults living with HIV admitted to hospital. This is the CASTLE trial: Computer Aided Screening for Tuberculosis in Low Resource Environments.

> Burke, R. M., Nyirenda, S., Twabi, H. H., Nliwasa, M., Joekes, E., Walker, N., Nyirenda, R., Gupta-Wright, A., Fielding, K., MacPherson, P., & Corbett, E. L. (2022). Design and protocol for a cluster randomised trial of enhanced diagnostics for tuberculosis screening among people living with HIV in hospital in Malawi (CASTLE study). *PloS one*, *17*(1), e0261877. https://doi.org/10.1371/journal.pone.0261877

Chapter 6 is the CASTLE results manuscript as prepared for publication, reporting the trial results and setting them in context. I show that the CASTLE intervention increased the number of people starting TB treatment (trial primary outcome), but had no effect on secondary outcomes of 56 day mortality,

same day TB treatment initiation or undiagnosed TB at discharge. This is an manuscript prepared for publication in a journal.

Chapter 7 sets out some of the notable and unanticipated challenges faced during the implementation of the CASTLE trial, mitigations adopted, and lessons learned. The major challenges with the CASTLE trial were: lower than initially anticipated recruitment, which was addressed early in the study period; the COVID-19 pandemic; the now well-documented lot-to-lot variation in performance with SILVAMP-LAM; the less well documented issue of variable performance with LF-LAM; and the lack of clinical action consequent to dCXR-CAD results.

Chapter 8 is a chapter of discussion, conclusions and future research needed. In this chapter I summarise the thesis and discuss the remaining important research and implementation questions about what might be effective to reduce inpatient mortality from advanced HIV disease and contribute towards goals of ending AIDS as a public health problem.

1.4 My role

I led the all of the research that is contained within this thesis, including being Principal Investigator of the CASTLE trial, although I am grateful for support and wisdom of many people especially my supervisors Prof Corbett and Prof MacPHerson.

I conceived the question for the systematic review, wrote the protocol, executed the database searches, reviewed papers for inclusion, extracted data and wrote the first draft of the paper. I worked with colleagues / co-authors for help with conceiving the review (Dr Nathan Ford at WHO as well as my supervisors) peer reviewing the search strategy (Russell Burke, LSHTM libraries), being a second reviewer for papers (Dr Hussein Twabi, Kamuzu University of Health Sciences) and for input and review of manuscript (all authors, particularly my supervisors).

For the analysis of time trends in HIV related hospital admission and deaths, I obtained data from Malawi Liverpool Wellcome data team. I cleaned the data, led the design of the statistical analysis, wrote all code and figures, and wrote the first draft of the manuscript. I had input from Prof MacPherson for help with correctly coding the statistical analysis, and from Dr Marc Henrion (Malawi Liverpool Wellcome Trust) about statistical methods, particularly about missing data handling.

I designed the CASTLE trial, initially as part of my (successful) funding proposal for this PhD. Whilst I led the design process, I am particularly grateful for the wisdom and input of my supervisors and Prof Katherine Fielding. My other co-authors provided valuable input to design – including Dr Marriott Nliwasa, Dr Saulous Nyriendra and Dr Liz Joekes. Prof Fielding provided input about statistical methods to ensure trial integrity. I wrote the trial protocol, the Standard Operating Procedures, the Statistical Analysis Plan and all the content for ethical approval from LSHTM and Kamuzu University of Health Sciences Research Ethics Committee – with review and input from Prof Corbett, Prof MacPherson and Prof Fielding. I designed all the trial Case Report Forms using Open Data Kit, and was assisted in back-up servers, archving and web hosting by data team at Malawi Liverpool Wellcome. With the help of Malawi Liverpool Wellcome Human Resources team, I recruited, training and provided directed supervision to the CASTLE trial research clinical officer (Timeo Mtenga) and research assistants (Ivy Missi and Gift Kazembe). I led the CASTLE trial team day to day, with advice and input as needed from my supervisors (which was particularly invaluable when navigating COVID impact on the trial). I managed data – blinded to trial arm allocation – and conducted interim data reports for the Data Safety Monitoring Committee (interim data reports did not split data by trial arm and did not do formal interim analyses). I worked with MLW Clinical Research Support Unit who provided trial monitoring to ensure integrity of research. I wrote code and I performed statistical analyses for trial outcomes, including all figures in the manuscript. I am grateful for help and code review from Prof Fielding, who also reviewed and provided input to the Statistical Analysis Plan. Prof MacPherson also had access to the full trial dataset and can verify results. I wrote the first draft of the CASTLE trial manuscript, with revisions, comments and input from my supervisors, Prof Fielding and other co-authors.

1.5 Ethical considerations

The CASTLE trial was reviewed and approved by London School of Hygiene and Tropical Medicine Research Ethics committee (reference #17799) and the University of Malawi Research Ethics Committee (reference #P/08/19/2772). All participants gave written (or thumbprint and witnessed) informed consent prior to any trial activities. Study monitoring was conducted the Malawi Liverpool Wellcome Clinical Research Support Unit. The trial was registered at clinicaltrials.gov (NCT04545164)..

1.6 Funding

Funding for this PhD, including the CASTLE trial, was from Wellcome via my Clinical PhD Fellowship (203905/Z/16/Z).

1.7 References

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2. Harries, A. D. *et al.* Act local, think global: how the Malawi experience of scaling up antiretroviral treatment has informed global policy. *BMC Public Health* **16**, 938 (2016).
3. Malawi Summary Sheet 2020-2021. *PHIA Project* https://phia.icap.columbia.edu/malawi-summary-sheet-2/.
4. AIDSinfo | UNAIDS. https://aidsinfo.unaids.org/.

Chapter 2: Background

2.1 Advanced HIV and outcomes for adults living with HIV admitted to hospital in Africa

2.1.1 Setting the scene: the HIV epidemic in Southern and Eastern Africa, and Malawi

Of the 38.4 million people worldwide living with HIV in 2021, 20.6 million live in Southern and Eastern Africa. In 2021 in Southern and Eastern Africa, 85% of people living with HIV knew their status, 88% of people who knew their status were on anti-retroviral therapy (ART) and 92% of those on ART were virally suppressed.[1] This coverage was made possible by development of low cost service delivery models taking a "public health approach" to ART scale-up.[2,3] The public health approach to ART uses a limited number of highly standardised (not individualised) diagnostic tests, ART regimens, and follow-up schedules, minimising the cost and level of skill needed to manage patients and thereby facilitating decentralisation of HIV care from a small number of hospital hubs (as was the case in the early 2000s) to a large number of primary care clinics, opening up access to ART for millions of people. The public health approach includes task shifting so that HIV testing and ART related tasks are performed by cadres of staff following protocols with shorter training.[2]

UNAIDS HIV epidemic estimates start from 1990, with an estimate of 4% adults age 15-49 in Southern and Eastern Africa living with HIV at that time;[1] HIV was prevalent in Southern and Eastern Africa before 1990 and reports of people with AIDS in Uganda and Democratic Republic of the Congo were

documented in scientific literature in 1980s.[4,5] Effective combination therapy for HIV was discovered in mid-1990s,[6,7] but it was not made available in the parts of the world most affected by HIV until much later.[8] Decades of increasingly concerted and coordinated effort from health providers, activists, funders, academics and people living with HIV were required to make ART available to people who needed it.[9] Notable dates include founding of Global Fund and the UN Declaration of Commitment on HIV/AIDS, both in 2001,[10,11] the"3 by 5" initiative to start three million people on ART by 2005,[12] launched in 2003 (at which time there were 14 million people living with HIV in Southern and Eastern Africa), and the Millennium Development Goals in 2005 to combat HIV/AIDS.[13] More recently, in 2016, WHO recommended ART should be offered to everyone living with HIV ("treat all")[14] and UNAIDS established goals for 90% of people living with HIV to know their status, 90% of those people to be on ART and 90% of those people to achieve viral load suppression (90-90-90 goals, revised to 95-95-95 in 2020).[15] In 2021 a UN adopted a high level resolution to "get back on track" to end AIDS by 2030.[16]

Malawi was one of the first countries to innovate and scale up the public health approach to ART. In Malawi the peak of new HIV infections occurred in 1994 (with 130,000 estimated new HIV infections that year) and HIV related deaths peaked in 2004 (estimated 81,000 AIDS-related deaths), with both peak infections and peak deaths preceding other Southern and Eastern Africa countries by several years.[1] In Malawi, a very limited amount of paid-for ART (using stavudine-based regimens) was available from 2001 onwards.[17] However the real change in Malawi was when ART began to be available free of charge in 2004 for people with low CD4 cell counts (e.g. adults with CD4 <200 cells/mm^3, later for everyone with CD4 < 350 cells/mm^3) or with WHO Stage 3 or Stage 4 opportunistic infections.[18] Lifelong ART for all pregnant and breastfeeding women (Option B+) was started in 2013, and ART for all people living with HIV regardless of CD4 cell count ("treat all") has been available since 2016. From 2018, Malawi introduced doltutegravir (DTG, an integrase strand reverse transcriptase inhibitor [INSTI] class ART drug) as its first line ART, in combination with tenofovir and lamivudine, and transitioned all people (including those stable on older ART regimens) to DTG-based ART. This pattern of ART timing, eligibility, availability and types of regimens in Malawi is similar to or slightly earlier to other countries in Southern and Eastern Africa region.[18]

In 2021, there were nearly one million people living with HIV in Malawi, with an estimated HIV prevalence among adults age 15-49 years of 7.7% (95% confidence interval [CI] 7.1 – 8.0%).[1] In Blantyre City and Zomba City where research for this thesis is based, HIV prevalence in 2020 was 17.0% (95% CI 15.9-18.1%) and 18.3% (15.7-21.3%) respectively.[19] Overall, in Malawi in 2021, 93% of people

living with HIV know their status, 97% of those are on ART and 83% of people on ART are virally suppressed. There are no sub-national estimates for the HIV treatment cascade.[1]

2.1.2 The persistent problem of advanced HIV

Despite the successes and the millions of years of life saved from the scale up of ART, advanced HIV disease (defined as CD4 cell count <200 cells/mm^3) remains a persistent problem. People with advanced HIV disease have a much higher risk than people with controlled HIV of opportunistic infections, HIV-associated cancers, other AIDS-related diseases, hospital admission and death.[20–22]

Worldwide, an estimated 4.3 million people have advanced HIV disease, of whom 2.5 million live in Africa.[23] The persistence of advanced HIV disease, despite well-developed ART programmes, is a major contributor to the 650,000 deaths (lower estimate 510,000 - upper estimate 860,000) from HIV worldwide, and 280,000 (lower estimate 230,000 - upper estimate 360,000) deaths in Southern and Eastern Africa in 2021.[1] Modelling of AIDS-related deaths is challenging due to lack of vital registration in many HIV-affected countries, lack of cause-of-death reporting,[24,25] and the need to extrapolate death rates from relatively small datasets [26] (which themselves has lost to follow up and missing data challenges[27,28]) to entire countries and regions. There is a large uncertainty around numbers with advanced HIV disease and death.[26,29,30] Spectrum – supported by UNAIDS - is the major model used to combine data on HIV prevalence surveys, sentinel surveillance, ART programme data and vital registration (were available) to model HIV incidence, prevalence and deaths - data related to AIDS deaths are particularly sparse.[29,30]

Several studies illustrate this problem of advanced HIV: One quarter of all people starting ART in Gabarone, Botswana, between 2015 and 2017 had CD4 <200 cells/mm^3 at ART initiation [31] and 17% of people newly entering HIV care as part of a large community based HIV study in rural Botswana between 2013 and 2018 had advanced HIV.[32] In the South African National Health Laboratory Service database of 3.99 million people entering HIV care between 2011 and 2016, 33% had advanced HIV disease.[33] Data from the IeDEA / COHERE collaborations show that in the 16 low income countries contributing data (all of which are in Africa), 31% of all participants starting ART in 2015 did so with CD4 <200 cells/mm^3.[34] In Malawi, data from first quarter of 2022 showed that 27% (3,039 / 11,091) of all CD4 counts reported to the Department of HIV / AIDS in Malawi were <200 cells/mm^3 (personal communication), although this may be biased due to indication for doing CD4 tests being based on clinical suspicion of treatment failure.

While many studies focus on CD4 counts at ART initiation, increasingly people with advanced HIV are not ART-naïve but have either stopped taking ART, are taking ART intermittently, or are taking ineffective ART. HIV virologic failure is defined as HIV-1 viral load > 1000 copies/mL whilst taking ART for at least six months;[35] persistent low CD4 counts in people taking ART are usually, but not always, found in conjunction with virologic failure. As an example, in the Western Cape, South Africa, 51.8% of people with CD4 < 50 cells/mm^3 recorded in National Health Laboratory Systems database were ART experienced – although there may be biases in eligibility for CD4 count measurement.[36] In a large, representative Zimbabwe Population Survey, 17% of all adults living with HIV had CD4 < 200 cells/mm^3, and 49.4% of those had been on ART for 6 months or more.[37] The hospital data (summarised below) also shows increases in the number and proportion of ART-experienced people (both people currently using ART, and people who previously started ART and then disengaged from care) with advanced HIV disease, in parallel with increased numbers of people starting ART in the population.

2.1.3 Advanced HIV, hospitalisations and in-hospital mortality of adults living with HIV in Africa

Hospital epidemiology related to HIV from Africa is relatively sparse. However, from the few cohorts available, the number of PLHIV becoming unwell and attending hospital has remained high in several countries in Southern and Eastern Africa, consistent with advanced HIV being an ongoing public health problem, even as ART has been successfully scaled up. This thesis focuses on adult admissions, and cannot be generalised to children, as the causes of admission, outcomes and – critically – prevalence and diagnostics for tuberculosis differ substantially between adults and children.[38] Accordingly, this introduction focuses on hospital epidemiology for adults. Table 2.1 gives an overview of relevant studies conducted in hospitals in Southern and Eastern Africa that recruited adult inpatients.

Sixty percent of adult hospital admissions to a general hospital in South Africa (GF Jooste, in Western Cape) were related to HIV in 2012-13, despite widespread ART availability in the community at that time.[39] Similarly, 50% and 42% of admissions to hospital in Lilongwe, Malawi between 2011 and 2012 and Kweneng East District, Botswana between 2015 and 2016, respectively, were related to HIV.[40,41] Ninety percent of adults admitted with suspected sepsis to emergency department of University Teaching Hospital, Lusaka in 2021 and 2013 were living with HIV in 2012-13,[42] and 41% of adults admitted with fever to seven hospitals in Malawi, Mozambique and Zimbabwe in 2018 to 2020 were HIV positive.[43]

Hospital, Country, and year	Population	Proportion living with HIV among all admissions	ART use among PLHIV	CD4 cell count comments	Virological suppression
Studies that recruited adults irrespective of HIV status					
G.F. Jooste, Cape Town, South Africa. 2012-2013.[39]	People admitted adult medical wards	60% (609/1013)	Current ART 45% (263/585),	CD4 < 200 cells/mm^3 65% (383/585)	VL suppression: 55% (142/263) of those on ART.
Kamuzu Central Hospital, Lilongwe, Malawi, 2011-2012.[40]	People admitted adult medical wards	50% (1174/2364 with known HIV status)	Current ART 58% (689/1174)	Not reported	Not reported
Scottish Livingstone Hospital, Kweneng East, Botswana, 2015-2017.[44]	People admitted to adult medical wards	48% (983/2024 with known HIV status)	Current ART 66% (653/983)	CD4 < 200 cells/mm^3 42% (377/898)	Not reported
Kamuzu Central Hospital, Lilongwe, Malawi, 2013[45]	People admitted to adult medical wards who survived their admission.	51% (1001/1962 with known HIV status)	Not reported	Not reported	Not reported
Tertiary hospital, North West Province, South Africa, 2014.[46]	People admitted to adult medical wards who survived admission.	168/293 (57%)	Current ART 52% (88/168)	Median CD4 56 cells/mm^3 in people newly diagnosed HIV, 182 in people already knew status	Not reported
University Teaching Hospital, Lusaka, Zambia, 2017-2018.[47]	Adults admitted to internal medicine who weren't critically ill.	67% (762/1183 with known HIV status)	Current ART 86% (206/239)	CD4 <200 cells/mm^3 59% (among those with CD4 measured, denominator not stated)	Not reported
Studies which recruited adults with sepsis / fever					
University Teaching Hospital, Lusaka, Zambia, 2012-2013.[42]	Adults in emergency department with fever syndrome	187/209 (90%)	107 / 209 (51%)	Median: 72 cells/mm^3 and 65 cells/mm^3 in each arm trial.	Not reported
Seven hospitals in Malawi, Mozambique, Kenya (FIEBRE study). Approx. 2018.[43]	Adults admitted to hospital with fever	383/926 (41%)	Not reported	Not reported	Not reported
Queen Elizabeth Central Hospital, Malawi, 2017-2018.[48]	Adults in emergency department meeting sepsis case definition	143/213 (67%)	Current ART 117/143 (82%)	52/84 (62%) people on ART >60months had CD4 <200 cells/mm^3	Not reported
Studies that recruited all inpatient adults living with HIV					
Homa Bay County Hosptial, Kenya, 2015 Centre Hospitalier de Kabinda, DRC, 2017[49]	Adults admitted to MSF-supported medical wards, living with HIV.	NA	ART > 6 months: Kenya 46% (155/331) DRC 57% (212/376)	CD4 < 200 cells/mm^3: Kenya 63% (210/331) DRC 56% (211/376)	VL suppression among those on ART >6 months who had VL measured: Kenya 37% (20/54) DRC 38% (65/171)
Chiradzulu District Hospital, Southern Region, Malawi, 2015-2017.[50]	Adults (>15 years) admitted to medical wards, living with HIV and not taking TB treatment at admission.	NA	Current ART 305/287 (81%)	CD4<200 cells/mm^3: 204/380 (54%)	Not reported
Zomba Central Hospital Malawi & Edendale hospital South Africa 2015-2017 (STAMP trial)[51–55]	Adults admitted to medical wards, living with HIV and not taking TB treatment at admission.	NA	Current ART: 92% (1021/1316) Malawi; 79% (840/1258) South Africa.	Median CD4 cell count: Malawi 219 cells/mm^3 South Africa 236 cells/mm^3	VL suppression among those on ART >6 months: 68% (534/786). Only measured in Malawi.
University Teaching Hospital, Lusaka, Zambia, 2018-2019.[56]	Adults living with HIV admitted to medical wards.	NA	184/200 (92%)	Only 39% had result documented. 75% (58/77) tests done were <350 cells/mm^3.	VL testing uncommon, 13/24 results showed viral suppression.

Table 2.1 Selection of studies relevant to either proportion of inpatients living with HIV, or advanced HIV disease among inpatients. VL = Viral load, DRC = Democratic Republic of Congo

Outcomes for adults and children living with HIV who are admitted to hospital are poor. In a systematic review and meta-analysis by Ford *et. al.,* of 19 published cohorts among adult PLHIV admitted to hospital in Africa between 2007 and 2015 (2007 was chosen as cut-off due to availability of ART), overall adult in-hospital mortality was estimated at 31% (95% CI 7% - 37).[38] The causes of admission and causes and death in that systematic review were overwhelmingly adjudicated to be AIDS-related causes - ascertainment of diagnoses varied by cohort, but mostly based on routinely available diagnostic tests and clinician diagnosis - suggesting that HIV status was not incidental to reason for hospital admission.[38] Not only was there a high risk of in-hospital death, but a further systematic review showed that even PLHIV who survived to discharge from hospital had a high risk of readmission (19%, 95% CI 15-22%) and death (23%, 95% CI 16-30%) within the subsequent year.[57] This same pattern of high in-hospital mortality and high mortality risk persisting even after discharge from hospital can be seen in participants of randomised trials recruiting the same cohorts of otherwise unselected adult hospitalised PLHIV, such as in the STAMP trial of urine TB diagnostics,[51] or trials recruiting people living with HIV and sepsis syndromes[42,48] or TB symptoms.[58]

Unsurprisingly, given the potent impact of viral load suppression on risk of death, advanced HIV disease and virologic failure are over-represented among people living with HIV who require hospital admission, as summarised in Table 2.1. Median CD4 counts are low even in recent hospital cohorts where ART use at hospital admission is more common – indicating that the issue of poor outcomes and high in-hospital mortality has not been solved simply by greater community availability of ART. For example, among adults taking ART for more than six months admitted to two hospitals in Kenya and Democratic Republic of Congo, the prevalence of virological failure among those with a viral load test was 63% and 62%, respectively – although not all participants had viral load measurement completed.[49] The high prevalence of virologic failure on ART is a particular challenge which adds extra complexity to hospital care. The group with virologic failure on ART probably represents a mix of people who are intermittently taking ART, who are taking ineffective ART (due to drug resistance or malabsorption) or who have persistent immunosuppression despite ART. In a study to develop and validate a risk score for mortality among people hospitalised with HIV associated TB, being ART experienced at admission to hospital was a risk factor for death[52] – i.e. conditional on being unwell enough to require hospital admission, reported ART use does not reduce risk of death. Although, in another large meta-analysis of people seriously ill with HIV-associated disseminated TB being ART experienced was neutral (neither predictive, nor protective) for risk of death.[59,60] In one study HIV-1 viral sequencing for predicted drug resistance among people with virological failure admitted to hospital in

Malawi showed that HIV drug resistance was common (resistance to at least two ART drugs in 83% of people taking ART with virologic failure), and associated with a risk of death.[53] That study was among people predominantly taking efavirenz-based ART; whether the finding of high levels of drug resistance and risk of death if resistance will hold in a population predominantly taking INSTI-based ART (which has a higher resistance bar) is uncertain.[61]

2.1.4 Tuberculosis/HIV co-infection

Since the beginning of the HIV epidemic, a strong association between HIV and incidence active tuberculosis disease has been noted.[62] For example, researchers noticed a 160% increase in tuberculosis admissions between 1983/84 and 1987/88 in a hospital in Thyolo, a rural area of Southern Malawi.[63] High HIV prevalence among people admitted to hospital with tuberculosis, particularly extrapulmonary tuberculosis, was reported as part of raising the alarm about HIV epidemic in Southern Africa in late 1980s.[64–66]

People living with HIV are substantially more suspectable to TB disease than HIV negative people.[67] The immunopathogenesis of HIV associated TB disease is incompletely understood. Unlike many other opportunistic infections, whilst people with low CD4 counts and advanced HIV disease are at highest likelihood of developing TB, the risk of developing TB disease increases shortly after HIV acquisition while CD4 counts remain high and the risk incompletely reduced by effective ART.[68] HIV infection clearly affects cell mediated immunity through depletion of CD4+ T cells, which impacts the ability of immune system to control TB.[69,70] HIV infection also effects other aspects of cell mediate immunity relevant to controlling TB – macrophage and dendritic cell antigen presenting activity, granuloma formation and neutrophil activation are all effected in HIV positive vs. HIV negative people with TB infection.[69,70] There may also be differences in innate immune responses to TB exposure, cytokine production and in B cell responses to TB infection in HIV positive compared to HIV negative people, although these are incompletely understood. In addition to having a higher incidence of pulmonary TB than HIV negative people, people living with HIV are at very substantially increased risk of extrapulmonary and disseminated TB disease than HIV negative people – probably due to immune dysfunction in granuloma formation and therefore failure of containment of initial TB bacilli leading to risk of haematogenous spread of TB. ART initiation leads to suppression of HIV viral replication and a subsequent increase in CD4 T cells – initially memory T cells (i.e. T cells specific to a particular antigen) and the pool of naïve CD4 cells.[69] This leads to an improved ability to control TB infection and people started on ART have a much lower risk of TB disease than people without ART. However, ART doesn't completely reverse the risk of TB disease in people living with HIV – this might be because CD4 T cell

responses remain persistently altered in people living with HIV or because ART doesn't fully restore all aspects of innate and cell mediated immune pathways.[67,70]

2.1.5 Tuberculosis is a major cause of in-hospital death

HIV associated tuberculosis remains a major cause of in-hospital death among adults living with HIV. Evidence for this claim is from meta-analysis of routine data, autopsy data and prospective cohort studies with diagnostic components, as discussed below.

In a systematic review and meta-analysis of published cohorts of adults living with HIV admitted to hospital between 2007 and 2015 by Ford *et. al.*, tuberculosis was estimated to be the reason for admission in 17% hospital admissions and the main cause of death in 27% of in-hospital deaths worldwide.[71] That review was of published cohorts using routinely available tuberculosis diagnostics, including clinical diagnosis (i.e. tuberculosis diagnosis made in the absence of microbiological confirmation).[71]

In a meta-analysis of nine autopsy series reports in adults living with HIV from Africa from 1988 to 2012, 43.2% of all autopsies showed tuberculosis to be present at death.[72] Most people who died in those autopsy series were in hospital at the time of their death and 91% of deaths with TB the pathologists concluded tuberculosis was the cause of death (rather than a co-morbid condition). The proportion of deaths due to tuberculosis in this autopsy series meta-analysis increased between 1992 to 2012 by approximately 5% every ten years. Five further autopsy papers published after this meta-analysis show similar results: see table 2.2

First author (year)	Summary of participants and methods	TB prevalence
Systematic review & meta-analysis		
Gupta et. al. 2015 [72]	Nine autopsy case series reports; papers from adults in Africa, between 1988 and 2012. Autopsy and TB diagnostic methods varied.	43.2% pooled prevalence of TB at death. In 88% of people with TB, TB was disseminated. 91% TB was cause of death. 50% undiagnosed at time of death. Increasing prevalence 1992 – 2012.
Relevant autopsy studies published post 2012		
Wong et. al. 2012[73]	39 adults who died at Charlotte Makexe Hospital Johannesburg (South Africa). 2009. Minimally invasive autopsy (needle biopsy) note a	26/39 (66%) had TB disease and 1 person disseminated *Mycobacterium avium* infection. All had extrapulmonary disease 17/27 (62%) had at least one other infectious or neoplastic cause of death (bacterial co-infection most common). 8 had Immune Reconstitution Inflammatory Syndrome in conjunction with tuberculosis
Karat et. al. 2016 [74]	34 adults initially enrolled in TB Fast Track trial (South Africa) 2012-2014. Participants were ambulant adults with CD4 <150 cells/mm^3 at time of trial enrolment, but 74% were in hospital at time of death. Minimally invasive autopsies note a	16/34 (47%) had TB disease. 14 has extrapulmonary disease 6 not on TB treatment at death 11 had concurrent cryptococcal or bacterial infection.
Bates et. al. 2015 [75]	101 HIV positive adults who died at Lusaka Teaching Hospital (Zambia) 2012-2013. A further 24 autopsies on HIV negative adults. Full post mortem.	66/101 (65%) had TB disease 33 had extrapulmonary disease 46 had a concurrent comorbid condition note b 13 had rifampicin-resistant TB (presumed MDR) on NAAT. None were on MDR-TB treatment. 78 people in total had TB disease (including autopsies in HIV negative adults) 20 /78 not on TB treatment at death
Garcia-Basteiro et. al. 2019 [76]	73 HIV positive adults who died at Maputo Central Hospital (Mozambique) 2013-2015. Full study also includes children, pregnant women (maternal deaths) and HIV negative non-pregnant adults. Full post mortem.	37/73 (50%) had TB disease note c 28 with disseminated TB 11 had a concurrent condition In all adults in study (including HIV negative), 41 people had proven histopathological and microbiological TB disease. 31/41 not were taking TB treatment at death.
Costales et. al. 2022 [77]	48 HIV positive adults and children who died in hospital in Moshi (Tanzania) 2016-2019. Full study also includes HIV negative adults and children. Mix of full post mortem and minimally invasive autopsy.	15/48 (31%) had TB disease. Among all adults and children (including HIV negative), 27 people had TB. 22 / 27 were not on TB treatment at death.

Table 2.2 Published studies reporting autopsy findings from adults living with HIV who died in hospital in Africa

BAL = Bronchoalveolar lavage, CSF = Cerebrospinal fluid. NAAT = Nucleic Acid Amplification Testing. MDR = Multi-drug resistance. In all studies, extrapulmonary disease category includes people who had both pulmonary disease and extrapulmonary disease.

(a) Minimally invasive autopsy involves taking samples from deceased people using needles and without removing or examining whole organs.

(b) The most common comorbid conditions were pyogenic pneumonia (21 people) and anaemia (12 people). Uncertain whether anaemia related to TB disease or a truly separate diagnosis.[78]

(c) Includes six people with TB detected on NAAT from post-mortem samples, but no histopathological features to support TB.

In a study conducted between 2012 and 2013 at GF Jooste hospital in Cape Town, South Africa, adult PLHIV admitted to medical wards for any reason were prospectively enrolled into a diagnostic study and had multiple samples taken for mycobacterial culture, including urine and induced sputum (mean of 5.6 tests per participant). Overall, 36% of participants had confirmed tuberculosis (this study did not include urine lipoarabinomannan diagnostic when reporting the initial cohort).[79,80] This study merits special mention because of the unusually rigorous microbiological testing done for tuberculosis. This study is discussed in more detail in the section about TB diagnostics for inpatient adults.

Further evidence from studies with well described TB testing (albeit a less comprehensive microbiological tuberculosis assessment than Lawn et. al.) which recruited a broad subset of hospitalised people, or are among high-risk outpatients also show high burdens of HIV-associated tuberculosis (Table 2.3). STAMP was a trial of urine TB screening in HIV positive hospital inpatients, which recruited participants between 2015 to 2017 in Malawi and South Africa; 16% of all participants in the trial arm with urine TB tests had microbiologically confirmed TB.[51] Several other hospital observational cohorts or studies that enrol a subset of all adults admitted to hospitals (e.g. adults with fever, adults with cough, adults with sepsis syndrome) have also shown very high burdens of microbiological confirmed tuberculosis.[42,43,58,81] Two major outpatient advanced HIV disease studies report systematic tuberculosis testing results: in the REALITY trial 5% of the entire cohort had died of tuberculosis within a year (based on expert panel review of adverse events), making tuberculosis the leading cause of death.[82] In the STATIS trial, 17% of participants in the test-guided TB treatment arm had either prevalent TB at enrolment or incident TB within one year of starting ART.[83]

Study	Population	TB diagnostic testing details	Proportion with TB
Extensive microbiological sampling, all adults with HIV			
Cape Town Study[80]	427 adults living with HIV, admitted to GF Jooste hospital 2012-2013. Recruited regardless of symptoms.	Mean 5.6 tests per person, included (induced) sputum for NAATs and culture, mycobacterial blood culture, urine NAAT. This paper does not include urine LF-LAM testing.	139 (32.6%) microbiologically confirmed TB. 115 (83%) had extrapulmonary TB. Clinical TB diagnoses not reported.
Malawi hospital cohorts which recruited all adults with HIV			
STAMP [51]	1287 adults living with HIV admitted to Zomba Central Hospital (Malawi) or Edendale hospital (South Africa) 2015-2017. Recruited regardless of symptoms. Table only includes people in the TB diagnostic intervention arm.	Sputum NAAT, urine NAAT, urine LF-LAM (by trial team) plus any other TB tests requested by usual care.	210 (16.3%) microbiologically confirmed TB 72 (5.6%) clinically diagnosed TB.
Heurga et. al. 2020 [50]	387 adults living with HIV admitted to Chiradzulu District Hospital, Malawi. 2015-2017. Recruited regardless of symptoms.	Sputum NAAT, sputum smear microscopy, urine LF-LAM, CxR.	119 (31%) microbiologically confirmed TB (80 had LF-LAM as only positive test).

			Clinical TB diagnoses not reported as distinct from microbiologically confirmed.[note a]
Kanyama et. al. 2022 [84]	438 adults living with HIV admitted to Kamuzu Central Hospital, regardless of symptoms. 2016-2017.	Urine NAATs, urine LAM done by study. Sputum NAATs according to usual care.	82/363 (22%) LF-LAM tests done were positive. 14/292 (5%) urine Xpert positive. 97 people started TB treatment (unable to determine confirmed vs. presumed).
Large hospital cohorts with well documented microbiological testing, selected adults with HIV			
LAM-RCT [58]	1257 adults living with HIV admitted to one of ten hospitals in South Africa, Zimbabwe, Tanzania, or Zambia, in TB diagnostic intervention arm. 2013-2014. Recruited people with one or more TB symptoms. Table only includes people in the TB diagnostic intervention arm	Urine LF-LAM. Plus all routine TB diagnostics, including NAATs, liquid culture, CxR and other radiological tests (varied from hospital to hospital)	648 (52%) started TB treatment. 342 (27%) confirmed not including urine LAM results, plus at least 94 (7.3%) further people with TB based on LF-LAM result[note b]
Griesel et. al. 2018[81]	484 adults admitted to GF Jooste or Khayelitsha hospital 2013-2014 who were seriously unwell, HIV positive and with a cough.	All received CxR, three sputum samples (induced if necessary). Extra-pulmonary samples sent for mycobacterial culture "when appropriate"	255 (53%) had culture positive TB. 34 further people (7%) started TB treatment clinically.
Lewis et. al. 2022 [48]	143 adults living with HIV admitted to QECH in Blantyre, Malawi with a sepsis syndrome 2017-2018. .	Mycobacterial blood culture, urine LF-LAM. Sputum NAAT "when there was a suspicion of pulmonary TB".	71 (50%) had microbiologically confirmed TB.
Andrews et. al. 2017 [42]	187 adults living with HIV admitted to Lusaka Teaching hospital 2012-2013 with possible sepsis.	Mycobacterial blood culture provided by study, plus usual care tests (not described in detail.)	43 (23%) had mycobacteraemia. 7 (4%) had sputum culture positive for TB, without mycobacteraemia.
Large outpatient advanced HIV studies with well documented microbiological testing,			
STATIS [83]	527 adults starting ART with CD4 <100 cells/mm3 in the TB-test-guided treatment arm.[note c] Ivory Coast, Cambodia, Vietnam, Uganda. 2014-2017. Table only includes people in the test-guided TB arm.	Sputum NAAT, urine LF-LAM and CxR at baseline visit (regardless of symptoms). At visits throughout study, participants asked about TB symptoms and if any symptoms had sputum NAAT, urine LF-LAM, CxR and "other appropriate symptom driven investigations".	99 (19%) had confirmed TB, includes prevalent TB at baseline or incident TB up to 48 weeks from enrolment.
REALITY [82]	1805 adults and children starting ART with CD4 cell count <100 cells/mm3. [NB. 96% are adults.] Uganda, Zimbabwe, Malawi, Kenya. 2013-2015	At baseline, participants evaluated for TB using a symptom checklist followed by investigation "following national guidelines". During study participation, deaths and adverse events ascertained by study team and causes reviewed by endpoint review committee based on available information / clinical narratives.	271 (15%) participants had prevalent TB at baseline. 80 participants (5% of total) died of tuberculosis by 48 weeks from trial enrolment.

Table 2.3 Selected studies which included systematic microbiological TB testing among adults living with HIV admitted to hospital or major outpatient advanced HIV disease studies.

NAAT = Nucleic Acid Amplification Test for TB, CxR = Chest Xray, LF-LAM = Lateral Flow LAM.

(a) In this study, during the first nine months of study LF-LAM results weren't reported back to clinical teams and in the second nine months results were reported (as national guidelines changed). Thus a substantial proportion of microbiologically confirmed TB (based on LF-LAM in first nine months) weren't treated for TB. Unable to determine number of clinical TB diagnoses as distinct from microbiologically confirmed.

(b) Specificity of LAM reported at 736/830 (i.e. 736 negative LF-LAM tests in 830 people with negative reference standard tests), see Table 2 in reference [58]. Implies 94 people had positive LF-LAM in absence of other positive TB tests. Unknown about positive LF-LAM tests in people without reference standard tests completed.
(c) Only considering the test-guided TB treatment arm, in the other trial arm everyone received TB treatment and had fewer TB diagnostic tests.

In summary, based on evidence from systematic review of hospital cohorts, autopsy studies, studies with extensive microbiological sampling in unselected hospitalised adults living with HIV, and other studies with well documented TB testing, tuberculosis has persistently been identified as the most common disease and the most common cause of death among adults living with HIV in hospital in Africa.

2.1.6 Health needs of adults with HIV admitted to hospital in Africa

Whilst tuberculosis is the major cause of death among adults with HIV admitted to hospital, it is not the only cause of death. In the Ford *et. al.* systematic review, AIDS-related illnesses (which included tuberculosis, as well as toxoplasmic encephalitis, cryptococcal meningitis, pneumocystis pneumonia and AIDS malignancies) were the leading cause of adult HIV positive hospital admission (63% of all admissions), with bacterial infections next most common (36% of admissions).[38] In that review, 43% of all people were on ART at the time of admission to hospital although some of these cohorts date back to a time when population ART coverage was much lower than present.[38] However, the more recent inpatients studies from Southern Africa[47–49,56] suggest that AIDS-related conditions associated with immunosuppression are still the major causes of admission for people living with HIV, even in the context of high ART coverage in the population. The high prevalence of AIDS-related admissions and deaths reflects a complex of mix of people who never started ART (undiagnosed HIV or diagnosed but never linked to care), people who started ART and then disengaged from care for whatever reason, who are intermittently taking ART, who are taking ineffective ART (due to drug resistance or malabsorption) or who have persistent immunosuppression despite ART. From 2018 onwards, many countries, including Malawi have switched large numbers of people to DTG-based ART (from efavirenz-based), the public health impact of widespread use of DTG on population-level viral suppression, drug resistance, incidence of hospital admission and HIV-related deaths remain unclear.

There are several interlinked areas of research priorities related to hospital care for adults living with HIV in Southern Africa.

1. In the era of well-established “treat all” ART programmes, what is the incidence of HIV positive hospital admission and in-hospital death?
2. Among adults living with HIV admitted to hospital, what are the most common causes for admission and death? Are they still mostly AIDS-related? And what proportion are incidental to HIV status?

3. Are there any interventions that can be broadly applied to all or most adults living with HIV admitted to hospital to reduce death? This could include diagnostic interventions.
4. Are there disease-specific interventions or ways to improve management of major opportunistic infections, such as disseminated TB, cryptococcal meningitis and severe bacterial infections?
5. How can hospitals be better linked to communities and primary care clinics? This to facilitate early intervention in people with advanced HIV disease to reduce risk of serious illness, facilitate timely referral to hospital if needed and quality care post discharge?
6. How should ART be managed in seriously unwell inpatients, what is the role for viral load testing and how should virological failure in context of serious illness be managed?

This thesis addresses the first three of these questions. Research and implementation related to improving in-hospital care for people living with HIV should occur against a background of tackling social determinants of HIV and advanced HIV disease, HIV testing and ART services and progress towards universal healthcare (see discussion chapter 8).

2.2 Diagnosis of HIV-associated tuberculosis

The second part of this introductory chapter focuses on the diagnosis of HIV-associated TB, with a particular emphasis on hospital inpatient adults.

HIV associated tuberculosis can be difficult to diagnose and is particularly challenging in settings with relatively limited laboratory and radiology capacity, such as Malawi. The most common form of tuberculosis is pulmonary tuberculosis, defined as tuberculosis affecting lung parenchyma or tracheobronchial tree. Extrapulmonary tuberculosis is tuberculosis anywhere other than lungs; including lymph node tuberculosis, pleural or pericardial tuberculosis, tuberculosis meningitis, spinal tuberculosis, and tuberculosis affecting other organs. Disseminated tuberculosis is tuberculosis occurring in two or more non-contiguous body sites, with presumed haematogenous spread. Pulmonary tuberculosis can – and frequently does – coexist with extrapulmonary or disseminated tuberculosis.[72] Whilst all forms of tuberculosis are more common in people living with HIV, and in people with low CD4 counts compared to those with higher CD4 counts, disseminated tuberculosis is particularly common in people with advanced HIV. For example, in the autopsy series meta-analysis from 1988 and 2012, TB was disseminated in 88% of people with TB disease at autopsy.[72] Unlike pulmonary tuberculosis, which has a variety of clinical manifestations including relatively indolent forms, disseminated tuberculosis can be rapidly fatal even with prompt recognition, high quality care, and anti-tuberculosis treatment.[59,85]

Broadly speaking, HIV-associated tuberculosis can be diagnosed through two pathways – systematic protocol-guided screening, or individualised clinically-guided diagnostic investigation, although lines between the two pathways are often blurred. Table 2.4 illustrates features of the two pathways.

Systematic protocol-guided screening	Clinically guided diagnostic investigations
• Tests systematically offered to everyone who meets criteria. Heterogeneity (of symptoms or risk of TB) within those who have meet criteria for screening usually not considered.	• Heterogeneous group may present for care and different people may have individualised testing strategies.
• Usually starts with a "triage test" or screen and proceeds to a "diagnostic test" if initial screen positive. [86] Tests offered and sequence of tests standardised.	• The tests and testing sequencing offered are not standardised and number and types of tests offered will depend on individual need.
• Triage test should ideally be highly sensitive, and diagnostic test highly specific.(note a)	• Information from highly specific tests integrated with non-specific tests and other clinical / radiological features. Decisions made after weighing up all available laboratory, radiology and clinical information.
• Relatively clear paths to enter and exit screening. If prescribed tests are positive, start treatment and, if negative, exit screening.(note b) Alternative diagnoses not usually relevant or considered.	• Can be difficult to decide when to stop testing. If tests are positive: start treatment. If tests are negative a decision has to be made about starting clinical treatment (i.e. making an empiric diagnosis), stopping testing and refuting diagnosis, or continuing to do more tests. Whether an alternative diagnosis has been or can be made is highly relevant.
• Not individualised. Highly suitable for task-shifting and delivery at scale.	• Highly individualised, needs trained and experienced clinicians and ideally should usually take into account individual values and preferences of patient.

Table 2.4 Features of diagnostic testing comparing systematic protocol-guided screening [87] and clinically-guided diagnostic investigation. See footnotes (overleaf) .

Footnotes to table 2.4 (previous page):
The distinctions between the two groups are not absolute.
[(a)] Urine LAM testing for all inpatients fits most of my description as a "systematic protocol-guided screening" test, but it is a not particularly sensitive and is not described by WHO Global TB programme as a "screening" test.
[(b)] Usually if tests are negative a person exits the screening pathway and is considered not to have the disease. In some situations, if symptoms persist someone who initially starts in a systematic protocol-guided screening pathway might be referred to clinically guided testing and diagnostic work-up.

WHO recommends systematic screening for tuberculosis for all PLHIV at every healthcare encounter, regardless of the reason for seeking care.[88] This initial screening step can use either symptom screening (recommended since 2011[89]) or C-reactive protein (CRP) measurement or chest X-ray (CxR) – with CRP and CXR being new recommendations from the 2021 WHO consolidated TB screening guidelines.[88] Symptom screening means asking for presence of one or more of cough of any duration, night sweats, weight loss or fever (WHO four symptom screen [WHO4SS]). CxR can be read by either human interpreter or by computer aided diagnosis (CAD) software. If a person has a positive screening test (i.e. presence of symptoms, high C-reactive protein or abnormal X-ray depending on which screening modality is being used) then they should proceed to a specific diagnostic test such as sputum NAAT.

WHO also recommend systematic use of urine lipoarabinomannan (LAM) testing of people living with HIV who have one more of; any TB symptom, low CD4 cell count (<100 cells/mm^3), or who are admitted to hospital and have CD4 cell count <200 cells/mm^3.[90] A recent individual patient data meta-analysis considered diagnostic accuracy of WHO4SS and CD4 cut-offs to guide use of urine LF-LAM among inpatients, highlighting relatively poor performance of WHO4SS and suggested recommending urine LF-LAM for all inpatients living with HIV would be more logical.[91] The LF-LAM recommendation describes a similar use case as the systemic protocol-guided screening as outlined in table 2.4, insofar as the recommendation is for urine LAM testing to be done systematically to everyone who meets the criteria without further clinician input. However, urine LAM testing differs from typical screening tests because the urine LAM test is much less sensitive than a typical screening test and thus a negative test does not rule out tuberculosis. To illustrate this point, the WHO Global TB Programme do not refer to urine LAM testing as "screening", and instead describe the use of urine LF-LAM as "assisting diagnosis" of tuberculosis.[90,92]

The other pathway for people living with HIV receiving a tuberculosis diagnosis is through clinically-guided diagnostic investigations. This is where a person presents to a healthcare setting because they are unwell or have symptoms and a clinician considers tuberculosis as a possible diagnosis. Typically, the pre-test probability in this pathway of tuberculosis is higher than in the screening pathway and the

challenge is identifying tuberculosis vs. another disease-causing symptoms, rather than tuberculosis vs. no illness (as in the systematic protocol-guided pathway). In this setting a variety of tuberculosis tests should be done (at minimum, a sputum NAAT would be usual), but tuberculosis diagnostic tests might be performed on multiple different specimens, depending on symptoms, radiological findings, ability to collect invasive samples and laboratory capacity. Information from non-specific laboratory tests such as presence or absence of anaemia, or cerebrospinal fluid biochemistry (in possible TB meningitis) might be combined with radiological tests, including CxR. In this clinical care setting, a clinician (ideally in conjunction with patient) will make decisions about how many and what types of TB diagnostics tests to do, will interpret test results considering all available evidence, and will decide about the risks and benefits of starting TB treatment in the absence of a positive diagnostic test (empiric TB treatment). In practice, the line between a systematic protocol-guided pathway and a clinician-directed pathway can become blurred, particularly in settings where there are insufficient clinicians to guide a clinician-guided pathway even among people who seek care because of their symptoms.[93,94]

Inpatients living with HIV in high tuberculosis burden settings are likely to benefit from tuberculosis diagnostics being delivered through both systematic protocol-guided screening and clinically-guided diagnostic investigation pathways. As discussed above, tuberculosis is extremely common in this setting and is often missed as a diagnosis (as shown in autopsy studies). Therefore, there should be systematic screening for tuberculosis for everyone, regardless of symptoms, similar to a screening pathway, but with a pre-test probability of disease that is unusually high for a typical screening programme. However, because everyone admitted to hospital has some illness that needs differentiated and ruling out tuberculosis is difficult, most adults admitted to hospital will benefit from additional clinician-directed tuberculosis testing and consideration of clinical tuberculosis treatment initiation.

2.2.1 Brief overview microbiological tests for HIV-associated tuberculosis

Broadly speaking, there are four types of microbiological tests currently available for detecting presence of *Mycobacterium tuberculosis* in patient samples: smear microscopy, mycobacterial culture, NAATs, and LAM testing.

Smear microscopy of sputum has been the mainstay of tuberculosis diagnosis for over a century, and is still the most widely used test globally, although no longer recommended by WHO as initial diagnostic test of choice.[92] Sputum (or other pathology specimens) are “smeared” onto a glass slide, with or without a prior concentration step, and stained with either Ziehl-Neelsen reagents or auramine. Slides

are examined under a microscopy for presence of absence of acid fast bacilli – using conventional light microscope for Ziehl-Neelsen stained slides, or Light Emitting Diode fluorescent microscope for auramine stained slides. It is not possible to distinguish *Mycobacterium tuberculosis* complex species from other non-tuberculous mycobacteria on smear microscopy. Smear microscopy has low sensitivity, notably for children and people living with HIV, and requires collection of sputum, which not all patients can produce. Fluorescent microscopy is more sensitive than Ziehl-Neelsen microscopy - the sensitivity of fluorescent microscopy compared to culture ranged from 52% to 97% in a systematic review.[95,96]

Mycobacterial culture and speciation is usually considered the reference standard for *Mycobacterium tuberculosis*. Culture can be highly sensitive and specific, with a single liquid culture having a sensitivity of 95% compared to a reference standard of four cultures in total, from two sputum samples collected at the same time.[97] However culture is technically complex and demanding, with turn around times that are too slow to be clinically useful for seriously ill patients. Processing is complex, requiring a Biolevel safety 3 laboratory, and presenting a potential biohazard to lab staff. Samples can easily be contaminated or overdecontaminated, leading to false negative results. Traditional methods of mycobacterial culture used solid Lowenstein-Jensen media which took up to eight weeks for a result. Newer semi-automated culture methods using liquid media Mycobacterium Growth Indicator Tubes (MGIT) produce faster results, with positive results in about three weeks and negative results at six weeks.[98] Culture is required for phenotypic drug sensitivity testing, although not all mycobacterial culture laboratories have drug sensitivity testing capacity, and for whole genome sequencing and non-automated genotyping. Even in high income countries, mycobacterial culture capacity is usually centralised in a small number of laboratories due to cost and quality control requirements.[99]

NAATs are polymerase chain reaction-based diagnostics that test for the presence of *M. tb* DNA in clinical samples. There are two automated NAAT platforms pre-qualified by WHO: Xpert (Cephid, USA); and Truenat (Molbio diagnostics, India). Both are contained systems where specimens are loaded into single-use cartridges following minimal processing, which are then inserted into a machine for analysis. Automated NAAT testing requires electricity and a lab infrastructure, but is much less complex than mycobacterial culture and much more sensitive than AFB microscopy.[96,100] Automated NAAT platforms can provide information about genotypic drug resistance – Xpert Ultra, Xpert MTB/rif and Truenat MTB-RIF test for presence of predicted rifampicin resistance. Compared to culture, Xpert MTB/rif has an estimated sensitivity for detecting *M.tb* of 85% and Xpert Ultra 91%.[96] Xpert Ultra has slightly lower specificity than Xpert MTB/Rif (specificity MTB/Rif 98% and Ultra 96% overall), particularly in people who have a history of previously treated tuberculosis (specificity MTB/rif 97% and Ultra 88%).[96] NAATs are now

recommended as initial tuberculosis diagnostic tests for everyone.[92] WHO also recommend using NAAT tests on non-sputum samples when extra-pulmonary tuberculosis is possible.[92]

Lipoarabinomannan (LAM) is a component of the mycobacterial cell wall and can be detected in urine of some people with tuberculosis, particularly those with disseminated TB.[79,85,101,102] Unlike other microbiological methods, detecting LAM antigen doesn't necessarily require either viable mycobacteria (as for culture), intact mycobacteria (as for acid fast bacilli microscopy) or significant quantities of mycobacterial DNA to be a present in a sample (as for NAATs). Urinary LAM antigens can be detected using lateral flow tests, based on single use cardboard strips or in simple self-contained plastic cassettes. Currently one LAM test is recommended by WHO (Determine TB LAM, Alere/Abbot USA, referred to by WHO and in this thesis as LF-LAM)[90] although several other high-sensitivity LAM tests are in development and at least one product is developed to the point of having a CE mark approved and being distributed for use in multiple research studies – although not yet commercially available (SILVAMP LAM, Fujifilm, Japan).[103] The Alere/Abbot LF-LAM product has to be read with reference to a "reference card", and a band has to be at least as intense as the lowest positive band to be considered positive (i.e. very faint lines on the test strip are not positive tests).[104,105] Urine LAM is not suitable for use in HIV-negative people because of low sensitivity.[90] Sensitivity in PLHIV improves markedly in people with lower CD4 cell counts, likely to reflect greater sensitivity for detecting disseminated than pulmonary TB.[106] The diagnostic strengths and limitations of urine LAM are discussed in detail below.

2.2.2 Diagnosing or refuting a diagnosis of HIV-associated tuberculosis

None of the currently available diagnostic tests for TB have perfect accuracy, and test accuracy tends to be lower in people living with HIV (compared to HIV negative people) and in extrapulmonary TB (compared to pulmonary TB). This means that defining a reference standard to divide a population into confirmed TB vs. not TB is challenging. The measured diagnostic accuracy (sensitivity, specificity, negative predictive value, and positive predictive value – see table 2.5) of novel diagnostic tests will depend on the comprehensiveness of the reference standard used to divide a population into TB negative and TB positive, and thus define false negative and false positives of the index test.

Term	Definition
Reference standard	The best available test or set of tests, used to define who truly does and truly doesn't have TB. May be a composite standard (involving several tests), and may include a clinician component. In TB diagnostic evaluation (particularly for extra-pulmonary TB) it is common to use both a "microbiological reference standard" and a "clinical reference standard".
Index text	The test being evaluated.
True positive (TP)	Reference standard positive, index test positive
False positive (FP)	Reference standard negative, index test positive
True negative (TN)	Reference standard negative, index test negative
False negative (FN)	Reference standard positive, index test negative
Sensitivity	Proportion of index tests positive in people who truly have disease. i.e. TP / (TP + FN)
Specificity	Proportion of index tests negative in people who truly do not have disease. i.e. TN / (TN + FP)
Negative predictive value (NPV)	Proportion of people with a negative index test who truly do not have disease i.e. TN / (TN + FN)
Positive predictive value (PPV)	Proportion of people with a positive index test who truly do have disease i.e. TP / (TP + FP)
Pre-test probability	Chance that a person being evaluated using index test has the disease. For diagnostic evaluations applied to a group of people, the average pre-test probability within the group is equivalent to the prevalence of disease in that group.

Table 2.5 Glossary of terms used in diagnostic evaluation.[107]

Appropriate reference standards depend on the use and setting of a new diagnostic tool – particularly the pre-test probability of TB and the likelihood of pulmonary vs. extrapulmonary TB. Many studies of tests intended to screen large numbers of people for pulmonary TB use a reference standard of a single sputum culture, and sputum culture has been recommended as a reference standard for smear-replacement TB tests (ideally more than one sputum culture, but one is considered acceptable).[86,108] Most of the chest Xray evaluations discussed below use results of a single sputum culture to define TB positive vs. TB negative. While a single sputum culture might be an appropriate reference standard in a setting with a low pre-test probability of TB, a single sputum culture is not perfectly sensitive at defining pulmonary TB. For example, in a study of 1462 participants a single sputum MGIT culture has a 95% sensitivity compared to a reference standard of four cultures total from two specimens, dropping to 85% sensitive among participants with negative sputum smears.[97]

In settings with a very high pre-test probability of TB and a high probability of extrapulmonary TB – such as inpatients living with HIV - defining a reference standard for TB is challenging. In this setting a reference standard should have multiple diagnostic tests, from multiple anatomical sites.[86,109,110] Even in a setting with a well functioning culture laboratory and the ability to collect invasive samples (such as via bronchoscopy or lymph node biopsy) refuting a TB diagnosis is difficult in inpatients living with HIV. Recommendations for research studies evaluating diagnostic accuracy are that both a "microbiological reference standard" and a "clinical reference standard" should be used.[109,110] Even then, diagnostic test accuracy studies should anticipate a substantial proportion of participants where TB disease status

cannot be determined (due to missing laboratory results, lost to follow up or clinical uncertainty).[109] A single sputum culture is an insufficient reference standard among people living with HIV in hospital. Several studies have shown that, unsurprisingly, doing more TB tests usually results in a greater yield of microbiologically confirmed TB.[80,93,94,111,112] The implication is that an insufficient reference standard could lead to falsely defining someone as TB reference standard negative, which could have subsequent effects on whether a positive index test is considered a false positive or a true positive and consequently reported diagnostic accuracy of the index test.[113] See below for detailed discussion of the Cape Town study which has one of the most through microbiological reference standards.

In most non-research settings TB is diagnosed (or excluded) on a combination of clinical suspicion and a limited number of microbiological tests, following a clinician-guided diagnostic investigations. For people in hospital, as outlined above, this can be especially challenging because of the poor sensitivity and specificity of "typical" symptoms[80,88,114,115], poor availability and long turn-around times of mycobacterial culture based tests, the wide array of different anatomical sites where *Mycobacterium tuberculosis* could be present to sample from, and low sensitivity from any single microbiological test.[93,116]

The Cape Town study (parent paper reference Lawn et. al 2015,[80] samples or data used in several other reports[79,85,117,118]; table 2.6) used extensive mycobacterial culture sampling from otherwise unselected population of adults living with HIV admitted to hospital. The researchers recruited 427 adults living with HIV admitted to GF Jooste Hospital, irrespective of symptoms or reason for admission. From those 427 people, 1,745 samples were taken and 2,391 microbiological tests for tuberculosis were done (a single sample could be tested more than once, for example by culture and NAAT). Samples included sputum (spontaneously expectorated or inducted), blood, urine, and other body fluids, such as pleural fluid or lymph node aspirate, depending on clinical presentation. In total, 139 people (32%) had microbiologically confirmed tuberculosis.

Study	Rationale and relationship to Cape Town Study
Lawn et. al. 2015 [80]	This is the parent study. Conducted between 2012-2013 in G.F. Jooste hospital in Cape Town. 427 people recruited adults living with HIV not currently on TB treatment. Recruited irrespective of symptoms. 139 people had confirmed TB. Multiple samples (mean 5.6 per person) taken for mycobacterial culture and NAATs, including blood culture, urine NAATs, multiple sputum samples, induced sputum and other samples as clinically indicated. Urine samples stored for future work.
Lawn et. al. 2017 [79]	Study about yield of urine LAM testing, LF-LAM tests done retrospectively from frozen samples. Many reported results are on 413 people who had reference standard TB microbiological results from samples from two of more anatomic sites and available LF-LAM urine results (i.e. excluding 14 people who couldn't have TB status classified). 136 of these 413 people had confirmed TB. Uses the pre-2014 Alere LF-LAM reference card, positive LAM results are Grade 2 or higher intensity.(note a)
Kerkhoff et. al. 2017 [85]	Assesses prevalence of TB mycobacteriaemia (as proxy for disseminated TB) and diagnostic performance of LF-LAM to detect mycobacteraemia.. Restricted to 410 participants from original cohort, excludes 17 people who did not have a mycobacterial blood culture taken.
Broger et. al. 2019 [117]	Analysis of biobanked samples with LF-LAM and SILVAMP-LAM, using samples from three biobank cohorts. ("Cohort 2" in the paper is the Cape Town study). Frozen urine samples re-tested with LF-LAM specifically for this study, and with SILVAMP-LAM. Uses the current (post-2014) Alere LF-LAM where a grade 1 or band or higher are positive results.(a) Concordance with this set of LF-LAM results from earlier set of LF-LAM results in Lawn et. al. 2017 not reported. The main number of participants in "cohort 2" in tables published paper is 362. However there is data in the appendix results for a further 46 people with "unclassifiable" TB status and 10 people with missing urine sample, giving data for 420 people. See figure 2.2. I assume the reason for their being 420 people in this analysis and 427 in the parent paper is that seven people are excluded for having TB diagnosed but treatment not started before admission. Parent paper only excludes people already taking TB treatment, Broger et. al. excludes people with any diagnosis of TB.
Kerkhoff et. al. 2020 [119]	Cohort "A" in this letter is the Cape Town study. This is analysis of SILVAMP LAM tests preformed in Broger et. al 2019. This letter focuses on extra-pulmonary vs. pulmonary TB, concluding SILVAMP-LAM especially useful in extra-pulmonary TB.
Broger et. al. 2020 [103]	This is an individual patient data meta-analysis of diagnostic performance of SILVAMP-LAM which includes data from Broger et. al. 2019.
Barr et. al. 2020 [60]	This is an individual patient data meta-analysis of prevalence of mycobacteraemia, which includes data from Cape Town study.

Table 2.6 Overview of studies which have used samples or data from the Cape Town parent study

in chronological order of publication.
a: Illustration of the pre-2014 and post-2014 reference cards for LF-LAM is in figure 2 in Pandie et. al. (reference [105], publisher license doesn't include permission to reproduce in thesis). Grade 1 on post-2014 reference card reportedly corresponds to Grade 2 on pre-2014 reference card.[105]

The researchers then looked at the performance of an admission screen for tuberculosis, using urine LF-LAM samples and sputum samples collected in first 24 hours of admission.[79] They showed that only 73/139 (53%) of people who were truly positive for tuberculosis would have had their tuberculosis diagnosed on the basis of admission urine LF-LAM plus sputum NAAT – figure 2.1.

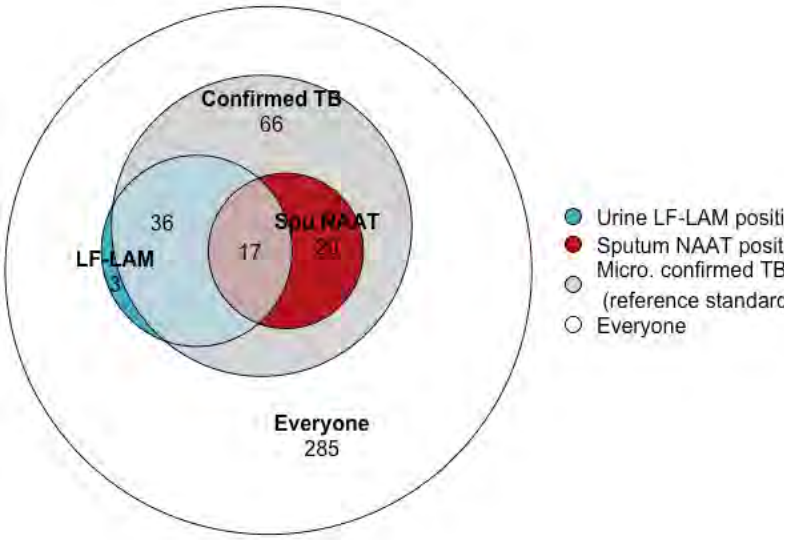

Figure 2.1 LF-LAM and sputum NAAT results in Cape Town Study

Data from Lawn et. al.[79] Tuberculosis reference results (grey circle, confirmed tuberculosis if any one or more samples taken across whole of hospital admission positive not including LF-LAM results). Positive sputum NAAT (red circle) and positive urine LAM (blue circle) results taken in first 24 hours for 427 adults living with HIV admitted to hospital.

This study illustrates two points; firstly that defining a reference standard for diagnostic accuracy for index tuberculosis tests in inpatients is difficult. For instance, if the reference standard for urine LAM had been based on a single sputum NAAT gathered at admission, most (36/53) of the true-positive urine LAM tests would have been misclassified as false-positive or removed from analysis for not having a reference standard result. The second point is more important for public health: even a "good" admission screen for tuberculosis using the best currently available tests and combining a urine test for disseminated tuberculosis and a sputum test for pulmonary tuberculosis would still miss nearly half of all people with microbiologically confirmed tuberculosis (66/139 missed).

Difficultly in producing sputum a major issue in inpatient settings, particularly when sputum induction is not available.[120] In that Cape Town study, only 158/427 (37%) of participants produced sputum within 24 hours even with sputum induction facilities and research nurse time being available. Sputum scarcity has also been shown in other studies.[51,109]

2.2.3 Use of chest Xray (CxR) for tuberculosis diagnosis, including Computer Aided Diagnosis (CAD)

Chest X-ray has been used a diagnostic tool for TB for decades and is recommended by WHO for TB diagnosis, including for clinical diagnosis of TB if microbiological tests are unavailable or negative.[14,88,121] Chest X-ray can have high sensitivity for pulmonary tuberculosis, including in HIV co-infection,[81,122–124]

particularly if an experienced radiologist is interpreting the films. Chest x-ray continues to play an important role in TB diagnosis in high-income settings in combination with other radiological examination (such as computed tomography) and microbiological tests. Although chest x-ray has been used for many years as a diagnostic tool, widespread implementation in low-resource / high tuberculosis prevalence settings has been limited by poor access to high quality equipment and expert radiologists, low specificity (leading to over-diagnosis of TB if chest x-ray alone is used) and high inter-reader variability.[121] Currently, many countries, including Malawi, have low coverage of radiology services, including lack of trained radiologists.[125] For example, in the Malawi arm of the STAMP TB tuberculosis diagnostic trial in 2015 – 2017 only 23% of PLHIV admitted to hospital had a chest X-ray.[51] Chest X-ray can be used either in a tuberculosis systematic protocol-guided screening pathway, to determine who should have a specific microbiological diagnostic test or in a tuberculosis clinician-guided diagnostic pathway setting, to provide a piece of evidence to be considered in light of clinical setting and other tests to help confirm or refute a tuberculosis diagnosis.

Computer-assisted diagnosis (CAD) software for chest x-ray interpretation – artificial intelligence algorithms used to classify digital images - are available, and can be integrated within digital x-ray units to provide immediate interpretation.[126–138] Systematic review and individual patient data meta-analysis of available evidence and two large multi-center evaluations (not included in meta-analysis) studies showed that Computer Aided Diagnosis and human readers can achieve similar sensitivity and specificity[126,139] : Table 2.7. According to meta-analysis for WHO guidelines, pooled sensitivity for digital chest Xray with Computer Aided Diagnosis (dCXR-CAD) for use as a TB triage test in people attending outpatient medical care was 0.90 to 0.91 and specificity 0.25 to 0.79. For CxR with a human reader flagging “any abnormality” (a sensitivity-maximizing approach, as opposed to asking human readers to flag only “abnormality likely due to TB”) the equivalent sensitivity was 0.89 to 0.96 and specificity 0.36 to 0.63.[88] Table 2.5 summarizes individual patient data metanalyses and large studies, and Figure 2.2 illustrates area under curve (AUC) for test performance of five human radiographers and CAD4TBv6 compared to sputum NAAT reference standard in one study.

Meta-analyses or large studies of CXR-CAD		
Study	**Participant characteristics**	**Threshold and point estimates of diagnostic accuracy.**
Tavaziva et. al. 2022 [137]	Individual patient data meta-analysis of four source studies.[128–130,133,140] All were studies in symptomatic people referred or self-referred for TB testing. Reference standard sputum culture or NAAT. 3727 radiographs. 17% people living with HIV. 17% people had proven TB.	With threshold set to be 90% sensitive, equivalent specificity (%): CAD4TBv6: 56.9 Lunit: 54.1 qXR: 60.5 Using the same threshold as needed to be 90% sensitive in whole population, applied to people living with HIV only (618 radiographs): CAD4TB: Sens 80.4, Spec 52.0 Lunit: Sens 86.4, Spec 45.2 qXR: Sens 78.9, Spec 51.6
Kik et. al. 2022 (pre-print) [(a)] [136]	Individual patient data metanalysis of six source studies (references [141–143], the other three studies have no references cited and presumably are unpublished sources). All screening studies, including HIV-risk screening and national prevalence surveys. Reference standard sputum culture or NAAT. 1753 radiographs 30% people had confirmed TB [(b)] 8% participants living with HIV. 55% had TB symptoms.	With threshold set to be 90% sensitive, equivalent specificity (%): CAD4TBv6: 34.9 Lunit 54.5 qXR 47.7 At same-sensitivity as radiologist determined "active TB likely" (which was 94% sensitive), equivalent specificity (%): Radiologist: 45.6 CAD4TBv6: 22.4 Lunit: 41.0 qXR: 34.6
Qin et. al. 2019 [(c)] [126]	1196 Outpatients in Nepal and Cameroon, presenting to clinics with symptoms. Reference standard sputum NAAT. 3.2% HIV positive (4.6% in Cameroon, 1.4% in Nepal). 9.2% sputum NAAT positive (2% in Cameroon, 18% in Nepal)	At ROC01[(d)], sensitivity and specificity (%): CAD4TB: Sens 0.91, Spec 0.84 Lunit: Sens 0.87, Spec 0.89 qXR: Sens 0.88 , Spec 0.89 Nepal: At same-sensitivity as radiologist determined "any abnormality" (which was 96% sensitive), equivalent specificity: Radiologist: 0.48 CAD4TB: 0.69 Lunit: 0.60 qXR: 0.65 Cameroon: At same-sensitivity as radiologist determined "any abnormality" (which was 80% sensitive), equivalent specificity: Radiologist:0.74 CAD4TB: 0.9 Lunit: 0.94 qXR: 0.95
Qin et. al. 2021 [144]	23,854 participants at three TB screening centres in Bangladesh. 15.3% sputum NAAT positive HIV testing not done.	At same-sensitivity as radiologist determined "any abnormality" (which was 89% sensitive), equivalent specificity: Radiologist: 0.63 CAD4TBv7: 0.76 Lunit 0.70 qXR: 0.77 Inferred DR: 0.65 JF CxR: 0.64

Table 2.7 Evaluations of dCXR-CAD for tuberculosis screening

CAD4TB = CAD4TB manufactured by Delft Imaging Systems, Netherlands. Inferread DR by Infervision (China), Lunit = Lunit INSIGHT CxR for Chest Radiography (South Korea), qXR by Qure.AI (India), JF CXR-1 by JF Healthcare (China). NAAT = Nucleic Acid Amplification Test for TB.

(a) Pre-print, not currently peer reviewed.

(b) This IPD meta-analysis included all TB positive radiographs in source studies, and negative radiographs selected at random from source studies. TB prevalence was 30% among radiographs evaluated, not 30% in populations screened.

(c) Published after data gathered for Tavaziva IPD, referenced in Tavaziva, but not included in meta-analysis.

(d) **"**ROC01 is the main of the ROC that was closest to coordinates (0,1), the perfect classification

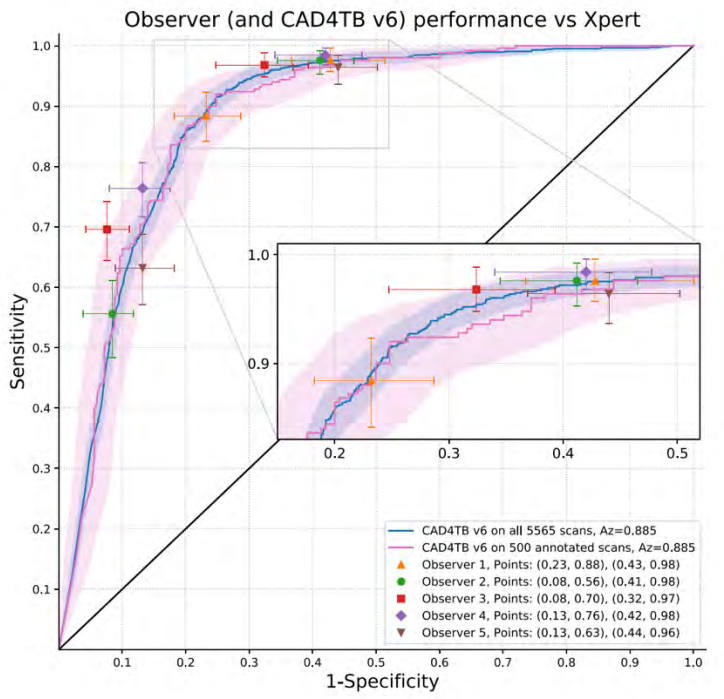

Figure 2.2: Performance of CAD4TBv6 vs. human radiologists
Blue line is sensitivity and specificity at different thresholds from all 5,665 radiographs interpreted by CAD and pink line is from the same 500 radiographs that humans read. Points show sensitivity and specificity for five human readers. There are two points for each human reader, one showing sensitivity and specific when the human reader was asked to flag "any abnormality" and one with sensitivity and specificity where the human reader to asked to flag "likely TB" CxRs Reference standard is sputum NAAT.
From Murphy et. al., which was a study in Pakistan of people with presumptive referred or who self-referred to a Chest Xray diagnostic center.[135]

As discussed above, the reference standard of a single NAAT test might under-estimate the true prevalence of tuberculosis and therefore increase the apparent false positive proportion of chest X-ray (as interpreted by both CAD and human radiologists). Almost all available studies evaluate CAD in a systematic protocol-guided screening setting, comparing to a microbiologic reference standard (usually one sputum test). To my knowledge, only one large study has evaluated dCxR-CAD for screening comparison to clinical reference standard, using CAD4TBv5. In that study true TB was determined by a specialist physician teams, and of the 87 people with true TB, 61 had TB microbiologically confirmed.[130] This means that had a microbiological reference standard been used, 26 people with high CAD scores would have been treated as false positive rather than true positive.

WHO currently recommends that CAD systems can be used in place of human readers for tuberculosis screening.[88] To date, one published randomised trial has investigated the impact of dCXR-CAD

screening on patient outcomes, rather than diagnostic accuracy. The PROSPECT study was in a primary healthcare centre in Blantyre, Malawi, and showed that tuberculosis screening using CXR-CAD to triage to sputum Xpert testing plus provider-initiated HIV testing reduced time to tuberculosis diagnosis.[145]

There is less evidence about utility of CxR in a clinician-guided diagnostic workup setting or hospital inpatient setting, partly because the difficulties of defining a reference standard. One relevant study recruited 484 sequential HIV positive adults patients admitted to two hospitals in Western Cape, South Africa with cough of any duration and WHO "danger signs" (i.e. any one or more of tachycardia, tachypnoea, fever or unable to walk).[81] Every participant had three sputum samples and one blood culture for mycobacterial culture and NAAT, plus other extrapulmonary samples for mycobacterial culture as appropriate. Fifty three percent of participants (255 people) had culture positive tuberculosis, and a further seven percent (34 people) were treated for tuberculosis but had no positive cultures. An expert radiologist assessed the chest X-rays and considered that 36% were "likely tuberculosis", 54% were "possible tuberculosis" and 10% were "tuberculosis unlikely". Out of a series of signs, symptoms and laboratory findings, a chest Xray which was "likely tuberculosis" had the strongest association with culture-proven TB (odds ratio of 11.1 for culture-proven tuberculosis compared to a chest X-ray with "tuberculosis unlikely"). A medical officer's chest Xray interpretation of "likely tuberculosis" remained a strong predictor of culture-proven tuberculosis but this was less accurate than the expert radiologist (OR 9.4 for a medical officer "tuberculosis likely" vs. "tuberculosis unlikely" for association with culture-proven tuberculosis).[81] A second relevant study assessed the use of CAD for tuberculosis (using QureAI) in a case-control design among a predominantly HIV-negative hospital population of inpatients who had a chest X-ray and at least one microbiological sputum test for tuberculosis during their hospital admission at a tertiary hospital in India.[134] Thirty four percent of the participants had microbiologically confirmed tuberculosis. The CAD software had a sensitivity of 71% and a specificity of 80% using a threshold cut-off score of 0.81 (designed to maximise sensitivity and specificity), while human radiologist readers had a sensitivity of 56% for a specificity of 80%, for an interpretation of "TB screen positive". Both these studies have the limitation that people who did not produce sputum were excluded, and the India study has the limitation that the reference standard was a single sputum only (such that person with one out of one sputum sample being NAAT negative was considered "not tuberculosis" for the purpose of Xray scoring).

These studies illustrate that even among a group of patients with a high likelihood of chest X-ray abnormalities (both tuberculosis and non-tuberculosis abnormalities), that chest Xray continues to

have an important role in distinguishing people with tuberculosis from people without tuberculosis. Because everyone admitted to hospital is unwell, tuberculosis diagnosis in this setting is likely to be more difficult as for the most part the challenge is not to distinguish tuberculosis from no pathology, but to distinguish tuberculosis from other causes of illness, many of which may also cause abnormal chest X-rays.

For PLHIV who are unwell and require hospital admission WHO recommends chest x-ray (if available) as part of the initial diagnostic work up. Chest x-ray is further recommended for people who are Xpert MTB/Rif negative or where Xpert MTB/Rif is unavailable as part of further investigations for TB, in conjunction with empiric tuberculosis treatment.[14]

2.2.4 Use of urine LAM for tuberculosis diagnosis

Urine LAM testing is recommended by WHO for certain people living with HIV: everyone with TB symptoms, everyone seriously unwell, all inpatients with CD4 cell count <200 cells/mm^3 and all outpatients with CD4 cell count < 100 cells/mm^3.[90] There is one commercially available LAM test and it is recommended by WHO Global TB Programme, LF-LAM, manufactured by Alere/Abott (USA).

Several diagnostic accuracy studies have shown that among people living with HIV with advanced immunodeficiency, urine LAM testing with AlereLAM is quite specific, but not particularly sensitive – suitable to "rule in" but not "rule out" tuberculosis. Among inpatients with HIV, a 2019 Cochrane review estimated overall sensitivity of urine LAM as 62% and specificity 84%.[106] Specificity was higher (95%) but sensitivity was lower (35%) among studies which recruited all people living with HIV (regardless of TB symptoms, and including ambulant outpatient participants). Sensitivity is higher in people with lower CD4 cell counts, compared to higher CD4 cell counts. The relatively low specificity is probably related to a combination of shortcomings in sensitivity of the reference standard, meaning that a true positive LAM gets misclassified as a false positive because the reference standard is insufficiently sensitive and "real" false positive results. Regarding the possibility of "real" false positive results, we know that urine LF-LAM and urine NAATs have poor concordance despite both tests detecting parts of *M. tb* in urine and that cross-reactivity of LAM antibodies with bacteria other than *M. tb* is possible. A recent study of people with advanced HIV and signs and symptoms of meningitis admitted to hospital in Uganda showed poor concordance between urine Xpert Ultra testing and urine LF-LAM; 20% (48 of 243 people) had a positive urine LF-LAM test, 12% (29 people) had *M. tuberculosis* detected by Xpert Ultra in urine, but only 6% (14 people) were positive by both

tests.[146] In an older study, LAM tests in sputum (rather than urine) using an older ELISA assay that is no longer commercially available (Clearview-TB-ELISA, Inverness Medical Innovations, USA), showed that there was cross-reactivity between LAM and some mouth flora bacterial organisms such as *Nocardia* and *Actinomyces* species.[110,147] Findings from two newer studies incorporating LF-LAM alongside SILVAMP-LAM that were conducted concurrently with the trial reported in this thesis (CASTLE) – with results not available as CASTLE was being designed and conducted – are discussed in chapter 7 alongside discussion of CASTLE results.[148,149]

Unlike for most tuberculosis diagnostic tests, in addition to diagnostic accuracy studies,[150] there is evidence from two randomised trials that screening using LF-LAM reduces deaths in inpatients living with HIV. LAM-RCT was a unblinded multi-country trial in South Africa, Tanzania, Zambia and Zimbabwe between 2013 and 2014.[58] Adults living with HIV who had tuberculosis symptoms and were admitted to hospital were randomly assigned to screening using urine LAM plus routine tests vs. routine tests only. This showed an absolute risk reduction of 4% in 56 day mortality (95% CI -7% to -1%) – from 25% mortality in no-LAM group to 21% mortality in LAM group.[58] STAMP was a double-blind randomised trial in Malawi and South Africa from 2015 to 2017. Adults living with HIV admitted to medical wards were enrolled irrespective of tuberculosis symptoms and were randomly assigned to tuberculosis screening using sputum Xpert only, or to screening with sputum Xpert plus urine Xpert plus urine LAM. Results were reported back to clinicians as "TB screen positive", "TB screen negative" or "no TB samples received", without reporting whether urine plus sputum or sputum alone was tested. Overall, STAMP was consistent with a mortality reduction from urine screening, absolute risk difference -2.8% (95% CI -5.8% to +0.3%). This difference was statistically significant in people with tuberculosis symptoms, anaemia or CD4 cell count < 100cells/mm^3.[51]

2.2.5 High sensitivity urine LAM tests

Urine LF-LAM can improve diagnosis of disseminated tuberculosis and it has been shown to be clinically effective at reducing deaths. However, we also know LF-LAM is imperfectly sensitive and will be negative for a lot of people with true positive TB diagnoses. Accordingly, research in improving sensitivity of urine LAM testing and developing second generation urine LAM tests has been a priority. There are several high-sensitivity urine LAM tests in development, but SILVAMP-LAM (Fujifilm Corporation, Japan) is the most well developed. It has been used in several research studies and has a CE mark, although is not commercially available yet. At the time of designing and conducting this thesis research, several other prospective SILVAMP-LAM studies were ongoing, and have now reported

results.[148,149] This introduction section sets out knowledge when CASTLE was designed – the results of the two other studies are discussed in chapter 7 (CASTLE challenges) and chapter 8 (Discussion).

SILVAMP-LAM uses a pair of monoclonal antibodies to LAM and a silver amplification step to make band visible. Like LF-LAM, SILVAMP-LAM comes in a single-use self-contained lateral flow kit that requires no electricity.[117] While LF-LAM is small cardboard strips, the SILVAMP-LAM kits are slightly larger plastic cassettes and require two extra steps – taking 60 minutes from sample to result (compared to 25 minutes for LF-LAM).

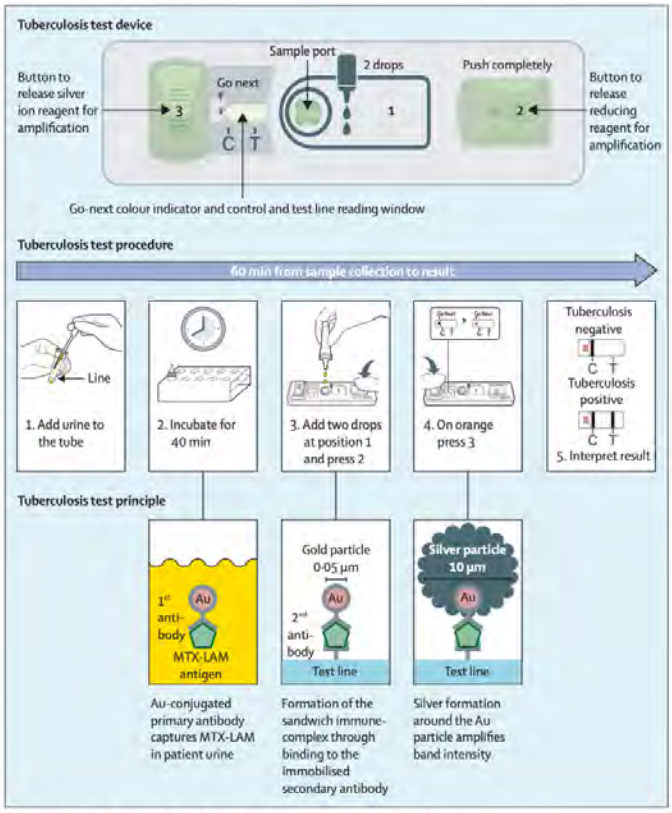

Figure 1: **Fujifilm SILVAMP TB LAM test device, procedure, and principle**
One antibody binds to tetra-arabinoside and hexa-arabinoside structures in the arabinan domain of lipoarabinomannan and the other antibody targets MTX-Man capping motifs of lipoarabinomannan (MTX-Man refers to mannose caps further modified with a 5-methylthio-D-xylofuranose residue).[24] Au=gold. C=control line. MTX-LAM=5-methylthio-D-xylofuranose-lipoarabinomannan. T=test line.

Figure 2.3: SILVAMP-LAM test device procedure and principle.

Reproduced from Broger et. al. [117] under terms of CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

SILVAMP-LAM accuracy was estimated using bio-banked urine from three South African cohorts of hospitalised adults living with HIV (including the Cape Town study[80] which has already been discussed).[117] Compared to a microbiological reference standard, SILVAMP-LAM was 70.4% sensitive and 90.8% specific (by contrast LF-LAM was 42.3% sensitive and 95.0% specific). Compared to a composite clinical / microbiological reference standard, SILVAMP-LAM was 64.9% sensitive and 95.7%

specific. The authors of that paper include an figure showing the relative yields of LF-LAM, SILVAMP-LAM and sputum Xpert in the biobanked samples from the Cape Town study – recreated below. [117]

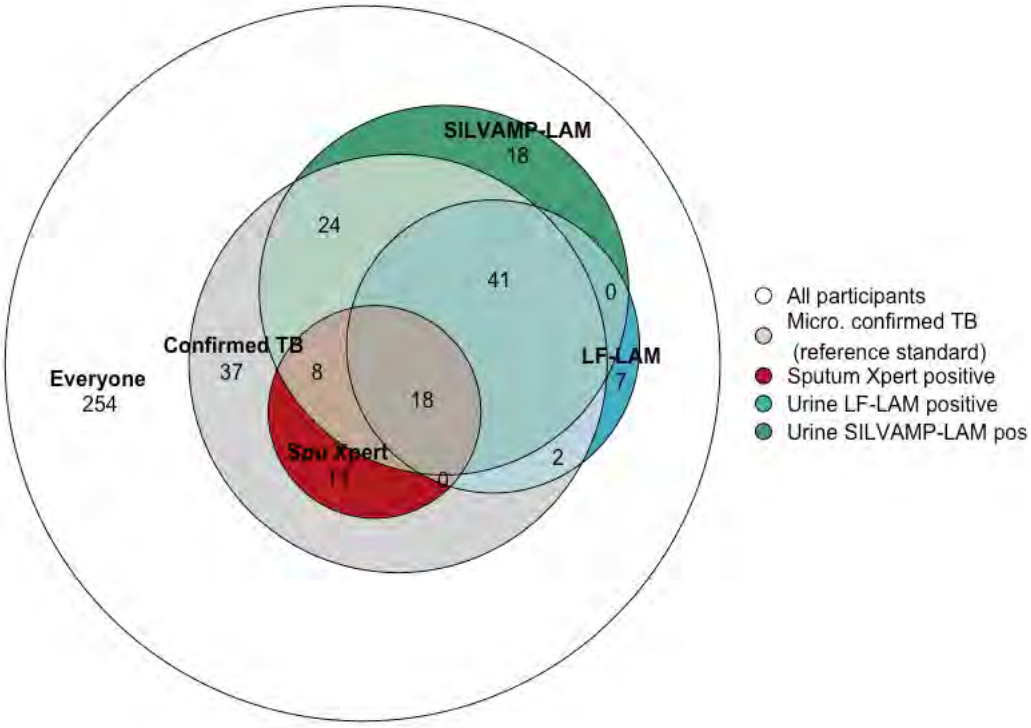

Figure 2.4: Yield of urine SILVAMP-LAM, urine AlereLAM, sputum Xpert from frozen biobanked urine, among adult inpatients living with HIV (recruited regardless of symptoms or CD4 count).

Notes on data for this figure:[79,117]

- Cape Town parent study reported data for 427 people, this study reports on 420 people. Seven people were excluded from Broger's analysis, presumably for having TB diagnosed pre-admission but treatment not started.
- I could not find information in Broger manuscript about why there were 139 people with confirmed TB in parent study, and 141 in Broger study.
- Neither LF-LAM not SILVAMP-LAM tests are included in the microbiological reference standard.
- LF-LAM tests reported here tests performed by Broger and colleagues (i.e. the LF-LAM results are a new test on the same sample, from an aliquot thawed years after LF-LAM tests done in the parent study). Concordance between original LF-LAM results from the Lawn et. al. paper and these tests from different aliquots of the same original sample are not reported.
- Broger et. al. paper states that there were 7 false positive LF-LAM tests and 18 false positive SILVAMP-LAM tests but not whether any of those were the same people, I have assumed they are different people for reconstructing this diagram. Note that the Euler diagram in the original paper (Figure 4) only includes overlap of test results in people with TB confirmed by microbiological reference standard, not false positive results. These are included in this diagram on basis of tables in manuscript.

SILVAMP-LAM has been shown to be accurate in other studies in South Africa,[117] Ghana,[151] Zambia,[152] and Vietnam (Vietnam study unpublished but data included in meta-analysis[103]). A meta-analysis of the three South Africa cohorts plus Ghana and Vietnam studies showed SILVAMP-LAM sensitivity of 70.7% (95% CI 59.0 – 80.8%) and specificity of 90.9% (95% CI 87.2-93.7%) compared to a microbiological reference standard.[103]

2.2.6 The need for studies of clinical impact and utility

From the above discussion, it is clear that tuberculosis has remained a leading cause of death among adults living with HIV admitted to hospital in Africa from the 1990s onwards. Tuberculosis is often difficult to diagnose and can also be difficult to exclude as a diagnosis. Diagnosis is particularly challenging in inpatient settings due to suboptimal diagnostic tests, high pre-test probability, patients who often can't produce sputum, the high proportion of disseminated and extrapulmonary tuberculosis meaning that clinical samples can be difficult to obtain or paucibacterial when obtained, and high early mortality meaning that waiting for response to other non-tuberculosis treatments may not be appropriate. An admission screen with sputum NAAT and urine LF-LAM is recommended by WHO, and tuberculosis screening using LF-LAM has been shown to reduce mortality in subgroups in two randomised trials. However, as shown in the Cape Town study (Figure 2.1) an admission screening with sputum NAAT and urine LF-LAM will still miss a substantial proportion of people with microbiologically-confirmed tuberculosis.

Chest Xray – particularly with expert radiologist interpretation – can achieve good sensitivity for identifying likely pulmonary tuberculosis and may be useful to help guide empiric tuberculosis treatment. Computer aided diagnosis achieves accuracy similar to that of expert radiologists in a systematic protocol-guided screening approach in primary care; however, data from inpatient settings is very limited. SILVAMP-LAM is a high sensitivity urine LAM test that may identify more people with tuberculosis than LF-LAM. The combination of dCXR-CAD plus SILVAMP-LAM is an attractive combination to rapidly and sensitively identify both pulmonary tuberculosis and disseminated tuberculosis. Whilst diagnostic accuracy studies using extensive microbiological sampling are necessary to estimate the extent to which true burden of active tuberculosis is under ascertained by routine systems, studies of clinical utility and diagnostic impact based on improving patient-important outcomes (notably: TB diagnoses made, time to TB treatment and death) are very important to generate evidence needed to inform policy and cost-effectiveness.

The overall aim of this thesis is therefore to contribute to the research evidence base to reduce mortality among adults living with HIV admitted to hospital, with a focus on improving tuberculosis diagnostics. In Chapter 3, I systematically review the literature to investigations interventions that have been shown to reduce mortality among adults living with HIV admitted to hospital. Secondly, in Chapter 4, I review patterns of incidence of HIV related hospital admission and death in Blantyre,

Malawi, between 2012-2018, during a time of substantial ART provision scale up. Thirdly, in Chapters 5 and 6, I conceived, designed, conducted, and analysed the CASTLE trial – Computer Aided Screening for Tuberculosis in Low Resource Environments. CASTLE is a randomised trial to assess whether enhanced tuberculosis diagnostics using SILVAMP-LAM plus LF-LAM plus dCXR-CAD can increase the number of people started on tuberculosis treatment (primary outcome) and reduce mortality, reduce undiagnosed tuberculosis, and increase same-day tuberculosis treatment initiation. Chapter 7 discusses unanticipated challenges faced during CASTLE trial, including diagnostic test limitations and Chapter 8 summarises conclusions and the next research steps needed to reduce AIDS deaths among people living with HIV admitted to hospital.

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Chapter 3:

A systematic review of interventions to reduce deaths among people living with HIV admitted to hospital in low and middle income settings.

3.1 Introduction to research paper

As outlined in chapter 2, people living with HIV admitted to hospital are at high risk of death, either during their hospital stay or shortly after discharge.

In this chapter, I systematically reviewed the literature to identify papers published since 2003 that evaluated interventions to reduce mortality among adults living with HIV admitted to hospital in low and middle income countries. I included studies that recruited all people admitted, and others which recruited a subset based on characteristics such as symptoms, people newly diagnosed with HIV or people with low CD4 counts. I excluded studies which only recruited participants with a defined aetiological diagnosis in addition to HIV (for instance, I excluded studies of treatments for cryptococcal meningitis).

I identified only ten studies published since 2003 that evaluated interventions applicable to a broad group of adults with HIV admitted to hospital. The main interventions tested centred around improving diagnosis of tuberculosis (three studies), reducing barriers to ART initiation, or speeding up ART initiation (four studies), early goal directed therapy for suspected sepsis (two studies) and one before-after study related to empiric tuberculosis treatment (one study). There was benefit from improving tuberculosis screening using urine tuberculosis tests and from one of the ART initiation studies. A protocol for early goal directed therapy for suspected sepsis was harmful.

Consistent with the literature overview in chapter 1, the studies in this systematic review reveal a very high death rate for enrolled participants and a very high prevalence of tuberculosis. Overall mortality was 23% (range 15% to 70%) and prevalence of microbiologically confirmed tuberculosis ranged from 15% (all PLHIV in ITU in South Africa regardless of reasons for admission) to 40% (PLHIV with TB symptoms who had urine LAM testing in multi-country study).

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PLOS GLOBAL PUBLIC HEALTH

RESEARCH ARTICLE

Interventions to reduce deaths in people living with HIV admitted to hospital in low- and middle-income countries: A systematic review

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Abstract

People living with HIV (PLHIV) admitted to hospital have a high risk of death. We systematically appraised evidence for interventions to reduce mortality among hospitalised PLHIV in low- and middle-income countries (LMICs). Using a broad search strategy with terms for HIV, hospitals, and clinical trials, we searched for reports published between 1 Jan 2003 and 23 August 2021. Studies of interventions among adult HIV positive inpatients in LMICs were included if there was a comparator group and death was an outcome. We excluded studies restricted only to inpatients with a specific diagnosis (e.g. cryptococcal meningitis). Of 19,970 unique studies identified in search, ten were eligible for inclusion with 7,531 participants in total: nine randomised trials, and one before-after study. Three trials investigated systematic screening for tuberculosis; two showed survival benefit for urine TB screening vs. no urine screening, and one which compared Xpert MTB/RIF versus smear microscopy showed no difference in survival. One before-after study implemented 2007 WHO guidelines to improve management of smear negative tuberculosis in severely ill PLHIV, and showed survival benefit but with high risk of bias. Two trials evaluated complex interventions aimed at overcoming barriers to ART initiation in newly diagnosed PLHIV, one of which showed survival benefit and the other no difference. Two small trials evaluated early inpatient ART start, with no difference in survival. Two trials investigated protocol-driven fluid resuscitation for emergency-room attendees meeting case-definitions for sepsis, and showed increased mortality with use of a protocol for fluid administration. In conclusion, ten studies published since 2003 investigated interventions that aimed to reduce mortality in hospitalised adults with HIV, and weren't restricted to people with a defined disease diagnosis. Inpatient trials of diagnostics, therapeutics or a package of interventions to reduce mortality should be a research priority.

Trial registration: PROSPERO Number: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019150341.

Competing interests: ELC, KF and AGW are authors of the STAMP trial which is included in this review. There are no other competing interests.

Introduction

Advanced HIV disease is a persistent public health challenge [1]. Expanded access to HIV testing and antiretroviral therapy (ART) has saved millions of lives globally, and probably averted many hospital admissions [2]. However, people living with HIV (PLHIV) continue to make up a disproportionate number of inpatient admissions in many high-HIV-burden countries, and of inpatients living with HIV many have advanced HIV disease (CD4 cells under 200 cells/mm^3) [3–6]. Increasingly, inpatients admitted with advanced HIV disease have been previously diagnosed and started on ART, rather than being newly diagnosed with HIV, but have either stopped taking ART or have treatment failure [7–10], often related to virological resistance [3,11]. Under these circumstances, the causes and outcomes of hospital admissions may be relatively unchanged by the availability of ART in communities, even if hospitalisation rates and the proportion of admissions accounted for by PLHIV decline [4,12].

PLHIV who require admission to hospital are an important population who have a very high risk of death [13] and whose care needs are likely to be different from ambulatory PLHIV due to clinical acuity, high probability of opportunistic infections, and high risk of death. Overall in-hospital mortality risk for PLHIV was 20% in a systematic review [13]. For people who survive initial hospital admission, death within 12 months of discharge from hospital was 14.1% in another review [10]. In 2017 WHO released the first guidelines for management of advanced HIV [14] which were incorporated into consolidated ART guidelines in 2021 [15]. These guidelines acknowledged that the majority of available evidence informing interventions for managing patients with advanced HIV relates to ambulatory ART-naïve participants, and that more research is required to evaluate the optimal interventions for PLHIV with treatment failure and inpatient management [14].

To identify interventions with potential to reduce mortality in this group we systematically reviewed the existing literature relating to interventions aiming to reduce mortality in adult hospitalised PLHIV in low- and middle-income countries (LMICs). We aimed to review interventions broadly applicable to all or many hospitalised patients, rather than interventions offered to a sub-group where a definite aetiological diagnosis was already made.

Methods

Search strategy

The protocol and search strategy are available online at PROSPERO (CRD42019150341). We used a broad search strategy (S1 Appendix) to identify studies that recruited hospitalised HIV positive participants [13,16]. We searched MEDLINE, EMBASE OVID, and Cochrane Central databases and included papers published after 1 January 2003 as this was when ART became available in low-income countries [17]. We also hand-searched abstracts from Conference on Retroviruses and Opportunistic Infections (CROI) and International AIDS Society (IAS) between 2015 and 2021. The initial search was on 9th October 2019, with an updated search on 23rd August 2021.

Eligibility criteria

Studies were eligible for inclusion if they reported results from a trial or study with a comparator arm (i.e. both randomised and non-randomised designs) that recruited inpatient adults (aged 15 years and older) living with HIV and where death was reported as an outcome (either inpatient mortality or measured over any specified time period following enrolment). We excluded studies solely in surgical or obstetric wards. This review was limited to adults, given the substantially different causes of paediatric HIV related hospital admissions [13].

We included studies with both inpatients and outpatients, both HIV positive and HIV-negative participants, and both adults and children, provided it was possible to disaggregate the data for inpatient adults living with HIV. We restricted studies to those conducted in LMICs as defined by the World Bank in 2019 [18]. Studies in sub-populations that required a specific aetiological diagnosis to have been made by clinicians in addition to HIV were excluded (for example, trials of participants with cryptococcal meningitis) as the aim here was to investigate broadly applicable interventions aimed at improving all-cause mortality. This was a change from original protocol which stated trials about interventions for some specific diagnosis would be included. This change was reflected in an amendment to the PROSPERO protocol on 20th March 2020.

Screening and data extraction

Two reviewers (RMB and HT) screened titles and abstracts against inclusion criteria using Rayaan software [19]. For the October 2019 search both reviewers assessed all title-abstracts and full text reviews independently in duplicate. For the August 2021 extension, 10% of title-abstracts and all full text articles were reviewed in duplicate. Differences between reviewers were resolved by discussion and consensus including a third reviewer (PM). Where studies included participants who met our review inclusion criteria but results were not disaggregated (most commonly, when both inpatients and outpatients were included), we contacted the authors by email to ask for disaggregated results. We extracted data from eligible studies on study year, location, trial design, intervention, comparator, median CD4 cell counts, proportion of participants on ART, proportion with tuberculosis, mortality outcome definition and deaths by trial arm.

Risk of bias assessment

We used the Cochrane Risk of Bias (ROB) 2 tool to assess study quality of individually randomised studies [20]. For cluster randomised trials we used Cochrane Risk of Bias (2016) [21]. For non-randomised studies we used the Cochrane ROBINS-I tool (Risk Of Bias In Non-randomised Studies of Interventions) [22].

Synthesis of results

Results are presented in narrative format, with summary measures as reported in the relevant papers or by authors' communication. We report adjusted estimates if these were performed by authors. Where no summary measure was reported for the population and outcome of interest, we calculated unadjusted absolute risk differences from grouped data. No meta-analysis was performed as study interventions and populations were too diverse. We grouped trials by the population recruited–whether all PLHIV in hospital, newly diagnosed PLHIV or PLHIV with additional set of symptoms or other criteria (for example, PLHIV with tuberculosis [TB] symptoms).

Results

After removing duplicates, we identified 16,421 studies from our initial search, from which 262 articles were selected for full text screening. In an updated search in August 2021, we identified 3,549 further articles for title-abstract screening and 64 for full text review. Nine published studies and one conference abstract were included in our qualitative synthesis (Fig 1).

Table 1 summarises the characteristics of included studies. Three studies were conducted in Zambia, three in South Africa and one study each in Malawi, Uganda, Tanzania, Zimbabwe, Brazil, China and Mexico (two of the ten studies were multi-country). There were 7531

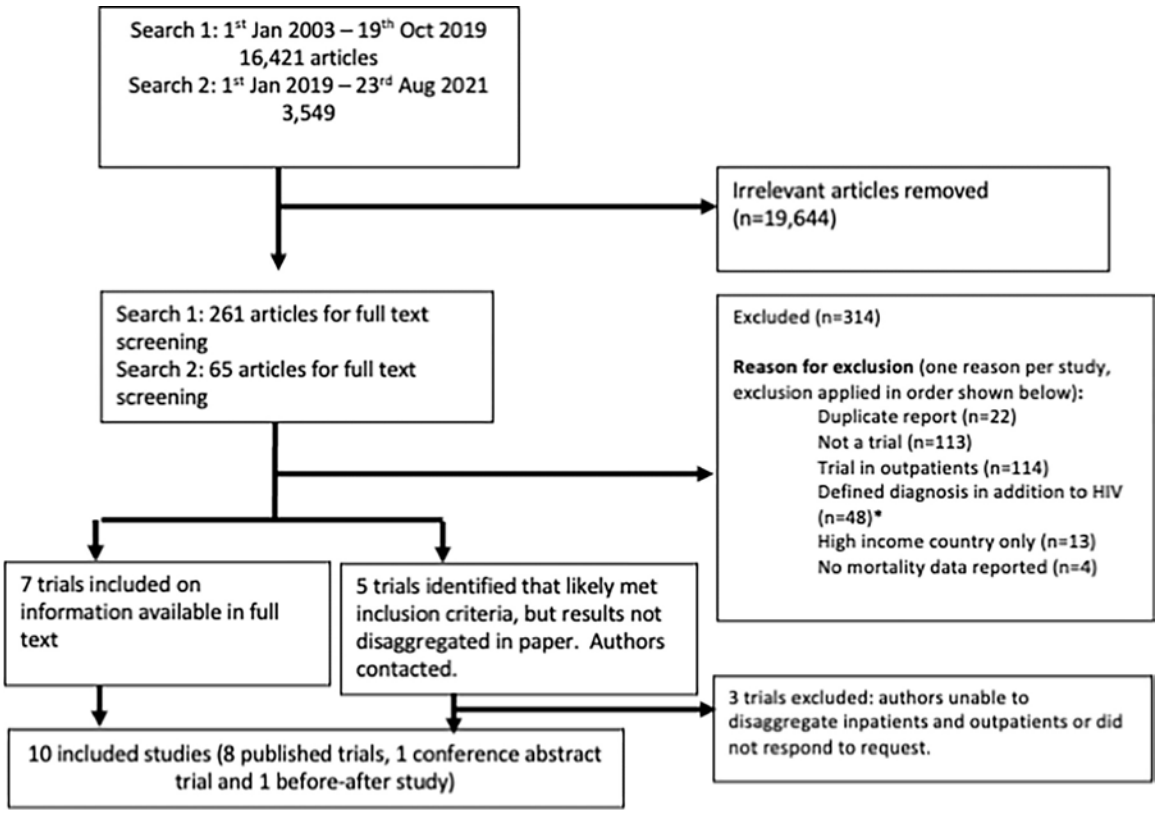

Fig 1. PRISMA diagram. *Defined diagnoses were cryptococcal meningitis (29 studies), TB (including TB meningitis, TB pericarditis and TB IRIS, 10 studies in total), bacterial meningitis (2 studies), Pneumocystis jirovecci pneumonia (2 studies), and bacterial pneumonia, toxoplasmic encephalitis, visceral leishmaniasis, Kaposi-sarcoma IRIS, and progressive multifocal leukoencephalopathy (1 study each).

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participants in total (range 58 to 2574 participants per study). Median CD4 count ranged from 40 cells/mm^3 in a Zambian trial that recruited patients with suspected severe sepsis during 2012, to 227 cells/mm^3 in a trial that recruited all PLHIV admitted to general medical wards in Malawi and South Africa during 2015–2017. For six studies not restricted to newly diagnosed HIV, the proportion of participants on ART ranged from 16% (in South Africa, recruitment between 2008–2009) to 72% (in Malawi and South Africa, recruitment 2015–2017). Overall, 23% of participants had died by the end of study follow up: follow up duration ranged from time of discharge from hospital to 12 months from enrolment.

Table 2 summarises the interventions assessed and their impact on mortality. Risk of bias assessments are detailed in S1 Appendix.

Interventions relevant to all people living with HIV admitted to hospital

Only one study, the STAMP trial (2018), recruited all PLHIV admitted to adult medical wards regardless of presenting signs or symptoms. Gupta-Wright and colleagues randomly allocated HIV positive inpatient participants to receive systematic screening for tuberculosis with either urine lipoarabinomannan (LAM) plus urine Xpert MTB/Rif (Xpert) plus sputum Xpert, or with sputum Xpert alone [23]. Overall, the difference in mortality at 56 days post enrolment between the two arms was consistent with survival benefit in the group with urine TB testing (adjusted risk difference for death [aRD] -2.8%, 95% CI -5.8% to 0.3%, not statistically

Table 1. Characteristics of included studies.

Study	Design	Year (s) of trial	County or countries	Setting	Population (whole trial)	Number participants (whole trial) *	Number participants who were adult inpatients with HIV	Median CD4 counts (cells/mm^3) #	% on ART at enrolment	Intervention	Comparator	Primary outcome	Mortality outcome details (if not primary outcome)
Studies in all inpatients with HIV													
Gupta-Wright et al. (STAMP) (2018) [23]	Individually randomised. Double blind.	2015–2017	Malawi and South Africa	2 hospitals (1 DGH / 1 referral)	Inpatient adults living with HIV (regardless of symptoms)	2574	2574	227	72%	Systematic screening for TB using urine LAM and sputum Xpert MTB/rif	Systematic screening for TB using sputum Xpert MTB/rif alone.	All-cause mortality at 56 days.	NA
Studies in newly diagnosed inpatients with HIV													
Wanyenze et al. (2013) [24]	Individually randomised. Not blinded.	2008–2011	Uganda	1 referral hospital.	Inpatient and outpatient adults newly diagnosed with HIV.	965	342	Not stated	NA	"Enhanced linkage to care". This involved counselling, supported disclosure, accompaniment to ART clinic.	Usual care. This involved giving an appointment card for ART clinic on discharge.	Receipt of HIV care. Defined as attending ART clinic.	All cause mortality at 90 days and at 12 months.
Wu et. al. (2017) [25]	Cluster randomised, hospital as unit of randomisation. Not blinded.	2015	China	12 hospitals (all DGHs)	Inpatient and outpatient adults newly diagnosed with HIV.	487	338	Not stated	NA	"One4all programme". Involves using rapid tests for HIV confirmation, CD4 count and HIV viral load in parallel.	Usual care. Usual care involved step-wise at reference labs with long turn around time.	"Completeness of testing" within 30 days. Defined as having HIV antibodies, CD4 and viral load available and post-test counselling.	All cause mortality at 90 days and at 12 months.
Boniatti et. al. (2020) [26]	Individually randomised. Not blinded.	2012–2015	Brazil	Intensive care unit at one hospital.	Adults admitted to ICU newly diagnosed with HIV with CD<350 or AIDS defining illness.	115	115	37 in intervention group, 49 in control group.	NA	Starting ART within 5 days of enrolment in trial.	Starting ART after discharge from ICU.	All-cause mortality prior to discharge from hospital.	In ICU morality and 6-month mortality also reported.
Peralta-Prado et. al. (2021) [27]	Individually randomised. Not blinded.	Not stated	Mexico	1 hospital	Adults admitted with an "opportunistic disease" (details no specified)	58	58	56	NA	"Immediate ART" (median of 2 days from enrolment to ART in this group)	"Conventional delayed ART" (median of 11 days to ART in this group)	Survival at 360 days	NA
Studies in inpatients with HIV and TB symptoms													
Holtz et al. (2011) [29]	Before and after study. Not randomised or blinded.	2008–2009	South Africa	3 hospitals (1 DGH, 1 private, 1 hybrid private/ DGH)	Adult inpatients living with HIV who had "suspected pulmonary tuberculosis" and WHO danger signs.	525	525	122 pre-intervention group, 74 in control group.	16%	Management "according to the 2007 WHO recommended algorithm for diagnosis and treatment of smear-negative pulmonary tuberculosis".	Management "according to standard practice".	Continued stay in hospital at 7 days and survival at 56 days after admission.	All cause mortality at 56 days.

(*Continued*)

Table 1. (Continued)

Study	Design	Year (s) of trial	County or countries	Setting	Population (whole trial)	Number participants (whole trial) *	Number participants who were adult inpatients with HIV	Median CD4 counts (cells/mm^3) #	% on ART at enrolment	Intervention	Comparator	Primary outcome	Mortality outcome details (if not primary outcome)
Peter et al. (LAM-RCT) (2016) [28]	Individually randomised. Not blinded.	2013–2014	South Africa, Zambia, Tanzania, Zimbabwe	10 hospitals (mix of DGH and referral)	Inpatient adults living with HIV with any WHO TB symptom.	2528	2528	84	48%	Systematic urine LAM testing plus routine diagnostic TB tests.	Routine diagnostic TB tests only. (These varied between sites).	All-cause mortality at 18 weeks.	NA
Studies in inpatients with HIV and signs and symptoms consistent with sepsis													
Andrews et al (2014) [30	Individually randomised. Not blinded.	2012	Zambia	1 referral hospital emergency department.	Adults with suspected infection + SIRs + organ dysfunction.	109	88	70 in control group, 40 in intervention group.	38%	"Early goal directed therapy". This was up to 4 litres IV fluid and dopamine and/or blood transfusion in selected patients.	Usual care.	In-hospital all-cause mortality.	NA
Andrews et al (2017) [31]	Individually randomised. Not blinded.	2012–2013	Zambia	1 referral hospital emergency department.	Adults with suspected infection + SIRS + hypotension. Participants with hypoxia or tachypnoea were excluded.	209	187	65 in control group. 72 in intervention group.	57%	"An early resuscitation protocol for sepsis". This was up to 4 litres IV fluid and dopamine and/or blood transfusion in selected patients.	Usual care	In-hospital all-cause mortality.	NA
Studies in inpatients with HIV mechanically ventilated in intensive care													
Calligaro et al. (2015) [32]	Individually randomised. Not blinded.	2010–2013	South Africa	Intensive care units at 4 hospitals (mix DGH and referral).	Mechanically ventilated adults with either HIV (regardless of symptoms) or HIV negative with TB symptoms.	341	115	122 in control group, 190 in intervention group.	28%	Local Xpert + culture respiratory secretions.	Smear microscopy plus culture of respiratory secretions preformed at a reference lab.	Proportion of culture-positive participants started on TB treatment 48 hours after enrolment.	All cause mortality in intensive care, in hospital, at 28 days, and at 90 days.

DGH = District General Hospital; SIRS Systemic Inflammatory Response Syndrome; LAM Lipoarabinomannan.

* Several trials included inpatients and outpatients, or inpatients with and without HIV.

Only if reported for inpatients only (some trials which included both inpatients and outpatients didn't report this data).

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Table 2. Effect of interventions to reduce mortality among adult PLHIV inpatients.

Study	Intervention	Comparison	Outcomes for adult PLHIV admitted to hospital, disaggregated from main trial population where necessary.				LTFU (%)	Overall risk of bias #	Source of presented data and effect estimate.
			Time-period over which mortality was ascertained	Mortality in comparison / control arm	Mortality in intervention arm	Measure of association between intervention and mortality outcome and 95% confidence interval *			
All PLHIV									
Gupta-Wright et al. (STAMP) (2018) [23]	Systematic urine LAM and sputum Xpert MTB/rif screening for TB.	Systematic screening for TB with sputum MTB/rif alone.	56 days	272/1287 (21%)	235 / 1287 (18%)	aRD -2.8%, 95% CI -5.8% - 0.3%). Adjusted for site only.	29 (1.1%)	Low	Published study.
Newly diagnosed PLHIV									
Wanyenze et al. (2013) [24]	Enhanced linkage to care (see for description)	Usual care	3 months 1 year	22 / 168 (13%) 36/168 (21%)	30 / 174 (17%) 47 / 174 (27%)	RD +4% [-3% to + 12%] RD + 6% [-3% to + 15%]	87 (9%)^	Some concerns	Disaggregated mortality outcome from author. Effect estimate calculated from grouped data.
Wu et. al. (2017) [25]	One4All programme (see text for description)	Usual care	1 year	98 / 185 (53%)	54 / 153 (35%)	RD -18% [-28% to -7%]	34 (6.9%)^	Low	Disaggregated mortality outcomes from published paper. Effect estimate calculated from grouped data, ignoring clustering (may be falsely narrow).
Bonaiatti et. al. (2020)	Early ART start (within 5 days)	ART start after ICU discharge.	In hospital	38 / 57 (69%)	37 / 58 (64%)	RD +3% [-15% to +20%]	5 (4.3%)	Some concerns	Published study, effect estimate calculated.
Peralto-Prado et. al. (2021)	Early ART	Deferred ART	360 days	2/ 28 (7%)	4 / 30 (13%)	RD -6% [-22% —+9%]	Not stated	Some concerns	Published study, effect estimate calculated.
PLHIV with signs and symptoms of TB or with presumptive TB.									
Holtz et al. (2011) [29]	"Implementation of WHO 2007 TB treatment guidelines"	Usual care prior to institutional implementation of WHO 2007 TB treatment guidelines.	In hospital At 8 weeks	30 / 338 (9%) 108 / 338 (32%)	12 / 187 (6%) 31 / 187 (17%)	RR 0.57 (0.328–1.14) aRR 0.46 (0.30–0.70) Adjusted for hospital and baseline CD4 count.	No participants LTFU	High	Published study.
Peter et al. (LAM-RCT) (2016)	Systematic urine LAM testing plus routine diagnostic TB tests.	Routine diagnostic TB tests only.	56 days	317 / 1271 (25%)	261 / 1257 (21%)	aRR 0.83 (0.73–0.96) Adjusted for country of recruitment.	117 (4.6%)	Some concerns	Published study.
PLHIV with signs of symptoms consistent with sepsis									
Andrews et al (2014) [30]	Early Goal Directed Therapy for sepsis with hypotension (see text)	Usual care	In-hospital	29 / 46 (63%)	29 / 42 (69%)	RR 1.10 (0.81–1.48)	6 (5.5%)^	Low	Disaggregated mortality outcome and effect estimate from published paper.

(*Continued*)

Table 2. (Continued)

Study	Intervention	Comparison	Outcomes for adult PLHIV admitted to hospital, disaggregated from main trial population where necessary.				LTFU (%)	Overall risk of bias #	Source of presented data and effect estimate.
			Time-period over which mortality was ascertained	Mortality in comparison / control arm	Mortality in intervention arm	Measure of association between intervention and mortality outcome and 95% confidence interval *			
Andrews et al (2017) [31]	Early Goal Directed Therapy for sepsis with hypotension (see text)	Usual care	In hospital	29 / 93 (31%)	46 / 94 (49%)	RR 1.57 (1.09–2.26)	15 (7.2%)^	Low	Disaggregated mortality outcome and effect estimate from published paper.
PLHIV who are mechanically ventilated									
Calligaro et al. (2015) [32]	Systematic TB screening using local Xpert MTB/rif testing.	Systematic TB screening using central reference lab culture.	28 days 90 days	12 / 30 (40%) 12 / 30 (40%)	13 / 37 (35%) 16 / 37 (43%)	RD -5% (-28% to 18%) RD 3.2% (-20% to 27%)	11 (3.2%)^	Low	Disaggregated mortality data from author, effect estimate calculated.

* Measure of association is according the primary outcome for paper or main mortality outcome reported (where mortality is not a primary trial outcome). Were there is no measure of association in the published paper (usually because PLHIV or inpatients were a subgroup) we calculate an estimated risk difference and 95% confidence interval from grouped data, ignoring clustering if relevant.

Risk of bias assessed using Cochrane ROB 2019 for randomised trials and Cochrane ROBINS-i for non-randomised studies.

^ Applies to the whole trial population and not just inpatient adults with HIV (unable to disaggregate LTFU).

Abbreviations: a = adjusted, OR = Odds Ratio, RR = Risk Ratio, HR = Hazard Ratio, RD = Risk difference, LTFU = Lost to follow up.

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significant). In three pre-specified sub-groups there was a survival advantage in the group screened with urine diagnostics. These were in participants with CD4 cell counts <100 cells/mm^3 (aRD -7.1%, -13.7% to -0.4%), participants with haemoglobin < 8g/dL (aRD –9.0%, –16.6% to –1.3%), and participants with tuberculosis in differential diagnosis (aRD –5.7% –10.9% to –0.5%). Tuberculosis was microbiologically confirmed in 16% (210/1287) of participants randomised to the urine diagnostic arm, based on combined results from the admission urine and sputum Xpert screen plus other usual care diagnostics on clinician request. This was a blinded trial, with low risk of bias.

Interventions relevant to newly diagnosed PLHIV hospital inpatients

Four studies evaluated interventions relevant to people newly diagnosed with HIV at the time of admission.

Wanyenze and colleagues (2013) randomised participants who tested HIV positive at a tertiary referral hospital in Uganda to standard vs. enhanced linkage to care [24]. Enhanced linkage to care involved counselling to reduce barriers to linkage to care, optional assisted disclosure to people who could provide social support, in-person introduction to clinic location and clinic staff and a reminder by phone call or home visit about their scheduled ART clinic visit, which for inpatients was following discharge. Approximately one-third of patients were inpatients at the time of randomisation. For all participants (outpatients and inpatients) there was no difference between standard linkage to care and enhanced linked to care (aHR 0.97, 95% CI 0.70–1.36). The authors provided data for participants recruited as inpatients or

from emergency departments at our request. Compared to standard linkage to care, enhanced linkage to care did not alter mortality at one year, although the confidence interval was wide (unadjusted risk difference [RD] +6%, 95% CI -3% to +15%). There were some concerns of bias due to completeness of follow up (9% of participants were lost to follow up).

Wu and colleagues (2017) evaluated an intervention package that removed laboratory test barriers to timely ART initiation [25]. Twelve hospitals in China were randomised to either usual care or to implementation of the "One4All" intervention. All people (inpatients and outpatients) newly diagnosed with HIV on the basis of a single screening lateral flow HIV test in each hospital were recruited to the study. The intervention involved parallel rapid HIV antibody confirmatory testing, point of care CD4 count and viral load measurement at baseline. Usual care involved sequential testing for these three tests, with long turnaround times and requiring multiple clinic visits for blood tests, as was standard of care at the time. The hospital policies were that results of these tests were required before ART could be commenced. In the intervention arm required tests were complete in a median of 12 days from enrolment, compared with 58 days in usual care arm, meaning that the intervention reduced the time before ART was started. By 12 months 35% (54/153) of inpatient participants in the One4All group had died, compared to 53% (98/185) in the control group; this corresponded to an unadjusted RD for mortality of -18% (95% CI, -28% to -7%). This confidence interval was calculated from grouped data and ignores effects of clustering. This trial had a low risk of bias.

Boniatti and colleagues (2020) investigated early (within five days from enrolment) compared to late (after ICU discharge) ART start among ART-naïve HIV positive participants admitted to ICU in Brazil with low CD4 count or a AIDS-defining illness [26]. Timing of ART initiation made no difference to death by six months, RD +3% [-15% to +20%] for early ART compared to late ART. Forty-three percent (51/118) of people had diagnosed tuberculosis, including microbiologically confirmed and clinically diagnosed TB. There were some concerns of bias due to loss to follow up. In a conference abstract, Peralta-Prado and colleagues report results of a trial which randomly allocated 58 adult PLHIV with opportunistic infections not on ART to immediate vs. delayed ART in hospital (median 2 days to ART initiation in immediate arm and 11 days in deferred arm) [27]. There was no difference in survival at one year from enrolment.

Interventions relevant to people based on defined symptoms and signs

We identified five studies that evaluated interventions targeted towards patients with specific constellations of clinical symptoms and signs.

Two studies were conducted among PLHIV with signs or symptoms of TB. In the LAM-RCT study, Peter and colleagues (2016) randomised participants with any WHO TB symptom (cough, fever, weight loss or night sweats) in ten hospitals in four countries (Zambia, Zimbabwe, South Africa and Tanzania) to either urine LAM screening or routine non-LAM diagnostic tests for TB [28]. Urine LAM screening reduced all-cause mortality at eight weeks (RD -4%, 95% CI -7 to -1%). TB was microbiologically confirmed in 26% (664/2528) of all participants by TB culture or Xpert (corresponding to 29% (664/2333) of all participants who had at least one Xpert or culture sample taken). There were some concerns of bias due to the unblinded design.

In a non-randomised before-and-after study conducted in 2008–2009, Holtz and colleagues (2011) investigated the effect of implementing the WHO 2007 smear-negative and extrapulmonary TB diagnosis and treatment guidelines for PLHIV at three hospitals in South Africa [29]. Hospitalised PLHIV were recruited if they were seriously ill (one or more WHO "danger signs"), had TB symptoms or a chest X-ray consistent with TB and negative sputum smear

microscopy (the authors refer to this as meeting definition for smear negative TB, although in the pre-intervention part of the study less than half of participants actually received a TB diagnosis). In the pre-intervention group (August 2008 to February 2009), usual care varied and was not guided by any protocol; in the intervention period (March to December 2009) clinicians were trained and asked to manage patients according to the WHO 2007 algorithm which recommended empirical initiation of TB treatment. The percentage of participants initiating TB treatment was 46% (157/338) in the pre-intervention cohort and 100% (187/187) in the intervention cohort. Mortality was much lower in the post-intervention cohort compared to pre-intervention, with an adjusted hazard ratio (aHR) for death up to 56 days from enrolment of 0.46 (95% CI: 0.30–0.70) after adjustment for baseline CD4 count and hospital. This was an unblinded before-and-after study with serious risk of bias.

Two studies recruited patients with signs and symptoms consistent with sepsis and hypotension; both were RCTs conducted in one hospital in Zambia that evaluated the use of early goal-directed therapy in the emergency department. In both trials, adults attending the hospital emergency department were eligible regardless of HIV status, but the majority were HIV positive. In their first trial Andrews and colleagues (2014) recruited participants with signs and symptoms consistent with sepsis and organ dysfunction between February and July 2012 [30]. In the second trial (Andrews et al, 2017) [31] recruitment was restricted to people attending the emergency department between October and December 2013 with hypotension, but without hypoxia or tachypnoea. The intervention protocol mandated up to four litres intravenous fluid, and vasopressors or blood transfusion in participants with refractory hypotension and anaemia respectively. In both trials, a higher proportion of participants in the intervention groups died compared to those in the control groups: risk ratio (RR) 1.05, 95%CI 0.79–1.41 for the first trial (not statistically significant), and RR 1.46, 95% CI 1.04–2.05 for second trial. The authors note that limited access to intensive care unit level monitoring or mechanical ventilation for patients may have contributed to harm from aggressive fluid resuscitation. Among all participants in both trials (including a small proportion HIV-negative), 27% (81/297) had microbiologically confirmed TB. These trials both had some concerns of bias due to the unblinded design, although this would have likely had the effect of bias towards the null.

One trial (Calligaro et al, 2015) investigated a TB screening intervention comprising Xpert MTB/Rif testing compared to smear microscopy done in a centralised laboratory among ventilated patients in ICU in South Africa, regardless of symptoms or reason for ICU admission [32]. The prevalence of microbiologially confirmed TB was high (15%, 13/86 participants overall across both trial arms). Use of Xpert compared to smear was not associated with a difference in mortality between groups at 90 days (RD +3%, 95% CI -20% to 27%), and this trial was terminated early due to Xpert becoming standard of care in South Africa. This trial was at a low risk of bias.

Discussion

People living with HIV who are unwell enough to require hospital admission likely have healthcare needs that are substantially different to people attending outpatient services, due to clinical acuity, extremely high probability of opportunistic infections and high risk of poor outcomes [10,13]. Whilst the numbers of people living with HIV being admitted to hospital are likely decreasing due to community availability of ART, and newer ART regimens [12], people in hospital contribute disproportionately to AIDS deaths. For example, about one quarter of all AIDS related deaths in Blantyre, Malawi in 2018 occurred in a government hospital [12]. There is no evidence of improvement in outcomes for PLHIV admitted to hospital over time [10,12,13]. While the WHO 2017 guidelines for advanced HIV disease and 2021

consolidated guidelines [14,15] apply to both inpatients and outpatients, most of the evidence considered for those guidelines was from outpatient studies, and there are currently no differentiated recommendations to reflect the particular needs of inpatients. We identified ten publications from 2003 (nine randomised trials and one before-and-after study) that investigated interventions aimed broadly at reducing mortality among PLHIV admitted to hospital in LMICs, although several of these were interventions that are superseded by more recent ART guidelines. Four consistent findings in studies in review were: high risk of death, with early mortality ranging from 20% (at 56 days, all PLHIV admitted to medical wards in South Africa and Malawi) [23] to 70% (by six months, PLHIV in intensive care in Brazil) [26]; a high prevalence of microbiologically confirmed TB; high prevalence of signs of critical illness; and low CD4 counts even in studies where many participants are on ART. The finding of low CD4 counts persisted in more recent studies even though many participants in more recent studies [23,28,31] reported already knowing their HIV status and taking ART (Table 2). Interventions were aimed at intensified TB diagnosis or treatment (four studies), reducing perceived or actual barriers to initiation of ART (two studies), rapid inpatient ART start (Two studies), and fluid-based management of sepsis (two studies).

TB is a major cause of death in PLHIV admitted to hospital, as shown in previous reviews of hospitalised HIV cohorts [13,33] and autopsy series [34]. Consistent with this: *M. tuberculosis* was the single most common blood culture pathogen in the sepsis trials in Zambia (isolated from 35% of positive blood cultures); microbiologically confirmed tuberculosis was diagnosed in 16% of urine-screening-arm STAMP trial participants (unselected PLHIV admitted to medical wards in Malawi and South Africa) [23]; 27% of urine screening arm LAM-RCT participants (PLHIV inpatients with TB symptoms in Tanzania, South Africa, Zambia and Zimbabwe); and 15% of PLHIV ventilated in Intensive Treatment Unit (ITU) in South Africa (regardless of reason for ITU admission). Four of the ten studies investigated TB-related interventions [23,28,29,32]. Two multi-country randomised trials [23,28] investigated TB urine diagnostic interventions and reported substantially increased proportions of inpatients treated for TB as well as modest mortality reductions. These results led to a strong recommendation from WHO in 2019 [35] that urine LAM testing should be used to test all inpatients with CD4 $<$200 cells/mm^3, signs and symptoms of TB or who are seriously unwell [35–37]. Although we did not identify any trials investigating empirical TB treatment specifically for inpatients, one non-randomised study in South Africa [29] reported large survival gains from implementing the 2007 WHO algorithm for critically ill PLHIV with TB symptoms; which in effect substantially increased the number of people starting TB treatment without clinical confirmation of TB. However, this study was before widespread availability of sputum Xpert and urine LAM testing for TB. Both TB prevalence and risk of death from TB vary considerably by level of health service for PLHIV [38], thus these results support the need for randomised trials investigating inpatient empirical TB treatment, despite the lack of benefit shown for outpatients [39–41]. Such studies could be combined with investigation of more intensive TB regimens, for instance high dose rifampicin, and would ideally use pragmatic eligibility criteria aiming to recruit large numbers of inpatients, as for LAM-RCT and STAMP, rather than aiming for highly pre-screened and/or immunosuppressed participants that can cause critical recruitment problems [42] and applicability concerns.

A high proportion of participants in all trials had clinical signs of critical illness. Nearly half of all emergency department attendees in the Zambian trials met the case definition for sepsis [30,31] The definitions of sepsis used for these studies are similar to WHO danger signs used to identify need for hospitalisation. In the STAMP trial, 21% of all participants (all PLHIV in medical wards) had one or more WHO 'danger signs' at the time of recruitment [23].

Of the six studies that included people regardless of ART status, there was a pronounced increase from the earlier to the later trials in the proportion of participants already being on ART, reflecting global efforts to scale-up diagnosis and treatment as part of UNAIDS Fast-track HIV elimination strategy. Most trials reported median CD4 count and proportion of participants on ART, but without disaggregating CD4 counts by ART status. Median CD4 counts were low, ranging from 40 cells/mm^3 for a 2012 study with ART at admission in 38% of participants [32] to 227 cells/mm^3 for a 2015–2017 study with ART at admission in 72%. [23] Low median CD4 count despite high ART coverage suggests a high prevalence of underlying ART treatment failure, confirmed for patients admitted to the Malawi site of the STAMP trial [11]. Given the high mortality for admitted PLHIV, we recommend that investigation and management of potential ART treatment failure should be considered as a matter of urgency for inpatients, ideally informed by trials investigating health outcomes from diagnostic interventions providing early data on viral load, genotyping, and investigating optimal timing of ART regimen change following inpatient admission. Such trials could inform current WHO HIV treatment failure guidelines that are mainly based on data from ambulant outpatients.

Two of the included trials [24,25] addressed real or perceived barriers to starting ART; one showed a mortality benefit and one didn't. These were all before 2016 "treat all" recommendations and some of the barriers that were being overcome (such as availability of CD4 counts to determine ART eligibility) are no longer standard of care [15]. Two small studies of very early vs. slightly delayed ART showed no difference in mortality (with wide confidence intervals).

Limitations to our present study include trials for which our population of interest (hospitalised PLHIV) represented only a proportion of the entire trial population, leaving trials underpowered for inpatient mortality. In two papers, authors were unable to disaggregate the inpatients and outpatients thus these were not included [43]. We did not include trials that were restricted to people in hospital who already had a specific diagnosed opportunistic infection (for example cryptococcal meningitis). We had initially intended to include interventions for people with certain diagnosed opportunistic infections but after starting the search we amended the inclusion criteria (reflected in changes at PROSPERO registration) for three reasons. First, we were initially including several small drug trials of varying quality and were thereby duplicating work of other reviews (for example, cryptococcal meningitis, where clear guidelines based on a systematic review about appropriate management already exist). Second, because, microbiologically or pharmacologically, treatment for diagnosed opportunistic infections isn't likely to substantially vary between inpatients and outpatients and inpatients aren't necessarily a distinct population–although they have a higher risk of death. Finally, interventions delivered to a small subset of inpatients are unlikely to have large public health benefits, and our focus was on interventions or a package of interventions that could be recommended generally for hospitalised adults to broadly reduce risk of death.

In summary, we found relatively few trials to inform recommendations for interventions or a package of interventions to offer as part of a package of care to adults living with HIV admitted to hospital. There were two studies of urine TB diagnostics which showed mortality reductions in people with TB symptoms, but no studies of other diagnostic interventions. Understanding the likely impact of combined diagnostic interventions such as urine LAM, cryptococcal antigen tests, rapid CD4 count and viral load measurements would help to guide investment in inpatient management. This could be similar to studies such as REMSTART [44] and REALITY [45] which informed the 2017 WHO recommended package of care for advanced HIV [14] while the package could be delivered to both inpatients and outpatients with advanced HIV, the evidence is from trials in outpatients. There is a need for evidence to optimise the timely diagnosis and management of potential ART treatment failure in inpatients—people who are unwell enough to be admitted to hospital and who are currently taking

ART might have worse outcomes than those not on ART, so this is a priority [46]. Whilst a trial in Uganda over a decade ago didn't show a difference in survival for people offered a linkage to care package (many of whom weren't eligible for ART at the time) vs. no linkage to care, more evidence in the "treat all" era about the best strategies to start, restart or change ART, or otherwise address ART treatment failure, and effectively link people from hospitals to primary care should be another priority. Given the high prevalence of TB among inpatient PLHIV and sub-optimal sensitivity of urine LAM tests, trials of empirical anti-infective treatment of critically ill PLHIV would be helpful, for instance empirical TB treatment with or without additional antibiotics for severe bacterial infection.

Conclusion

Overall mortality was 23% (range 15% to 66%) for participants in this heterogeneous group of inpatient studies from different continents, with different recruitment criteria and different follow up durations. TB screening using urine-based diagnostics reduced mortality, and there was a suggestion of benefit from empirical TB treatment among inpatients that should be investigated further despite the lack of benefit shown for outpatients. Aggressive fluid administration for people who are critically ill should generally be avoided especially where intensive care facilities are not readily available. PLHIV who require hospitalisation should be managed with urgency, with results from trials specific to inpatients ideally used to guide optimal management and improve patient outcomes.

Supporting information

S1 Appendix. Search strategy and ROB Medline search strategy, and risk of bias assessments.
(DOCX)

S2 Appendix. PRISMA checklist.
(DOCX)

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Appendix 1: Search strategy

Medline search strategy

1	Terms related to HIV	1. exp HIV/ 2. exp HIV Infections/ 3. (HIV or human immunodeficiency virus or HIV-1 or HIV-2 or AIDS or Acquired Immunodeficiency or acquired immun* deficiency).mp. 4. 1 or 2 or 3
2	Terms related to inpatients (a)	5. Inpatients/ 6. hospital*.mp. 7. patient*.mp. 8. inpatient*.mp 9. (admission* or readmission*).mp. 10. (admit* or readmit).mp. 11. exp Hospitalization/ 12. discharge*.mp 13. 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12
3	Filter for clinical trials (b)	14. Randomi?ed controlled trial.pt. 15. controlled clinical trial.pt. 16. randomi?ed.ab. 17. placebo.ab. 18. clinical trials as topic.sh. 19. randomly.ab. 20. trial.ti. 21. 14 or 15 or 16 or 17 or 18 or 19 or 20 22. exp animals/ not humans.sh. 23. 21 not 22
4	1 and 2 and 3 and 4	24. 4 and 13 and 23
5	Date of publication filter	25. limit 24 to yr="2003 -Current"

Embase search strategy

1	Items related to HIV	1 exp Human immunodeficiency virus/ 2 exp Human immunodeficiency virus infection/ 3 (HIV or human immun??deficiency virus or HIV-1 or HIV-2 or HIV-i or HIV-ii AIDS or Acquired Immunodeficiency or acquired immun* deficiency).mp 4 or/1-3
2	Items related to inpatients	5 exp hospital patient/ 6 exp hospitalization/ 7 hospital*.mp. 8 patient*.mp. 9 inpatient*.mp 10 (admission* or readmission*).mp. 11 (admit* or readmit*).mp. 12 discharg*.mp. 13 or/5-11
3	Items related to trials (d)	14 (((((random$ or factorial$ or crossover$ or cross over$ or cross-over$ or placebo$ or doubl$) adj blind$) or singl$) adj blind$) or assign$ or allocat$ or volunteer$).mp. 15 exp double blind procedure/ 16 exp crossover procedure/ 17 exp randomi?ed controlled trial/ 18 exp single blind procedure/ 19 or/14-18
4	Combining themes	20 4 and 13 and 19

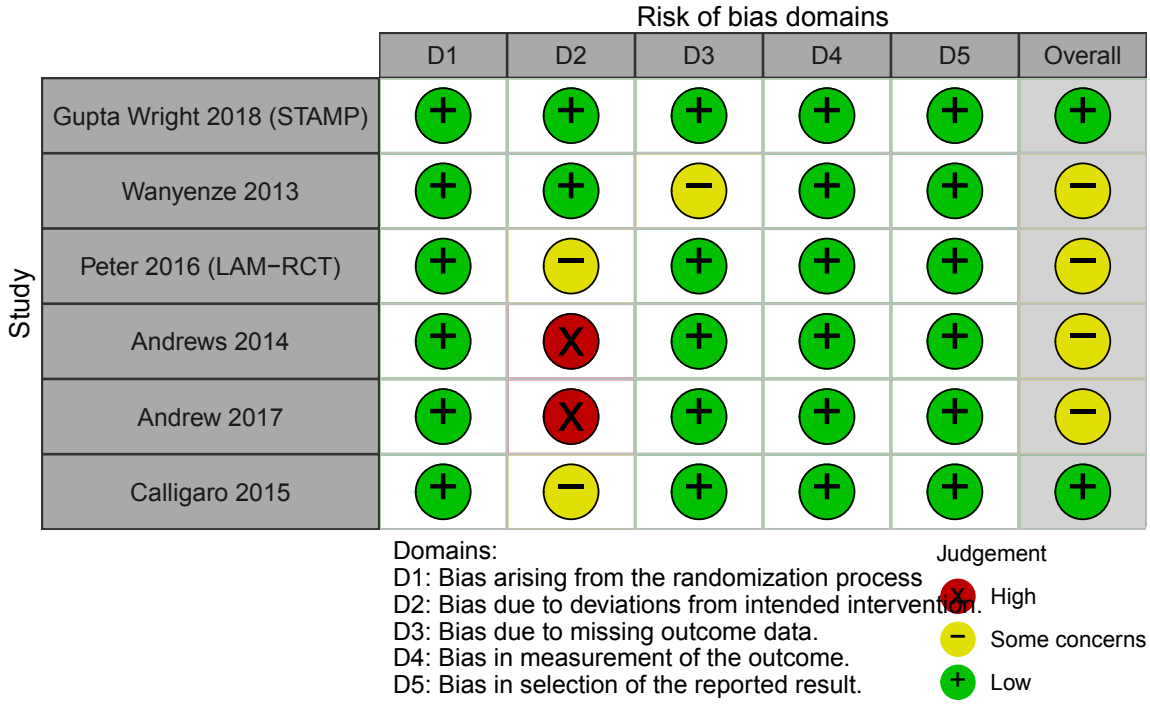
5	Date of publication filter	21 Limit 20 to yr="2003 – Current"

Cochrane Central Search Strategy

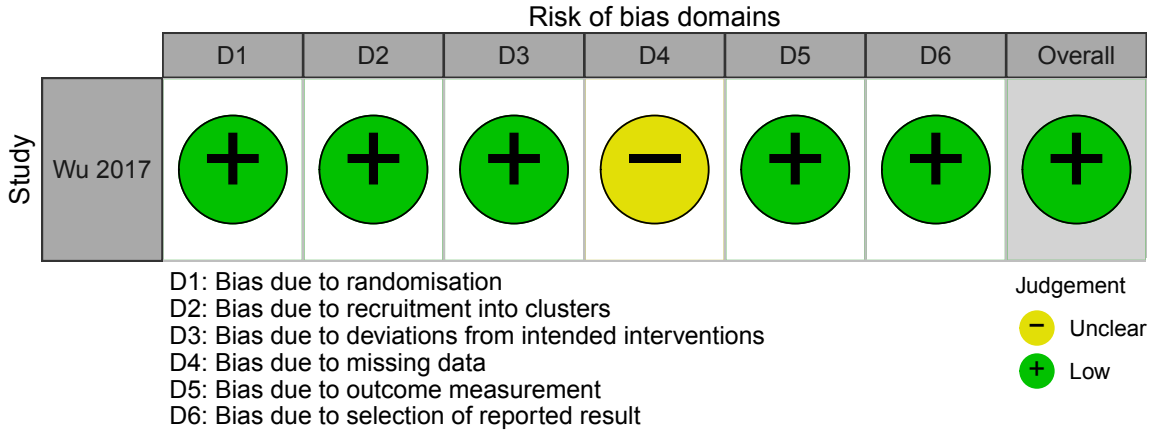
1	Items related to HIV	1 MeSH descriptor: [HIV] explode all trees 2 MeSH descriptor: [HIV infections] explode all trees 3 #1 or #2
2	Items related to inpatients	4 MeSH descriptor [Hospitalization] in all MeSH products 5 MeSH descriptor: [Inpatients] this term only 6 (admission* or readmission*): ti,ab,kw 7 (admit* or readmit*):ti,an.kw 8 (discharge*):ti,ab.kw 9 (patient* or inpatient*):ti,ab,kw 10 #4 or #5 or #6 or #7 or #8 or #9
3	Combining themes	11 #3 and #10
4	Date of publication filter	With publication date from Jan 2003 and Dec 2019

Appendix 2: Risk of bias summary

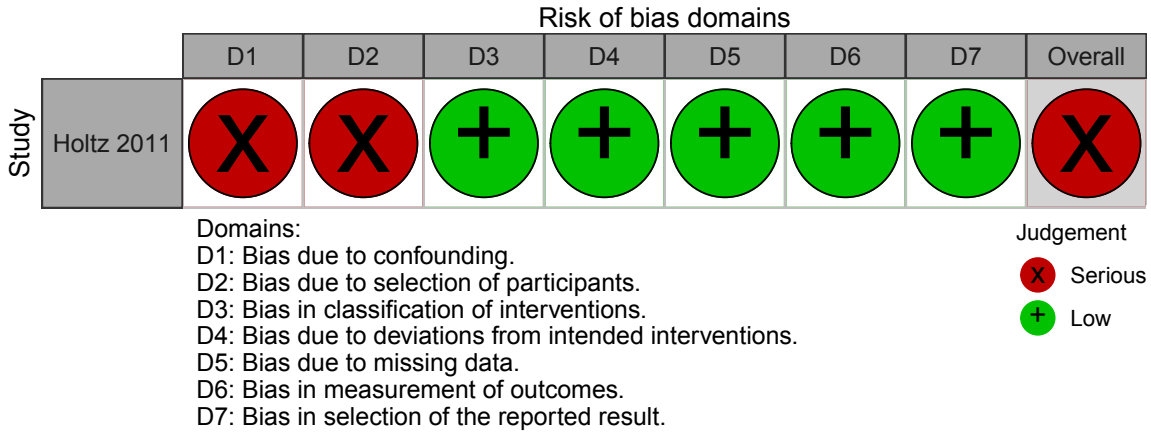
Cochrane ROB2.0 (2019) for individually randomised trials



Cochrane ROB 2.0 (2016) for cluster randomised trial



Cochrane ROBINS-I (2016) for non-randomised study



Chapter 4: Incidence of HIV-positive admission and inpatient mortality in Malawi (2012–2019)

4.1 Introduction

Chapters 2 and 3 clearly demonstrate that, among people living with HIV admitted to hospital in Southern and Eastern Africa, there is a high risk of mortality and, despite being the leading cause of death, TB remains challenging to diagnose. Very few interventions aimed at reducing mortality in PLHIV admitted to hospital have been rigourously evaluated. However, much of the available data comes from years, or decades ago, and before the availability of newer ART regimens, and high population coverage of testing and treatment of HIV. There is urgent need to investigate the epidemiology of HIV-associated hospital admission in this modern era.

In this chapter I use data from the Blantyre City Census and enhanced admission surveillance data from Queen Elizabeth Central Hospital in Blantyre, to look at the population level incidence of HIV-associated hospital admission.

I show that from 2012 to 2019 there was a reduction in HIV associated hospital admission in Blantyre in all age and sex groups, with an estimated 10,818 fewer hospital admissions in these seven years compared to a counterfactual scenario where there was no decline and the incidence of admission stayed the same as it was in 2012 throughout whole period (95% confidence interval 10,068 – 11,568). However, I also show that in-hospital mortality for hospitalised people living with HIV was 23.5%, and that this remained unchanged across this seven year period. This chapter uses an enhanced surveillance system from Queen Elizabeth Central Hospital, but the data available is relatively sparse with no data on reasons for admission, CD4 counts or HIV viral load. There is no follow up beyond hospital discharge, so post-discharge early mortality is not captured.

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SECTION A – Student Details

Student ID Number	1604611	**Title**	Dr
First Name(s)	Rachael Mary		
Surname/Family Name	Burke		
Thesis Title	Improving outcomes for adults living with HIV admitted to hospital in the era of high antiretroviral therapy coverage		
Primary Supervisor	Prof Elizabeth L Corbett		

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?	AIDS		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I cleaned the data, led the design of the statistical analysis, wrote all code and figures and wrote the first draft of the manuscript. I had input from Prof MacPherson for help with correctly coding the statistical analysis, and advice from Dr Marc Henrion (Malawi Liverpool Wellcome Trust) about statistical methods, particularly about missing data handling.

SECTION E

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Date	13 March 2023

Incidence of HIV-positive admission and inpatient mortality in Malawi (2012-2019): a population cohort study

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HIV, epidemiology, hospital, mortality, ART, temporal trends

Abstract

Objective

To investigate trends in population incidence of HIV positive hospital admission and risk of in-hospital death among adults living with HIV between 2012 and 2019 in Blantyre, Malawi.

Design

Population cohort study using an existing electronic health information system ('SPINE') at Queen Elizabeth Central Hospital and Blantyre census data.

Methods

We used multiple imputation and negative binomial regression to estimate population age- and sex-specific admission rates over time. We used a log-binomial model to investigate trends in risk of in-hospital death.

Results

Of 32,814 adult medical admissions during Q4.2012-Q3.2019, HIV status was recorded for 75.6%. HIV-positive admissions decreased substantially between 2012 and 2019. After imputation for missing data, HIV positive admissions were highest in Q3.2013 (173 per 100,000 adult Blantyre residents) and lowest in Q3.2019 (53 per 100,000 residents). An estimated 10,818 fewer than expected people living with HIV (PLHIV) (95%CI 10,068-11,568) were admitted during 2012-2019 compared to the counterfactual situation where admission rates stayed the same throughout this period. Absolute reductions were greatest for women aged 25-34 years (2,264 fewer HIV-positive admissions, 95%CI 2,002-2,526). In-hospital mortality for PLHIV was 23.5%, with no significant change over time in any age-sex group, and no association with ART use at admission.

Conclusions

Rates of admission for adult PLHIV decreased substantially, likely due to large increases in community provision of HIV diagnosis, treatment and care. However, HIV-positive in-hospital deaths remain unacceptably high, despite improvements in ART coverage. A concerted research and implementation agenda is urgently needed to reduce inpatient deaths among PLHIV.

Introduction

The Joint United Nations Programme on HIV/AIDS (UNAIDS), national country HIV programmes and many other actors in the HIV community share a common goal to end AIDS as a public health problem by 2030. In sub-Saharan Africa, great progress has been made towards goals of achieving 95% of people living with HIV knowing their status, 95% of those who know their status to be taking antiretrovial therapy (ART), and 95% of those of those taking ART to have undetectable HIV viral loads. Malawi is one of countries worst affected by the HIV epidemic, with estimated adult HIV prevalence in 2019 of 8.9% nationwide and 17.7% in Blantyre City.[1] In the past two decades the Malawi national HIV programme has made excellent progress in providing HIV testing, ART and other HIV care services; in 2019, 90% of all PLHIV in Malawi knew their HIV status, 88% of those who knew their status were taking ART and 92% of those on ART were virally suppressed.[2]

Despite increasing population ART coverage, the number of PLHIV becoming unwell and attending hospital has remained high in several countries in Southern and Eastern Africa. For example, 60% of hospital admissions to a general hospital in South Africa were related to HIV in 2012-13, despite widespread ART availability in the community at that time.[3] Similarly, 50% and 42% of admissions to hospital in Lilongwe, Malawi between 2011 and 2012 and Kweneng East District, Botswana between 2015 and 2016, respectively, were related to HIV.[4,5] Another study found that 83% and 97% of PLHIV admitted to hospitals in Kenya and DRC respectively had advanced immunosuppression (CD4 <200 cells/mm^3).[6] In general, hospital epidemiological data related to HIV in Southern and Eastern Africa is sparse. In Johannesburg, South Africa, 39% of people initiating ART in 2017 had CD4 <200, indicating that advanced HIV remains a persistent challenge.[7–9]

We used routine hospital data and city census data to investigate changes in HIV-positive hospital admissions to adult medical wards over time in Blantyre, Malawi, where there is only one public hospital serving the population, acting as both District General hospital and a tertiary referral hospital. The primary objective was to assess time trends in the incidence (i.e. number of hospital admissions per 100,000 population) of HIV-positive hospital admission for Blantyre residents between 2012 and 2019. The secondary objective was to investigate whether hospital admission outcomes (died vs. discharged from hospital alive) for people living with HIV (PLHIV) have changed over time.

Methods

Setting

Blantyre district contains the second largest city in Malawi (Blantyre City) and it's surrounding periurban / rural area. At the 2018 census, Blantyre district had a population of approximately 1.2 million people with a median age 17 years.[10] One main government hospital (Queen Elizabeth Central Hospital, QECH) provides free secondary and tertiary care to the population of Blantyre, including inpatient medical care. There are some smaller private (including private-not-for-profit) hospitals accessed by a small sub-set of the population who can afford the fees, but the vast majority of people living in Blantyre rely on QECH exclusively for inpatient care. QECH provides a range of general medical services, HIV testing (provider-initiated testing and counselling [PITC]) and ART. QECH has 120 general adult medical beds and this capacity hasn't substantially changed between 2012 and present.

Population and data sources

Since late 2009 adult medical admissions to QECH have been recorded in in an electronic surveillance system (Surveillance Programme of IN-patients and Epidemiology [SPINE]) by data clerks working on both of the medical admissions wards.[11] For all patients admitted to the ward, data clerks recorded: sex, age, neighbourhood of residence, date of admission, HIV status, ART status and outcome (discharge from hospital alive vs. died prior to discharge). Individual patients are not linked over time, and results of CD4 cell counts or HIV viral load tests are not recorded. ART status was ascertained from medical notes or patient-held record ("health passport") during admission. Quality is assured by reconciling admissions with government paper ledgers, nurses' paper records and data clerks physically walking around bed spaces each morning. There was some interruption to SPINE data collection in 2011–2012, so we included medical admissions recorded by SPINE from October 2012 to September 2019. We removed duplicate records, records for people under 15 years old and records for inpatients who reported residing outside of Blantyre. We assumed that those with missing location data lived in Blantyre.

The government of Malawi conducted population censuses in 2008 and 2018. Mid-quarter population estimates for Blantyre (combining "Blantyre urban" and "Blantyre rural" districts) for each quarter between October 2012 and September 2019 were calculated by linear interpolation and extrapolation, by 10-year age group and sex.

Statistical analysis

Characteristics of patients admitted to QECH medical wards were summarised using percentages, and compared to interpolated Blantyre census data. Where data on HIV status, ART and outcome were

147 missing in SPINE, we used multiple imputation by chained equations (using the 'mice' package in R)
148 with predictive mean matching to impute missing data.[12] Variables used for imputation were HIV
149 status, age group, quarter-year, sex and outcome. Missing ART status for the small number of people
150 who reported being HIV positive was also imputed based on above variables. Since ART status
151 missingness is conditional on HIV status missingness, we did not impute ART status for people who
152 had missing or unknown HIV status in SPINE. For the secondary outcome assessing assosciations with
153 in-hospital death we assumed that everyone who was HIV positive (based in imputation) but had an
154 unknown or missing HIV status in SPINE was not taking ART – this was not relevant for the primary
155 outcome of incidence of admission. We imputed 25 datasets (reflecting the ~25% missingness of ART
156 status), and combined model outputs across all 25 datasets using Rubin's rules.[13–15] Sensitivity
157 analyses were performed by conducting complete case analysis; for HIV-related admission incidence
158 analysis, complete case analysis is equivalent to assuming all participants with unknown HIV status
159 were HIV-negative.
160
161 We estimated the incidence of HIV-positive and HIV-negative admission to hospital among Blantyre
162 residents per quarter-year between Q4.2012 and Q3.2019 overall, and separately for each age group-
163 sex-quarter strata. To investigate trends in admission over time, we fitted a negative binomial
164 regression model (because the data were overdispersed) with interactions between age group, sex
165 and quarter, and a natural cubic spline term with three knots for annual quarter. Age group and sex
166 were included as interaction variables in the models *a priori* because there are sex and age-group
167 specific differences in HIV incidence, prevalence, and access to testing and ART services. We
168 performed sensitivity analyses using the Poisson and gamma response distributions, and separately
169 without spline terms.
170
171 To quantify the magnitude of change in admissions over the study period overall, and for each age
172 group-sex strata, we calculated the expected number of admissions under the counterfactual
173 condition where the incidence of HIV positive admission remained constant as the model predicted
174 for Q4.2012 (ie. the first quarter of observation) over the entire study period, and subtracted from the
175 model-predicted number of admissions. Confidence intervals were estimated using parametric
176 bootstrap resampling.
177
178 Temporal trends in the risk of inpatient death were analysed using a generalised linear model with
179 log-binomial link function to approximate risk of death. Age group and sex were included as
180 interaction variables *a priori*. We investigated whether adding ART use at admission (including ART

used as an interaction variable with age, sex and quarter-year) improved model fit using Akaike information criteria.

Ethical approval, funding and data sharing

Use of anonymous electronic data (from SPINE project) was approved by QECH hospital research committee. Individual patient consent for anonymised secondary analysis was not sought.

All code for analyses, Blantyre census dataset, datapoints from figures and a 'synthetic' (i.e. artificial data that mimics properties of real data) dataset for hospital admissions are available online at https://rachaelmburke.github.io/hivhospital/. Synthetic data was created using synthpop package.[16] Further details including how to access real data are included in data sharing statement.

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Results

During the 28 quarters between October 2012 and September 2019, there were 32,814 medical admissions to QECH among adults (age >= 15 years) who resided in Blantyre (median quarterly admissions 154 per 100,000 people). There were a further 5,511 people admitted to QECH who reported residing outside of Blantyre, and their data were excluded from this analysis. Fifty percent (16,408) of these were known to be HIV-positive, and in 24% (7,996) of admissions, HIV status was unknown (Table 1).

Incidence of HIV related admission

The median number of known HIV-positive admissions (i.e. before imputation for missing HIV status) to QECH per quarter-year was 592 (80 per 100,000 Blantyre population). It was highest in Q3 2014 (767 known-HIV admissions, 110 per 100,000) and lowest in Q2.2019 (343 known HIV admissions, 44 per 100,000) in Q2 2019. In contrast, known HIV-negative admissions were at their lowest towards the start of the study period, with 160 admissions (23 per 100,000 population) in Q3 2013 and highest in Q3.2019 with 482 admissions (61 per 100,000). The number of admissions with unknown or missing HIV status decreased throughout the study period, with a 695 admissions with HIV status missing or

unknown in Q3 2017 (102 per 100,000) and 104 HIV unknown admissions in Q1.2017 (13 per 100,000). The proportion of people currently taking ART among known PLHIV admitted to hospital increased from 66% (363/550) in Q4 2012 to 94% in Q3 2019 (372/402); the denominator includes those who knew their HIV status prior to admission and those newly diagnosed in hospital, but not those who had missing or unknown HIV status recorded. Supplementary Figures 1A-C show HIV status, absolute number and population incidence of admissions over time. The adult Blantyre mid-year census population was 577,893 in 2008 and was 764,323 in 2018. The estimated population in February 2016 (i.e. the mid study period) was 722,377 (Supplementary Table 1B).

Table 1 Characteristics of adult medical admissions to Queen Elizabeth Central Hospital, Malawi, Q4 2012 – Q3 2019, and population demographics of Blantyre in Feb 2016 (midpoint Q3 2012 – Q3 2019)

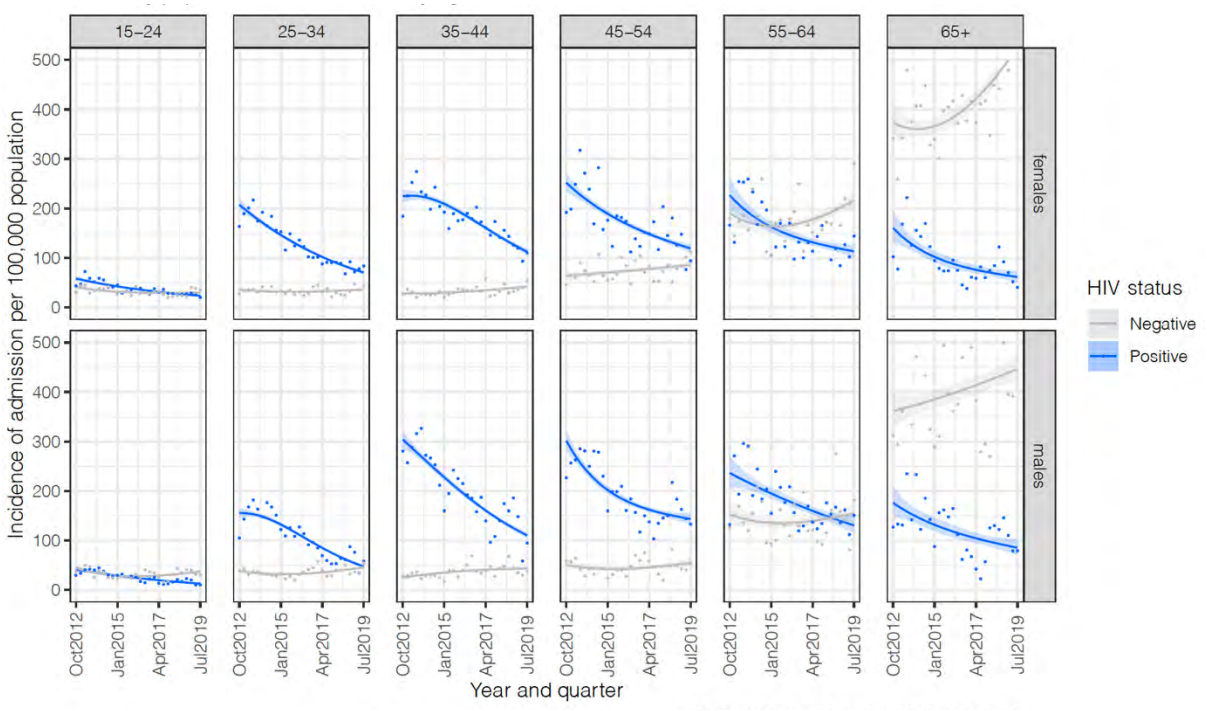
	Adult medical admissions Oct 2012 to Sept 2019 (N=32,814)	**Blantyre population estimates Feb 2016 (N=722,377)**
Age (years)		
15-24	4,808 (14.7%)	270,260 (37.4%)
25-34	8,404 (25.6%)	197,589 (27.4%)
35-44	8,161 (24.9%)	131,376 (18.2%)
45-54	4,074 (12.4%)	60,267 (8.3%)
55-64	2,968 (9.0%)	32,416 (4.5%)
65+	4,399 (13.4%)	30,469 (4.2%)
Sex		
Females	16,618 (50.6%)	361,988 (50.1%)
Males	16,196 (49.4%)	360,389 (49.9%)
HIV status		
Negative	8,410 (25.6%)	
Positive	16,408 (50.0%)	
Missing or unknown	7,996 (24.4%)	
ART status (HIV positive only)		
Currently taking ART	13,074 (79.7%)	
Not currently taking ART	3,050 (18.6%)	
Missing or unknown	284 (1.7%)	
Outcome from hospital admission		
Alive	24,056 (73.3%)	
Dead	6,071 (18.5%)	
Missing or unknown	2,687 (8.2%)	

Multiple imputation and modelling trends in incidence of HIV-related hospital admission

After using multiple imputation to impute HIV status for the 24% (7,996/32,814) of people where it was unknown, estimated true HIV positive admissions were highest in Q3.2013 with 1169 admissions (173 per 100,000) and lowest in Q2.2019 with 417 admissions (53 per 100,000). If we assume that all those with missing or unknown HIV status in SPINE but who were HIV positive based on imputation were not taking ART, then ART coverage increased from 48% in Q4.2012 to 76% in Q3.2019.

Using regression modelling with parameters averaged across 25 multiply-imputed datasets, we estimate that the true number of HIV-positive hospital admissions between Q4.2012 and Q3.2019 (inclusive) was 21,170 (95% confidence interval [CI] 20,411–21,928). Between October 2012 and September 2019, the modelled trend of incidence of HIV-positive hospital admission in Blantyre decreased in all age and sex groups (Figure 2). In sensitivity analysis, this overall finding was robust to reclassification of missing HIV data (Table 2 and Supplementary Figures 2A and 2B), and to model specification (Supplementary Figures 3A-3D).

Figure 1: Population incidence of hospital admission to medical wards QECH by HIV status Q3 2012 – Q3 2019.



If the age group- and sex-specific incidence of HIV related hospital admissions had stayed the same throughout the period October 2012–September 2019 as it was in Q4.2012, then we would have

252 expected to see 31,988 (95% CI 31,229–32,746) HIV-positive admissions, taking into account the
253 increasing population of Blantyre. Therefore, we estimate that there were 10,818 (95% CI, 10,060–
254 11,577) fewer HIV-positive admissions during this period than there would have been under
255 counterfactual scenario where incidence of admission had remained constant during this period
256 (Table 2). This is equivalent to 33.8% fewer HIV-positive admissions (95% CI 32.3% to 35.4%).
257
258 The greatest reductions in absolute numbers of admissions compared to expected number of
259 admissions had there been no change in population incidence of admission were in women aged 25–
260 34 years old and men aged 35–44 years old. The smallest magnitude of absolute decline in admissions
261 were in men aged 55–64 years old and men age 65+ (Figure 2 and Supplementary Table 2).
262
263 These estimates were robust to reclassification of missing HIV status. If all admissions with missing
264 HIV status were considered to be HIV-negative we estimate there would have been 3,854 (95% CI:
265 3,453 to 4,255) fewer HIV-positive admissions (equivalent to a 19.0% decrease), and if all admissions
266 with unknown HIV status were considered HIV-positive, there would have been 13,865 (95% CI: 13,050
267 to 14,681) fewer admissions (equivalent to a 36.2% decrease). In the sensitivity analysis scenario
268 where all patients with missing HIV status were classified as HIV-negative, while overall HIV-positive
269 admissions decreased, but there was no decrease in admissions among women aged 45 years or older,
270 nor among men aged 65 years or older (Supplementary Table 2 and Supplementary Figure 2B).
271
272 During this period the incidence of HIV negative hospital admissions stayed the same or increased in
273 all age and sex groups (Figure 2) and increased substantially among those 65 years or older.
274
275

Table 2: Estimates of magnitude of reduction of HIV-related admissions

	Model predicted HIV-related admissions (Q4. 2012 – Q3. 2019), 95% confidence interval)			
Scenario	**Number of HIV-related admissions predicted if incidence was the same throughout period as it was in Q4.2012 (A)**	**Estimated number of HIV-related admissions from regression model (B)**	**Absolute number fewer HIV-related admissions (A-B) ***	**Relative percentage decline in HIV related admissions (A-B / A) ***
HIV status imputed when missing	31,988 (31,268 to 32,708)	21,170 (21,109 to 21,230)	10,818 (10,093 to 11,544)	33.8% (32.3 to 35.4%)
All HIV unknown / missing positive	38,270 (37,457 to 39,082)	24,404 (24,344 to 24,465)	13,865 (13,050 to 14,681)	36.2% (34.8 to 37.6%)
All HIV unknown / missing negative	20,262 (19,863 to 20,660)	16,408 (16,372 to 16,443)	3,854 (3,453 to 4,255)	19% (17.3 to 20.7%)

* Compared to counterfactual if admission incidence had stayed the same as it was in Q4.2012.
95% confidence intervals estimated through parametric bootstrapping of 25 multiply-imputed datasets

Outcomes for PLHIV admitted to QECH

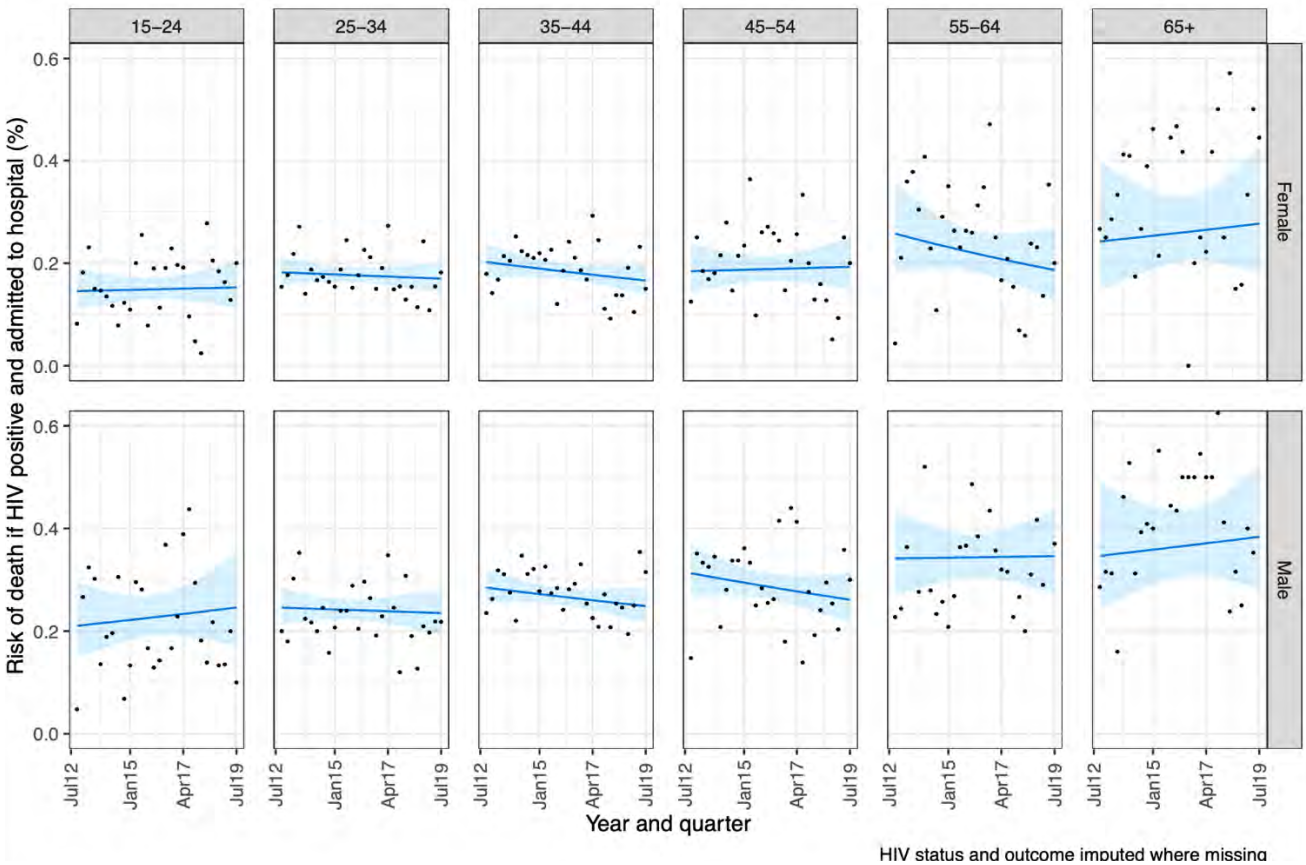
Overall, 18.5% (6,071/32,814) of adults admitted to QECH died during their admission, and a further 8% (2,687 / 32,814) had unknown outcome or missing outcome data. After multiple imputation, we estimate the proportion of adult medical inpatients who died to be 20.3% overall and 23.5% among PLHIV (Table 3). Supplementary Table 3A and 3B show outcomes by age group and sex.

Table 3: Outcome of hospital admission (dead or discharged alive) by HIV and ART status

	Alive	**Dead**	**Outcome missing**	**Overall**
Data without imputation				
HIV negative	6767 (80.5%)	952 (11.3%)	691 (8.2%)	8410 (100%)
HIV positive (overall)	11387 (69.4%)	3276 (20.0%)	1685 (10.3%)	16408 (100%)
HIV positive, ART status unknown	177 (62.3%)	53 (18.7%)	54 (19.0%)	284 (100%)
HIV positive, not on ART	2200 (72.1%)	558 (18.3%)	292 (9.6%)	3050 (100%)
HIV positive, on ART	9070 (69.4%)	2665 (20.4%)	1339 (10.2%)	13074 (100%)
HIV status unknown or missing	5842 (73.1%)	1843 (23.0%)	311 (3.9%)	7996 (100%)
TOTAL	24056 (73.3%)	6071 (18.5%)	2687 (8.2%)	32814 (100%)
Data with imputation (mean of 25 imputations)				
HIV negative	9935 (85.4%)	1701 (14.6%)	-	11636 (100%)
HIV positive (overall)	16249 (76.7%)	4929 (23.3%)	-	21178 (100%)
HIV positive, no ART status as HIV status imputed (likely no ART)	3495 (73.3%)	1275 (26.7%)	-	4770 (100%)
HIV positive, not on ART	2482 (79.5%)	640 (20.5%)	-	3122 (100%)
HIV positive, on ART	10272 (77.3%)	3014 (22.7%)	-	13286 (100%)
TOTAL	26184 (79.8%)	6630 (20.2%)	-	32814 (100%)

Risk of inpatient death did not change over the study period overall, or within any age-sex subgroups (Figure 2 and Supplementary Table 4). This finding was robust to sensitivity analyses for misclassification of HIV status and outcome (complete case analysis - Supplementary Figure 4). Reported ART use at admission did not affect the risk of in-hospital death and did not improve the model fit (Supplementary Figure 5); Akaike information criteria statistics were higher in models that included ART as a covariate in all 25 imputed datasets. Risk of death was higher for people living with HIV than people without HIV in all age and sex groups (Table 3, Supplementary Table 3, and Supplementary Figure 6).

Figure 2: Risk of inpatient death among PLHIV if admitted to Queen Elizabeth Central Hospital, Malawi, Q3 2012 – Q3 2019. Log-binominal model.



Discussion

We used electronic inpatient records and national census data to show that between 2012 and 2019, per capita rates of HIV-positive medical admissions in Blantyre, Malawi decreased substantially. There were an estimated 10,818 (95% CI: 10,068 to 11,568) fewer HIV-positive admissions to the single public hospital than would have been expected if admission rates had been unchanged from the last quarter of 2012. These data were adjusted for population growth, and excluded tertiary admissions referred from districts outside of Blantyre. The likely driver was ART scale-up, with substantial increases in community ART coverage during this time, consistent with the observed increase in the proportion of HIV-positive patients already on ART at the time of admission. Once admitted, however, mortality remained extremely high with 23.5% of PLHIV dying before discharge, no obvious improvements over time, and no benefits from being on ART at the time of admission. High in-patient mortality following medical admission in Africa is a critical issue that needs to be investigated and addressed urgently.

The substantial reduction in admission rates is an encouraging finding, and is congruent with other data which indicate that the proportion of people living with HIV in Blantyre who know their status, are on treatment, and are virally supressed and therefore not experiencing medical complications has increased considerably between 2012 and 2019,[2] a tremendous testament to the Malawian National HIV Programme. Alternative explanations for our findings are less likely. Queen Elizabeth Central Hospital is the single government hospital for the city, and care has remained free of charge and available to the population with no substantial changes or prolonged disruption to services during this time. Of note, this analysis ends in September 2019, before any COVID-19 related disruption. Incidence of HIV-negative hospital admissions stayed the same or increased in every age and sex group during this time, consistent with investments in health system strengthening and indicating that the decline in HIV positive admissions is not a data capture issue.

To put these results into context; estimated national adult HIV prevalence in Malawi was relatively static between 2012 and 2019, although AIDS deaths and new HIV infections fell, concurrent with rising coverage of ART.[17] There are limited subnational HIV estimates for Blantyre derived from Naomi / Spectrum model, with estimates available for March 2016 and December 2019 only.[18,19] Similar to the national picture, Blantyre adult HIV prevalence was largely unchanged: 17.0% in March 2016 and 16.7% in December 2019. Blantyre ART coverage increased, from 60.1% in March 2016 to 73.6% in December 2019; which is similar to our observed ART coverage. Nationally, the peak of AIDS related deaths in Malawi was in 2004 with 71,000 deaths, several years before the SPINE database was

instituted. Between 2012 and 2019 (i.e. the dates of this analysis) national HIV related deaths declined from 24,000 annually to 13,000, with steeper declines at the start of this time period. There are no subnational estimates for deaths. As a rough estimate - assuming that the proportion of HIV related deaths in Blantyre vs. rest of Malawi is the same as the proportion of people living with HIV in Blantyre vs. rest of Malawi - in 2018 between one quarter and one third of all Blantyre HIV related deaths occurred in QECH and were captured in this analysis. Our hospital observations are consistent with the modelled national and subnational trends – this analysis provides a further demonstration from empiric longitutinal data (rather than modelled data) of the impact of ART on the HIV epidemic in Malawi.

Once admitted to medical wards the risk of in-hospital death remained high and unchanged throughout the seven-year study period, being 23.5% for HIV-positive medial inpatients and 14.5% for HIV-negative inpatients, once missing HIV status and outcomes were imputed. Although ART coverage among PLHIV admitted to hospital increased substantially between 2012-19 (commensurate with increasing population ART coverage), taking ART at admission did not alter risk of death. The impact of virological failure in this cohort can only be inferred, as HIV viral load measurement on admission is not currently supported by the routine medical services, and data on HIV viral loads are not routinely captured. Studies that have measured HIV virologic failure among people in hospital have shown similarly high mortality and high levels of proven HIV virologic failure among people admitted to hospital. In the STAMP trial in 2015 to 2017 in Zomba Central Hospital, Malawi (in a nearby district to Blantyre), 32% of all PLHIV admitted to hospital had confirmed HIV virologic failure and this was associated with increased risk of death.[20] Other African studies report a high prevalence of HIV virologic failure among PLHIV admitted to hospital; 63% and 62% in Kenya and Democratic Republic of Congo, respectively.[6] In a predictive model developed using STAMP data and validated on cohorts from another multi-centre trial and a cohort in Kenya, use of ART at admission to hospital was associated with increased risk of death by two months from admission.[21] In the present analysis, use of ART made no difference to risk of in-hospital death.

At the start of the study period slightly less than half of all people with HIV were taking ART. It is possible that, for those that survived the acute illness that precipitated admission, effective ART could be started and outcomes may be relatively favourable. By the end of the study period three quarters of HIV positive peole admitted to hospital were taking ART. If a substantial proportion of those on ART had HIV virologic failure and were not switched to effective ART, then they may be discharged with their acute illness treated, but the underlying immunosuppression that precipitated the illness unresolved. At present, WHO guidelines for managing confirmed or suspected HIV virologic failure do

not distinguish between stable ambulatory outpatients and unwell patients admitted to hospital, and recommend enhanced adherence counselling following identification of an elevated HIV viral load.[22] There are scant data to address this issue or provide guidance as which groups of people require urgent ART switch and in which groups of people adherence counselling and repeat viral load may be appropriate.

In a meta-analysis of PLHIV admitted to hospital, AIDS-related conditions (including tuberculosis and cryptococcal meningitis) and severe bacterial infections were the most common causes of admission and death,[23] consistent with previous data from QECH about cause of admission,[11] and suggesting that for most people living with HIV their HIV status is not incidental to the reason for hospital admission. Two trials have shown that urine-based TB diagnostics reduce deaths of PLHIV in hospital,[20,24] and several trials have shown effectiveness of newer antifungal treatments for cryptococcal meningitis.[25,26] However, there are no trials of pragmatic management protocols (which might include a package of diagnostics), or of interventions to optimise management of virological failure among people in hospital. In the era of universal ART coverage, PLHIV admitted to hospital should be managed with great urgency, given their high risk of imminent death, and we urge more trials to produce evidence-based pragmatic management protocols similar to those recently developed for patients with low CD4 counts.[27,28]

There are some limitations to this work. We do not have information on cause of admission or cause of death for those who died. Similarly, we do not have information on HIV viral loads or CD4 counts, to be able to measure prevalence of advanced HIV or HIV virologic failure directly. There was no follow up beyond length of hospital stay to ascertain mortality in the immediate period after admission. Malawi has very recently switched first-line ART to a dolutegravir-based regimen, away from reliance on non-nucleoside reverse transciptase inhibitors (NNRTIs), including switching those who are stable on NNTRI-containing ART regimens; the switch occurred in 2019, but this is too early to observe if this will have causes any change in HIV related hospital admissions. QECH has a large outpatient ART service, so it is possible that people who were taking ART were more likely than those not on ART to be admitted to hospital (either due to emergency referral from ART clinic or from being familiar with services available at the hospital). This might mean that the proportion of inpatients taking ART is higher than the proportion of all people who are sick (but don't access QECH hospital care) who are taking ART.

In conclusion, the incidence of HIV-positive hospital admission in Blantyre has substantially reduced in the seven years between Q4.2012 to Q3.2019, in keeping with impressive gains in coverage of HIV

testing, treatment and care in Malawi during this period. However, PLHIV who were admitted to hospital continued to experience extremely high in-hospital mortality that did not change throughout this period. This suggests that advanced HIV and HIV-related complications remain persistent clinical and public health challenges, even as large improvements are made in providing HIV testing and care services to the majority of community members in Malawi. Interventions to reduce deaths in PLHIV following admission to hospital, including prompt management of HIV virologic failure in unwell and unstable patients, are an urgent research priority.

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List of tables and figures

Data sharing statement

All code for analysis and the Blantyre Census population denominator data is freely available online at https://rachaelmburke.github.io/hivhospital/. Unrestricted access to the SPINE dataset cannot be provided due to risk of reidentification of individuals. Instead a "synthetic" dataset is provided, created using 'synthpop' package in R statistical software. Synthetic data is artificial data that mimics some properties of the real data. It is intended to be used to be able to run and understand our code, but is not suitable for use in further analyses.

The Malawi Liverpool Wellcome data department may be able to facilitate access to the real SPINE dataset and can be contacted on data@mlw.mw. Permission from QECH hospital is likely to be required. The first (rachael.burke@lshtm.ac.uk) and last author (peter.macpherson@lstmed.ac.uk) can also be contacted to enquire about how to access SPINE data.

The dataset used for this cannot be analysis is anonymous and contains six variables (age, date of admission, sex, HIV status, ART status and outcome). Some of these combinations of variables include only one person and there is a theoretical risk of re-identification and disclosure of HIV status. Therefore it cannot be shared without restriction.

Pre print server

A earlier version of this manscript is available on SSRN pre-print server ("Preprints with the lancet")

Burke, Rachael Mary and Henrion, Marc Y. and Mallewa, Jane and Masamba, Leo and Kalua, Thokozani and Khundi, McEwan and Gupta-Wright, Ankur and Rylance, Jamie and Gordon, Stephen B. and Masesa, Clemens and Corbett, EL and Mwandumba, Henry C. and MacPherson, Peter, Incidence of HIV-Related Hospital Admission and Inpatient Mortality in Malawi (2012-2019): A Population Cohort Study. Available at SSRN: https://ssrn.com/abstract=3751320 or http://dx.doi.org/10.2139/ssrn.3751320

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Developed concept of study: RMB, MYRH, JR, HM, PM. Responsible for data collection and curation: JM, LM, CM. Performed or assisted with statistical analysis: RMB, MYRH, MK, PM. Wrote first draft: RMB, PM. Contributed to writing manuscript and putting research in context: RMB, JM, LM, AGW, JR, TK, SBG, HM. All authors approved final draft.

This is supplementary material related to paper

Burke, Rachael M.[a,b]; Henrion, Marc Y.R.[b,c]; Mallewa, Jane[d]; Masamba, Leo[d]; Kalua, Thokozani[e]; Khundi, McEwan[b,f]; Gupta-Wright, Ankur[a]; Rylance, Jamie[b,c]; Gordon, Stephen B.[b,c]; Masesa, Clemens[b,c]; Corbett, Elizabeth L.[a,b]; Mwandumba, Henry C.[b,c]; Macpherson, Peter[a,b,c] **Incidence of HIV-positive admission and inpatient mortality in Malawi [2012–2019]**, AIDS: July 01, 2021 - Volume - Issue - doi: 10.1097/QAD.0000000000003006

Supplementary figures 1A – B Absolute numbers and population incidence of admission to QECH, by HIV status.

Supplementary tables 1A and 1B Measured population of Blantyre, Malawi at census in 2010 and 2018, and interpolated / extrapolated population at Q4.2012 and Q3.2019.

Supplementary figures 2A and 2B Sensitivity analyses of incidence of admission, with different missing data handling choices.

Supplementary table 2 Reduction in admission to QECH by age group and sex.

Supplementary figures 3A – D Modelled and observed population incidence of HIV related hospital admission, using various different choices of model (Poisson distribution, Gamma distribution, negative binominal without splines, negative binomial with splines with five knots)

Supplementary table 3 Risk of death if admitted to QECH by age group, sex and HIV status.

Supplementary figure 4 Risk of death if living with HIV (complete cases analysis).

Supplementary figure 5 Risk of death by quarter year, age group, sex and ART status.

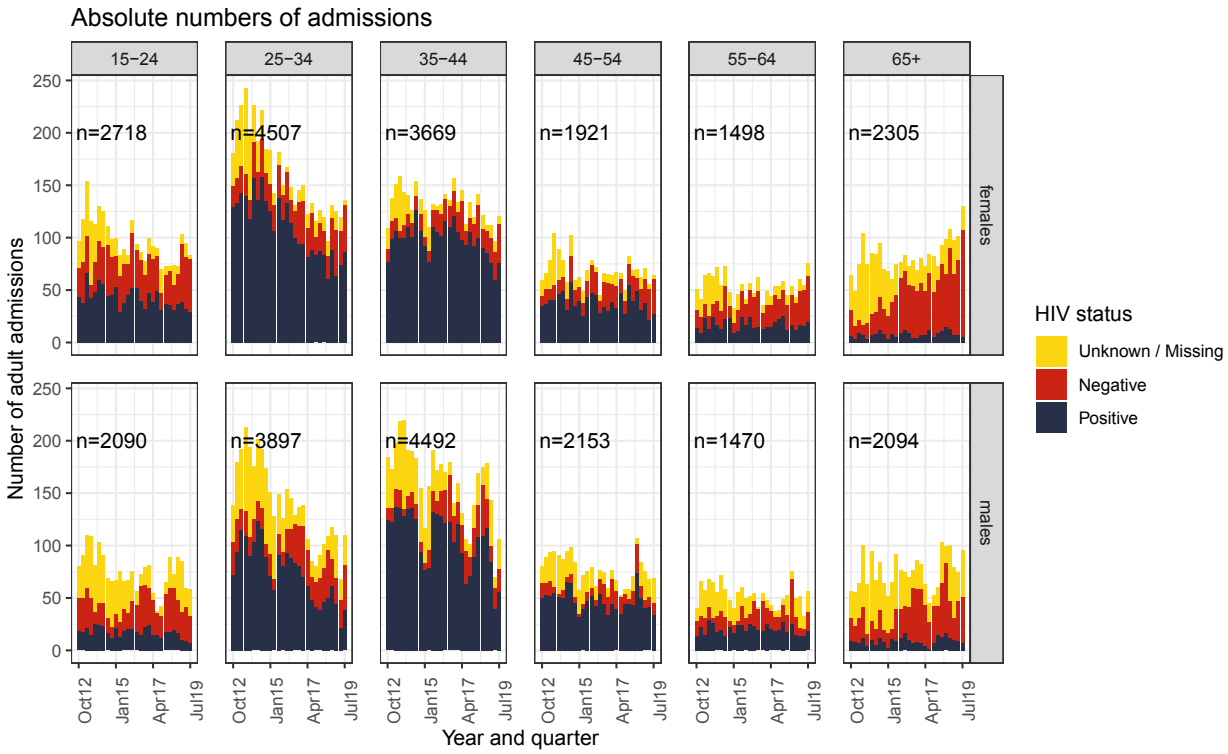
Supplementary figure 6 Risk of death by quarter year, age group and HIV status.

Supplementary table 4 Trend by quarter year in risk of death if living with HIV and admitted, by age and sex.

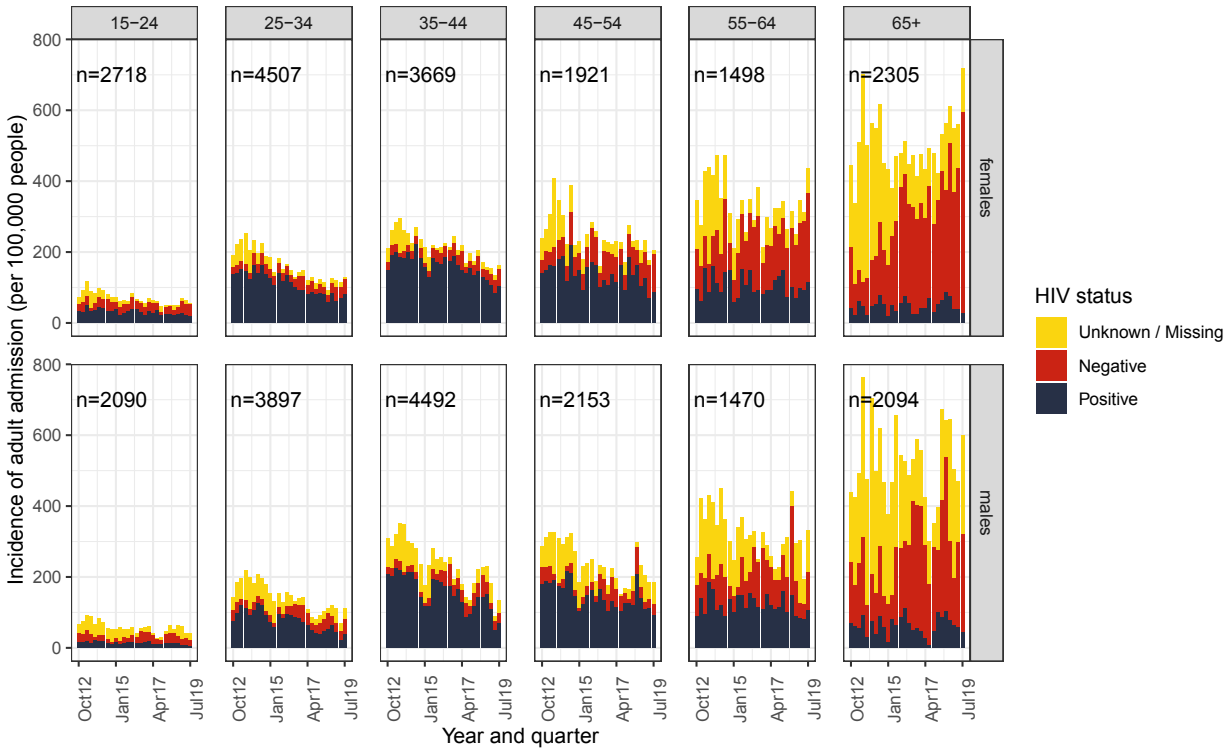
Information about people who resided outside of Blantyre (and are excluded from this analysis)

Supplementary Figure 1: Admissions by age, sex, HIV and quarter-year

S. Figure 1A: Absolute numbers of admissions, stacked bar chart of crude data.



S. Figure 1B: Population level incidence of admissions, stacked bar chart of crude data.



Supplementary table 1: Blantyre census for 2008 and 2018

S Table 1A: Measured population Blantyre (includes Blantyre City and Blantyre Rural administrative districts) at 2008 and 2018 census

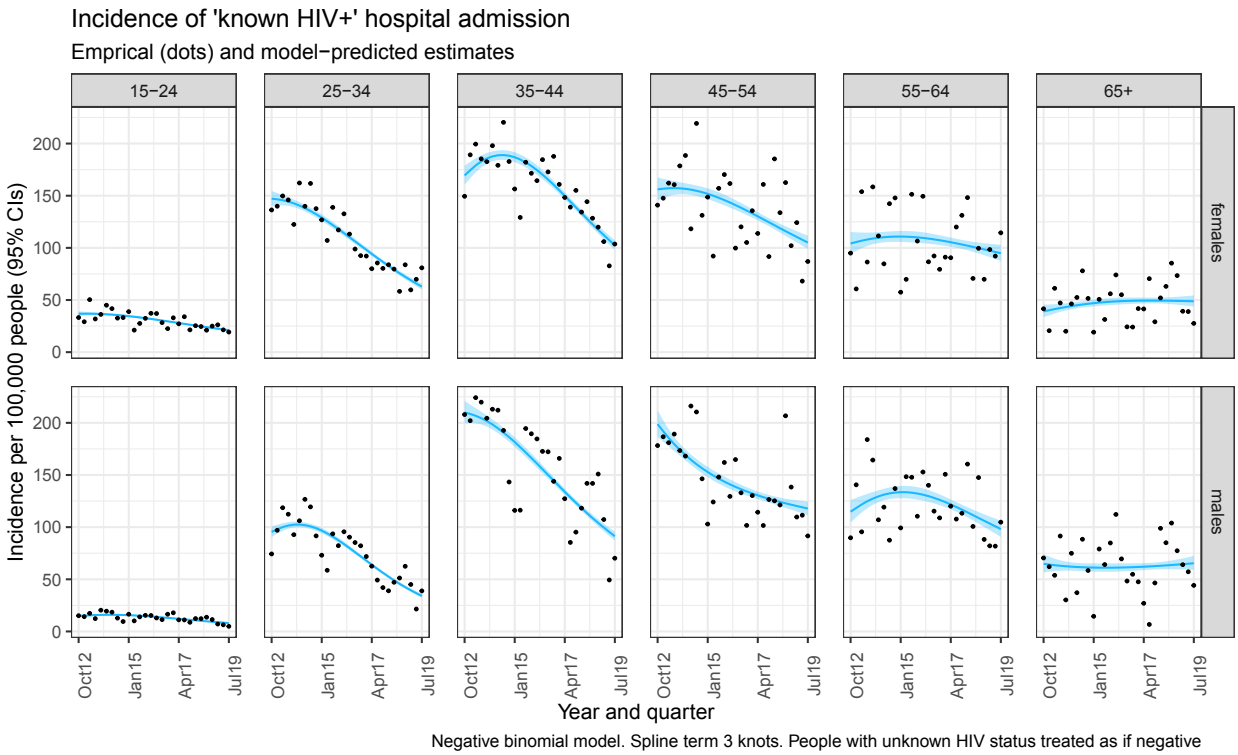
ageg10	sex	2008-04-01	2018-04-01
15-24	females	115573	146543
15-24	males	103290	138639
25-34	females	86762	104200
25-34	males	96314	97602
35-44	females	36968	69311
35-44	males	46176	76067
45-54	females	20676	29912
45-54	males	22000	35461
55-64	females	12915	16974
55-64	males	14529	16887
65+	females	11962	17441
65+	males	10728	15286

S Table 1B: Population Blantyre at start (Q4.2012) and end (Q3.2019) study period [calculated using linear interpolation and extrapolation]

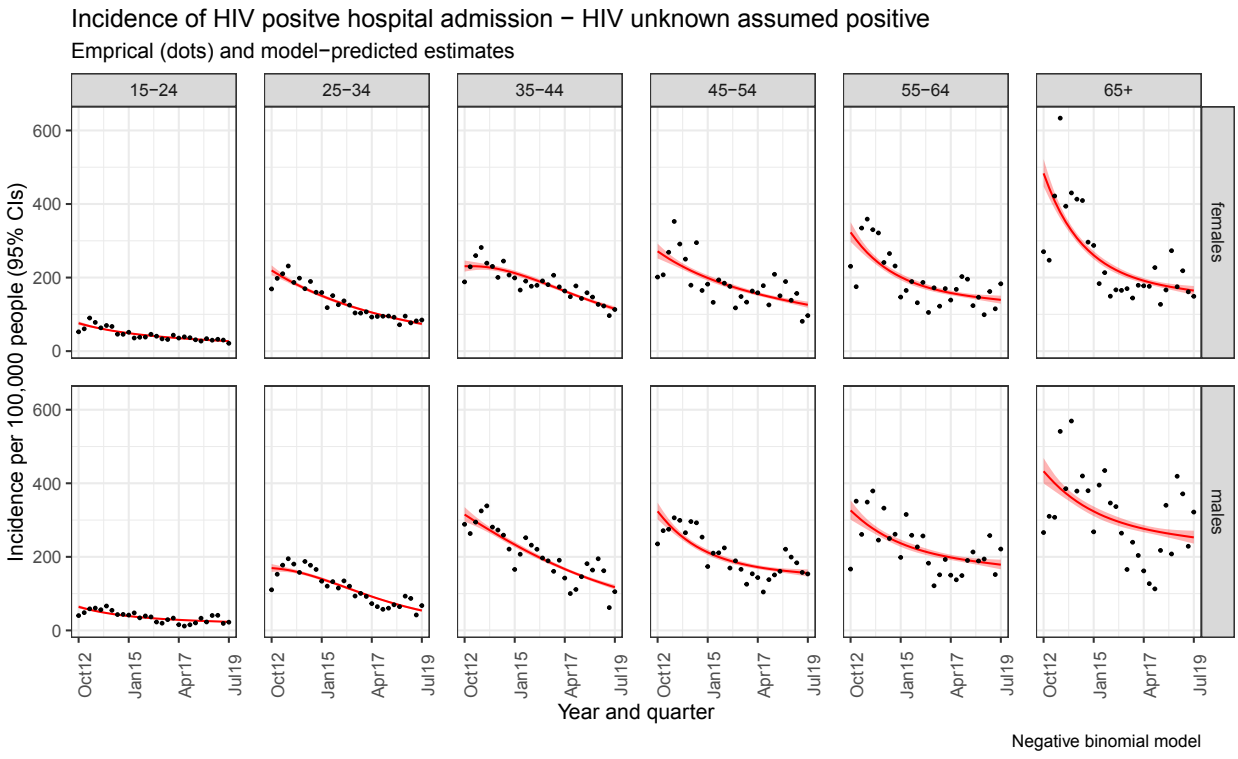
ageg10	sex	2012-10-01	2019-07-01	change
15-24	females	129510	150378	14%
15-24	males	119197	143016	17%
25-34	females	94609	106359	11%
25-34	males	96894	97762	1%
35-44	females	51523	73319	30%
35-44	males	59627	79772	25%
45-54	females	24832	31058	20%
45-54	males	28058	37129	24%
55-64	females	14743	17476	16%
55-64	males	15591	17180	9%
65+	females	14427	18121	20%
65+	males	12778	15851	19%

Supplementary figure 2: Sensitvity analysis for incidence (imputation)

S. Figure 2A: Everyone with HIV status unknown / missing is treated as if HIV negative



S. Figure 2B: Everyone with HIV status unknown / missing is treated as if HIV positive

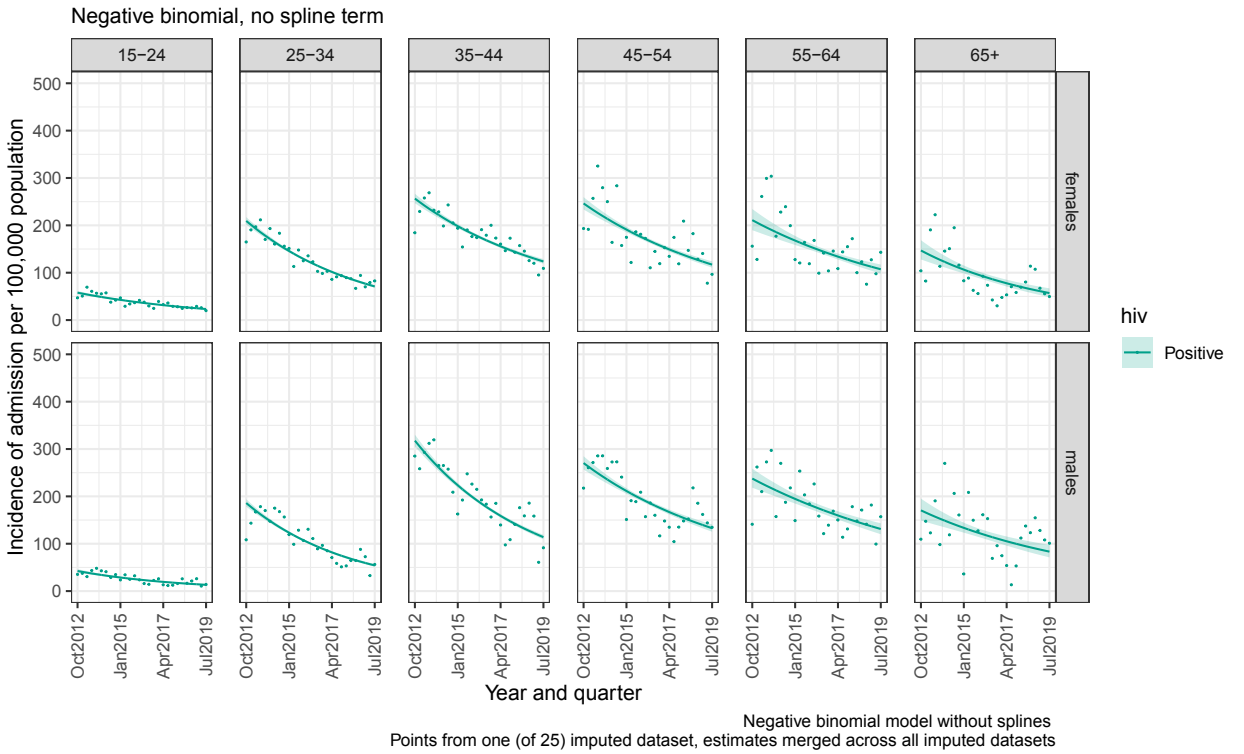


Supplementary Table 2: Reduction in admission to QECH by age group and sex

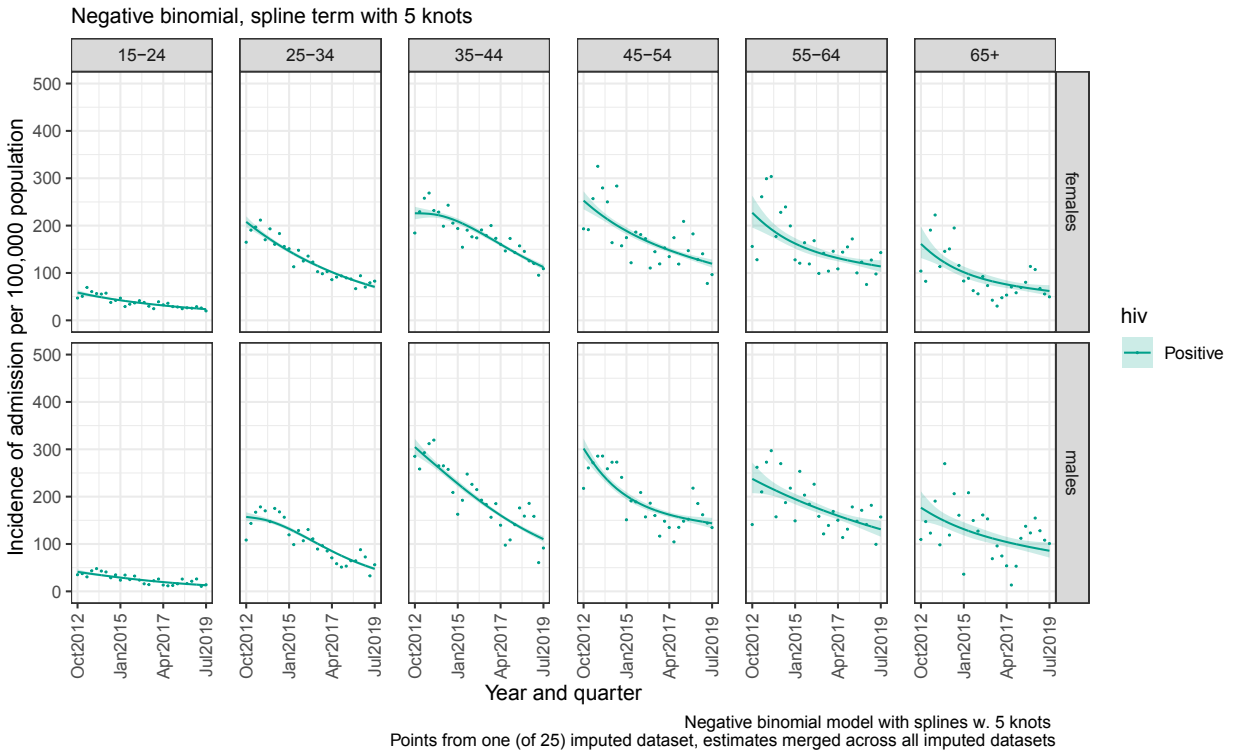
		HIV status imputed when missing		HIV unknowns assumed negative		HIV unknowns assumed positive	
Age group	Sex	Absolute number	Relative decline	Absolute number	Relative decline	Absolute number	Relative decline
15-24	females	815 (612 to 1019)	0.356 (0.299 to 0.414)	253 (164 to 342)	0.175 (0.125 to 0.226)	1248 (1049 to 1447)	0.42 (0.381 to 0.459)
15-24	males	599 (417 to 782)	0.398 (0.325 to 0.472)	66 (19 to 113)	0.121 (0.045 to 0.198)	1039 (879 to 1198)	0.441 (0.403 to 0.479)
25-34	females	2264 (1940 to 2589)	0.388 (0.354 to 0.422)	1078 (873 to 1283)	0.26 (0.223 to 0.297)	2474 (2096 to 2851)	0.401 (0.364 to 0.437)
25-34	males	1330 (1065 to 1595)	0.314 (0.271 to 0.357)	530 (392 to 669)	0.204 (0.161 to 0.246)	1489 (1194 to 1784)	0.322 (0.279 to 0.366)
35-44	females	844 (609 to 1080)	0.215 (0.168 to 0.262)	223 (61 to 385)	0.075 (0.025 to 0.126)	895 (632 to 1157)	0.222 (0.171 to 0.273)
35-44	males	2169 (1828 to 2509)	0.366 (0.329 to 0.402)	1121 (911 to 1331)	0.274 (0.236 to 0.311)	2255 (1877 to 2633)	0.367 (0.328 to 0.406)
45-54	females	635 (481 to 790)	0.322 (0.269 to 0.375)	149 (59 to 239)	0.122 (0.058 to 0.187)	713 (559 to 866)	0.336 (0.287 to 0.384)
45-54	males	1025 (836 to 1213)	0.372 (0.329 to 0.415)	497 (381 to 614)	0.274 (0.227 to 0.321)	1119 (909 to 1329)	0.379 (0.334 to 0.423)
55-64	females	337 (178 to 497)	0.33 (0.226 to 0.435)	-6 (-55 to 43)	-0.013 (-0.119 to 0.094)	595 (476 to 714)	0.409 (0.36 to 0.457)
55-64	males	267 (114 to 420)	0.246 (0.139 to 0.353)	-30 (-82 to 21)	-0.058 (-0.162 to 0.047)	461 (338 to 583)	0.308 (0.251 to 0.364)
65+	females	308 (156 to 459)	0.421 (0.297 to 0.544)	-37 (-65 to -8)	-0.207 (-0.398 to -0.016)	1081 (911 to 1252)	0.491 (0.451 to 0.531)
65+	males	225 (102 to 348)	0.319 (0.2 to 0.438)	9 (-26 to 43)	0.033 (-0.096 to 0.162)	498 (366 to 630)	0.287 (0.233 to 0.341)

Supplementary figure 3A: Sensitivity analysis for incidence (choice of model)

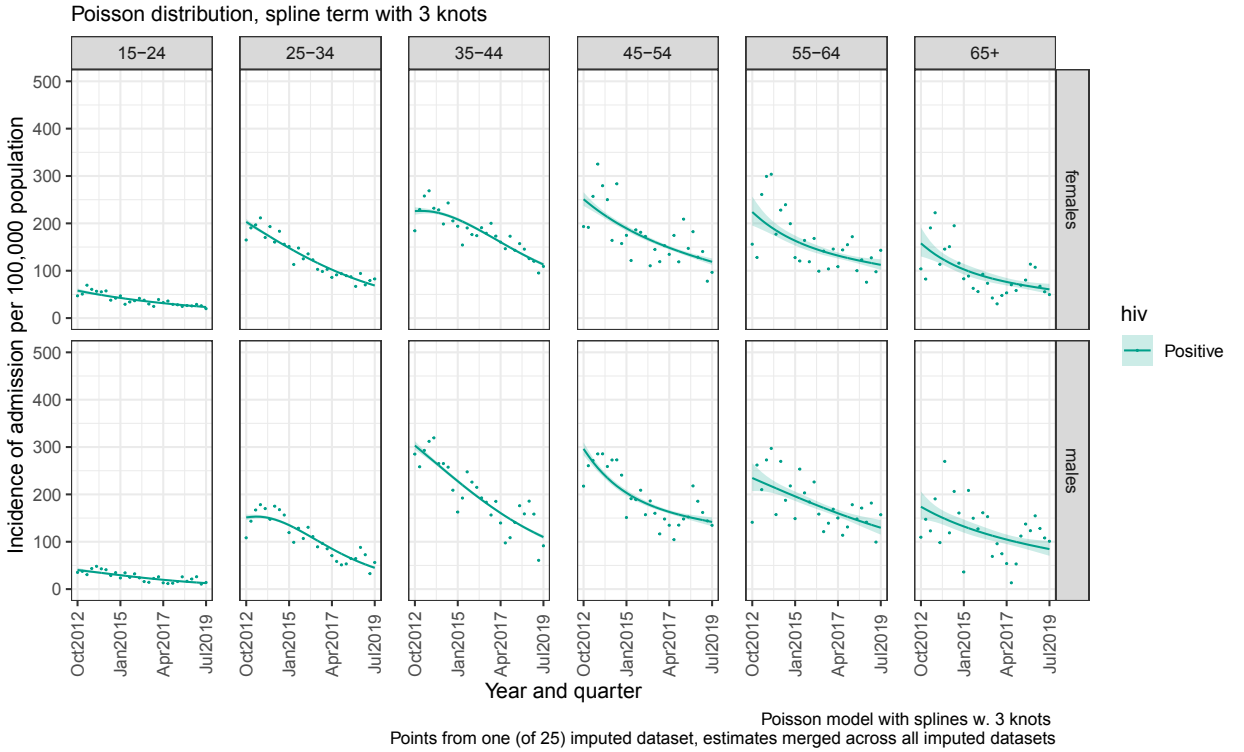
S. Figure 3A: Negative binomial distribution without spline terms



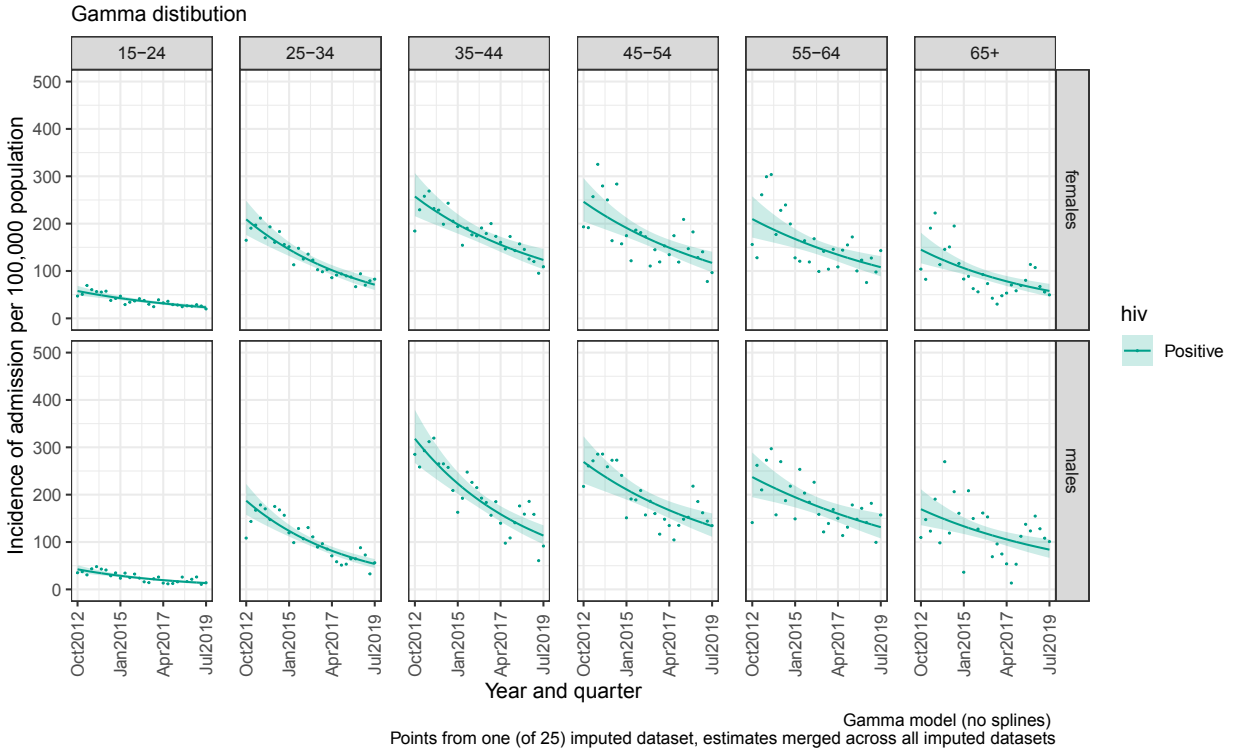
S. Figure 3B: Negative binomial distribution with 5 knots in spline



S. Figure 3C: Poisson distribution, 3 knot splines



S. Figure 3D: Gamma distbution, no splines



Supplementary table 3: Death risk by age group and sex

Table 3A: Crude Data

hivart		(Missing) (N=2687)	Died (N=6071)	Discharged alive (N=24056)
HIV negative	**ageg10**			
	15-24	73 (4.1%)	124 (7.0%)	1575 (88.9%)
	25-34	66 (4.2%)	135 (8.6%)	1366 (87.2%)
	35-44	96 (8.5%)	123 (10.9%)	909 (80.6%)
	45-54	79 (9.5%)	104 (12.6%)	645 (77.9%)
	55-64	119 (11.1%)	143 (13.4%)	809 (75.5%)
	65+	258 (12.6%)	323 (15.8%)	1463 (71.6%)
	sex			
	females	403 (8.6%)	448 (9.6%)	3814 (81.8%)
	males	288 (7.7%)	504 (13.5%)	2953 (78.9%)
HIV positive, ART status unknown	**ageg10**			
	15-24	3 (15.8%)	2 (10.5%)	14 (73.7%)
	25-34	22 (22.9%)	18 (18.8%)	56 (58.3%)
	35-44	20 (19.6%)	16 (15.7%)	66 (64.7%)
	45-54	3 (8.3%)	6 (16.7%)	27 (75.0%)
	55-64	6 (30.0%)	7 (35.0%)	7 (35.0%)
	65+	0 (0.0%)	4 (36.4%)	7 (63.6%)
	sex			
	females	24 (20.2%)	19 (16.0%)	76 (63.9%)
	males	30 (18.2%)	34 (20.6%)	101 (61.2%)
HIV positive, not on ART	**ageg10**			
	15-24	15 (4.0%)	73 (19.4%)	288 (76.6%)
	25-34	115 (10.8%)	168 (15.8%)	783 (73.5%)
	35-44	110 (11.0%)	179 (17.9%)	712 (71.1%)
	45-54	32 (8.5%)	87 (23.2%)	256 (68.3%)
	55-64	11 (7.1%)	31 (19.9%)	114 (73.1%)
	65+	9 (11.8%)	20 (26.3%)	47 (61.8%)
	sex			
	females	123 (9.0%)	195 (14.2%)	1055 (76.8%)
	males	169 (10.1%)	363 (21.6%)	1145 (68.3%)
HIV positive, on ART	**ageg10**			
	15-24	138 (10.8%)	184 (14.5%)	951 (74.7%)
	25-34	389 (9.8%)	748 (18.8%)	2838 (71.4%)
	35-44	487 (10.6%)	965 (20.9%)	3160 (68.5%)
	45-54	202 (10.2%)	430 (21.7%)	1346 (68.0%)
	55-64	82 (9.6%)	230 (26.8%)	545 (63.6%)
	65+	41 (10.8%)	108 (28.5%)	230 (60.7%)
	sex			
	females	739 (10.2%)	1210 (16.7%)	5315 (73.2%)
	males	600 (10.3%)	1455 (25.0%)	3755 (64.6%)
HIV status unknown	**ageg10**			
	15-24	29 (2.1%)	179 (13.1%)	1160 (84.8%)
	25-34	54 (3.2%)	328 (19.3%)	1318 (77.5%)
	35-44	41 (3.1%)	331 (25.1%)	946 (71.8%)
	45-54	42 (4.9%)	223 (26.0%)	592 (69.1%)
	55-64	46 (5.3%)	233 (27.0%)	585 (67.7%)
	65+	99 (5.2%)	549 (29.1%)	1241 (65.7%)
	sex			

hivart		(Missing) (N=2687)	Died (N=6071)	Discharged alive (N=24056)
	females	125 (3.9%)	641 (20.1%)	2431 (76.0%)
	males	186 (3.9%)	1202 (25.0%)	3411 (71.1%)

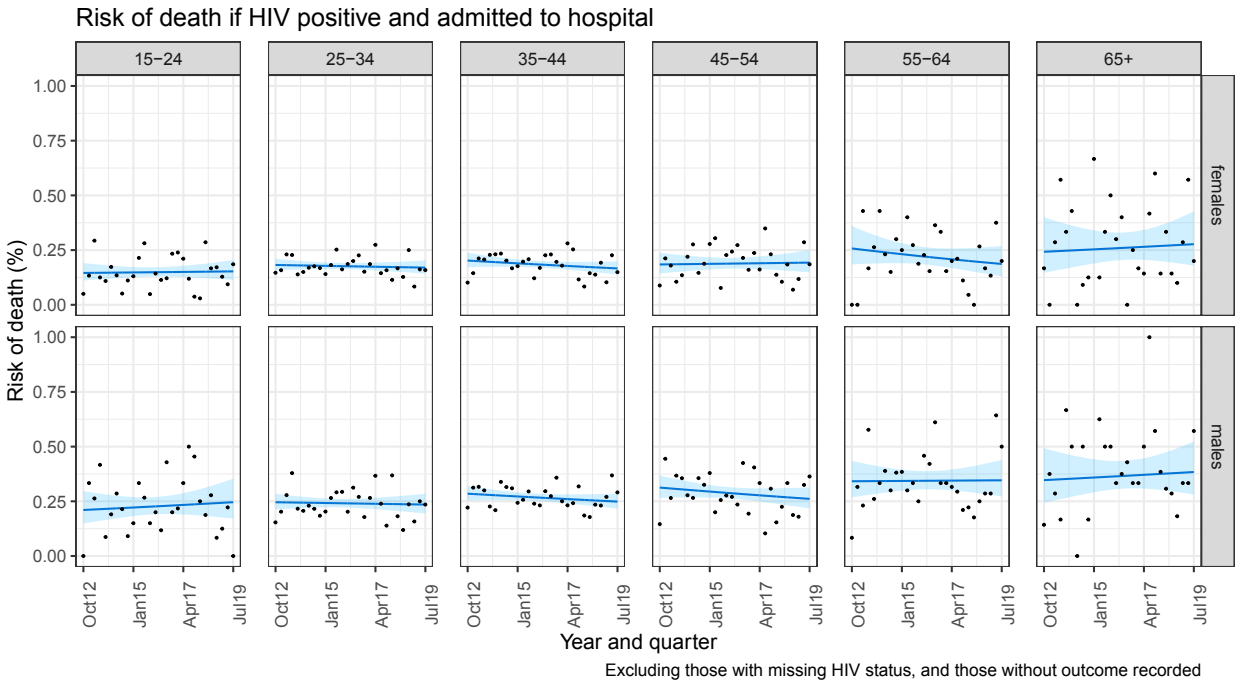
Table 3B: After imputation

Table 2: After imputation for missing data

hivart		Died (N=6656)	Discharged alive (N=26158)
HIV negative	**ageg10**		
	15-24	185 (7.5%)	2280 (92.5%)
	25-34	178 (9.2%)	1763 (90.8%)
	35-44	158 (11.7%)	1193 (88.3%)
	45-54	158 (15.6%)	855 (84.4%)
	55-64	245 (16.6%)	1234 (83.4%)
	65+	784 (22.5%)	2707 (77.5%)
	sex		
	females	782 (12.8%)	5321 (87.2%)
	males	926 (16.4%)	4711 (83.6%)
HIV positive by imputation, assume no ART for model	**ageg10**		
	15-24	135 (20.0%)	540 (80.0%)
	25-34	299 (22.5%)	1027 (77.5%)
	35-44	312 (28.5%)	783 (71.5%)
	45-54	190 (28.3%)	482 (71.7%)
	55-64	157 (34.4%)	299 (65.6%)
	65+	185 (41.9%)	257 (58.1%)
	sex		
	females	408 (23.2%)	1351 (76.8%)
	males	870 (29.9%)	2037 (70.1%)
HIV positive, not on ART	**ageg10**		
	15-24	75 (19.8%)	303 (80.2%)
	25-34	198 (18.1%)	898 (81.9%)
	35-44	213 (20.7%)	816 (79.3%)
	45-54	95 (25.0%)	285 (75.0%)
	55-64	33 (20.9%)	125 (79.1%)
	65+	25 (32.1%)	53 (67.9%)
	sex		
	females	227 (16.2%)	1174 (83.8%)
	males	412 (24.0%)	1306 (76.0%)
HIV positive, on ART	**ageg10**		
	15-24	211 (16.4%)	1079 (83.6%)
	25-34	863 (21.4%)	3178 (78.6%)
	35-44	1114 (23.8%)	3572 (76.2%)
	45-54	473 (23.5%)	1536 (76.5%)
	55-64	236 (27.0%)	639 (73.0%)
	65+	134 (34.5%)	254 (65.5%)
	sex		
	females	1402 (19.1%)	5953 (80.9%)
	males	1629 (27.5%)	4305 (72.5%)

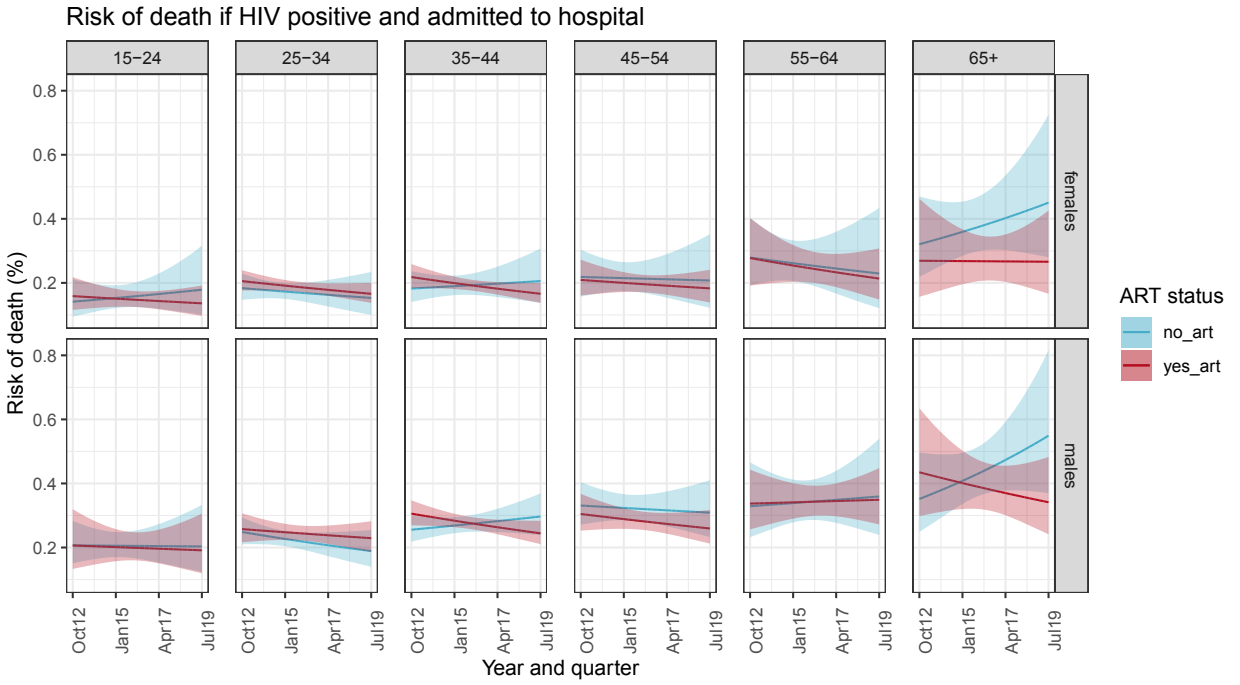
Supplementary figure 4: Sensitivity analysis risk of death

Complete case analysis, HIV and outcome unknown status removed

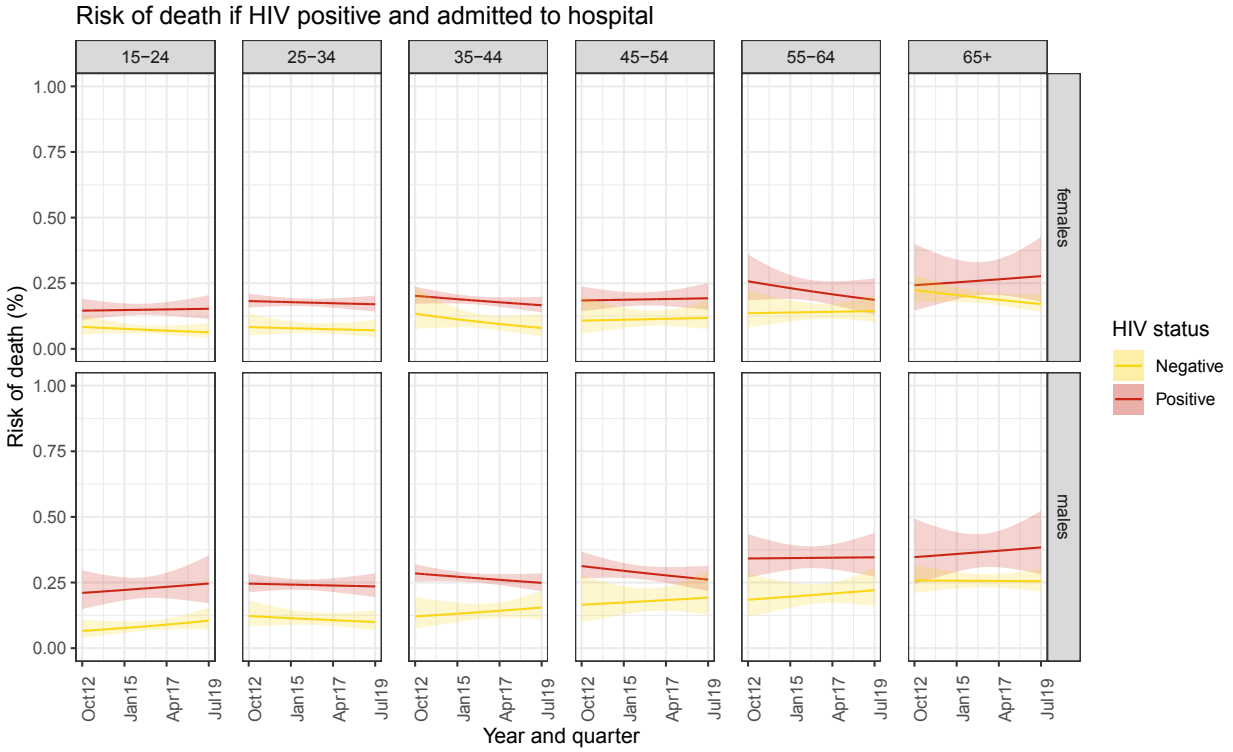


Supplementary figure 5: Risk of death including ART as a covariate

Plot model-predicted risk of death from model incorporating age group, sex, quarter-year and ART status



Supplementary figure 6: Risk of death over time including HIV negative



Supplementary table 4: Trend in outcome by age and sex

Using "emtrends" from "emmeans" package. In every age and sex group, confidence intervals for trend by quarter cross null effect.

ageg10	sex	q.trend	SE	df	asymp.LCL	asymp.UCL
15-24	females	0.0004729613	0.0011636125	Inf	-0.001807677	2.753600e-03
25-34	females	-0.0010007736	0.0008266785	Inf	-0.002621034	6.194864e-04
35-44	females	-0.0017293607	0.0009121361	Inf	-0.003517115	5.839319e-05
45-54	females	-0.0011786709	0.0013566146	Inf	-0.003837587	1.480245e-03
55-64	females	-0.0039595377	0.0020453824	Inf	-0.007968414	4.933821e-05
65+	females	0.0008105217	0.0027584539	Inf	-0.004595949	6.216992e-03
15-24	males	-0.0008936541	0.0016541745	Inf	-0.004135777	2.348468e-03
25-34	males	-0.0012515457	0.0010450936	Inf	-0.003299892	7.968002e-04
35-44	males	-0.0016206534	0.0009252838	Inf	-0.003434176	1.928696e-04
45-54	males	-0.0010379190	0.0013394894	Inf	-0.003663270	1.587432e-03
55-64	males	0.0005027680	0.0020536922	Inf	-0.003522395	4.527931e-03
65+	males	0.0015549194	0.0026720492	Inf	-0.003682201	6.792040e-03

Information about people who resided outside Blantyre

5,511 people were excluded from analysis due to residence outside Blantyre. They had similar characteristics to people who lived in Blantyre.

HIV status of those inside and outside of Blantyre

HIV	Blantyre	elsewhere
Unknown / Missing	24.4% (7996)	25.3% (1392)
Negative	25.6% (8410)	36.2% (1996)
Positive	50.0% (16408)	38.5% (2123)

Age distribution of those inside and outside of Blantyre

ageg10	Blantyre	elsewhere
15-24	14.7% (4808)	17.1% (942)
25-34	25.6% (8404)	23.0% (1269)
35-44	24.9% (8161)	21.6% (1193)
45-54	12.4% (4074)	12.8% (704)
55-64	9.0% (2968)	9.9% (543)
65+	13.4% (4399)	15.6% (860)

Sex distribution of those inside and outside of Blantyre

sex	Blantyre	elsewhere
females	50.6% (16618)	53.9% (2971)
males	49.4% (16196)	46.1% (2540)

Outcomes of those inside and outside of Blantyre

outcome	Blantyre	elsewhere
1. Survived to discharge	73.3% (24056)	71.6% (3946)
2. Died in hospital	18.5% (6071)	23.3% (1283)
3. Missing / unknown	8.2% (2687)	5.1% (282)

Chapter 5: Design and protocol for CASTLE trial

5.1 Overview and links with other chapters

This chapter outlines the protocol and methods for the CASTLE trial. The objective for the CASTLE trial is as follows:

To determine if, compared to usual care alone, enhanced TB diagnostics using Fujifilm high sensitivity lipoarabinomannan (LAM) urine testing plus digital chest X-ray with computer aided diagnosis (DCXR-CAD) plus usual care can increase the proportion of adults living with HIV admitted to hospital who are started in TB treatment.

CASTLE is a cluster randomised trial, where clusters are days of admissions. The rationale for the cluster randomised design, the rationale for the primary and secondary outcomes and the sample size are outlined in the manuscript for this chapter, published in PLoS One.

The appendices to this thesis include further relevant information for CASTLE trial

Appendix 1: CASTLE Protocol
Appendix 2: CASTLE Data Safety and Monitoring Committee charter
Appendix 3: CASTLE Data Analysis Plan

In this chapter, I outline the design and protocol for CASTLE trial. The protocol paper is published in PloS One, and this chapter contains this paper plus a linked published edit to correct a mistake in the description of the primary trial outcome.

5.1 Summary of design

Table 5.1 Summary CASTLE trial

Trial title	Computer Aided Screening for Tuberculosis in Low Resource Environments (CASTLE)
Short title	CASTLE study
Trial Design (methodology)	Single site (Zomba Central Hospital) cluster randomised trial with two trial arms and a third nested observational enhanced diagnostic cohort that will not contribute to trial outcomes (4:4:1 allocation, randomised by admission day).
Trial population	HIV positive adult patients admitted to medical wards at Zomba Central Hospital. Unit of randomisation will be admission day.
Planned sample size	102 clusters per trial arm (approximately 306 participants). A further 26 clusters in enhanced diagnostic cohort (approximately 78 participants). Total of 230 clusters with approximately 690 participants.
Follow up duration	56 days (eight weeks) from day of recruitment
Recruitment period	Pilot phase: 31st January 2020 - 13th March 2020 Randomised trial: Started 2nd September 2020, aim to finish December 2021
Trial intervention	Digital Chest x-ray with Computer Aided Diagnosis (DCXR-CAD) and urine high sensitivity lipoarabinomannan (FujiLAM) screening performed on participants admitted on days assigned to trial intervention arm. Numerical X-ray TB score, interpretation of score ("Pulmonary TB likely" or "Pulmonary TB not likely") and FujiLAM results are appended into patient's notes. X-ray imaging available for clinical team review on study computer in order to inform TB treatment decision making. If a participant's CAD score indicates "TB likely", they will have sputum taken for Xpert Mtb/Rif. DCXR-CAD and FujiLAM are in addition to usual care in the intervention arm. The control arm is assigned to usual care alone. Usual care includes all TB diagnostics routinely available at Zomba hospital, including, but not limited to, conventional (film) Xray and urine AlereLAM on treating clinician request. In addition to two trial arms, a third smaller arm (1 in 9 of all days randomised) will consist of days randomised to diagnostic cohort. This group will have extra tests related to HIV and infectious disease, and have more in depth clinical information collected. They will not contribute to trial outcomes.

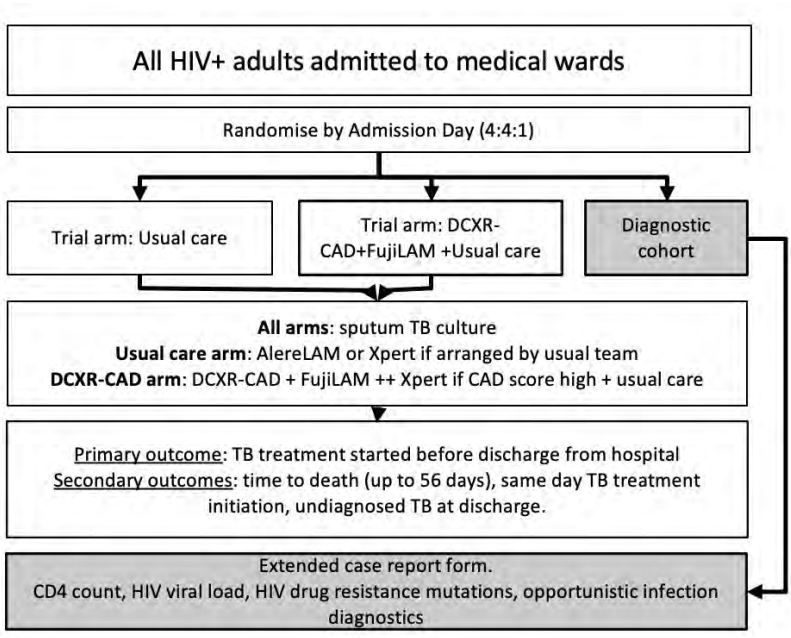


Figure 5.1: Schematic summarising CASTLE trial

5.2 Trial rationale

The CASTLE trial primary outcome is TB treatment initiation, and it is clear to see how enhanced TB diagnostics might lead to an increased TB treatment initiation. However, death is the more relevant outcome – although a secondary outcome because of feasibility and trial size.

The rationale for how an enhanced TB treatment diagnostic intervention might reduce all cause mortality is as follows:

- As discussed in chapter 2, tuberculosis is the leading cause of inpatient death (see section 2.1.5, and table 2.2 and 2.3 which summarises evidence) and is often undiagnosed at death.
- Other leading causes of death of adults living with HIV admitted to hospital in Africa include severe bacterial infections and non-TB AIDS related causes [1] such cryptococcal meningitis.[2]
- TB disease in people living with HIV is often severe and causes complications related to local disease (such as respiratory failure and pulmonary haemorrhage for pulmonary TB or raised intracranial pressure and encephalopathy for TB meningitis[3]) and to disseminated spread (which can include a hypotensive sepsis-like presentation[4]). The end process for severe TB disease is multi-organ failure as mode of dying.
- Some people with TB disease and admitted to hospital will be so far along their disease trajectory that even where TB disease is identified and treatment initiated promptly upon admission, they may nonetheless already have irreversible organ failure that will lead to death (particularly in a setting with limited critical care capability).[5] On the other hand, there is some evidence from community based surveys that some people self-cure TB [6] and even without diagnosis and treatment, mortality of HIV-associated TB is unlikely to be 100% - although this is difficult to quantify as is ethically impossible to gather epidemiological studies on people living with HIV where TB treatment is withheld.[7] It is reasonable to assume however than on aggregate, prompt diagnosis and treatment initiation for people admitted to hospital with HIV-associated TB will substantially reduce mortality in this group compared to a counterfactual situation were TB was present but undiagnosed and untreated.
- Evidence from STAMP [8] and LAM-RCT [9] trials (as well as indirect evidence from a before and after study of increasing TB treatment initiation in hospitals in South Africa[10]), shows important reductions in all-cause mortality concurrent with an increase in the proportion of people starting TB treatment. This supports our understanding that TB disease is an important cause of death, often underdiagnoses and that improving diagnosis and treatment can reduce all-cause mortality.
- It is possible – although more speculative – that early TB diagnostics and access to chest X-ray might improve care for non-TB conditions through making other diagnoses (such as heart failure or pneumonia) via chest X-ray.

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Student ID Number	1604611	**Title**	Dr
First Name(s)	Rachael Mary		
Surname/Family Name	Burke		
Thesis Title	Improving outcomes for adults living with HIV admitted to hospital in the era of high antiretroviral therapy coverage		
Primary Supervisor	Prof Elizabeth L Corbett		

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Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the CASTLE trial, initially as part of my (successful) funding proposal for this PhD. Whilst I led the design process, I had advice and input from my supervisors and Prof Katherine Fielding to design. My other co-authors provided valuable input to design – including Dr Marriott Nliwasa, Dr Saulous Nyriendra (particularly about practical implementation of the trial in Zomba Central Hospital) and Dr Liz Joekes (around X-ray interpretation). Prof Fielding provided input about statistical methods to ensure trial integrity. I wrote the trial protocol, the Standard Operating Procedures, the Statistical Analysis Plan and all the content for ethical approval from LSHTM and Kamuzu University of Health Sciences Research Ethics Committee. I designed all the trial Case Report Forms using Open Data Kit, and was assisted in back-up servers, archving and web hosting by data team at Malawi Liverpool Wellcome. I wrote the first draft of this protocol paper manuscript, which was reviewed by all co-authors.

SECTION E

Student Signature	[redacted]
Date	13 March 2023

Supervisor Signature	[redacted]
Date	13 March 2023

PLOS ONE

STUDY PROTOCOL

Design and protocol for a cluster randomised trial of enhanced diagnostics for tuberculosis screening among people living with HIV in hospital in Malawi (CASTLE study)

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Abstract

Background

People living with HIV (PLHIV) have a high risk of death if hospitalised in low-income countries. Tuberculosis has long been the leading cause of admission and death, in part due to suboptimal diagnostics. Two promising new diagnostic tools are digital chest Xray with computer-aided diagnosis (DCXR-CAD) and urine testing with Fujifilm SILVAMP LAM (FujiLAM). Neither test has been rigorously evaluated among inpatients. Test characteristics may be complementary, with FujiLAM especially sensitive for disseminated tuberculosis and DCXR-CAD especially sensitive for pulmonary tuberculosis, making combined interventions of interest.

Design and methods

An exploratory unblinded, single site, two-arm cluster randomised controlled trial, with day of admission as the unit of randomisation. A third, smaller, integrated cohort arm (4:4:1 random allocation) contributes to understanding case-mix, but not trial outcomes. Participants are adults living with HIV not currently on TB treatment. The intervention (DCXR-CAD plus urine FujiLAM plus usual care) is compared to usual care alone. The primary outcome is proportion of participants started on tuberculosis treatment by day 56, with secondary outcomes of mortality (time to event) measured to to 56 days from enrolment, proportions with undiagnosed tuberculosis at death or hospital discharge and comparing proportions with enrolment-day tuberculosis treatment initiation.

analysis, decision to publish, or preparation of the manuscript.

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

Discussion

Both DCXR-CAD and FujiLAM have potential clinical utility and may have complementary diagnostic performance. To our knowledge, this is the first randomised trial to evaluate these tests among hospitalised PLHIV.

Introduction

Adults living with HIV who require admission to hospital in high-HIV prevalence, low-income countries have a very high risk of death with 31% pooled in-hospital mortality in a meta-analysis of African hospital cohorts 2007–2015 [1]. Advanced HIV and high mortality among people living with HIV (PLHIV) admitted to hospital is a persistent problem, despite improvements in antitretroviral thereapy (ART) regimens and widespread availability of ART at primary care [2]. Tuberculosis (TB) is the leading cause of hospital admission and inpatient death [3]. Suboptimal TB diagnostics contribute substantially to this problem: in an autopsy series meta-analysis, 48% of HIV positive people who died in hospitals in Africa had TB, and half of this was undiagnosed during life [4]. Two trials have demonstrated that these deaths are, at least in part, preventable: LAM-RCT [5] and STAMP [6] trials evaluated the first commercial urine lipoarabinomannan (LAM) lateral flow test for diagnosing TB among hospitalized people living with HIV (PLHIV). These trials showed increased TB diagnosis and reduced mortality in people with TB symptoms and other high-risk groups when urine TB diagnostics were used [5,6].

Urine testing for LAM using Determine TB LAM (AlereLAM, Alere/Abbott, USA) has been shown to reduce mortality when used in addition to sputum testing with Xpert MTB/Rif (Cepheid, USA) [5,6]. However, admission screening with sputum Xpert and AlereLAM will still fail to identify a substantial proportion of people who truly have TB. A study in Cape Town showed that, among 427 PLHIV sequentially admitted to inpatient wards, a third (139 people) had microbiologically confirmed TB when provided with enhanced mycobacterial-culture based diagnosis from multiple samples. Only 53% (73/139 people) had their TB diagnosed from urine LAM or sputum Xpert on samples collected in first 24 hours of admission [7].

Newer TB diagnostic tools hold promise to improve TB diagnosis and clinical outcomes among adults living with HIV admitted to hospital. The Computer Aided Screening for Tuberculosis in Low Resource Environments (CASTLE) trial will evaluate two new TB diagnostic tests used together. These tests are digital chest X-ray with Computer Aided Diagnosis (DCXR-CAD) [8,9] and a new high sensitivity urine LAM test (Fujifilm SILVAMP TB LAM [FujiLAM], Fujifilm Corporation, Japan) [10,11].

DCXR-CAD uses a computer software algorithm to analyze chest X-ray images and provide a probabilistic score of TB likelihood. Sensitivity and specificity compared to sputum Xpert or culture reference standard are similar that that of expert radiologists [8]. FujiLAM is a high sensitivity urine test for presence of LAM in urine, using a pair of monoclonal antibodies to LAM and novel silver amplification step. FujiLAM kits are self-contained lateral flow kits which require no electricity and no additional equipment. FujiLAM has been shown to be more sensitive than the AlereLAM (70.7% vs. 34.9% sensitive when compared to microbiological reference standard) [10,11].

Both of DCXR-CAD and FujiLAM appear promising in diagnostic accuracy studies, and using a combination of DCXR-CAD to sensitively screen for pulmonary TB disease and FujiLAM to detect disseminated TB is an attractive combination among inpatients. To our

knowledge, there has only been one trial assessing patient relevant outcomes from use of DCXR-CAD (in outpatients in Malawi) [12] and no completed trials assessing outcomes from use of FujiLAM. Trials of clinical effectiveness of new diagnostic tools are important as the impact of clinicians' testing practice, TB treatment decision-making and patient outcomes are unknown.

Protocol

Study design

The CASTLE Study is an unblinded, single site, two-arm (4:4 recruitment) cluster-randomised controlled trial, where clusters are day of admission. There is a third smaller enhanced diagnostic cohort that does not contribute to trial outcomes, so that the overall allocation ratio is 4:4:1 to usual care trial arm: intervention trial arm: diagnostic cohort, respectively. Table 1 summarises the trial schedule according to SPIRIT guidelines [13].

Study hypothesis

The CASTLE Study will test the hypothesis that TB screening using FujiLAM and DXCR-CAD for all adults living with HIV requiring admission for any reason to medical wards at hospital will increase the proportion of people started on TB treatment. As secondary objectives we will assess: time to death, measured up to 56 days from randomisation; the proportion of people with undiagnosed microbiologically confirmed TB at death or discharge from hospital (see below for study specific definition); and proportion starting TB treatment in 24 hours from enrolment—recognising that limited study power means that these two secondary outcomes are more properly considered Phase 2 than Phase 3.

Table 1. CASTLE trial schedule (SPIRIT guidelines).

	STUDY PERIOD					
	Allocation	**Enrollment**	**Post-allocation**			**End of participant f'up**
TIMEPOINT**	**8am each day**	**t = 0**	**t = 24 hours**	***t = end of hospital admission (up to 56 days)***	***t = ~6–8 weeks***	***t = 56 days***
ENROLMENT:						
Eligibility screen		X				
Informed consent		X				
INTERVENTIONS:						
INTERVENTION GROUP: dCXR-CAD, urine LAM, sputum Xpert (if CAD high)		X				
BOTH GROUPS: Usual care (see description in manuscript)		X				
BOTH GROUPS: Sputum sample for culture		X				
ASSESSMENTS:						
On ART? Presence/absence TB symptoms? Was TB in differential diagnosis? Able to walk unaided?		X				
Started on enrollment day TB treatment?			X			
Started on TB treatment during hospital admission?				X		
Discharge from hospital alive vs. in-hospital death?				X		
Enrollment mycobacterial culture results (culture and identification takes 6–8 weeks)					X	
Alive or dead at 56 days?						X

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Study site and population

The CASTLE Study will be conducted at Zomba Central Hospital (ZCH), a combined secondary / tertiary hospital in Southern Region, Malawi, which serves both urban and rural populations and has sixty adult medical inpatient beds. This was the Malawi site of the STAMP trial of urine TB screening (using AlereLAM, conducted between 2015–2017) [6]. In STAMP Malawi arm the overall prevalence of microbiologically confirmed TB was 17% (113/656 people), in the group allocated to receive urine-based diagnostics.

Usual care TB diagnostics available at ZCH include sputum smear microscopy, Xpert MTB/Rif, urine AlereLAM and plain film radiography with radiographers and non-radiologist clinicians to interpret films: all these are available on clinician request. Comprehensive HIV care for inpatients is available and includes routine provider-initiated HIV testing and counselling for all admissions, and CD4 count and viral load on clinician request.

CASTLE study participants are HIV-positive (confirmed by review of patient held medical records or lateral flow testing) adults aged 18 years or older who are admitted to medical wards at ZCH for any reason. Exclusion criteria include people who: are currently taking TB treatment; have taken TB treatment in the past six months; are unable or unwilling to provide consent to be in the trial; and/or have been in admitted to wards for more than 18 hours at the time of recruitment to the trial.

Randomisation, blinding, definition of clusters

Days will be randomly assigned in a 4:4:1 ratio to one of three arms. The arms are usual care, TB diagnostics intervention (DCXR-CAD and FujiLAM) and enhanced diagnostic arm.

Randomisation is done by computer algorithm, using varying block sizes (blocks are nine, 18 or 27 days), with allocations placed into sequentially numbered opaque, sealed envelopes. Participants will be recruited up to 3pm each day. People admitted to wards after 3pm will be eligible for recruitment on the following day, providing less than 18 hours has elapsed since their admission time. This is in order to be able to complete all study interventions on the same day as recruitment.

Because of the nature of the study and the interventions offered, it is not possible to mask participants or research assistants to allocation groups. However, as far as possible, the investigators will be blinded to allocation groups until final analysis and data will be cleaned without reference to trial arm allocation.

Interventions

All participants (regardless of trial arm) will have a single sputum sample for mycobacterial culture using Mycobacterial Growth Indicator Tubes (Bactec MGIT, BD). Positive results will be communicated to participants and Zomba district TB team as soon as available.

Usual care arm. Participants allocated to usual care will receive clinician directed care, using any of the facilities available at ZCH (as above).

Intervention arm (DCXR-CAD and FujiLAM). Participants allocated to the intervention arm will receive a DCXR with CAD score (CAD4TBv6.0, Delft, Netherlands) and urine FujiLAM (Fujifilm Corporation, Japan) and AlereLAM testing. If the CAD score is ≥ 60, a single sputum for Xpert testing will be collected by study team. The CAD score, urine LAM results and Xpert result (if CAD score high) will be recorded in the participant's medical file and the chest X-ray image will be made available on a computer on the ward. This is in addition to usual care (as above).

Enhanced diagnostic cohort. The enhanced diagnostic cohort contributes to better understanding of case-mix, but not trial outcomes. Participants in the enhanced diagnostic

cohort (one out of every nine cluster days) will receive DCXR-CAD, FujiLAM and AlereLAM. They will also have a blood sample drawn for CD4 cell count, HIV viral load (both of which are available, but neither of which are routine in usual care), bacterial blood culture and serum CrAg performed immediately with results reported back to clinical team as soon as available. Blood will be stored for batch testing for Pro-calcitonin (PCT), CRP, and HIV drug resistance mutations (if HIV virus detected). Since May 2021, all participants (in any arm) can opt into having a blood sample taken for targeted HIV viral load. Prior to May 2021 only participants in diagnostic cohort had blood samples taken by the trial team for HIV viral load, although HIV viral load measurement has always been available upon clinical request as part of usual care.

Definitions

TB treatment initiation. TB treatment initation is as recorded in Malawi National TB register paper ledger at Zomba hospital. It is measured up to midnight on day of hospital discharge.

Time of TB treatment. Time TB treatment is dispensed from pharmacy to participant (or to the participant's guardian or ward nurse).

Microbiologically confirmed TB. At least two positive Acid Fast Bacilli (AFB) smears or one or more Xpert Mtb/Rif positive or one or more culture positive for Mycobacterium tuberculosis on any specimen or a positive urine LAM result.

Undiagnosed TB. Refers specifically to participants who do not have a microbiological diagnosis of TB made on the basis of study or usual care samples, and have not been empirically started on TB treatment following a clinical diagnosis of TB, and have culture-positive Mycobacterium tuberculosis on study sputum culture taken at recruitment.

Trial outcomes, assessment and analysis

The primary outcome is the proportion of participants started on TB treatment between recruitment into the trial and discharge from hospital, including on the same day as discharge. Analysis of the primary outcome will be done on an intention to treat basis, with all participants allocated to trial groups included and analysed in the group to which they were randomized.

The secondary trial outcomes will be time to death due to any cause, measured up to 56 days from enrolment in the trial; proportion of people starting TB treatment within 24 hours of enrolment in the trial; and proportion of people with undiagnosed microbiologically-confirmed TB at discharge from hospital (undiagnosed TB has a specific definition for this trial, above). The mortality outcome (the only outcome measured after discharge from hospital) will be ascertained predominantly by phonecalls to participants or a representative nominated by the participant, with home tracing for those who don't respond to phonecalls or who don't have a phone number.

Adjustment to effect estimates will be made to take account of clustering of outcome by day of admission. The full statistical analysis plan will be finalised before completion of enrolment, approved by the Data Safety and Monitoring Committee, and made publically available.

Rationale for primary outcome

With TB being the most common cause of inpatient death among PLHIV, mortality provides the ideal outcome for TB diagnostic trials, as for the LAM-RCT and STAMP trials. For the single site CASTLE trial it is not feasible to recruit enough patients to be confident to have statistical power to detect a mortality benefit as primary outcome; instead the trial is focused on the

more immediate and common outcome of increasing the proportion of inpatients started on TB treatment. TB treatment initiation is directly impacted by enhanced TB diagnostics, allowing a smaller trial comparing usual care and enhanced TB diagnostic arms. We consider this outcome as important for two main reasons. Firstly, whilst practices around empiric TB treatment (i.e. treatment in the absence of microbiological confirmation) vary substantially in different settings, in most settings TB is probably underdiagnosed among severely ill PLHIV in hospital. This is most notably demonstrated in autopsy series meta-analysis where 46% of people with TB were not diagnosed ante mortem, and similar findings in two more recent autopsy studies [4,14,15]. Secondly, both LAM-RCT and STAMP increased TB treatment initiation in the enhanced TB diagnostics arms, from 47% to 52% in LAM-RCT and from 15% to 22% in the urinary diagnostic arm of STAMP, consistent with a causal role in the reduced mortality [5,6]. As such, increasing TB treatment initiation is likely to be an important step towards further reduced mortality, provided that accuracy is maintained. TB treatment initiation is the primary outcome of the CASTLE trial, with mortality as a secondary outcome.

Sample size

We plan to recruit 102 clusters per trial arm (i.e. 102 clusters in each of usual care and intervention arms), with approximately 306 participants per arm. We assume 10% of people in usual care arm will start TB treatment and hypothesise that the screening intervention could increase this to 18%. We assume a mean cluster size of three (i.e. three eligible participants admitted to hospital each day) and a coefficent of variation (k) of 0.005 (i.e. that clusters are relatively similar to each other). This will yield 82% power with 5% type 1 error to detect a difference between groups at least this large.

For the secondary mortality outcome, we hypothesise that 20% of participants will die within 56 days from enrolment. If the intervention time to death compared to usual care had hazard ratio of 0.6 and if there were a mean of four participants per cluster (i.e. four HIV-positive participants admitted to hospital per day) there would be 82% power to detect a mortality difference at least this large. If overall mortality is lower than 20%, the cluster size is smaller than four, or the effect size is lower power to detect a difference between arms is substantially lower.

In addition, we will recruit a further 26 clusters (approximately 78 people) in the enhanced diagnostic cohort, which will not contribute to trial outcomes.

The enhanced diagnostic cohort

The first objective of the enhanced diagnostic cohort is to provide contextual information on the trial population, both laboratory tests such as CD4 counts and HIV viral load and more detailed clinical information about causes of admission to hospital and clinical course in hospital. The second objective is to investigate the prevalence of HIV viral failure, and infectious diseases leading to admission and contributing to deaths among PLHIV admitted to hospital in Zomba.

To put the HIV virological failure question into context; Malawi has a well performing national HIV programme and in 2020 it is estimated that 78% of all people living with HIV were established on antitretoroviral therapy (ART); this was 86% of all those who knew their HIV status [16]. In 2015–2017 in an analysis from the STAMP trial, 32% of PLHIV admitted to adult medical wards in ZCH had HIV virologic failure and the vast majority had resistance mutations to one or more first line ART drugs (at the time, first line ART was EFV+3TC +TDF). In 2019 the majority of PLHIV in Malawi were switched from non-nucleoside-reverse-transcriptase inihibitor (NNRTI) containing first line ART to integrase inhibitor

containing ART (DTG+3TC+TDF), including those already stable on NNRTI containing regimens. As an exploratory aim, we intend to describe the prevalence of HIV virological failure among PLHIV admitted to hospital and the prevalence and types of drug resistance mutations in those with HIV virological failure, in the context of the roll out of dolutegravir containing ART.

Funding, ethics and regulatory information

The sponsor of the trial is the London School of Hygiene & Tropical Medicine (rgio@lshtm.ac.uk). The protocol was given ethical approval by the ethics committess at the University of Malawi College of Medicine (COMREC, reference P.08/19/2772) and the London School of Hygiene & Tropical Medicine (LSHTM REC, reference 17799). All participants in the trial will be asked to give individual written informed consent. For people who are illiterate, they will give witnessed thumbprint consent. All participants are adults and there will be no proxy consent (i.e. lack of capacity to consent to the trial is an exclusion criterion).

The trial is registered at clinicaltrials.gov (NCT04545164). The trial is funded by the Wellcome Trust, through a PhD fellowship awarded to RMB (203905/Z/16/Z). The funder will have no role in the design or conduct of the trial. Any important trial amendments will be approved by the above ethics committees, communicated to sponsor and trial regisration will be updated. There is an independent Data Safety and Monitoring Committee (DMSC) comprised of three independent members, who have an advisory role for the trial. The Clinical Research Support Unit at Malawi Liverpool Wellcome Trust will periodically visit to review the study conduct. Both LSHTM REC and COMREC will receive annual monitoring reports.

No data are associated with this article. During the trial, data will be collected electronically on tablets using OpenDataKit. During the conduct of the trial and afterwards participant identifiable information will be kept confidential and held on a secure database in Malawi at the Malawi-Liverpool-Wellcome Trust. After completion of the trial, and at time of publication of the trial, anonymous trial data will be made available on LSHTM data compass.

The full protocol (version 5.1, date 2020-10-30), consent forms (S1 Appendix), DSMC charter (S2 Appendix) and SPIRIT checklist (S3 Appendix) [13] are available as an Supplementary Information to this article.

Discussion

The CASTLE trial is a pragmatic cluster randomised trial of the effectiveness of the combination of two new TB diagnostic tests. Both tests have shown promise in diagnostic accuracy studies, are relatively affordable, and have quality certification (CE mark) from the European Union as diagnostic tests. However, to date, there are no studies of effect on patient-relevant outcomes in inpatients. People living with HIV admitted to hospital, particularly in low-income, high-HIV prevalence countries such as Malawi, have an unacceptably high risk of death. TB is the major cause of mortality and, as shown by STAMP and LAM-RCT trials [5,6], is at least partly preventable.

The CASTLE trial has some limitations. The main limitation is the lack of a robust microbiological reference standard for ascertaining which participants truly have TB, despite taking a single research culture sample. This is particularly relevant for the outcome of "undiagnosed TB at discharge". TB is extremely difficult to fully refute, as evidenced by both autopsy findings (half of all TB diagnosed among PLHIV in autopsy series meta-analysis was not diagnosed antemortem despite the people who died having been in contact with health services prior to death) [4,14,15], and that in clinical practice TB is often treated "empirically" (i.e. without microbiological confirmation) [17]. Anticipating that this intervention may increase the

amount of empiric TB treatment used, we chose to have one study sputum culture as a minimum objective TB reference, whilst acknowledging that a single sputum culture result is far from a definitive way to diagnose or refute TB. However, diagnostic accuracy studies with extensive microbiological reference testing have already been undertaken to evaluate both the diagnostic tests used, and the focus of this trial is the impact of introducing these tests on patient-relevant outcomes.

We cannot see any feasible way to have introduced masking in this trial, as clinicians will be able to review laboratory results and chest X-ray images. The outcomes for the trial (as with almost any diagnostic intervention) depend—to an extent—on healthcare provider behaviour subsequent to test results. It is possible that there may be an effect of clinician behaviour for participants in usual care arm, particularly through requesting more usual care TB tests. This may reduce the difference between arms compared to if fewer TB tests (e.g. AlereLAM, conventional chest Xray and sputum Xpert) were requested in usual care clusters. However, the effect of interest for the trial is the additional impact of DCXR-CAD plus FujiLAM over and above maximising the use of existing diagnostic tests.

The cluster randomised design (by admission day) is designed to enable batching of study tasks (for example, radiographer time) and to allow the trial to recruit a larger number of participants than might otherwise be possible with existing resources. It is also possible that clustering patients by day might be less likely to alter clinician behaviour for participants in the usual care only arm than individual randomisation, as clinicians will not be dealing with patients having had different TB testing simultaneously on the same admission ward round.

In summary, people living with HIV admitted to hospital in low income high-HIV-prevalence settings have a high risk of death and TB is the leading cause of both admission and death. The CASTLE trial is a pragmatic single site cluster randomised trial of the effectiveness of enhanced TB diagnostics offered to all PLHIV admitted to hospital using DCXR-CAD plus FujiLAM. We are hopeful that the CASTLE trial will provide policy-relevant evidence of the impact and effectiveness of introducing new TB diagnostic tools in order to move beyond diagnostic accuracy studies alone.

Supporting information

S1 Fig. CASTLE trial schedule (SPIRIT guidelines).
(DOCX)

S1 Appendix. CASTLE study protocol.
(PDF)

S2 Appendix. CASTLE data safety and monitoring committee charter.
(PDF)

S3 Appendix. CASTLE trial protocol SPIRIT checklist.
(DOCX)

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Conceptualization: Rachael M. Burke, Saulos Nyirenda, Hussein H. Twabi, Marriott Nliwasa, Elizabeth Joekes, Naomi Walker, Rose Nyirenda, Ankur Gupta-Wright, Katherine Fielding, Peter MacPherson, Elizabeth L. Corbett.

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Design and protocol for a cluster randomised trial of enhanced diagnostics for tuberculosis screening among people living with HIV in hospital in Malawi (CASTLE study)

Rachael M. Burke , Saulos Nyirenda, Hussein H. Twabi, Marriott Nliwasa, Elizabeth Joekes, Naomi Walker, Rose Nyirenda, Ankur Gupta-Wright, Katherine Fielding, Peter MacPherson , Elizabeth L. Corbett

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Abstract incorrect about timescale of measuring outcome

Posted by **rburke** on **17 Jan 2023 at 11:15 GMT**

In the abstract of this article (but not the relevant body text or the SPIRIT figure) we stated an incorrect timing for measurement of the primary outcome (TB treatment initiation). We stated the primary outcome was measured up to 56 days from enrolment. TB treatment initiation is actually measured up to discharge from hospital, including on day of discharge. The body text, SPIRIT figure (table 1), and the protocol (which is an appendix to this paper) are all correct. The trial registration at clinicaltrials.gov has the correct timescale. This is a regrettable error in the abstract of this trial protocol paper only and not a change in protocol. The primary outcome has never been TB treatment up to 56 days, it was always until discharge from hospital.

We contacted PLoS shortly after publication to explain our error and asked for a correction. PLoS guidance states; "You are also welcome to use the Commenting feature on your article to notify readers of any potential errors."

Competing interests declared: I am the first author of this paper and principal investigator for the CASTLE trial.

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Chapter 6: CASTLE trial results

6.1 Introduction

This chapter contains the results for the CASTLE trial, prepared as manuscript under submission.

I conducted the CASTLE trial between 2 September 2020 and 15 February 2022 among adults living with HIV admitted to medical wards at Zomba Central Hospital. As discussed in chapter 5 (Protocol), admission-days were randomly assigned to: enhanced diagnostics for TB using urine lipoarabinomannan (LAM) antigen test (SILVAMP-LAM, Fujifilm, Japan and LF-LAM, Alere/Abbot, USA), digital chest X-ray with computer aided diagnosis (dCXR-CAD, CAD4TBv6, Delft, Netherlands), plus usual care ("enhanced TB diagnostics arm"); or usual care alone ("usual care arm"). The primary outcome was TB treatment initiation during admission. Secondary outcomes were 56-day mortality, TB diagnosis within 24-hours, and undiagnosed TB at discharge, ascertained by culture of one sputum sample taken at admission. All participants had sputum culture at admission.

Between 2 September 2020 and 15 February 2022, we recruited 419 people. Four people were excluded post-recruitment, leaving 415 adults recruited during 207 randomly assigned admission-days in modified intention-to-treat analysis. At admission, 90.8% (377/415) were taking antiretroviral therapy (ART) with median (IQR) CD4 cell count 240 cells/mm^3 (124-440). In the enhanced diagnostic arm, median CAD4TBv6 score was 60 (IQR: 51-71), 4.4% (9/207) had SILVAMP-LAM-positive and 14.4% (29/201) had LF-LAM positive urine with three samples positive by both urine tests. TB treatment was initiated in 46/208 (22%) in enhanced TB diagnostics arm and 24/207 (12%) in usual care arm (risk ratio [RR] 1.92, 95% CI 1.20-3.08). There was no difference in mortality by 56 days (enhanced TB diagnosis: 54/207, 26%; usual care: 52/207, 25%; hazard ratio 1.05, 95% CI 0.72–1.53); TB treatment initiation within 24 hours (enhanced TB diagnosis: 8/207, 3.9%; usual care: 5/208, 2.4%; RR 1.61, 95% CI 0.53–4.71); or undiagnosed microbiological-confirmed TB at discharge (enhanced TB diagnosis, 0/207 (0.0%), usual care arm 2/208 (1.0%) (p=0.50).

I show that enhanced tuberculosis diagnostics using SILVAMP-LAM plus LF-LAM plus digital chest Xray with Computer Aided Diagnosis (dCXR-CAD) was feasible, and led to more people being diagnosed with tuberculosis compared to usual care alone. However there was no evidence that the intervention made a difference in risk of death by 56 days, number of people with undiagnosed microbiologically confirmed TB at discharge, or people starting TB treatment within 24 hours of study enrolment. In keeping with the evidence from systematic reviews, we observed a very high rate of death over the 56 days following hospital admission. CASTLE showed a high level of discrepancy in results between SILVAMP-LAM and LF-LAM, and this is discussed further in Chapter 7 along with discussion of challenges and unanticipated findings in CASTLE trial.

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Student ID Number	1604611	**Title**	Dr
First Name(s)	Rachael Mary		
Surname/Family Name	Burke		
Thesis Title	Improving outcomes for adults living with HIV admitted to hospital in the era of high antiretroviral therapy coverage		
Primary Supervisor	Prof Elizabeth L Corbett		

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Please list the paper's authors in the intended authorship order:	Rachael M Burke (1) (2) Saulos Nyirenda (3) Timeo Mtenga (1) Hussein H Twabi (2)(4)

	Elizabeth Joekes (5) Naomi F Walker (5) Rose Nyirenda (6) Ankur Gupta-Wright (1)(7) Marriott Nliwasa (2)(4) Katherine Fielding (8) Peter MacPherson (1)(2)(5)* Elizabeth L Corbett (1)(2) *
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)	I was the principal investigator of the CASTLE trial. I recruited, supervised and managed a three-person trial team (one clinical officer and two research assistants). I led the CASTLE trial team day to day, with advice and input as needed from my supervisors. This involved liason with MLW Clinical Research Support Unit who monitored the trial, MLW Data Department who hosted the data servers (although I designed the database), staff and management at Zomba Central Hospital and with LSHTM and Malawi ethics committees. I monitored data – blinded to trial arm allocation – and conducted interim data reports for the Data Safety Monitoring Committee (interim data reports did not split data by trial arm and did not do formal interim analyses). I wrote code and I performed statistical analyses for trial outcomes according the the Statistical Analysis Plan, including all figures in the manuscript. I wrote the first draft of the Statistical Analysis Plan, which was reviewed and agreed by Prof Fielding. I am grateful for help and code review from Prof Fielding, who also reviewed and provided input to the Statistical Analysis Plan. Prof MacPherson also had access to the full trial dataset and can verify results. I wrote the first draft of the CASTLE trial manuscript, with revisions, comments and input from my supervisors, Prof Fielding and other co-authors.

SECTION E

Student Signature	
Date	13 March 2023

Supervisor Signature	
Date	13 March 2023

Enhanced tuberculosis diagnosis with computer-aided chest X-ray and SILVAMP-LAM in adults with HIV admitted to hospital (CASTLE study): A cluster randomised trial

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Summary (40 words):

A diagnostic intervention of screening with urine SILVAMP-LAM / LF-LAM plus dCXR-CAD identified more hospitalised PLHIV with TB than usual care. There was poor concordance between LF-LAM and SILVAMP-LAM tests. Inpatient mortality for adults living with HIV remains unacceptability high.

Abstract

Background

People living with HIV (PLHIV) admitted to hospital have high mortality, with tuberculosis (TB) being the major cause of death. Systematic use of new TB diagnostics could increase timeliness and completeness of TB diagnosis, potentially improving outcomes.

Methods

We conducted a cluster randomised trial among adult PLHIV admitted to medical wards at Zomba Central Hospital, Malawi. Admission-days were randomly assigned to: enhanced diagnostics for TB using urine lipoarabinomannan (LAM) antigen test (SILVAMP-LAM, Fujifilm, Japan and LF-LAM, Alere/Abbot, USA), digital chest X-ray with computer aided diagnosis (dCXR-CAD, CAD4TBv6, Delft, Netherlands), plus usual care ("enhanced TB diagnostics arm"); or usual care alone ("usual care arm"). The primary outcome was TB treatment initiation during admission. Secondary outcomes were 56-day mortality, TB diagnosis within 24-hours, and undiagnosed TB at discharge, ascertained by culture of one sputum sample taken at admission. All participants had sputum culture at admission.

Findings

Between 2 September 2020 and 15 February 2022, we recruited 419 people. Four people were excluded post-recruitment, leaving 415 adults recruited during 207 randomly assigned admission-days in modified intention-to-treat analysis. At admission, 90.8% (377/415) were taking antiretroviral therapy (ART) with median (IQR) CD4 cell count 240 cells/mm^3 (124-440). In the enhanced diagnostic arm, median CAD4TBv6 score was 60 (IQR: 51-71), 4.4% (9/207) had SILVAMP-LAM-positive and 14.4% (29/201) had LF-LAM positive urine with three samples positive by both urine tests. TB treatment was initiated in 46/208 (22%) in enhanced TB diagnostics arm and 24/207 (12%) in usual care arm (risk ratio [RR] 1.92, 95% CI 1.20-3.08). There was no difference in mortality by 56 days (enhanced TB diagnosis: 54/207, 26%; usual care: 52/207, 25%; hazard ratio 1.05, 95% CI 0.72–1.53); TB treatment initiation within 24 hours (enhanced TB diagnosis: 8/207, 3.9%; usual care: 5/208, 2.4%; RR 1.61, 95% CI 0.53–4.71); or undiagnosed microbiological-confirmed TB at discharge (enhanced TB diagnosis, 0/207 (0.0%), usual care arm 2/208 (1.0%) (p=0.50).

Interpretation

Urine SILVAMP-LAM / LF-LAM plus dCXR-CAD diagnostics identified more hospitalised PLHIV with TB than usual care. Poor concordance between LF-LAM and SILVAMP-LAM urine tests requires further investigation. Inpatient mortality for adults living with HIV remains unacceptability high.

Introduction

People living with HIV (PLHIV) who are admitted to hospital are at very high risk of death during or shortly after hospital admission.[1–3] Tuberculosis (TB) is the most common reason for admission to hospital and inpatient death for PLHIV.[1,4–7] Difficulties in TB diagnosis contribute substantially to inpatient mortality, with up to half of people who died from HIV-associated tuberculosis in autopsy studies not diagnosed before death, despite contact with health services.[5,8] Urine lipoarabinomannan (LAM) testing using lateral flow LAM (LF-LAM, manufactured by Alere/Abbott, USA) has been shown in two trials to reduce eight-week all-cause mortality among high-risk groups admitted to hospital.[9–11] However, LF-LAM has suboptimal sensitivity, giving negative results in a substantial proportion of hospitalised PLHIV with confirmed TB.[12] A newer LAM test (SILVAMP-LAM, manufactured by Fujifilm, Japan) was reported to have higher sensitivity than LF-LAM in detecting disseminated TB.[13]

Computer-aided diagnosis (CAD) on digital chest X-ray (dCXR-CAD) is as accurate as expert radiologists in diagnosing pulmonary TB[14,15] and may shorten time to tuberculosis treatment initiation,[16] although radiological screening studies and CAD product development have mainly focused on outpatient or community settings to date.[17,18] Using dCXR-CAD to screen for likely pulmonary TB together with SILVAMP-LAM to diagnose disseminated TB may help to identify more people with TB among hospitalised PLHIV, with potential to improve clinical outcomes and reduce mortality.

We therefore conducted a randomised trial to determine the effectiveness of enhanced TB diagnostics (using dCXR-CAD plus SILVAMP-LAM plus LF-LAM) plus usual care on TB treatment initiation compared to usual clinician-directed testing among adults PLHIV admitted to hospital.

Methods

Study design and participants

We conducted a cluster randomised trial in Zomba Central Hospital, Malawi. Each cluster was an admission day, covering a 24-hour period between 3:00pm and 2:59pm. Clusters were randomised in a 4:4:1 ratio to: usual care alone; enhanced TB diagnostics plus usual care; or to a diagnostic cohort. Participants in clusters assigned to the diagnostic cohort did not contribute to trial outcomes and are not reported in detail here.

Participants were adults (aged 18 years and older), living with HIV, admitted to medical wards at Zomba Central Hospital less than 18 hours before recruitment to the trial, who were willing and able to give consent, and were not already taking treatment for TB disease. Potential participants were eligible for recruitment regardless of symptoms, reasons for admission, or ART use. We used a cluster randomised design so that when usual care clinical staff reviewed people newly admitted to hospital, all eligible participants on a morning post-admission ward round would receive the same intervention, and because it was an efficient use of limited radiographer, transport, and laboratory staff resources.

Randomisation and masking

The randomisation sequence was computer generated using 'randomizerR' package in R statistical software, using block randomisation with variable block size. An independent researcher generated the randomisation codes and put them into sealed opaque envelopes. Once the cluster allocation was revealed by opening the envelope each morning, participants, study staff and clinicians were not blinded to allocation. No interim analyses were done.

Procedures

All participants able to spontaneously produce sputum had one sample taken at recruitment for *M. tb* culture. Sputum culture results were communicated to participants and routine clinical staff when available, but since culture results take up to six weeks, these did not affect in-hospital TB treatment decisions.

Participants admitted on a day assigned to the usual care arm could receive any tests usually available at Zomba Central Hospital. This included: nucleic acid amplification testing for TB (Xpert MTB/Rif, Cephid, USA) on sputum and other samples; X-ray with interpretation by radiographers, medical officers or non-radiologist physicians; and urine testing with LF-LAM (Determine LAM, Alere/Abbot, USA). None of these tests were protocol-mandated or provided by the study; completion was dependent on request by routine clinical staff and tests were carried out by usual care staff.

Participants admitted on a day assigned to the enhanced TB diagnostics arm additionally received urine TB testing using SILVAMP TB-LAM (Fujifilm Corp, Japan) and LF-LAM, and a dCXR-CAD using CAD4TB v6 software (Delft imagining, Netherlands). When the CAD4TB score was ≥ 60, the study team would obtain a sputum sample for Xpert, in patients able to expectorate.[19] Study staff were

trained in urine LAM testing and interpretation of results, and all trial-provided urine LAM tests were read by two study staff members (non-blinded) to ensure consistency in test interpretation.

Test results were provided to clinicians and recorded on a sticker in participants medical records, with CXR available to view on tablet computer. Decisions about TB treatment initiation were made by routine clinical staff.

Outcomes

The primary outcome was TB treatment initiation before death or discharge from hospital (whichever happened first). The secondary outcomes were: mortality up to 56 days from enrolment; TB treatment initiation within 24 hours of enrolment; and undiagnosed TB, defined by discharge or death without initiation of TB treatment in a patient whose admission sputum culture grew *Mycobacterium tuberculosis*.

Statistical analysis

The sample size was 102 clusters per trial arm. We anticipated a median cluster size of three participants per day, an intra-cluster correlation coefficient (ρ) of 0.005, and that in the control arm 10% of participants would initiate TB treatment.[9] One hundred and two clusters per trial arm gave 80% power to detect a difference between arms at least as large as a relative increase of 1.8, equivalent to an absolute increase of eight percentage points.

For analysis of the primary outcome (TB treatment initiation), we estimated a risk ratio and 95% confidence interval comparing trial arms using a log-binomial regression model, with robust standard errors to account for clustering. We also calculated the absolute risk difference. For the secondary outcome of TB treatment initiation within 24 hours we used log-binomial regression, and for the survival outcome we used a Cox regression model to estimate a hazard ratio – both with robust standard errors to account for clustering. For the undiagnosed TB at discharge outcome we were unable to estimate a risk ratio due to having zero events in one arm; we therefore tested for a difference between arms by calculating a two-sided Fisher's exact p-value.

We had two pre-specified subgroups for the primary outcome: people with TB in the differential diagnosis at admission, and people with a CD4 count ≤100 cells/mm^3. TB in the differential diagnosis at admission was defined as TB included in the written differential diagnosis by the admitting clinician. For all outcomes, we conducted a modified intention to treat analysis, with people found to

be ineligible following recruitment removed from the outcome analysis. We did analyses in Stata (v15).

Ethics, funding, trial registration and data availability

We obtained ethical approval from London School of Hygiene & Tropical Medicine and the College of Medicine Research Ethics Committee, University of Malawi. The CASTLE trial is registered with clinicaltrials.gov (NCT04545164). CASTLE was funded by Wellcome. The funder had no role in the study design, data collection, data analysis, data interpretation or writing of manuscript. All participants gave written (or witnessed thumbprint) informed consent. Anonymised trial data and analysis code will be made available at LSHTM Data Compass.

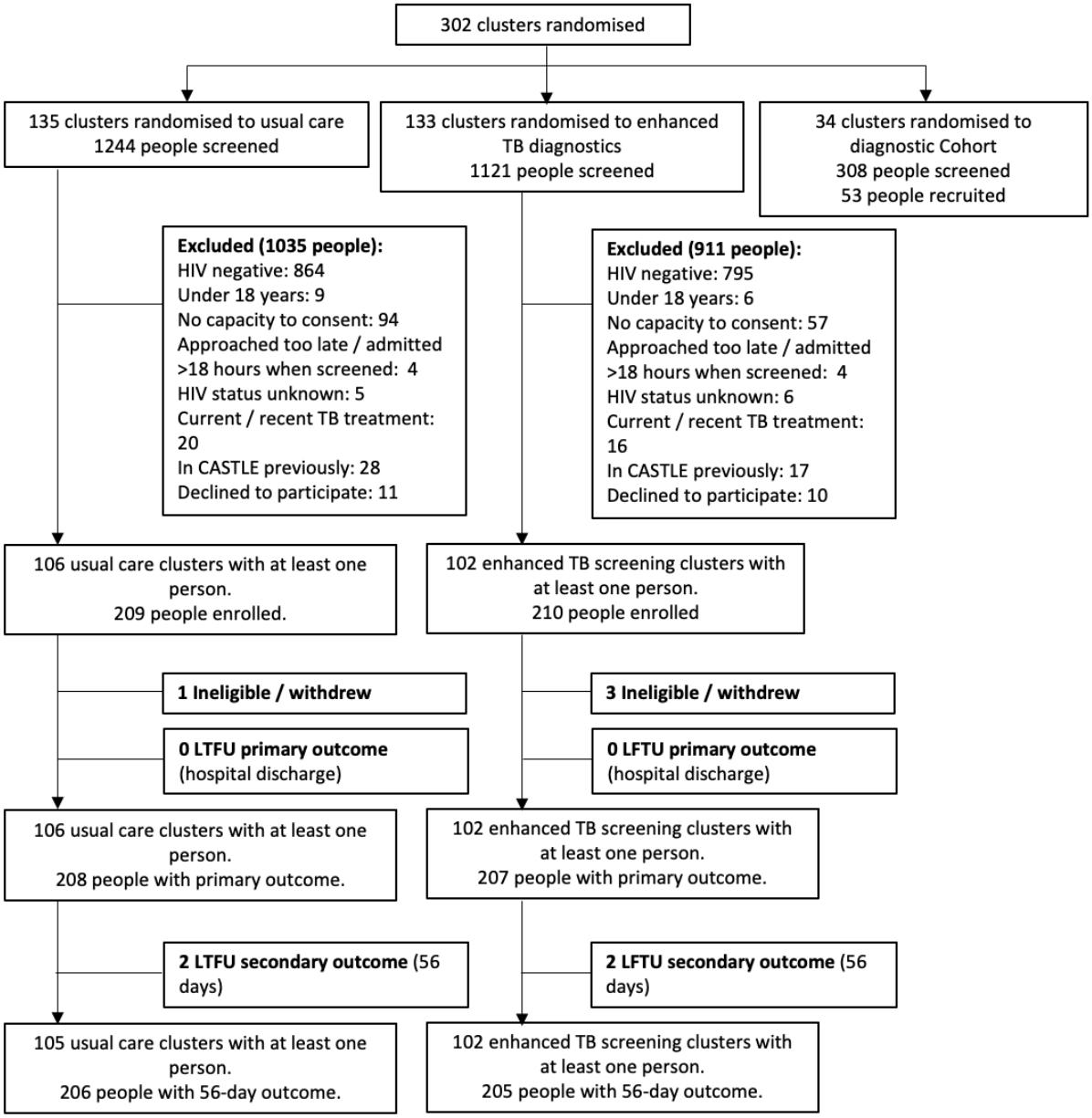
After the completion of this trial, data from two multi-country studies were published showing substantial lot-to-lot variation in the accuracy of SILVAMP-LAM.[19,20] In this trial, all SILVAMP-LAM tests were from batch numbers 19002 or 20004.

Results

Between 2nd September 2020 and 15th February 2022 we screened 2673 people admitted to adult medical wards, and recruited 419 trial participants in 208 clusters (admission days). A further 53 participants (28 clusters with at least one person) were recruited on days randomly assigned to the diagnostic cohort and will be described separately.

The main reason for non-recruitment was being HIV-negative, followed by not having capacity to consent to participate (Figure 1). Three participants were excluded after recruitment due to ineligibility (one in hospital >18 hours at recruitment, one HIV negative, one had been in CASTLE on a previous admission) and one participant withdrew, leaving 415 participants in the modified intention to treat analysis.

Figure 1: CONSORT diagram



Baseline characteristics are shown in Table 1. The median age was 41 years (interquartile range [IQR] 36 – 51 years) and 56.6% (235/415) were female. Most participants (78.6%, 326/415) had been taking ART for more than six months, the median CD4 count was 240 cells/mm^3 (where measured) and 18.9% (58/307) of people who had a CD4 cell count measured had values below 100 cells/mm^3.

Table 1: Characteristics of clusters and participants by trial arm

	Usual Care arm	Enhanced TB Diagnostics arm
Clusters		
Number of clusters randomised (including clusters where no-one was admitted)	135	133
Number of clusters included (clusters where at least one person was recruited)	106	102
Median size of cluster (min. – max.)	2 (1-5)	2 (1-5)
Participants		
Number of participants	208	207
Age in years (median, IQR)	42 (36 – 51)	41 (32 – 51)
Sex		
Men	84 (40.4%)	96 (46.4%)
Women	124 (59.6%)	111 (53.6%)
ART status		
Not on ART (new HIV diagnosis)	19 (9.1%)	14 (6.8%)
Interrupted ART	5 (2.4%)	2 (1.0%)
On ART < 6 months	20 (9.6%)	29 (14.0%)
On ART ≥ 6 months	164 (78.8%)	162 (78.3%)
Tuberculosis symptoms (self-reported)		
Cough	60 (28.8%)	62 (30.0%)
Fever	64 (30.8%)	65 (31.4%)
Night sweats	45 (21.6%)	41 (19.8%)
Weight loss	96 (46.2%)	82 (39.6%)
Any ≥1 tuberculosis symptom	148 (71.2%)	139 (67.1%)
Tuberculosis in differential diagnosis at admission*	62 (29.8%)	70 (33.8%)
Cannot walk unaided	105 (50.5%)	105 (50.7%)
CD4 cell count**		
Median cell/mm^3 (IQR)	288 (146 - 438)	220 (110 – 440)
< 100 cells/mm^3	27 (13.0%)	31 (15.0%)
≥ 100 cells/mm^3	131 (63.0%)	118 (57.0%)
Not measured / not recorded	50 (24.0%)	58 (28.0%)

* Tuberculosis in differential diagnosis at admission means the admitting (non-study) clinician had written tuberculosis as a possible diagnosis in the medical record.
** CD4 counts provided by the routine health service.

The median duration of hospital stay was six days (IQR: 3 – 9 days). By hospital discharge, 16.9% (70/415) participants had started TB treatment: 11.5% (24/208) in the usual care arm and 22.2% (46/207) in the enhanced TB diagnostics arm. The risk ratio for starting TB treatment was 1.92 (95% confidence interval [CI] 1.20–3.08) for intervention vs. usual care participants. The intra-cluster correlation coefficient was 0.088. Results were similar in prespecified subgroups analyses defined by CD4 count and whether TB was in the differential diagnosis at admission (Table 2).

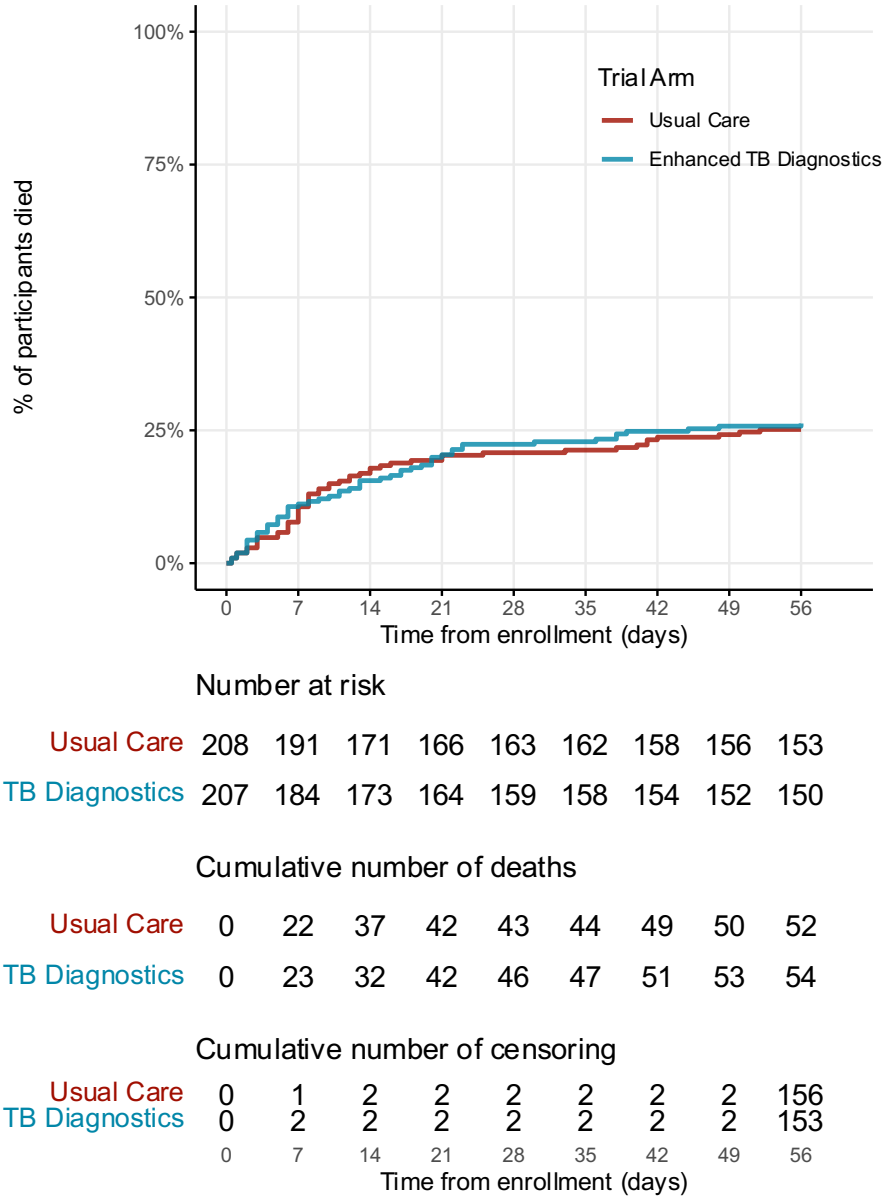
Table 2: Effect of intervention on trial outcomes

	Usual Care arm	Enhanced TB Diagnostics arm	Relative risk (95% CI)	Absolute risk difference (percentage points, 95% CI)
Primary outcome				
Tuberculosis treatment initiation during admission	24/208 (11.5%)	46/207(22.2%)	1.92 (1.20–3.08)	+10.7 (+3.34 to +18.0)
Primary outcome by subgroups				
TB in differential diagnosis at admission				
Yes	12/62 (19.4%)	19/70 (27.1%)	1.40 (0.76–2.60)	+7.79 (-5.88 to +21.4)
No	12/146 (8.2%)	27/137 (19.7%)	2.34 (1.24–4.64)	+11.5 (+2.95 to +20.0)
CD4 count				
< 100 cells/mm^3	5/27 (18.5%)	10/31 (32.3%)	1.74 (0.67–4.52)	+13.7 (-8.76 to +36.1)
≥ 100 cells/ mm^3	16/131 (12.2%)	27/118 (22.9%)	1.87 (1.05–3.34)	+10.7 (+1.30 to +20.0)
CD4 not measured	3/50 (6.0%)	9/58 (15.5%)	2.59 (0.74–8.93)	+9.52 (-1.71 to +20.7)
Secondary outcomes				
Undiagnosed tuberculosis at discharge[†]	2/208 (1.0%)	0/207 (0.0%)	Not estimated [†]	Not estimated [†]
Tuberculosis treatment within 24 hours of admission	5/208 (2.4%)	8/207 (3.9%)	1.61 (0.54–4.81)	+1.46 (-1.86 to +4.79)
Death by 56 days *	52/208 (25.0%)	54/207 (26.1%)	HR 1.05 (0.72–1.53)*	NA [¶]

* HR is a Hazard Ratio (time to event outcome for death).
[†] Not estimated because there were no events in the intervention arm. P value (Fisher's exact) for difference between arms is 0.50.
[¶] Not applicable because absolute risk not meaningful for a time to event outcome.
CI confidence interval

By 56 days from enrolment four participants were lost to follow-up: two in the usual care arm and two in the enhanced TB diagnostics arm. There was no difference in mortality, undiagnosed TB at discharge, or TB treatment with 24 hours of enrolment between the two trial arms (Table 2, and Figure 2).

Figure 2: Survival curve showing hazard of death by trial arm.



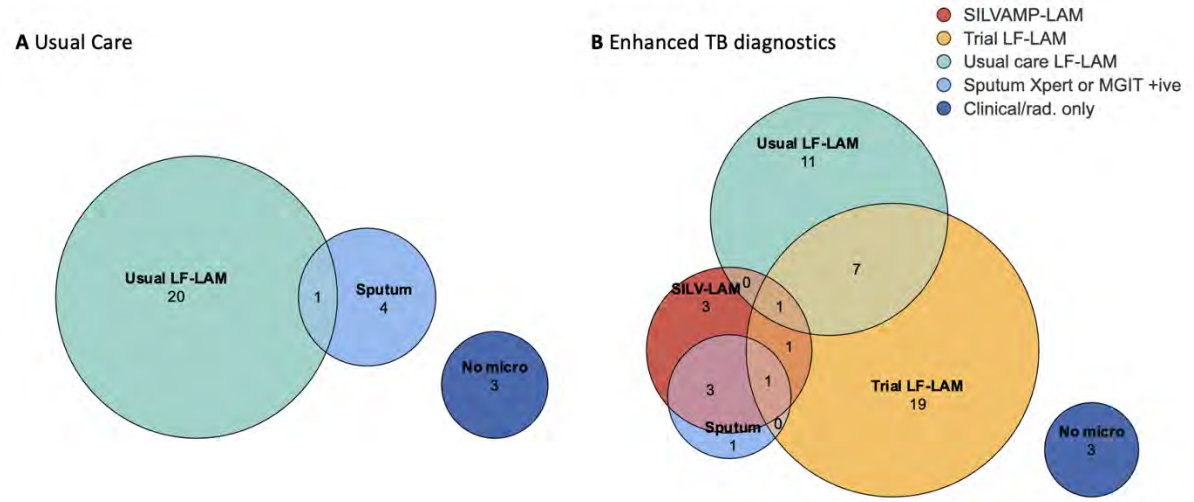
We obtained dCXR-CAD scores for 96.6% (200/207) and SILVAMP-LAM results for 97.1% (201/207) of participants in the enhanced TB diagnostics arm. CAD4TB scores were generally high: 51% (102/200) had a CAD4TB score greater than or equal to our pre-specified CAD4TB score cut-off of 60, and 8% (16/200) had a CAD4TB score ≥ 90 (see Appendix Figure S1)

At least one LF-LAM was performed in 38.5% (80/208) of participants in the usual care arm and in 97.1% (201/207) participants in the enhanced TB diagnostics arm. Sputum Xpert tests were done on 20.2% (42/208) people in the usual care arm and 40.6% (84/208) in the enhanced TB diagnostics arm, including 66% (67/102) participants with CAD4TB score ≥60. Seven sputum Xpert tests were positive (four in people with high CAD score, one in person with low CAD score and two in people in

usual care arm without CAD score). Sputum for cultures was taken on admission from 53.4% (111/208) and 55.6% (115/207) people in the usual care and enhanced TB diagnostics arms, respectively. Eight participants had a positive sputum culture on study admission sample, four in each arm (8/415, 1.9% of all participants or 8/226, 3.5% of all participants with sputum sent for culture).

We observed substantial discrepancies between results of LF-LAM and SILVAMP-LAM testing. Overall, 14.4% (29/201) LF-LAM tests done by the study team (i.e. not including "usual care" LF-LAM tests) were positive, and 4.5% (9/201) SILVAMP-LAM tests were positive, with poor concordance between tests (Table 3). Of the six people who had a positive urine test by SILVAMP-LAM but negative by LF-LAM, three had TB confirmed by a sputum microbiological test. Figure 3 summarises microbiological reasons for TB diagnosis in each trial arm, including for eight people (four in each arm) who had positive TB tests but did not start TB treatment. Further details are in Appendix Table S2 and S3 and Figure S2.

Figure 3: Positive TB tests (A) Usual care arm and (B) Enhanced TB diagnostics arm.



SILVAMP-LAM = SILVAMP LAM urine test manufactured by FujiFilm (Japan), LF-LAM = Determine LF-LAM urine test manufactured by Alere/Abbott (USA). Xpert = Xpert Mtb/rif rapid molecular diagnostic test, manufactured by Cephid (USA). Clinical/rad. only = participants who started TB treatment based on clinical or radiological findings, but without positive microbiological tests. Trial LF-LAM means LF-LAM testing done by CASTLE trial team. Usual LF-LAM means LF-LAM done by usual care team. Results shown for all participants who had at least one positive TB test or who started TB treatment. Includes 8 people (4 in each arm) who had a positive test but did not start TB treatment.

Table 3: Urine lipoarabinomannan results in the enhanced TB diagnostics arm participants

		Urine SILVAMP LAM results*				Totals
		Positive	Negative	Indeterminate	Not done	
Urine LF-LAM results	Positive Grade 2-4	1	6	0	0	**7**
	Positive Grade 1	2	20	0	0	**22**
	Negative	6	165	1	0	**172**
	Not done	0	0	0	6	**6**
Totals		**9**	**191**	**1**	**6**	**207**

* Results of testing fresh urine, both tests conducted on the same sample.

Discussion

In this cluster randomised trial, PLHIV admitted to hospital on days randomised to enhanced TB diagnostics had significantly increased likelihood of starting TB treatment during admission (22.2%) compared to usual care (11.5%), but no reduction in the high risk of death by 56 days from enrolment (25.0% usual care and 26.1% enhanced TB diagnostic arms). Enhanced TB diagnostics consisted systematic provision of two urine lipoarabinomannan antigen tests (SILVAMP-LAM and LF-LAM) and a digital chest X-ray with computer assisted reading (dCXR-CAD) on admission, followed by sputum Xpert MTB/RIF for people with chest X-ray abnormalities suggestive of TB who were able to produce sputum. In the usual care arm, all TB tests normally available at the hospital (including conventional chest X-ray, sputum Xpert and LF-LAM) could be requested by treating clinicians. Most of the increase of TB diagnosis appeared to be driven due to greater use of WHO recommended LF-LAM in the enhanced diagnostics arm and underuse of LF-LAM in the usual care arm. We also found that, SILVAMP-LAM identified substantially fewer positive results than LF-LAM, and did not demonstrate the high sensitivity that has been seen in previous studies

Since CASTLE trial finished, two large studies of SILVAMP-LAM diagnostic accuracy[19,20] that were running concurrently with CASTLE have published findings showing substantial lot-to-lot variability of SILVAMP-LAM. The batch of SILVAMP-LAM mostly used in CASTLE (#20004) was considered to have high specificity but low sensitivity.[19] As in CASTLE, poor concordance between LF-LAM and SILVAMP-LAM was seen.[20] Quality assurance for urine LAM testing has not been optimised. Unlike point of care HIV rapid tests, manufacturers do not supply control material that can be used for quality control testing, there are no well established or widely used standards for proficiency testing, and interpreting results for LF-LAM relies on visually comparing a colour band on a test strip to a band on a reference card.[21] Several studies have shown urine Xpert to be an unsuitable quality control for urine LAM.[22]

In CASTLE, only 37% of participants in our usual care arm had urine LF-LAM tests performed, despite this being policy for all PLHIV admitted to hospital with strong evidence from two randomised trials showing mortality reductions among hospitalised PLHIV.[23] Our results, and multi-country evaluations of SILVAMP-LAM, underscore the urgent need for routine proficiency and quality assurance systems for LAM-based tests, if LAM-based tests are to be scaled up more widely and interpreted with confidence.

To our knowledge, CASTLE was the first trial to investigate systematic testing with digital CXR-CAD for hospitalised PLHIV, for whom the pre-test probability of pulmonary TB is high, but so is the probability of other pulmonary infections and radiological abnormality not due to TB disease.[24] The software, CAD4TB, performs well for distinguishing TB from normal chest Xrays, but less well for distinguishing active TB from other pathologies, including bacterial pneumonia and COVID-19. CAD4TB should be considered a useful adjunct to be used alongside specific diagnostic tests and clinical judgment, and not a replacement for either.

TB has consistently been shown to be the leading cause of both hospital admission and death in African adults living with HIV, based on routine records review, autopsy studies and studies which use extensive microbiological diagnoses.[5–7] In autopsy studies, TB is often undiagnosed at the time of death. While systematic LF-LAM testing has shown to reductions in all-cause mortality, randomised trials in outpatient settings that have investigated the impact of sputum Xpert testing have mostly failed to show morbidity or mortality benefit.[25] A trial of enhanced TB diagnostics in hospitalised children with severe pneumonia also showed no mortality benefit, although the increase in number of children starting TB treatment in the intervention group was modest and not statistically significant.[26] As such, there is still considerable uncertainty as to the optimal screening, diagnostic, and management strategies for providing early TB diagnosis for inpatients and outpatients living with advanced HIV disease, suggesting the need for more intervention trials with patient-important outcomes beyond diagnostic accuracy.

Comparing CASTLE trial participants with a previous trial of TB screening (STAMP: 2015-2017) recruiting at the same hospital and with the same eligibility criteria, we observed lower number of daily admissions to medical wards for PLHIV, fewer people not on ART (20% STAMP, 8% CASTLE) but relatively similar low CD4 counts (median CD4 219 cells/mm^3 STAMP, 240 cells/mm^3 CASTLE). Whilst progress has been made in the between 2015 and 2020 with expanding HIV testing and starting ART

treatment in Malawi, little has changed for people admitted to hospital in terms of their very high risk of death. To achieve goals of ending AIDS, more focus on preventing deaths from advanced HIV are needed, particularly for people living with HIV admitted to hospital.[1,2] The overall mortality in CASTLE study of 25% by 2 months was similar to STAMP and other trials and studies in hospitalised populations,[1,9] showing that evidence-based interventions able to reduce this very high all-cause mortality among people living with HIV admitted to hospital are urgently needed.

CASTLE was a pragmatic and relatively small single-site trial, designed to provide preliminary estimates of the likely impact of introducing two TB diagnostic interventions with high potential to be scaled-up. Limitations include a sample size that was underpowered for our secondary outcome of death within 56 days. We observed far less than expected agreement between TB tests, with most patients started on treatment due to otherwise unconfirmed LF-LAM positive results. The COVID-19 pandemic likely affected both our case-mix and the diagnostic performance of dCXR-CAD in ways that cannot be clearly quantified and no longer apply. We also note a relatively low prevalence of culture-confirmed TB diagnosed through sputum culture, with only 3.5% of specimens culture positive. By comparison, 8.1% of sputum Xpert tests were positive when sputum was collected in a similar systematic way from STAMP trial participants. Population wide TB incidence is declining in Malawi, and it is possible that the true burden of TB as a cause of admission among PLHIV has indeed declined since 2015 and – if true – this might alter the risks and benefits associated with systematic TB screening.

Despite these limitations, we have shown that systematic testing with DCXR-CAD and two urine LAM tests is feasible. The lack of impact on mortality is disappointing, given that short term outcomes for adults living with HIV admitted to hospital in Southern and Eastern Africa, remain extremely poor.[1–3,27,28] Assuming that TB remains the major cause of hospital admission and death, quality assured TB diagnostics need to be developed and made available for inpatients, to support high quality clinical care including clinician judgement about empiric TB treatment. Existing tools - including LF-LAM - need to be fully implemented and appropriately supported. More research is urgently needed to understand causes of death in the context of ART scale-up, define appropriate packages of interventions for people with advanced HIV in hospital, support evidence-based interventions, and provide clearer treatment guidelines focused on severe bacterial infections, TB, and ART treatment failure.

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CASTLE trial appendix

Supplementary methods

Details on clusters and timing of recruitment

Each cluster was an admission day, covering a 24-hour period between 3:00pm and 2:59pm.

Screening and recruitment happened during office hours: people admitted to hospital in the late afternoon (after 3pm) or overnight could be recruited the following morning, but people admitted at weekends (before 3pm on Sunday) or before 3pm on public holidays were not recruited.

Each day's allocation was revealed by opening the envelope at 8am each morning. Participants admitted the previous evening (after 3pm) and during that day (up to 2:59pm) received the intervention according to the allocation.

Details on urine LAM testing

For participants in the enhanced TB diagnostics arm, urine LAM tests (SILVAMP-LAM and LF-LAM) were performed by CASTLE study staff, in a study room adjacent to medical ward. Study staff were provided with training in conducting and interpreting LAM results (LF-LAM and SILVAMP-LAM) with regular supervision. Urine LAM (both SILVAMP-LAM and LF-LAM) results were read by two study staff members to ensure consistency of judgement, although they weren't blinded to each other's reading. These results were recorded on a paper trial ledger, and the electronic study database in real time. They were also reported into participants medical records for use by routine clinical staff.

LF-LAM (but not SILVAMP-LAM) was also available through usual care. In this case, LF-LAM was conducted the routine clinical staff (not CASTLE staff) using their standard operating procedures. CASTLE were not involved in training or supervising LF-LAM tests done by routine staff team. The results for these LF-LAM tests were recorded in paper ledger from the Malawi Department of HIV/AIDS (not the CASTLE ledger). Results were collected from this ledger retrospectively periodically during CASTLE trial. For people in usual care arm, their only access to LF-LAM was through usual care. For people in enhanced TB diagnostics arm some participants had an LF-LAM result by both the CASTLE team, and by routine care team.

Table S1: Reasons for non-randomisation of clusters (days).

Between 2nd September 2020 and 15th February 2022 there were 532 days (inclusive). In total, 302 days were randomised (see CONSORT diagram), although not all of these became clusters because in some days no eligible participants were recruited.

In total, 1312 people were admitted to hospital at weekends, public holidays or other days that were not eligible for randomisation to a study cluster.

Reasons for non-randomisation of days and number of admission those days were as follows:

	Number of days not randomised	**Number of people admitted on those days**
Christmas period 2020 and 2021	28	Not measured
COVID study close (Jan 2021)	16	190
Saturday or Sunday	144	839
Friday before May 2021*	32	240
Public holidays (not including public holidays around Christmas and in Jan 2021)	10	43

* At the start of the CASTLE trial we didn't recruit on Fridays, to enable study team members to have time for administration and other tasks. Due to lower than anticipated recruitment, in May 2021, we amended the protocol to recruit on Fridays.

Table S2: TB tests performed

	Usual care arm	**Enhanced diagnostics arm**
dCXR with CAD score [(a)]	0 (0.0%)	200 (96.6%)
Urine SILVAMP-LAM	0 (0.0%)	201 (97.1%)
Urine LF – LAM [(b)]	80 (38.5%)	201 (97.1%)
Sputum Xpert	42 (20.2%)	84 (40.6%)
Sputum culture	111 (53.4%)	115 (55.6%)

Number of participants with at least one of the following TB test performed. dCXR-CAD = Digital Chest Xray with Computer Aided Diagnosis, SILVAMP-LAM = SILVAMP LAM urine test manufactured by FujiFilm (Japan), LF-LAM = Determine LF-LAM urine test manufactured by Alere/Abbott (USA). Xpert = Xpert Mtb/rif rapid molecular diagnostic test, manufactured by Cephid (USA).

[(a)] It was not possible to determine how many people had a conventional (non-digital) CxR.

[(b)] Includes either LF-LAM performed by trial staff or usual care.

Table S3: Urine LAM results by SILVAMP-LAM batch number

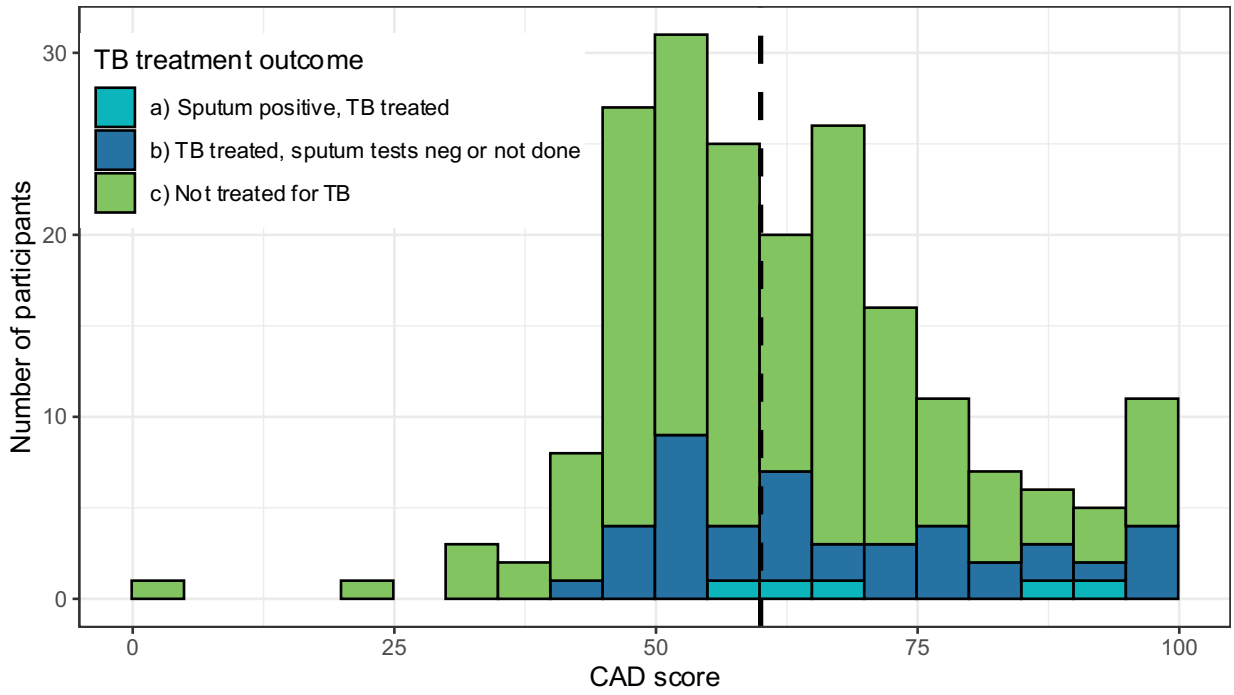
		LF-LAM		**Overall SILVAMP** (by batch)
		Positive (any grade)	Negative	
SILVAMP LAM				
Batch 19002 (n=51 tests)	Positive	2	2	**4/51 (8%)**
	Negative	9	38	47/51(92%)
Batch 20004 (n=151 tests)	Positive	1	4	**5/150 (3%)**
	Negative	17	127	144/150 (96%)
	Indeterminate	0	1	1/150 (1%)
	Overall LF-LAM	29/201 (14%)	172/201 (86%)	

Table S4: Sputum Culture Results

Usual care arm				
	Sputum Xpert			
Sputum Culture (MGIT)	a) Xpert negative	b) Xpert positive	c) Xpert not done	**TOTAL (MGIT culture)**
a) Negative	25	1	72	98
b) *M. tb*	1	1	2	4
c) MOTT	0	0	1	1
d) Contaminated	4	0	4	8
e) No sputum received in lab for culture.	10	0	87	97
Enhanced TB diagnostics arm				
	Sputum Xpert			
Sputum Culture (MGIT)	a) Xpert negative	b) Xpert positive	c) Xpert not done	**TOTAL (MGIT culture)**
a) Negative	54	0	38	92
b) *M. tb*	0	4	0	4
c) MOTT	0	0	0	0
d) Contaminated	10	1	8	19
e) No sputum received in lab for culture.	15	0	77	92

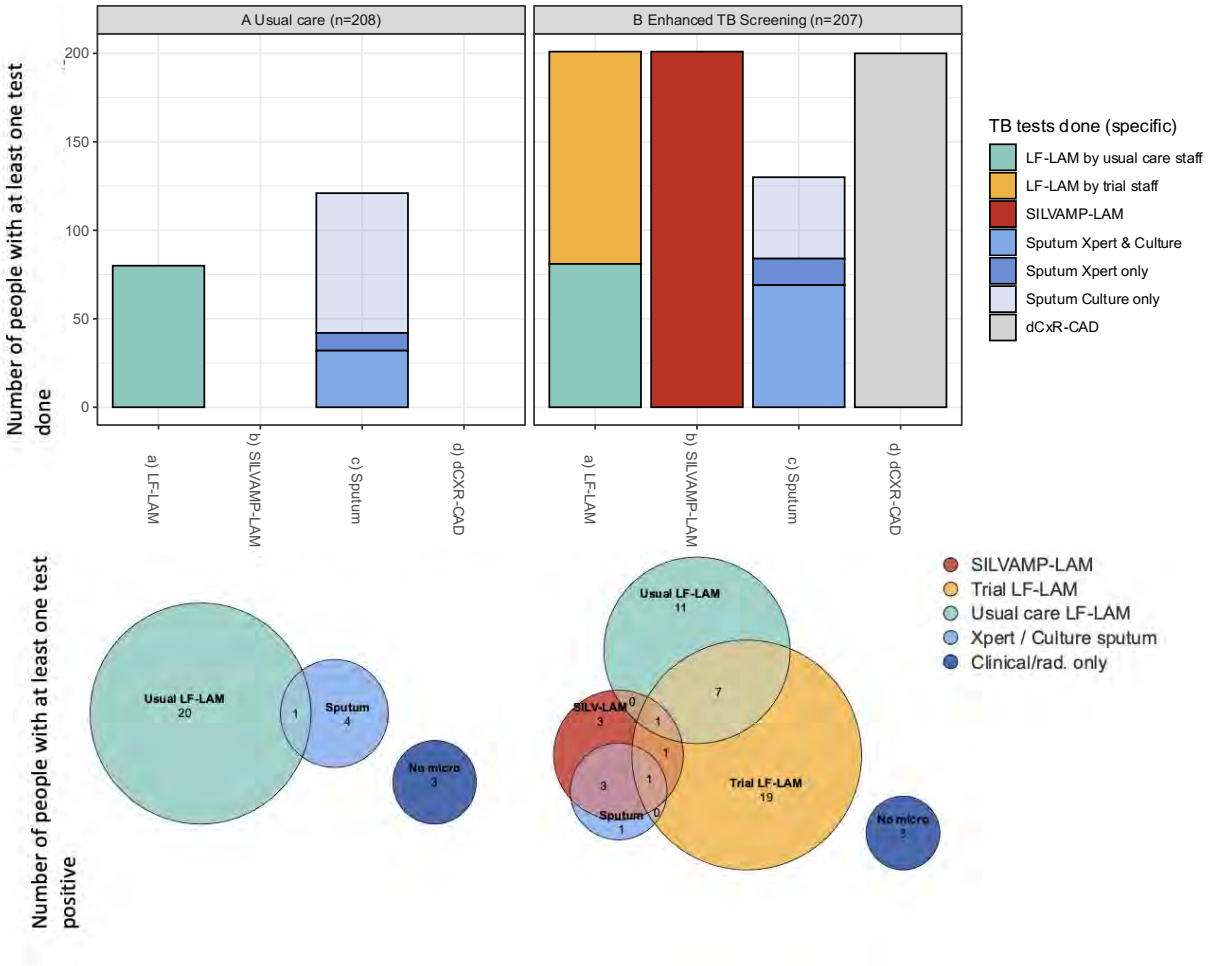
MOTT = Mycobacterium other than tuberculosis (not possible to provide further species information)

Figure S1: CAD4TB scores



CAD4TBv6 scores for 200 people in Enhanced TB diagnostics arm who had a valid score.

Figure S2: TB tests performed and results



"Sputum" circle contains Xpert and Culture results, combined together for ease of understanding Euler diagram.

Five people in each arm had at least one positive sputum test. Note that where multiple sputum tests were done – culture and Xpert – these were done on different sputum samples. In the usual care arm the five people with positive sputum results were as follows: one culture positive + Xpert positive, two culture positive + Xpert not done, one culture positive + Xpert negative and one culture negative + Xpert positive. In the enhanced TB diagnostics arm the five positive sputum results were as follows: four people had culture positive + Xpert positive and one person culture contaminated + Xpert positive.

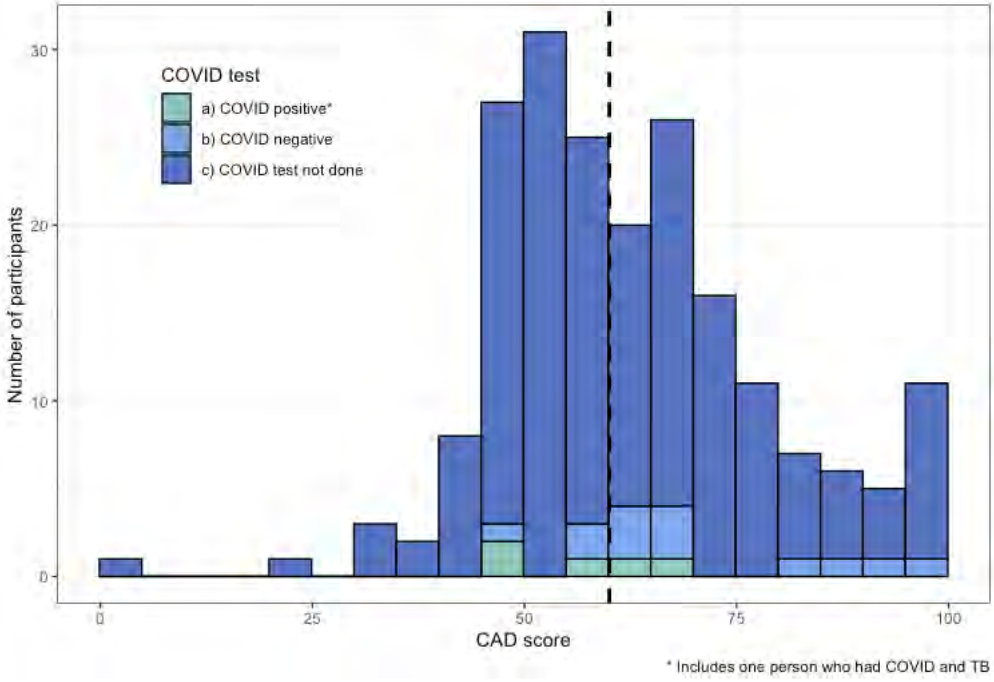
COVID-19 test results and CAD scores

COVID-19 test were available on request through usual care systems.
43/415 (10%) CASTLE participants had a documented, completed COVID-19 test with result recorded. The Zomba Central Hospital lab has both PCR (Cephid) and rapid lateral flow tests for COVID, which test was used was not recorded.
8/43 (19%) of these tests were positive.

One person had both COVID-19 and TB (TB diagnosis based on positive LF-LAM).

CAD scores by COVID-19 category for people in enhanced diagnostic arm are below.

Figure S3: CAD scores by COVID-19 test result



CD4 count and baseline TB symptoms

Malawi National HIV guidelines recommend LF-LAM for all PLHIV admitted to hospital. WHO Global TB programme guidelines recommend LF-LAM for PLHIV admitted to hospital who have either CD4 <200 cells/mm^3 or who have signs or symptoms of TB.

This table summarises which CASTLE participants would have been recommended to have LF-LAM by WHO guidelines (shaded categories are not recommened for LF-LAM by WHO Global TB Programme).

"Signs and symptoms of TB" means either reporting one or more of WHO four symptom screen to CASTLE trial team at baseline, or having "TB" written as a potential diagnosis by the clinician who admitted the participant to hospital.

Table S5: CD4 count and TB symptoms in CASTLE trial participants

	Signs and symptoms of TB		
	No	Yes	Total
CD4 count			
<200 cells/mm3	27	140	167
>=200 cells/mm3	43	97	140
CD4 count not done	31	77	198
Total	101	314	415

Mortality by LAM status and CAD score

Table S6: Death by 56 days by LF-LAM status [note a]

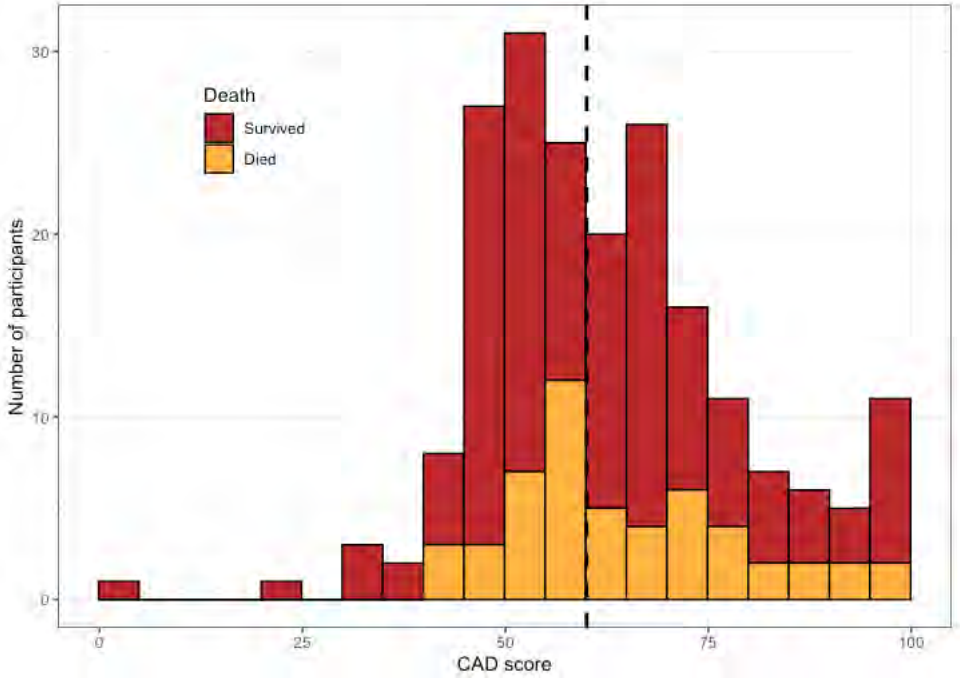
	Urine LF-LAM status LF-LAM positive [note b]	LF-LAM negative	LF-LAM not done
Usual care arm			
Survived [note c]	10 (48%)	43 (73%)	103 (80%)
Died	11 (52%)	16 (27%)	25 (20%)
Enhanced diagnostics arm			
Survived	28 (70%)	122 (76%)	3 (50%)
Died	12 (30%)	39 (24%)	3 (50%)

Notes:
a: Percentages are of died vs. survived within each arm and LAM result grouping.
b: For participants in usual care arm, only LF-LAM tests done are from usual care. For participants in enhanced diagnostic arm, LF-LAM positive includes positive results either by trial staff or through usual care (if a second LF-LAM test was done through usual care)
c: "Survived" includes two participants in each arm who were lost to follow up at 56 days, but alive when last in contact with trial team (which was at discharge from hospital).

Death by 56 days by CAD4TB score

There was no difference in mortality between participants with CAD score <60 or CAD score >=60. Among those with CAD<60 25/89 (26%) died by 56 days from enrolment, and among those with CAD >=60 27/102 (26%) died by 56 days.

Figure S5: CAD score by mortality outcome



Chapter 7: CASTLE trial challenges

This chapter sets out some of the notable and unanticipated challenges faced during the implementation of the CASTLE trial, mitigation strategies adopted, and lessons learned. It contains additional material to the supplemental results already presented in the Chapter six manuscript and it's appendix, including a detailed analysis of two other studies that used SILVAMP-LAM and LF-LAM testing and were conducted concurrently with CASTLE. The major challenges with the CASTLE trial were: lower than initially anticipated recruitment, which was addressed early in the study period; the COVID-19 pandemic; the now well-documented lot-to-lot variation in performance with SILVAMP-LAM; the less well documented issue of variable performance with LF-LAM; and the lack of clinical action consequent to dCXR-CAD results.

The concept for the CASTLE trial was developed in late 2018, as part of preparing my Wellcome grant application for this PhD. The detailed protocol was developed in mid to late 2019, following award of funding and registration as a PhD student. I implemented a short pilot phase (as described in the protocol) from 31st January to 13th March 2020. From 14th March to 13th August 2020, CASTLE was closed due to COVID-19 pandemic, in accordance with guidance from Malawi national authorities and the Malawi-Liverpool-Wellcome Programme. From 13th to 31st August 2020 there was a second short pilot phase to refamiliarise staff with procedures prior to starting recruitment into the CASTLE trial. The first participant was recruited and randomised into the CASTLE trial on the 2nd September 2020.

7.1 Recruitment lower than initially anticipated

I designed the CASTLE trial, initially using assumptions based on the STAMP trial,[1] which had been conducted in the same hospital (Zomba Central Hospital) 2015-2017.

The initial protocol approved by ethics committees at London School of Hygiene and Tropical Medicine and College of Medicine Research Ethics Committee specified mortality up to 28 days and TB treatment initiation as joint primary outcomes (two primary outcomes, not a composite measure). I anticipated recruiting seven people per cluster (recruitment day) for a total of 714 people in 102 clusters, although I planned a pilot phase because I anticipated potential uncertainty in numbers of HIV-positive hospital admissions due to the successful ART scale up in Malawi, as well as the switch to INSTI-containing ART regimens. The CASTLE pilot phase (2019) revealed that admissions to hospital were less common compared to during STAMP (2015-2017) with a median of three HIV positive adults admitted each day. This is consistent with data presented in Chapter 3 showing declining HIV related admissions to Queen Elizabeth Central Hospital 2012-2019. See section 7.3 (below) for more detailed comparison of the STAMP and CASTLE trials.

Having identified that the mortality primary outcome would likely be substantially underpowered, I redefined this to be a secondary outcome, retaining only TB treatment initiation as the primary outcome. These changes were discussed and agreed with my Data Safety Monitoring Committee members, supervisors and advisors and the relevant ethics committees, and protocols were amended and approved, and the new primary outcome was registered on clinicaltrials.gov before the first trial participant was recruited. This change in primary outcome to TB treatment alone is problematic, however, since increases in TB treatment initiation can be due to either appropriate treatment of patients with TB (true positive) or due to inappropriate treatment of patients without TB (false positive). Given the smaller number of likely participants available to be recruited to the trial and TB treatment as only primary outcome, CASTLE should be viewed as an early stage diagnostic evaluation trial, to evaluate the feasibility of introducing systematic DCXR-CAD, as well evaluating the combination of CXR plus a highly sensitive SILVAMP-LAM, as well as providing data on how these tests performed in a field setting; and the impact on clinician behaviour.

7.2 Urine LAM quality issues

The main unexpected finding in CASTLE was far greater discrepancy in results between SILVAMP-LAM and LF-LAM than reported previously.[2] CASTLE was not set up to be a diagnostic accuracy study and, importantly, the study design and outcomes precluded use of an extensive and standardised reference standard TB testing, since this would impact TB diagnostic decisions. Providing standardised detailed TB reference testing (such as urine NAAT testing) would be a different intervention and one that would probably not be easily scalable within health systems such as Malawi. As such, this unexpected discrepancy around SILVAMP-LAM and LF-LAM cannot be conclusively interpreted in CASTLE, but clearly needs discussion.

Evidence prior to CASTLE indicated that SILVAMP-LAM had substantially higher sensitivity than LF-LAM and similar specificity without the need to interpret colour intensity of faint lines.[2–5] However, in CASTLE, we found substantially more positive results from LF-LAM than SILVAMP-LAM. Overall, 14% (29/201) of CASTLE LF-LAM tests were positive, compared to 4% (9/201) of SILVAMP-LAM tests. Concordance between the two tests was poor with only three urine samples positive on both tests.

Two large multi-country prospective diagnostic accuracy studies conducted concurrently with CASTLE are relevant to compare with these results: one undertaken by FIND (Székley et. al.)[6] and available as a preprint; and one by Medicins Sans Frontiers (MSF) (Huerga et. al.).[7] These studies are useful to illuminate CASTLE findings – they are discussed in detail below, followed by a discussion of possible explanations for discrepant LAM results in CASTLE.

7.2.1 Concordance of LF-LAM and SILVAMP-LAM in the MSF and FIND diagnostic evaluation studies

Both the MSF study[7] and the FIND study[6] showed much greater discrepancies between FujiLAM and LF-LAM than anticipated. They also showed a large number of false positive LAM results for both SILVAMP-LAM and LF-LAM but this is likely to be a reflection – at least in part – of a relatively limited reference standard.

Details of population and microbiological and clinical reference standards for both the studies are summarised below (Table 7.1). In both settings, LF-LAM results did not form part of the microbiological reference standard, but were available to treating clinicians to form part of decision-making for TB treatment initiation, and as such likely to have influenced the clinical reference standards. Despite this, many participants with positive LF-LAM results in both these studies did not receive TB treatment (see figure 7.1, below), perhaps suggesting some lack of faith in – or action following – receipt of LF-LAM results by clinicians.

Study	Population	Reference tests	Definition of TB / not TB
Huerga et. al. (MSF)[7]	1575 outpatient adults living with HIV in Uganda, Kenya, Mozambique, South Africa. 1031 with TB symptoms, 544 no TB symptoms but CD4 <200 cells/mm^3.	Sputum NAAT Sputum culture Sputum induction available. Urine NAAT if unable to produce two sputum samples. Clinical evaluation Chest X-ray (read by clinician in real time to make clinical decisions, reviewed by radiologist at end of study) Ultrasound available in most centres on clinical request. Extrapulmonary samples for NAATs on request. Sputum smear microscopy for all participants in Uganda. LF-LAM results not part of microbiological reference standard but were available to clinicians making treatment decision (relevant for clinical reference standard)	**Microbiological reference standard** TB At least one culture or NAAT positive. Not TB No positive culture or NAAT **and** At least two negative cultures or NAAT (at least one of which must be sputum). Unclassified Did not have at least two samples for testing (at least one of which was sputum) **Clinical reference standard** TB At least one culture/NAAT positive **Or** Clinical decision to treat for TB **AND** positive smear microscopy, chest x-ray suggestive of tuberculosis, ultrasound or retinoscopy suggestive of tuberculosis, or clinical diagnosis of extrapulmonary tuberculosis. Not TB At least one negative culture/NAAT on at least one sample (sputum sample if symptoms, any sample if no symptoms) **and** radiologist chest X-ray interpretation not suggestive of TB **and** no decision to treat for TB. Unclassified Anyone not including in "TB" or "not TB" groups.
Székely et. al. (FIND)[6]	1624 inpatient and outpatient adults living with HIV in Malawi, South Africa, Tanzania, Thailand, Uganda, Vietnam, Zambia). Outpatients had TB symptoms, inpatients recruited regardless of symptoms. Any CD4 counts.	Urine NAAT (concentrated) Mycobacterial blood culture Sputum microscopy, culture and NAAT (at day 1,2, month 3, month 6). Non-study samples at clinician discretion. LF-LAM results not part of microbiological reference standard but were available to clinicians making treatment decision (relevant for clinical reference standard)	**Microbiological reference standard** TB At least one culture NAAT/positive Not TB No positive culture or NAAT **and** At least one negative sputum cultures or NAAT. Unclassified Did not have at least one sputum sample for testing. **Clinical reference standard** TB At least one culture/NAAT positive **Or** Clinical decision to treat for TB **AND** clinical response to treatment at 2-3 months. Not TB No positive culture/NAAT **AND** no symptoms at 2-3 months without TB treatment. Unclassified Anyone not included in "TB" or "not TB" groups.

Table 7.1 Summary of population, methods and definitions of reference standards for two multi-country diagnostic accuracy studies of urine LAM testing (SILVAMP-LAM and LF-LAM)

A

	N	TP	FP	FN	TN	Unclass.	N	TP	FP	FN	TN	Unclass.
	Micro. reference standard						**Clinical reference standard**					
Heurga (MSF)												
LF-LAM	1106	46	135	68	857	458	1261	62	57	101	1041	303
SILVAMP-LAM (overall)	1106	68	133	46	859	458	1261	78	108	85	990	303
SILVAMP-LAM lot 20004 note a	275	22	7	24	222	-	-	-	-	-	-	-
Szekly (FIND)												
LF-LAM	1615	89	123	203	1200	77	1398	146	43	336	873	294
SILVAMP-LAM	1615	160	197	132	1126	77	1399	218	125	264	792	293

B

	Micro. Reference standard	**Clinical reference standard**
Heurga et. al. (MSF)	TB note b: 10% (114/1106). Not TB note b: 90% (992/1106) Unclassed note c: 29% (458/1564)	TB note b: 13% (163/1261) Not TB note b: 87% (1098/1261) Unclassed note c: 19% (303/1564)
LF-LAM Positivity note d: 16% (254/1564)	Sens: 40% (46/114) Spec: 86% (857/992) PPV: 23% (46/199) NPV: 92% (857/925)	Sens: 38% (62/163) Spec: 95% (1041/1098) PPV: 52% (62/119) NPV: 91% (1041/1142)
SILVAMP-LAM (overall) Positivity note d: 16% (247/1564)	Sens: 60% (68/114) Spec: 87% (859/992) PPV: 33% (68/201) NPV: 95% (859/905)	Sens: 48% (78/163) Spec: 90% (990/1098) PPV: 41% (78/186) NPV: 92% (990/1075)
SILVAMP-LAM (lot #20004) Positivity: 22/275 (8%) note a	Sens: 48% (22/46) Spec: 97% (222/229) PPV: 75% (22/29) NPV: 90% (222/246)	Not reported
Székely et. al. (FIND)	TB note b: 18% (292/1615) Not TB note b: 82% (1323/1615) Unclassed note c: 5% (77/1692)	TB note b: 34% (482/1398) Not TB note b: 65% (916/1398) Unclassed note c: 17% (294/1692)
LF-LAM Positivity note e: 13% (212/1615)	Sens: 30% (89/292) Spec: 85% (1200/1403) PPV: 42% (89/212) NPV: 91% (1200/1323)	Sens: 30% (146/482) Spec: 95% (873/916) PPV: 77% (146/189) NPV: 72% (873/1209)
SILVAMP-LAM Positivity note e: 22% (357/1615)	Sens: 55% (160/292) Spec: 85% (1126/1323) PPV: 43% (160/366) NPV: 90% (1126/1258)	Sens: 45% (218/482) Spec: 86% (792/917) PPV: 64% (218/343) NPV: 75% (792/1056)

Table 7.2 Performance characteristics of LF-LAM and SILVAMP-LAM in two multicountry studies.

A Performance characteristics of LF-LAM and SILVAMP-LAM in two multi-country studies. N = Number of eligible participants with reference standard results, TP = True Positive, FP = False Positive, FN = False Negative, TN = True Negative

B Summary diagnostic performance (calculated from data in panel A).

Notes:

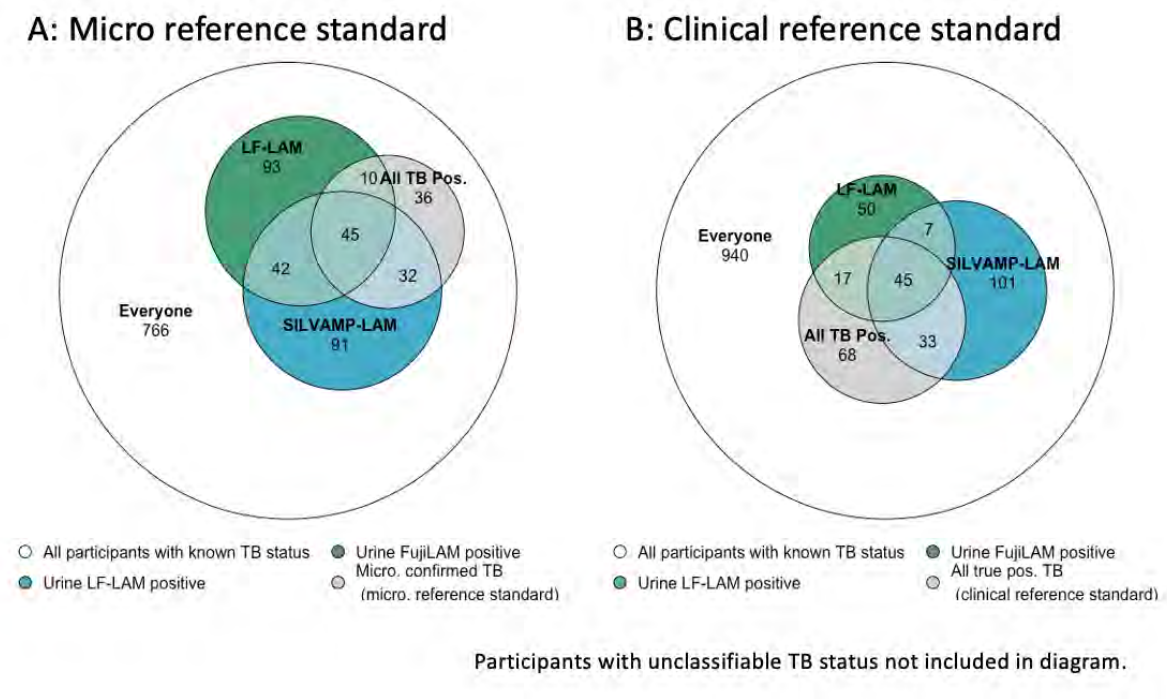
a: MSF study did not use lot #19002 so can't include here (lot #19002 was used for a quarter of SILVAMP-LAM test in CASTLE). FIND didn't report detailed breakdown by lot numbers.

b: Denominator for TB/not TB is among all participants with a classifiable TB status

c: Denominator for unclassified is all participants recruited

d: Includes LAM results from all participants tested, including people with unclassifiable reference standard results. Only excluded from denominator if no valid LAM result obtained.

e: Includes LAM results only from participants included in the microbiological reference standard. Urine LAM results for the 68 people unclassified in by microbiological reference standard are not reported in paper.



Heurga et. al (MSF)	LF-LAM positive	LF-LAM negative	
SILVAMP-LAM positive	96	151	**247**
SILVAMP-LAM negative	158	1159	**1317**
	254	**1310**	1564

Figure 7.1 and Table 7.3: Concordance of LF-LAM and SILVAMP-LAM in Heurga et. al. (MSF study)[7]

Note that Figure 1 contains test results for only people with classifiable status by Microbiological or Clinical Reference Standard. Table 7.3 contains urine LAM results for the whole population (including those with unclassifiable results)

Tables 7.1-7.3 and Figure 7.1 summarise results from the FIND and MSF studies (pooled across all SILVAMP-LAM lots used). Figure 7.1 shows the overlap in LF-LAM and SILVAMP-LAM results. The FIND study doesn't report concordance between SILVAMP-LAM and LF-LAM, so these cannot be shown as Euler diagrams or a 2x2 table, as the overlap is unknown.

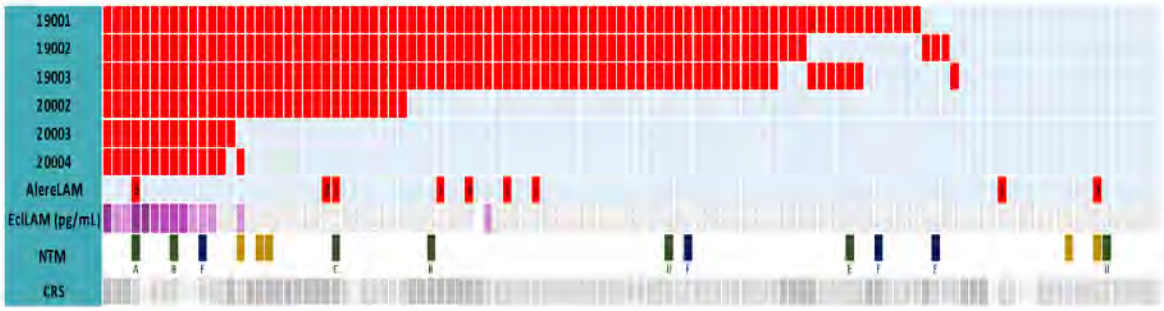
The noteworthy issues are that the overall positivity rate for LF-LAM and SILVAMP-LAM (across all lots) are similar to each other (whereas if SILVAMP-LAM was more sensitive a higher positivity rate would be expected), and the low concordance between LF-LAM and SILVAMP-LAM positive tests such that only about 40% of urine tests positive by any one LAM test were positive by the other (Figure 7.1).

In both the MSF and FIND studies, many more participants had positive LAM tests (by either point of care LAM test) than had positive microbiological reference tests, with positive predictive values (according to microbiological reference standard) less than 50% in each study and for each LAM test. As discussed in Chapter 2 (Background), there can be serious difficulties in defining a reference standard for TB and it can be challenging to evaluate an index test that is potentially more sensitive

than the reference standard, but this level of discordance – particularly in the FIND study, which included mycobacterial blood cultures and concentrated urine NAATs as part of the reference standard [6] – is unexpected and was not seen in earlier studies using biobanked urine samples (see Chapter 2, particularly Broger et. al.[2]).

In the FIND study the investigators conducted post-hoc laboratory analyses on stored urine samples to better understand discordant results. They used a highly a sensitive quantitative laboratory electrochemiluminescence LAM assay (EclLAM, Meso Scale Diagnostics, Rockville, MD, USA) to define a reference standard for LAM concentrations. Urine samples that were positive by SILVAMP-LAM at a site, but negative by reference standard (i.e. urine samples from difficult-to-classify participants), were re-analysed in controlled laboratory conditions by all four SILVAMP-LAM lots, LF-LAM, and the EclLAM assay. The results are reproduced below.

Figure 3. Exploratory comparison of FujiLAM positivity rates in 111 eMRS negative, FujiLAM positive samples from the study



eMRS, extended microbiological reference standard; NTM, nontuberculous mycobacteria; CRS, composite reference standard; Red cells indicate positive result and light blue cells indicate negative result on FujiLAM or AlereLAM. For AlereLAM positive results, the numbers further indicate line grade intensity (1–3). LAM concentration measured with EclLAM is illustrated on the purple scale from darkest to lightest: >200 pg/mL, 51–200 pg/mL, 11–50 pg/mL; diagonal stipe pattern indicates <limit of detection (11 pg/mL); Dark yellow – Full NTM speciation not done; green - slow growing mycobacteria; dark blue- fast growing mycobacteria; A- *M. simiae*; B- *M. intracellulare*; C- *M. avium*, D- *M. scrofulaceum*; D- *M. gordonae*; F- *M. fortuitum;* Dark grey- CRS positive, mid grey-CRF neg, light grey- unclassifiable.

Figure 7.2: Result of laboratory testing urine samples from FIND diagnostic accuracy study.[6]

Urine results for: SILVAMP-LAM (six lots, lot number on left hand side), LF-LAM ("AlereLAM"), reference standard quantative LAM ("EclLAM", in purple). Presence of non-tuberculois mycobacteria in sputum samples from that participant, and clinical reference results in rows at bottom.
Reproduced from Szekley et. al. under CC-BY-NC license.
SILVAMP-LAM lot #20004 (bottom row of SILVAMP LAM #s) was the main lot used in CASTLE, with some of lot #19002 also used.

Figure 7.2 shows that whilst lot #20004 (the main lot used in CASTLE) had a lower positivity rate than other lots (and is referred to as “low sensitivity” in pre-print), it maintained excellent specificity with respect to the EclLAM laboratory assay. The other SILVAMP-LAM lots had positive results in many cases where there was no LAM detected by laboratory assay. Also of note is that LF-LAM (AlereLAM) results in these urine samples exhibit extremely low sensitivity and specificity with respect to the EclLAM (1/9 LF-LAM positives also positive on EclLAM, and only 1/14 samples positive by EclLAM also LF-LAM positive). The numbers are small, and this is a group of samples specifically selected for being challenging to classify (rather than arising from a representative patient population), but nonetheless the diagnostic performance of LF-LAM is seems particularly poor even under laboratory conditions.

7.2.2 Possible reasons for discordant LAM results in CASTLE

CASTLE was not a diagnostic accuracy study and it is difficult to be definitive about reasons for discordant LAM results, but possible considerations are outlined below and CASTLE diagnostic test results (from chapter 6) are reproduced here for ease of reference.

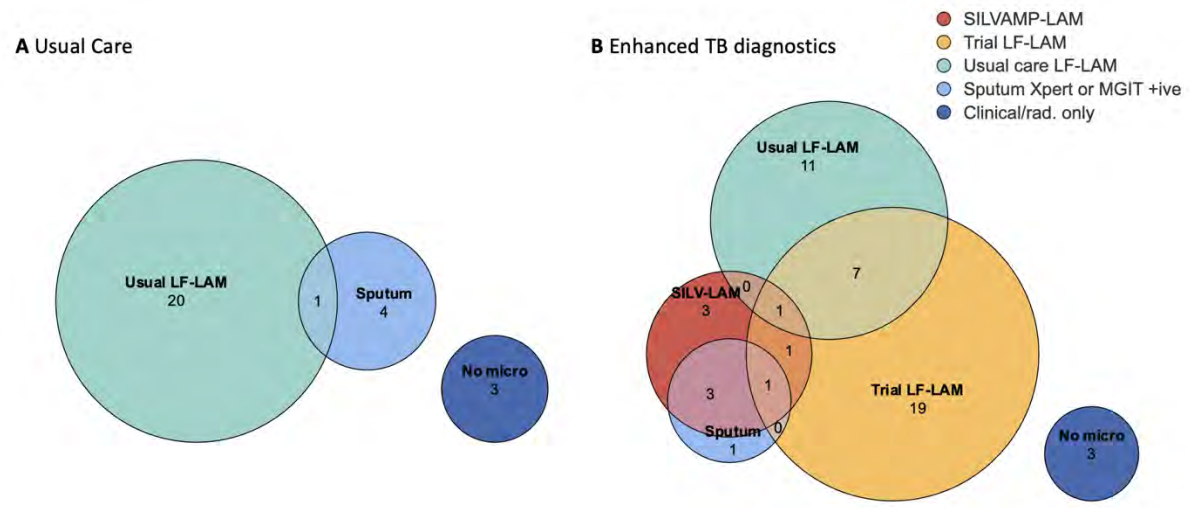


Figure 7.3: Positive TB tests (A) Usual care arm and (B) Enhanced TB diagnostics arm in CASTLE, reproduced from chapter 6

SILVAMP-LAM = SILVAMP LAM urine test manufactured by FujiFilm (Japan), LF-LAM = Determine LF-LAM urine test manufactured by Alere/Abbott (USA). Xpert = Xpert Mtb/rif rapid molecular diagnostic test, manufactured by Cephid (USA). Clinical/rad. only = participants who started TB treatment based on clinical or radiological findings, but without positive microbiological tests. Results shown for all participants who had at least one positive TB test or who started TB treatment. Includes 8 people (4 in each arm) who had a positive test but did not start TB treatment.

		Urine SILVAMP LAM results				Totals
		Positive	Negative	Indeterminate	Not done	
Urine LF-LAM results	Positive Grade 2-4	1	6	0	0	**7**
	Positive Grade 1	2	20	0	0	**22**
	Negative	6	165	1	0	**172**
	Not done	0	0	0	6	**6**
Totals		**9**	**191**	**1**	**6**	**207**

Table 7.4 : Urine lipoarabinomannan results in the enhanced TB diagnostics arm participants in CASTLE study, reproduced from chapter 6.
Results of testing fresh urine, both tests conducted on the same sample.

- **Low sensitivity of SILVAMP-LAM lot**

  Both the FIND and MSF studies showed that SILVAMP-LAM lot #20004 was a lower sensitivity lot than other lots used in the studies;[6,7] this was the lot used for most of CASTLE (150/201 SILVAMP-LAM tests). Unexpectedly low sensitivity of SILVAMP-LAM used in CASTLE will likely have contributed to discrepant results, especially if LF-LAM was more sensitive. In the MSF study, SILVAMP lot #20004 reportedly had 48% sensitivity (compared to overall 40% sensitivity for LF-LAM).[7]

- **Operator reading LF-LAM sub-optimal / overreading "faint lines"**

  LF-LAM results must be read with reference to a card inset and faint lines that are less intense than the lowest line on the reference card classified as negative (or invalid) results. The interpretation of faint lines is subjective, and "over-reading" of faint lines could lead to false positive interpretations, as has been described in other settings.[8,9] To mitigate this risk in CASTLE all LF-LAM results from trial team were double-read by two research assistants (although not blinded), and staff were trained about the interpretation of faint lines.

  The 14% overall positivity rate for trial LF-LAM tests was the same as observed in the Malawi arm of the STAMP trial of LF-LAM (14.3%, 94/656 tests),[1] similar to the inpatient positive rate in the FIND evaluation (18.6%, 125/672),[6] and lower than three other HIV positive inpatient Malawi LF-LAM studies (26%, 100/383 in Chiradzulu District Hospital[10], 22%, 81/363 in Kamuzu Central Hospital[11] and 48%, 70/143 in Queen Elizabeth Central Hospital). This suggests that systematic over-reporting of faint lines (leading to false positive LF-LAM results) is less likely as an explanation for discrepant SILVAMP-LAM / LF-LAM results in CASTLE, although a possibility remains that inconsistent reading of faint lines may have contributed to discrepant results.

- **Cross reactivity, or other reasons for false positive results**

  LAM tests (LF-LAM and SILVAMP-LAM) can be positive in people with non-tuberculous mycobacteria.[12,13] However, only one person in CASTLE had a mycobacterium other than tuberculosis on sputum culture, suggesting that there isn't a very high prevalence of non-tuberculous mycobacteria among CASTLE participants – although not everyone produced sputum for culture. There is some evidence from early research in the development of LAM tests indicating cross reactivity between antibodies to LAM and various mouth flora, which was identified using a LAM ELISA kit made by Alere that is no longer commercially available.[14] Cross reaction with perineal or urine bacteria causing false positive results in one of LF-LAM or SILVAMP-LAM (but not the other) is a further possible contributing factor to discordant results.

- **Relatively high CD4 counts in CASTLE**

  In the Malawi 2022 HIV guidelines, LF-LAM is recommended for all people living with HIV admitted to hospital.[15] The WHO Global TB programme guidelines also recommend LF-LAM for inpatients with a CD4 of <200 cells/mm^3 or who have TB symptoms,[16] so have a slightly more restrictive approach than Malawi national guidelines. Of note, a recent individual patient data meta-analysis study concluded that the additional CD4 and symptom criteria to guide LF-LAM testing within HIV-positive inpatients have limited utility / diagnostic accuracy and recommended it would be more effective to test all HIV positive inpatients.[17]

  In CASTLE the median CD4 cell count was 240 cells/ mm^3, when measured (CD4 counts were conducted by usual care teams, not the trial and as such were sometimes not done). This is higher than some other inpatient cohorts (see Chapter 2) and means that over half of CASTLE participants had a CD4 count higher than recommended by WHO (but not the Malawi HIV programme) for systematic LF-LAM testing inpatients people without TB symptoms. In CASTLE, 43 people (10% of the trial population) had both CD4 >200 cells/mm^3 and no signs of symptoms of TB at admission, a further 31 people (7%) had no CD4 count measured and no TB signs or symptoms – these people met Malawi National recommendation for LF-LAM testing, but not WHO recommendations.

  LF-LAM is known to have reduced sensitivity in people with higher CD4 cell counts,[18] probably because the likelihood of dissemination and haematogenous spread (including to kidneys) of *M. tb* is less likely with higher CD4 counts.[19] Thus the relatively higher CD4 counts (compared to most other LF-LAM evaluation studies) in the population tested in CASTLE might have contributed to poorer diagnostic performance of LF-LAM because the proportion of participants

who truly had disseminated TB was lower than in a cohort with a lower median CD4 count, leading to an increased false positive rate.

- **Poor test performance of LF-LAM / quality assurance process not well developed**
  For LF-LAM, I used LAM strips procured through a mixture of normal Malawi government channels, supplemented by LF-LAM locally supplied through commercial Malawian distributors, as needed. CASTLE staff were trained and had regular supervision in interpreting LAM results (LF-LAM and SILVAMP-LAM), and results were reviewed and agreed by two readers (although not blinded). All manufacturer instructions for use were followed and records maintained in paper log - the manufacturer's instructions for use do not specify use of external controls (there is an internal control strip on the lateral flow kit). There are no available external positive or negative controls for LF-LAM (or SILVAMP-LAM). This is unlike HIV testing, where external positive controls are available from manufacturers of test kits (e.g. Abbott for Determine HIV-1 test kits) and testing is supported by well-developed quality assurance materials from the USA Centers for Disease Control.[20] Urine NAAT testing is not a suitable control for urine LAM as there are known to be wide discrepancies between urine NAAT positivity and urine LAM positivity (even though both measure components of *M. tb* present in urine).[6,21]

  Our quality assurance for LAM focused on the tester competency, and is equivalent or more stringent that what happens in usual care, but lack of full quality assurance framework (including batch testing with external quality control samples) means it is difficult to be entirely clear about the diagnostic accuracy of LF-LAM.

In summary, SILVAMP-LAM has been shown to have unacceptably high lot-to-lot variability, which severely hampers utility of the test, and has delayed or halted the planned 2023 WHO Guideline Development Group meeting. In CASTLE, most of the SILVAMP-LAM test were on a lot now known to have low sensitivity but high specificity, so it is difficult to draw inference from CASTLE about what would have been the impact on TB treatment initiation and secondary outcomes, had a high-sensitivity / high-specificity SILVAMP lot been used been used. In addition, we showed a high rate of LF-LAM positivity in the setting with relatively low rates of positivity of highly specific TB tests (sputum NAAT and SILVAMP-LAM). Critically, the lot of SILVAMP-LAM used in CASTLE (150/201 CASTLE tests were done using lot #20004) did have high specificity and may have had better performance than LF-LAM in CASTLE.

Particularly given the lot-to-lot variation with SILVAMP-LAM which has paused WHO guidelines process, and given that alternative urine LAM products are in very early stages of development, Alere/Abbott LF-LAM will likely be the major TB urine diagnostic product in use for the near future. As such, further research on LF-LAM cross-reactivity with other bacterial in urine or other reasons for false-positives is important, as is a robust toolkit for quality assurance to support, along the lines of that which exists for point of care HIV testing. This should include manufactures making external positive and negative control material available, and possibly a digital reader to reduce subjective interpretations of line color intensity. This would support high quality LF-LAM testing and may contribute to uptake of LF-LAM testing.[22]

7.3 Comparison between CASTLE and STAMP

TB incidence and prevalence in Malawi are declining, as demonstrated by TB prevalence surveys in Blantyre: in the 2013 Malawi National TB prevalence survey, TB prevalence was 452 per 100,000 adults overall[23] with prevalence much higher in urban areas and Southern region of Malawi (~1000 per 100,000 adults). In contrast, in a 2019 prevalence survey for a cluster randomised trial, TB prevalence was 215 per 100,000 adults.[24] TB case notifications in Blantyre are also falling, as shown through Blantyre enhanced TB surveillance system.[24]

We also know that the incidence of HIV related hospital admission has been substantially reducing in recent years, as seen in Chapter three and also related to CASTLE trial recruitment issues detailed above (section 7.1). However, it is unclear whether - conditional on being unwell enough to require hospital admission - TB prevalence in people hospitalised will have reduced over time. As discussed in Chapter 2 (Background) and Chapter 8 (discussion), TB remains extremely common in hospitalised PLHIV, evidenced by studies with extensive reference standards, systematic reviews and autopsy studies.

CASTLE was not a diagnostic accuracy study and I do not think that the microbiological testing (one sputum culture attempted for all participants) is sufficient to draw clear conclusions about TB prevalence, however some comparison with the STAMP might be helpful in illuminating potential emerging trends in TB and HIV hospital epidemiology. The STAMP RCT was a two country study, with the Malawi site in Zomba Central Hospital from 2015-2017. STAMP was also not a diagnostic accuracy study, but like CASTLE had some microbiological TB tests as part of their trial interventions.

	STAMP Malawi arm			CASTLE		
	Usual	**Intervention**	**All**	**Usual**	**Intervention**	**All**
Location	Zomba Central Hospital, Southern Region, Malawi					
Population	Adults living with HIV admitted to medical wards (irrespective of ART use or reason for admission)					
Dates	Oct 2015- September 2017			September 2019 – February 2022		
1st line ART in Malawi	EFV + TDF + 3TC (treat all recently started)			DTG + TDF + 3TC (treat all well established)		
TB diagnostics implemented by trial (not including usual care)	Sputum NAAT	Sputum NAAT, urine NAAT, urine LF-LAM		Sputum culture	Sputum culture, urine LF-LAM, urine SILVAMP-LAM, sputum NAAT if CAD score >= 60	
Number of participants	660	656	**1316**	208	207	**419**
HIV indicators						
CD4 cell count done	657 (99.5%)	656 (100%)	**1313 (99.8%)**	158 (76%)	149 (72%)	**307 (74%)**
CD4 cell count < 100 cells/mm^3	178/660 (27.1%)	187/656 (28.5%)	**365/1316 (27.8%)**	27/158 (17.1%)	31/149 (20.1%)	**58/307 (18.9%)**
Median CD4	222	218	**219**	288	220	**240**
Current ART > 6 months	416 (63.0%)	398 (60.7%)	**814 (61.9%)**	164 (78.8%)	162 (78.3%)	**326 (78.6%)**
Current ART < 6 months	99 (15.0%)	108 (16.5%)	**207 (15.7%)**	20 (9.6%)	29 (14.0%)	**49 (11.8%)**
Interrupted ART	17 (2.6%)	13 (2.0%)	**30 (2.3%)**	5 (2.4%)	2 (1.0%)	**7 (1.7%)**
Never ART	128 (19.4%)	137 (20.9%)	**265 (20.1%)**	19 (9.1%)	14 (6.8%)	**33 (7.8%)**
Urine tests						
LF-LAM positive (by trial only) note a	NA	94/651 (14.4%)	**NA**	NA	29/201 (14.4%)	**NA**
LF-LAM positive (by trial or usual) note a	NA	94/651(14.4%)	**NA**	21/208 (10.1%)	40/207 (19.3%)	**61/415 (14.7%)**
SILVAMP-LAM positive (by trial)	NA	NA	**NA**	NA	9/201 (4.5%)	**NA**
Urine Xpert positive (by trial)	NA	37/651 (5.7%)	**NA**	NA	NA	**NA**
Sputum tests						
Sputum obtained NAAT or culture note b	277 (42%)	252 (38.4%)	**529 (40.2%)**	111 (53.4%)	130 (62.8%)	**241/415 (58%)**
Sputum NAAT or culture positive (as % all participants) note c	20/660 (3.0%)	22/656 (3.4%)	**42/1316(3.2%)**	5/208 (2.4%)	5/207 (2.4%)	**10/415 (2.4%)**
Sputum NATT or culture positive (as % those tested) note c	20/277 (7.2%)	22/252 (8.7%)	**42/529 (7.9%)**	5/111 (4.5%)	5/130 (3.8%)	**10/241 (4.1%)**
Sputum obtained for NAAT note d	277 (42%)	252 (38.4%)	**529/1316 (40.2%)**	42/208 (20%)	84/207 (40%)	**126/415(30%)**
Sputum NAAT positive (as % those tested)	20/277 (7.2%)	22/252 (8.7%)	**42/529 (7.9%)**	2/42 (5%)	5/84 (6%)	**7/126 (6%)**
TB diagnoses						
TB diagnosed – micro confirmed (including LF-LAM) note e	31 (5.7%)**	113 (17.2%)	**144 (10.9%)**	25 (12.0%)	47 (22.5%)	**72 (17.3%)**
TB diagnosed – micro confirmed (not including LF-LAM)	31 (5.7%)**	63 (9.6%)***	**94 (7.1%)**	5 (2.4%)	10 (4.8%)***	**15 (3.6%)**
TB diagnosed – including clinical	68 (10.3%)	126 (19.2%)	**194 (14.7%)**	28 (13.5%)	50 (24.0%)	**78 (18.8%)**
TB treatment started	65 (9.8%)	120 (18.3%)	**185 (14.1%)**	24 (11.5%)	46 (22.2%)	**70 (16.9%)**
Outcome						
Died in hospital	77 (11.7%)	71 (10.8%)	**148 (11.2%)**	33 (15.9%)	36 (17.3%)	**69 (16.6%)**
Died by 56 days	161 (24.4%)	137 (20.9%)	**298 (22.6%)**	52 (25.0%)	54 (26.1%)	**106 (25.5%)**

Table 7.5 Comparison of STAMP and CASTLE

Notes (overleaf):

a: In both CASTLE and STAMP, urine LF-LAM tests were conducted by the trial team for those in intervention arm only. In CASTLE (but not STAMP), LF-LAM was also available through usual care and recommended by Malawi 2022 National HIV guidelines for all PLHIV admitted to hospital. In the intervention arm of CASTLE, some people had LF-LAM from both CASTLE team and through usual care.

B: In STAMP, all participants were asked to submit sputum for NAAT testing, and no culture was done. In CASTLE all participants were asked to submit sputum for culture. In addition, NAAT testing could be accessed through usual care if requested by routine staff for any participants, and in intervention arm if CAD score was >=60 then CASTLE team would attempt to obtain sputum for NAAT. This is number of participants with at least one sputum sample (STAMP = Xpert only, CASTLE = Xpert and culture both attempted, reported as submitting sputum if at least one of these were done).
C: Denominator is everyone who submitted sputum, including 27 people in CASTLE with contaminated cultures (including as negative)
d: Only NAATs (not culture), as these tests impacted participant TB management. In STAMP as no culture was done, this is equivalent to above rows
e: In STAMP, includes results from urine Xpert (intervention arm only, 21 micro confirmed positive on urine Xpert only) and in CASTLE includes results from SILVAMP-LAM (intervention arm only, 5 micro confirmed on SILVAMP-LAM only).

In general, CD4 cell counts were slightly higher in CASTLE than in Malawi site of STAMP, and a greater percentage of participants were taking ART in the CASTLE trial. In the STAMP Malawi site, 3.2% of all participants had positive sputum NAAT (which was 7.9% of all participants who submitted sputum), whereas in CASTLE, 2.4% of all participants had a positive NAAT or culture (4.1% of participants who submitted sputum, as more participants produced sputum in CASTLE than in STAMP). The proportion of people with positive LF-LAM tests was identical in the two studies (14.4%).

The sputum culture and NAAT tests are likely to be highly specific, as are SILVAMP-LAM tests (as lot #20004 is highly specific); overall in CASTLE 15 people (3.6%) had microbiologically confirmed TB by a test other than LF-LAM (five in usual care arm all on sputum NAAT or culture and ten in enhanced TB diagnostics arm with six of those positive by SILVAMP-LAM but not sputum NAATs/culture). In STAMP, 94 people (7.1%) had TB diagnosed by a test other than LF-LAM.

The numbers of participants are small, and the diagnostic tests offered in both STAMP and CASTLE are insufficient to be a true reference standard for TB. Nonetheless it is possible that the overall prevalence of TB in CASTLE was lower than in STAMP, and this may have contributed to the lack of demonstrated mortality benefit in a TB focused intervention. This is for two reasons, firstly as the true prevalence of TB disease goes down, the false positive rate of any TB test will increase and thus test performance of TB diagnostics (positive predictive value) will be less good. Secondly, since the group of people who will benefit from a TB diagnostic intervention are those people who have TB disease, a lower TB prevalence means fewer people in the group stand to benefit (see discussion in Chapter 8 about the lack of mortality benefit).

7.4 COVID-19 impact

The COVID-19 pandemic had considerable effects on the CASTLE trial. Practically, the start of CASTLE was delayed by five months, and for six weeks in the middle of the trial (December – January 2021) recruitment was paused for safety reasons (mandated by the MLW Programme) due to COVID waves in Malawi. Whilst some of the sample size issues and reduced admission to hospital compared to STAMP (2015-2017) pre-date COVID-19, it was clear that the COVID-19 pandemic contributed to reduced numbers of people presenting to hospital overall and reduced numbers of people being admitted to medical wards, as some people were diverted from emergency department to COVID-19 specific wards (and therefore not eligible for recruitment into CASTLE). Medical staffing at Zomba Central Hospital was disrupted due to staff illness and requirements to cover COVID wards. Finally, COVID epidemic waves may have impacted the utility of symptom screening or chest X-ray scoring to distinguish TB from other reasons for hospital admission.

I considered carefully, with others in the trial team, whether to include COVID-19 testing as a trial intervention. The advantage of having protocolised COVID-19 testing would have been that we could have clearly put dCXR-CAD scores into the context of the COVID-19 epidemic. However, in 2020, COVID-19 rapid test kits were difficult to obtain and import into Malawi, and needed to be prioritised for clinical rather than research use. PCR was not widely available, and was expensive. There was also a large among of worry, fear and stigma related to COVID-19 among the general public and hospital patients and relatives (initially related to mandatory isolation in “container clinics” following a positive COVID-19 result), such that the acceptability of research COVID-19 testing was likely to be very low and participants may have declined CASTLE trial participation. TB and COVID-19 co-infection was likely, particularly if people developed nosocomial COVID-19 during their interactions with the health system leading up to hospital admission, so there was a risk of diagnostic overshadowing and missing the diagnosis of TB or other treatable opportunistic infections if COVID-19 test were positive.

Accordingly, we did not offer COVID-19 testing for CASTLE participants and it is possible some high CAD score X-rays might have been due to COVID-19 that went undiagnosed. However, it was always the case that in this setting high CxR CAD scores could be due to respiratory pathology other than TB (such as PJP, bacterial pneumonia, cardiogenic pulmonary oedema) and for this inpatient population, CAD scores require interpreting in light of the whole clinical picture, including but not limited to possible COVID-19. COVID-19 testing (PCR and rapid tests) were available on clinician request from usual care in Zomba Central Hospital for most of CASTLE, after the first few months of scarcity, albeit with occasional stock outs. Only 43 people in CASTLE had recorded results of COVID-19 test in their medical

records, eight of which were positive (three usual care, five enhanced diagnostics arm). CAD scores for the five people with COVID-19 positive PCR ranged from 45 to 65.

With the Malawi-Liverpool-Wellcome Programme, I worked in partnership with Zomba Central Hospital to support their COVID-19 work (including lab testing in usual care), as far as possible. We helped supply Personal Protective Equipment and other consumables for all staff, offered COVID-19 testing in the Malawi-Liverpool-Wellcome laboratory on clinician request (in the early months of the pandemic, before test kits were procured for Zomba Central Hospital laboratory) and provided oxygen tank refill supplies from the oxygen factor at Queen Elizabeth Central Hospital in Blantyre.

7.5 Actions consequent to chest X-rays

I determined the threshold to say a chest Xray was "TB likely" based on previous experience in outpatient chest X-ray studies and reviewing chest Xrays in the pilot period and discussion with an experienced radiologist. Chest X-ray images, CAD colour overlay highlighting abnormal areas, and the numeric CAD score were all available to treating clinicians, alongside the interpretation of "TB likely" vs. "TB not likely". If a CAD score was 60 or higher (our threshold), the CASTLE trial team would attempt to obtain a sputum NAAT.

The CASTLE trial used CAD4TBv6, which is one of the more established CAD softwares for dCXR, and gives a numeric score for how likely TB is. I considered using an alternative CAD product (specifically, Qure.AI from qXR, India) which produces an artificial-intelligence written summary akin to a radiologist report, in addition to a TB score. However issues with offline use and practical implementation of Qure.AI in a relatively remote hospital site without an internet connection meant I opted for CAD4TB, which has more robust offline implementation and more stable version control. Whether a written report output rather than just a score would have been helpful in this setting is unknown. CAD4TB has challenges with changing TB thresholds with different versions (e.g. CAD4TBv7 should be used with lower threshold to trigger action than CAD4TBv6[25,26]) but gives stable scores within a version.

I anticipated having chest X-rays routinely available to everyone would lead both to more confirmed TB cases (through prompting more sputum tests) and to a greater use of empiric TB treatment. My initial concern was over-diagnosis of TB based on X-rays and early teaching to staff in medical department on CxRs and CAD focused around that high CAD scores were not highly specific for TB and could be a diagnostic adjunct but not to prove TB diagnosis. However, we saw that in both CASTLE arms, empiric TB diagnoses and treatment initiations were rare (only three in each arm); availability of chest X-ray therefore potentially prompted fewer empiric TB diagnoses than anticipated. Participants with higher

CAD scores received sputum NAATs from trial team, where possible, but because so few NAAT tests were positive, this did not drive substantially more TB treatment initiations. We were unable to gather information on the number of people with departmental (conventional / film) chest X-rays in the usual care arm as this information wasn't captured in a ledger and chest X-rays were not always recorded in medical notes.

Most of the care in Zomba Central Hospital was done by clinical officers under the supervision of physicians, who are available for advice five days a week, but physician ward rounds occur only three times per week. It is possible that clinical officers didn't feel empowered to make TB treatment decisions in the absence of microbiological confirmation. Further work about implementing dCXR-CAD in inpatient or other very high TB prevalence settings should address whether and how CAD can be incorporated into clinical decision-making about empiric TB treatment (see Chapter 8 about use case of dCXR-CAD in inpatient setttings).

7.6 Summary

In summary, CASTLE was a relatively small and pragmatic trial to assess feasibility and impact of introducing systematic use of enhanced TB diagnostics into care for hospitalised people living with HIV. I showed an increase in the number of people diagnosed with TB and starting TB treatment, but probably no impact on mortality by 56 days. Limitations and challenges during CASTLE trial arose from lot-to-lot variation of SILVAMP-LAM, and that most of our SILVAMP-LAM tests came from a low sensitivity lot. I also identified a high positivity rate on LF-LAM that was not confirmed on SILVAMP-LAM or sputum NAAT tests. These issues have also been seen in two large diagnostic accuracy evaluations of SILVAMP-LAM that also tested samples using LF-LAM and raise the possibility that LF-LAM may have been less specific than anticipated. I also showed a relatively low prevalence of empiric TB diagnoses, suggesting that DCXR-CAD scores and availability of digital Xrays to view, were not prompting major changes in clinician behaviour. CASTLE was conducted during the COVID-19 pandemic, and this may have impacted the recruitment rate and sample size, as well as altered pattern of CAD4TB scores, although only 8 people in CASTLE were known to have positive COVID-19 tests, most of who had CAD scores below (43 people with COVID-19 tests done). I showed that urine LAM testing and DCXR-CAD interventions were feasible to perform in this setting, gave rapid results on the first day of admission and lead to more TB diagnoses being made.

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Chapter 8: Discussion and conclusions

8.1 Summary of thesis

High mortality among adults living with HIV admitted to hospital is an urgent public health problem. Effective interventions for this group are still needed to achieve goals to end AIDS as a public health problem by 2030[1], and to get to zero AIDS-related deaths.[2]

In Chapter 3, I identified relatively few trials that had investigated interventions to reduce all-cause mortality among hospitalised adults with HIV. Advanced HIV disease is a priority area for many national HIV programmes, international organisations, and organisations that provide support to HIV programmes. WHO has recommended a package of care for Advanced HIV Disease (AHD) since 2017.[3] Most of the evidence to

inform the AHD package of care comes from randomised trials among ambulatory outpatients with low CD4 cell counts, rather than people who are already seriously ill. Interventions evaluated in these trials have included: enhanced prophylaxis for TB, bacterial infections, fungal infections and helminths (REALITY[4]); screening and prophylaxis for cryptococcal meningitis with community based support (REMSTART)[5]; and the TB FAST TRACK,[6] REMEMBER,[7] and STATIS [8] trials, which showed no benefit of protocol-guided empiric TB treatment. Whilst many of the interventions in the WHO package of care for AHD are relevant and should be provided to people in hospital, there is little evidence for interventions specifically targeted to this hospitalised group.[9,10] The systematic review in Chapter 3 showed that urine LAM screening (which is now part of the WHO-recommended AHD package of care) can reduce mortality, while protocol-driven fluid resuscitation for people with sepsis was harmful. Randomised trials evaluating ART initiation strategies for people in hospital showed mixed evidence that, in any case, has been superseded by changes in ART initiation criteria; one trial showed mortality benefit from simplifying the steps needed prior to ART initiation in China, and one showed no difference in mortality with supporting people in outpatient ART initiation post-discharge in Uganda. However, these results are of limited relevance now that treat all and rapid ART initiation is recommended.

To my knowledge, no other trial investigating interventions to a general HIV positive inpatient population have been registered or published since my systematic search (Chapter 3) was completed, although several trials are ongoing or recently completed investigating specific treatments for TB meningitis,[11,12] disseminated TB[13] and cryptococcal meningitis.[14] Since this thesis was conceived, more effort and attention from policymakers and WHO has been devoted to people living with HIV who are seriously unwell, and a WHO policy brief with a focus on hospital management was published in March 2023.[9] Overall, the question how to optimise HIV in-patient management remains a major gap in the public health evidence-base. Few trials of interventions to reduce mortality in inpatient PLHIV have been conducted – as such, this remains an urgent global research priority in order to reduce AIDS deaths.

In Chapter 4, I analysed the ongoing high mortality among adults admitted to hospital in Queen Elizabeth Central Hospital Blantyre, Malawi and showed that, whilst population incidence of admission of PLHIV declined substantially between 2012-2019, in-hospital mortality risk for those who were admitted remained unchanged over this time period at 23%.[15] Inpatient HIV surveillance data – and indeed data on HIV-related deaths in general – remains very sparse.[16,17] More research on the epidemiology of HIV-associated hospital admission in this era of high coverage of ART – at minimum studies of medical record reviews, but ideally enhanced by improved pathology and radiology diagnostic capacity and autopsies for people who die – is much needed. Inpatient surveillance data focused on HIV could support updated estimates of HIV-positive

mortality, and help to develop research priorities, recommendations and guidelines for the management of ART failure in people who are seriously unwell and admitted to hospital. Whilst ending AIDS deaths clearly involves large scale interventions to reduce HIV transmission and undiagnosed/untreated ART, it also must involve providing clinical care to those at the highest risk of imminent death, and being able to monitor and track our progress in improving clinical care.

In the CASTLE trial (Chapters 5, 6 and 7), I showed that providing a package of enhanced TB diagnostics was feasible and increased the proportion of people admitted to hospital who were diagnosed with TB and started TB treatment, but that this probably did not affect mortality. The confidence interval around the mortality outcome was wide, reflecting the small sample size, which was underpowered for this outcome. In CASTLE, 25% of participants died by 56 days from enrolment, again highlighting the ongoing problem of high mortality among people living with HIV admitted to hospital in Malawi.

Chapter 7 provides more context and detailed discussion around the discrepancy in urine LAM results seen in both in CASTLE and in other recent SILVAMP-LAM diagnostic accuracy studies. I also discussed some reasons why dCXR-CAD might not have led to substantial change in behaviour: there were low levels of TB confirmation by sputum NAAT even in people with high CAD scores, and high CAD scores did not appear to prompt consideration of empiric TB treatment by clinicians. In this chapter, I discuss the what the likely true TB burden among hospitalised PLHIV was, and possible reasons for the lack of mortality difference in CASTLE.

Looking at the bigger picture of deaths related to advanced HIV disease, priority research questions arising include: In countries with well established “treat all” ART programmes, what is the incidence of hospital admission and what are the most common causes of hospital admission and death in people living with HIV? What are the optimal ways to diagnose and treat important opportunistic infections and provide supportive care? How should ART be managed in people who are seriously unwell not on ART, or with virological failure? How should hospital care be linked to primary care so that people with advanced HIV disease can be supported? All these questions are important to provide optimal care for adults living with HIV admitted to hospital, and therefore improve outcomes for this high-risk group of people admitted to hospital, and end AIDS deaths.

8.2 TB diagnostic tests in hospitalised PLHIV: accuracy and treatment decision making

The major unexpected findings in CASTLE were substantial discrepancy between SILVAMP-LAM and LF-LAM, and that relatively few TB diagnoses were made on either clinical or radiological grounds. Details about urine LAM (SILVAMP-LAM and LF-LAM) diagnostic accuracy, particularly in context of other recent studies and action consequent to dCXR-CAD scores were discussed in Chapter 7.

8.2.1 Use case for CXR-CAD

Chest X-ray can have clinical utility among inpatients, aside from its use in TB diagnosis.[18–20] High quality digital chest X-ray images might have assisted with making a wider range of diagnoses (such as bacterial pneumonia, *P. jirovecci* pneumonia or left sided heart failure, thereby improving patient care, although there is no direct evidence for this from CASTLE. Almost all previous CAD studies have been conducted among outpatient populations,[21–23] making CASTLE an important contribution to the literature. Further work is planned to compare dCXR-CAD scores to radiologist interpretation among CASTLE participants. For the inpatient HIV-positive population, systematically setting CAD thresholds and action that should be taken based on CAD scores is challenging. In CASTLE, most people with high (≥60) CAD scores did not have NAAT or culture positive sputum (73/102 negative NAAT or culture, 25/102 no sputum produced), and one person with CAD score below the cut off did have NAAT and culture positive sputum (their CAD score was 58) – see Figures in supplementary material in Chapter 6 (CASTLE results).

In community based surveys, dCXR-CAD can be used as an initial triage test, and those with a high CAD score are offered diagnostic testing (definitive test) with a sputum NAAT as part of a systematic protocol-guided screening.[24,25] But this approach isn't suitable for inpatients for a number of reasons illustrated in CASTLE: high CAD scores are commonly caused by conditions other than TB that are co-prevalent in inpatients (i.e. triage test might have limited positive predictive value); having pulmonary TB but a negative diagnostic sputum NAAT is common (i.e. the definitive test has poor negative predictive value); and pulmonary and extra-pulmonary disseminated TB frequently occur in people with lower CAD scores (i.e. the triage test has limited negative predictive value). In addition, having a triage test to prioritise access to a scarce definitive diagnostic makes sense when the prevalence of the condition is low and accordingly the number needed to screen is very high. In HIV positive inpatient settings the number needed to screen with a definitive diagnostic NAAT to detect one person with TB is already relatively low because the prevalence of TB is high.[26] Finally, in CASTLE we used a relatively high CAD threshold of ≥60 to report "TB is likely" and even then, half of participants had a positive (i.e. high) CAD score at a threshold of ≥60. If we used a CAD as a triage test with a threshold optimised in community studies of 47 (see references [22,27]), 88% of CASTLE participants (175/200) would have had a

positive CAD score / triage test. Whilst this would improve sensitivity, a triage test that is positive in nearly 90% of people is of limited use.

Therefore, experience from the CASTLE trial indicates that the optimal use case for chest Xray-CAD from in inpatients with HIV in high TB burden countries is as an adjunct to clinical decision-making in a clinician-guided diagnostic workup (see Chapter 2 for discussion of screening vs. diagnostic work up pathway). Since 2021, WHO has recommend that sputum NAATs are used for TB screening in all inpatients living with HIV, if the prevalence of TB is thought to be over 10%.[24,26] The evidence from CASTLE suggests that using CAD as a triage test step prior to undertaking a definitive NAAT sputum test among inpatients is likely not an efficient nor effective use of resources. Rather, a high CAD score should be a trigger for detailed clinician consideration of TB perhaps to provide impetus to try to obtain further sputum or non-sputum samples for NAAT testing (such as sampling of pleural fluid). In the right clinical context, a high CAD score should also prompt clinical consideration of empirical TB treatment, even if microbiological tests are negative - see discussion below about TB prevalence and negative microbiological tests. Further work about dCXR-CAD in inpatient or very high TB prevalence settings could address whether and how CAD scores can be used to inform clinical decision making about TB treatment in the absence of microbiological confirmation.

8.2.2 Understanding true TB burden

As outlined in Chapter 2 and above, ruling out TB in a population with a high pre-test probability of TB – such as HIV-positive inpatients - is challenging. It is very difficult to know what proportion of people with negative microbiological results (particularly when only few microbiological tests have been done) truly have TB. Figure 8.1 is an illustration of the importance of prevalence (or pre-test probability) for interpreting test results. For a hypothetical test with 90% sensitivity and 97% specificity, in a setting where TB prevalence was 1% (such as community-based TB active case finding), the chance of someone with a negative test truly having TB is 0.11%. If that same hypothetical test was applied to a population with a TB prevalence of 30% (such as an HIV+ inpatient setting), on average, a person with a negative test would have a 4.2% chance of having the disease. Of course, the chance of any individual within that high-prevalence group actually having TB will depend on other factors specific to that individual such as CD4 count, symptoms etc. – the hypothetical scenario illustrates the overall average probability.

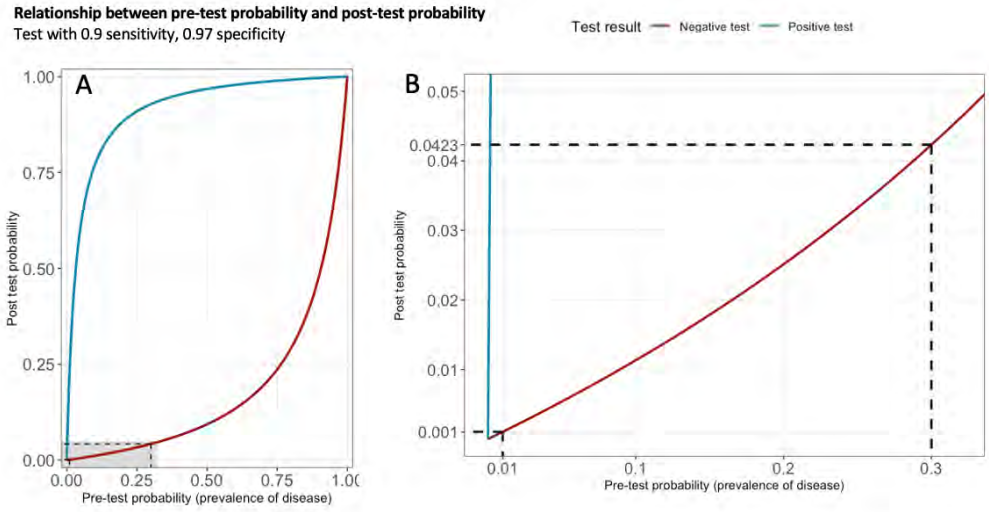

Figure 8.1 Illustration of relationship between pre-test and post-test probability.
Illustration of relationships between pre-test probability (prevalence, x axis) and post test probability (y-axis) and positive and negative test results (blue curve and red curve) for a hypothetical test with 0.9 sensitivity and specificity. Panel A shows whole range of pre-test and post test probability, panel B shows selected range (highlighted in grey in panel A) to illustrate post-test probability given a negative test and pre-test probabilities of 1% and 30%.

One way to determine true TB prevalence is to undertake extensive and repeated sampling and microbiological TB testing, but these studies are expensive and challenging to conduct. The Cape Town Study (methods in Lawn et. al.,[28] biobanked samples from this cohort have been used in several publications [29–33]) is one of the few studies that have undertaken extensive microbiological sampling from a hospital population - as discussed in Chapter 2. Key findings were that an admission screen in the first 24 hours admission comprising one sputum NAAT plus one urine LF-LAM would only identify 52% of people with microbiologically-confirmed TB (microbiological confirmation on the basis of all samples taken during hospital admission). In the Cape Town study the negative predictive value of having a negative urine LF-LAM and either a negative sputum NAAT or being unable to obtain a sputum for testing in first 24 hours of admission was 81% (i.e. 19% of people with negative or not available tests would still have microbiologically proven TB – see Chapter 2 and Figure 2.1).* Whilst the Western Cape in 2012 is not representative of the rest of Southern Africa in 2022, the point is that in a high TB pre-test probability group, if a small number of TB tests are attempted and results are negative, or samples not obtained (such as in STAMP or CASTLE) this does not define "true negative" TB status.

Further evidence supporting the difficulty in diagnosing TB in inpatient adults living with HIV comes from autopsy studies. A systematic review of autopsy studies conducted up to December 2013 showed TB prevalence of 43.3% in HIV-positive adults who died in sub-Saharan Africa, with 40% undiagnosed and

* Negative predictor value calculated as follows; 66 people who have TB according to microbiological reference standard but have negative or not done LF-LAM and sputum NAAT in first 24 hours (i.e. false negative). There are 285 people who have negative or not done LF-LAM and sputum NAAT and are not TB by microbiological reference standard (i.e. true negative). Negative predictor value is false negative / (false negative + true negative) – 66 / (285+66) = 81%. See figure 2.1 in Chapter 2. Reference [28]

untreated at time of death.[34] Most people in the studies were in hospital at the time of death and therefore had access to TB diagnostics; although most studies were conducted before widespread availability of NAATs. Five more recent autopsy case series also show similar patterns of high prevalence of missed TB disease, presumably for the most part despite investigations antemortem (see Table 2.2 in Chapter 2).[35–38]

A third piece of evidence around difficulty in determining TB prevalence in inpatients is TB comes from England in 2021.[39] This pattern probably applies to many high income, low TB prevalence settings, but the availability of high-quality surveillance data from England supports confidence in epidemiological estimates. In 2021, 40% of all people starting TB treatment in England did so without culture confirmation and thirty percent without any smear, NAAT or histological diagnosis.[39] This is in a setting where there is ready access to mycobacterial culture facilities and ability to collect samples for culture from invasive sampling, for example bronchoscopy and endobronchial ultrasound guided needle aspiration sampling. While the report doesn't distinguish between negative tests and tests not done, it is reasonable to assume that most of those people were diagnosed with TB despite negative microbiological tests (rather than having no tests) and therefore the negative predictive value of having negative tests is imperfect.

On the basis of the Cape Town study, autopsy studies, and knowing that in high income countries it is relatively commonly for people to be diagnosed with TB based on clinical grounds despite negative microbiological tests, I consider it likely that some people in CASTLE had true positive TB, despite negative tests. On the other hand, it is also possible (see Chapter 7) that some of the people with LF-LAM positives may not have truly had TB, although this risk is difficult to quantify. It is also possible that the overall risk of true TB was lower among CASTLE participants (2019-2022) than STAMP participants (2015-2017). The relative scarcity of clinically diagnosed TB in CASTLE, particularly in participants with very abnormal CAD scores, is notable and it is probably likely that some of these participants had true TB, but were not promptly treated by clinicians.

8.3 Why the lack of demonstrated mortality benefit in CASTLE?

At a conceptual level, to have shown reduced deaths, the CASTLE intervention would have required all four of the following to be true.

1. TB was a major reason for people becoming ill and requiring hospital admission.
2. In absence of the CASTLE intervention, TB would often not be diagnosed (and therefore not be treated).

3. The CASTLE intervention increased the number of people with TB who received a diagnosis and TB treatment, and there wasn't a substantial "Hawthorne" effect causing TB diagnoses to also have increased in the control arm.
4. In the people who received a TB diagnosis and treatment because of the CASTLE intervention, TB treatment was effective at preventing death.

Finally there would have to be a sufficiently substantial effect size, for there to be a large enough difference in deaths between arms to be able to observe a difference, given the relatively small size of the CASTLE trial.

Each possible explanation, beginning with addressing sample size, is discussed in turn below.

8.3.1 POSSIBLE EXPLANATION 1: A small true difference in arms existed but sample size was too small for this secondary outcome.

In order to have 80% or more power to detect a difference in death between arms, assuming very small design effect (minimal clustering), four admissions per day, and a 56 day mortality in usual care arm of 20% the hazard ratio for death would have to be at least 0.6 (as outlined in p.29 CASTLE protocol, see Appendix). The actual CASTLE cluster size was smaller, with a median of two participants per cluster rather than four, meaning the trial was underpowered even for a very large mortality effect (i.e. a hazard ratio of 0.6 or greater). As such, CASTLE primarily is about feasibility of the intervention and effect on TB diagnosis, rather than a definitive trial of mortality impact.

The 95% confidence interval for CASTLE intervention for hazard of death by 56 days is large, and contains values consistent with both benefit and harm. Therefore, it is possible that there was a true small magnitude beneficial or harmful effect from the CASTLE intervention that was not demonstrated because the sample size was small. Overall, CASTLE showed that the intervention did not lead to a large survival benefit (or cause large harm) but also did not rule out a true small difference in either direction.[40]

8.3.2 POSSIBLE EXPLAINATION 2: TB is not a substantial cause of death and/or not often missed

As discussed above, most evidence suggests that TB (including disseminated TB) continues to be the major cause of hospital admission and in hospital death in people living with HIV in Southern Africa. However, it is noteworthy that TB prevalence and incidence have been decreasing in Malawi.[41,42] The decline in TB incidence in Malawi is thought to be due in large part to increasing coverage of ART, with additional contributions from TB screening, care, and prevention activities.

The CASTLE population was adults living with HIV who were sick enough to require hospital admission. Even if TB incidence was declining in the overall general adult population, it's not clear that TB would necessarily be less common among people who are unwell and in hospital, although the incidence of hospital admission itself might reduce as seen in Chapter 4. A diagnostic study among people admitted to hospital in Blantyre in 2017-2018 with sepsis showed that TB was the most common cause of sepsis (76/162 [47%] of participants with an identified cause, 76/225 [34%] of all participants), although this was almost all based on urine LF-LAM tests.[43] Furthermore, the evidence from autopsy and other studies suggest that TB is commonly missed as a cause of admission or diagnosis.[34–37] Given the low incidence of empiric TB treatment in CASTLE (see below), we can assume that true TB diagnoses must be sometimes missed.

8.3.3 POSSIBLE EXPLANATION 3: People who truly had TB still had their diagnosis missed in intervention arm

The rate of empiric TB diagnosis and treatment in CASTLE was relatively low, particularly in comparison to the LAM-RCT trial,[44] and the South African arm of STAMP trial [45,46] (where approximately a quarter and a tenth of all adults living with HIV started empiric TB treatment, respectively). Only six people (1.5% participants, three in each arm) had TB treatment started without positive microbiological test in CASTLE. This was similar to the Malawi site of the STAMP trial, where only 13 people (2%) in the intervention arm (i.e. the group that had access to LF-LAM) had empirically diagnosed TB. It is therefore likely that some people who truly had TB in the intervention arm still had their TB diagnosis missed and therefore didn't benefit from the intervention.

This explanation is likely to play a small role, in that the CASTLE intervention could never have been expected to identify all the people with TB who were previously missed and some people with true positive TB would still have been missed due to suboptimal sensitivity of TB diagnostics. However, I would still have expected substantially fewer people to have a missed TB diagnosis in the intervention arm compared to the control arm (since overall more people had TB diagnosed), so this is not likely to be the major explanation.

8.3.4 POSSIBLE EXPLANATION 4: People started TB treatment (who wouldn't have without intervention) but were very sick at point of TB initiation and died despite TB treatment

Supplementary material to Chapter 6 shows that people diagnosed with TB were more likely than others to die, a finding that has been shown previously (e.g. people with LF-LAM positivity are at extremely high risk of death).[47–49] Whilst early TB treatment is clearly better than delayed or no TB treatment, it is not 100% effective at preventing death from HIV associated TB. Disseminated TB – even when diagnosed and treated – is still a deadly condition.[48,49] The CASTLE intervention is only one link in the causal chain to reduced deaths. In my opinion, deaths despite TB treatments among people who truly have TB is probably a major factor for why CASTLE intervention did not show reduced mortality.

8.4 What is driving mortality in people living with HIV admitted to hospital and what can be done?

8.4.1 High mortality from disseminated TB

In CASTLE, as in other studies, being diagnosed with TB and having a positive LF-LAM is correlated with risk of death (see Chapter 6 Appendix).[43,44,47,49–51] Even when identified and treated, disseminated TB is condition that often rapidly leads to death. Currently, treatment recommendations for disseminated TB are the same as pulmonary TB: 2RHZE/4RH.[52] Ongoing work investigating enhanced treatment for people with disseminated TB includes a randomised trial of adding steroids and/or high dose rifamapcin plus levofloxacin to standard TB treatment in a 2x2 factorial design (NEW-STRAAT TB).[13] An effective therapeutic intervention to reduce mortality from disseminated TB would be a very welcome step in reducing overall in-hospital mortality among people with advanced HIV disease.

8.4.2 High mortality from other opportunistic infections

Whilst TB (pulmonary, extra-pulmonary and disseminated) is the most common cause of hospital admission and death,[16,53] other opportunistic infections contribute substantially to mortality. Dedicated focus on developing and trialling new effective treatments for cryptococcal meningitis culminated with the AMBITION trial published in 2022,[54] and WHO treatment guidelines have been rapidly updated to recommend use of the AMBITION regimen.[55] Cryptococcal meningitis research and implementation of new treatments has been aided by the existence of a highly sensitive and specific diagnostic test, albeit one that requires invasive sampling.[56] Unlike disseminated TB and severe bacterial infections, with cerebrospinal fluid cryptococcal antigen testing it is relatively straightforward to know with confidence who does and does not have cryptococcal meningitis and therefore who will benefit from treatment – with an impressive >99% sensitivity and specificity for cryptococcal antigen lateral flow testing on cerebrospinal fluid, compared to culture.[57] Widespread implementation of the AMBITION treatment regimen, including widening access to liposomal amphotericin and flucytosine, in line with 2022 WHO guidelines is needed to realise the potential of scientific research advances to lead to reduced mortality.[58]

For important opportunistic infections other than TB or cryptococcal meningitis however, there is a less clear path to improving outcomes. Severe bacterial infections have been identified as the third most common cause of adult HIV hospital admissions in a systematic review.[16] As seen in Chapter 3, two trials in people with signs and symptoms of sepsis showed that implementing a package of care involving fluid resuscitation increased mortality.[59,60] Many of these people had severe bacterial infection, with a 20% having a positive blood culture for a non-tuberculosis bacterial pathogen.[60] Optimising early diagnostic and treatment pathways for severe bacterial infections in adults and children is important. In particular, strategies to reduce

mortality for people with infections against the backdrop of rising antimicrobial resistance are vitally important.[61–65] This will likely include both increasing access to broader spectrum antimicrobials in countries, districts and hospitals where people who need the medicines are located (i.e. ensuring geography and poverty don't prevent access to needed carbapenems), whilst also restricting access to only those people most likely to benefit (i.e. clinically restricting use of carbapenems) in order to combat antimicrobial resistance.[63,66,67] This should go alongside improving diagnostics for severe bacterial infections and antimicrobial resistance.[68–72] WHO recently convened a expert panel on severe bacterial infections in people living with HIV, with some recommendations for further research, including need for tiered antibiotic guidance for primary care and hospitals and the need for better surveillance for anti-microbial resistance.[61]

Other than TB, cryptococcal meningitis and severe bacterial infections, important opportunistic infections presenting as acute illness requiring hospitalisation include cerebral toxoplasmosis,[73] *Pneumocystis carnii* pneumonia,[74] disseminated CMV,[75] severe disseminated Kaposi sarcoma[76] and, in some settings, visceral leishmaniasis.[77] There is very little evidence about the prevalence of many of these opportunistic conditions among people living with HIV admitted to hospital in Africa, in part due to suboptimal diagnostics. In many parts of the world, fungal pathogens other than cryptococcal meningitis are common opportunistic infections.[78,79] WHO recently recommended that testing for histoplasmosis should be offered to people living with HIV with signs and symptoms of histoplasmosis, particularly in areas which are known to be hyperendemic.[80] . Recent research advances showed that two investigational chemotherapy regimens were non-inferior to standard care paclitaxel for advanced Kaposi sarcoma.[81] WHO recently summarised existing recommendations in a Policy Brief on providing care for people living with HIV who are seriously unwell.[9]

8.4.3 High prevalence of immunosuppression

In CASTLE, and most other hospital studies (see Chapter 2), most of the people admitted to hospital with opportunistic infections have underlying immunosuppression. The median CD4 cell count in CASTLE participants was 240 cells/mm^3, where measured. In this contemporary setting, highly immunosuppressed people are a heterogeneous group, including: a (declining) proportion of people who never knew their HIV status and/or never started ART; people who started ART but stopped or interrupted ART; people taking ART intermittently with or without erratic engagement in care; people taking ART with good concordance, but with malabsorption; people who are taking ART but have drug resistance mutations, which mean the ART is ineffective; and people with immune discordance who have low CD4 despite suppressed viral load.[82–85] These clinical states are not necessarily mutually exclusive, and for some people the experience of becoming unwell and being admitted to hospital might change their ART status (for example, some people might be unable to collect ART refills due to feeling sick, or conversely, some people who had stopped taking ART because they

felt well might start again when they get sick). This pattern of unwell, immunosuppressed people being on ART is shown in the systematic review of trials in PLHIV in hospital (Chapter 3), in Queen Elizabeth Central Hospital (Chapter 4), and in the CASTLE trial (Chapter 6). Since 2017 WHO guidelines have recommended rapid ART initiation within seven days for people living with HIV not taking ART, including in hospital. Despite these guidelines, a study in Uganda showed only half of people with a new in-hospital HIV diagnosis started ART before discharge.[86] For people taking ART with virologic failure (i.e. taking ART for more than six months and HIV viral load >1000 copies/mL), WHO recommend immediate switch if the person is not on an integrase-inhibitor containing regimen,[87] but to do adherence counselling and repeat viral load if they are taking an integrase inhibitor containing ART. The current WHO guidance doesn't differentiate between people who are stable with virologic failure detected routinely vs. those who are seriously ill with virologic failure. Whether differentiated approaches are needed is a research priority.

We know from several trials, including CASTLE, that early deaths occurring after hospital discharge but within eight weeks of hospital admission are common (approximately half of all deaths by eight weeks from admission in both STAMP [45] and CASTLE occurred after discharge), which suggests that underlying conditions have not been resolved at discharge. A systematic review showed that post-discharge death and re-admission are common.[17] More research on the causes, consequences, and methods to tackle disengagement from HIV services is needed, including how to optimise the interface between hospital and community services at the point of discharge from hospital. This applies both to people newly-started on ART, and people in whom ART virologic failure has been identified, to address next steps to re-establishing viral suppression (whether by ART switch or adherence counselling and repeat viral load).

8.4.4 A vicious cycle

Hospital care for people living with HIV sits within a wider health and social eco-system, including access to HIV testing, ART initiation and universal health care (or lack thereof). Whilst the whole cascade of care is important, attention should be given to the vicious cycle around care for people attending primary care with advanced HIV, admission to hospital, hospital management, and transitioning back to community based care in order to prevent deaths.

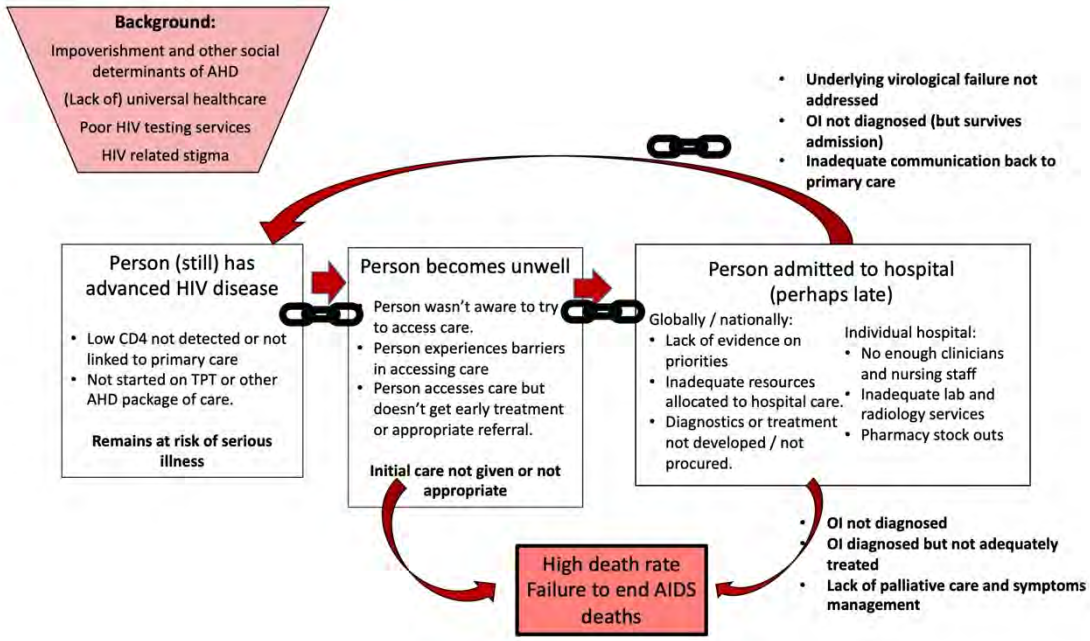

Figure 8.2: Illustrating some of the links in the vicious cycle chain leading from poor recognition and management of Advanced HIV Disease to in-hospital mortality, re-admissions and AIDS deaths.

When advanced HIV disease is not detected, or not addressed by the WHO recommended package of screening and preventive / pre-emptive treatment, people remain at risk of developing severe complications of HIV and becoming unwell. If a person becomes unwell and primary care clinics can't give appropriate and timely treatment or referral, the person will continue to deteriorate.[37,88] Late referrals and late presentations to care might mean that even if effective diagnosis, treatment and care is offered at hospital it might be too late to prevent death.[48] Even for those who survive their hospital admission, if the underlying causes of illness aren't addressed by the time of discharge and effective ART isn't started, high risk of death will persist following discharge from hospital and the cycle of ineffective community-based care for people with advanced HIV disease may continue – as illustrated in Figure 8.2. Intervening at a hospital-level to provide effective and high-quality care may break one link in the vicious cycle, but to be most effective the whole cycle of care for people with advanced HIV should be addressed.

8.5 Future research directions

8.5.1 Research arising from CASTLE

Following the CASTLE trial manuscript there are several planned additional manuscripts and additional research using collected data and samples, to build on my PhD work presented in this thesis. I will analyse findings from the diagnostic cohort including utility of serum C-reactive protein and procalcitonin to diagnose TB and bacterial infections. I will collaborate with several radiologists to look in more details at a comparison of CAD scores and radiologist interpretation of chest X-rays. And I have planned descriptive epidemiology exploration of immunosuppression and HIV virological failure including collaborating with colleagues to do drug level measurements and determining predicted drug resistance mutations in participants with high viral load. Looking ahead, I am part of nascent advanced HIV disease in hospital research consortium with partners in South Africa, Malawi, Uganda and Botswana, we are in the early stages of developing an trial around a package of care of diagnostics, empiric antimicrobial treatment, ART management and clinician decision aids for adults living with HIV admitted to hospital.

8.5.2 Policy and implementation of diagnostics in advanced HIV disease

There is a clear need for improved diagnostics for TB, including extrapulmonary and disseminated TB. The relatively disappointing SILVAMP-LAM results in CASTLE and other recent studies such as the FIND[89] and MSF evaluations[90] highlight the vital importance of high-quality manufacturing capacity and ongoing performance evaluation as part of quality assurance. WHO Global Tuberculosis Programme is joining the established WHO "prequalification" process, having previously used a different route to endorse products: ideally this will contribute to more transparent and standardised evaluation of recommended in-vitro diagnostics.[91] Countries need clear guidance on implementing LAM products, including LF-LAM, which should include an internationally-supported programme providing both proficiency testing for LAM users in all facilities implementing LAM routinely, and quality assurance for kits, with routine batch-testing to avoid any repetition of the problems of discrepant results. Better accuracy and more consistent use would also be supported by development of job-aides to supplement manufacturers Instructions-for-use (IFUs) for approved LAM products, and ideally work with manufacturers to optimise their IFUs and translations. For LF-LAM, this could include formative research (using for instance cognitive interviewing) to improve end-user operability and make the role of the reference card clearer; development of positive and negative control panels for quality assurance batch testing; and standardised end-user competency testing materials for proficiency testing. The SPI-RT programme and checklist from US Centres for Disease and Control for HIV testing quality assurance could serve as a useful model.[92]

Access to diagnostics relevant to advanced HIV disease at decentralised primary care locations or hospitals without a diagnostic laboratory on site is critical to improve reach and accessibility, but needs input from functioning centralised laboratories to support quality assurance and proficiency testing for near-patient testers at remote sites.[93] Tests themselves can be complex to perform and interpret, and unexpected test results are possible – particularly if batch-testing and proficiency testing are not in place or maintained.[94] Interpreting test results and appropriate consequent actions is not always straightforward. More research about best practice for task shifting diagnostics to decentralised sites to be conducted by minimally trained healthcare workers, and optimal approaches to linking remote sites and central laboratories for quality assurance of diagnostics would be welcome.[95–97] Likewise, more work on how to improve radiology services in hospitals without dedicated radiologists would be helpful, particularly whether it is possible to integrate CAD technology with teleradiology for specialist human input to aid complex clinical decision making (taking imaging and other clinical factors into account) that is not easily captured into an algorithm.[98]

8.5.3 Policy and implementation for hospital care

Many national HIV programmes and other organisations that support delivery of HIV care are implementing Advanced HIV Disease (AHD) care programmes, including differentiated service delivery approaches, where individuals identified as having advanced HIV disease have more intensive interventions in primary care than people with uncomplicated HIV.[99] This step should improve care in the first "box" of the vicious cycle (figure 8.2). [95,96,100,101] But there has been relatively less attention paid to inpatient care for advanced HIV disease. As outlined in the systematic review in Chapter 3, and above, primary research to identify evidence-based interventions to reduce deaths among people in hospital is urgently needed. This should go hand-in-hand with efforts to implement what is already known to be effective, and promote access – both in terms of helping individuals access hospital care, and ensuring hospitals access can access resources needed to provide the needed high quality care (such as access to ambisome for cryptotococcal meningitis treatment).[58] A recent WHO Policy Brief on Management of Advanced HIV Disease in people who are seriously unwell summarises existing guidance and research relevant to providing hospital care for people with advanced HIV disease and opportunistic infections.[9] Individualised guideline and research informed clinician-led care is not in opposition to a public health approach to ART delivery but is required for a subset of individuals (those who are seriously unwell) within the public health system.

8.5.4 Research priorities for the future

Research priorities aimed at improving outcomes from specific infectious disease (TB and cryptococcal meningitis, disseminated TB) were discussed above, including ongoing trials.

More generally, high-quality and detailed surveillance and epidemiology of the causes of HIV-related hospital admissions and deaths is a priority. As discussed in Chapter 2, there is a relative scarcity of information about the major causes of hospital admission, and many of the studies are older and before modern ART regimens and public health ART availability.[16,17] A detailed cohort study of consecutive people admitted to hospital with HIV, with availability of microbiological, histopathological and radiological tests, supported by autopsy examination and diagnostic testing for those who die would provide extremely valuable information about prevalence of opportunistic infections and the relative contribution of HIV-related vs. non-HIV related causes in this modern era of HIV care. Not only would this be helpful to guide research priorities, but it might be useful for determining pragmatic clinical diagnostic algorithms based on likelihood of underlying pathologies, along the lines of algorithms currently recommended for TB diagnosis in children.[102] Of course, relatively substantial regional variation would be expected based on endemic pathogens and adults and children would be expected to have different patterns of illness to adults, but this wouldn't obviate the usefulness of such a study.

Management of ART in seriously unwell individuals is another research priority. There is a guidance article about practice in managing ART in people with cryptococcal meningitis,[85] and some pragmatic guidance on ART in seriously unwell children in WHO guidelines,[103] but additional evidence is needed about the benefits and risks of early ART initiation in people seriously unwell before a clear diagnosis (or ruling out) of opportunistic infections has been made.[104,105] Management of virological failure, particularly in people who are already on integrase inhibitor regimens, is another area where research is needed to inform immediate management, including which patients should stay on their integrase inhibitor regimen and try to re-supress HIV viral load versus who should switch regimen straight away (and to what regimen should they switch).[87,106] This could be combined with research exploring reasons for virological failure, including suboptimal adherence, with a view to optimising interventions to promote adherence to ART following discharge from hospital. Implementation and programme research could focus on ways to arrange effective networks between primary care clinics and hospitals to promote equity and access to secondary care.

8.5.5 A virtuous cycle

Generating knowledge in public health through research is only useful if countries, funders, communities, and other relevant groups commit to implementing positive findings. Some of the links in the vicious cycle are related to research gaps where a technology is non-existent (for example, there are no single very good diagnostics for HIV-associated TB), some are implementation research gaps where the optimal methods of achieving something are not clear (for example, how best to support people to re-establish adherence to ART

following hospital admission with virologic failure), and others are gaps of lack of attention or funding (for example, access to evidence-based drugs for cryptococcal meningitis treatment).[58]

If research and implementation gaps can be addressed, and attention and resources directed to people with advanced HIV disease who are seriously unwell, links in the vicious cycle could be broken. If we focus on those people living with HIV who are either at very high risk of developing complications (advanced HIV disease with low CD4 but few or no symptoms) or have already developed complications (those who are seriously ill). Addressing this at hospital level and improving communication and referral between primary care and hospital can set up a "virtuous cycle" that could reduce deaths. One of the keys in this virtuous cycle is high quality hospital care, where conditions are diagnosed, and effective treatments started – illustrated in Figure 8.3.

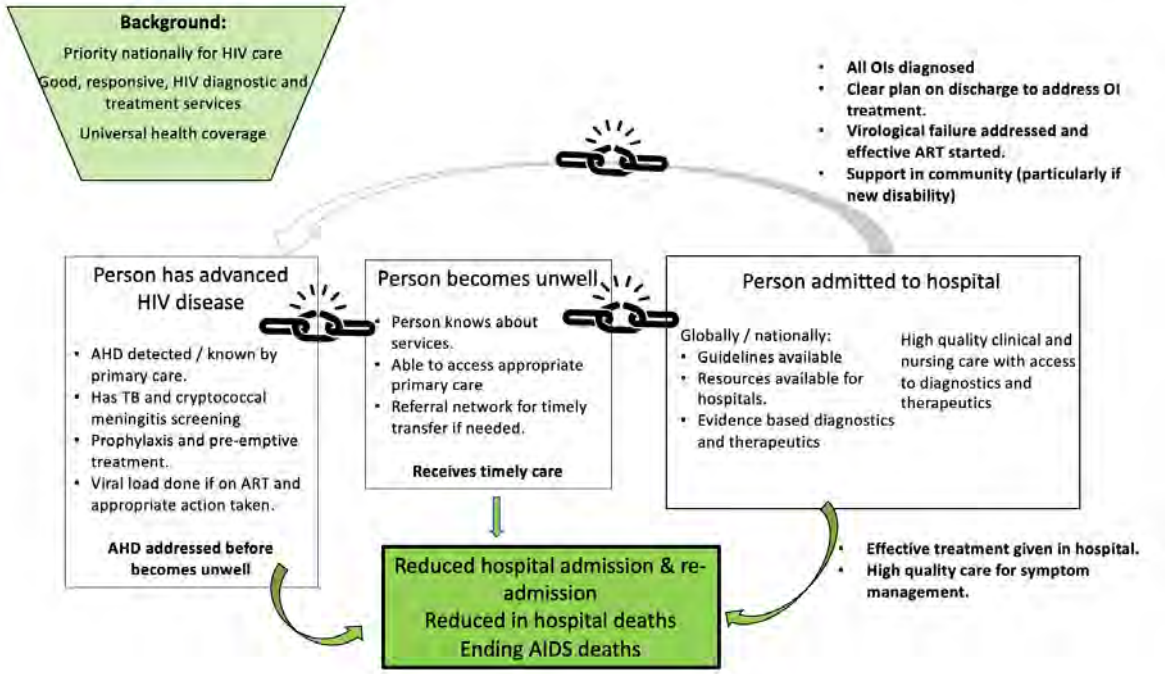

Figure 8.3 A virtuous cycle reducing deaths from advanced HIV disease at primary care and hospital levels.

8.6 Conclusions

The public health approach to HIV care, set out in 2006, has been instrumental in making ART accessible to large numbers of people and has saved millions of years of life.[107] As we approach twenty years of a public health approach to HIV, programmes and implementation research need to focus on filling the major gaps in evidence needed to manage the subset of individuals who remain at high risk of death because of advanced HIV disease or need for admission to hospital. Within a public health approach HIV care system, there should be scope for differentiated service delivery to recognise people at highest risk of poor outcomes and provide clinician-directed care for those who require this. We need to ensure that the care available can meet the needs of the subset of individuals that require complex or individualised clinical care – with attention paid to access and equity.

A package of care for people living with HIV admitted to hospital including appropriate diagnostics, management of ART and optimised treatments for opportunistic infections is important. The CASTLE enhanced TB diagnostic intervention may be a useful component of care for hospitalised adults, particularly if manufacturing issues with SILVAMP-LAM can be realised and if quality assurance for LF-LAM can be improved. Further work on clinical decision making for empiric TB treatment initiation, including chest X-ray information would be helpful. Although CASTLE did not demonstrate reduced mortality, it did lead to more bacteriologically confirmed TB treatment initiations. Other research on diagnostics, and treatments for disseminated TB and cryptococcal meningitis, together with research on how best to promote access and equity in hospital care and how to effectively link between hospital and community care is needed. Meeting the needs of people living with HIV who are seriously ill through high quality, safe, accessible hospital care is a critical component to achieving the goals of ending AIDS as a public health problem by 2030.

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Appendix A: CASTLE trial protocol

The CASTLE study

Computer Aided Screening for Tuberculosis in Low Resource Environments

Document: Study protocol

Version: 7.0

Author: Dr Rachael Burke

Date modified: 2021-10-13

Last modified by: Dr Rachael Burke

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigator team, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

TABLE OF CONTENTS

1. REGULATORY INFORMATION

Title: Computer Aided Screening for Tuberculosis in Low Resource Environments

Short title: CASTLE study

Research Organisation Ref: LOI 18.159 (LSHTM), LOI 510 (MLW)

Ethics Reference: 17799 (LSHTM), 2772 (COMREC)

Trial Registration Number: pending

Date and Version No: Version 6.0 2021-01-14

Principal Investigator: Dr Rachael Burke, Department Clinical Research, Faculty of Infectious and Tropical Disease, London School of Hygiene & Tropical Medicine

Sponsor: The London School of Hygiene & Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance and Integrity Office:
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Funder: Wellcome Trust.

Conflict of interests: We declare no conflicts of interest

Compliance: The trial will be conducted in compliance with the protocol, ICH GCP Guidelines and other relevant regulatory requirements applying in the countries in which the trial will be conducted.

Key trial investigators and contacts:

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Collaborator	Dr James Mpunga	Director, National TB Programme	
Collaborator	James Kandulu	Director Diagnostics, Ministry of Health	

2. ABSTRACT / EXECUTIVE SUMMARY

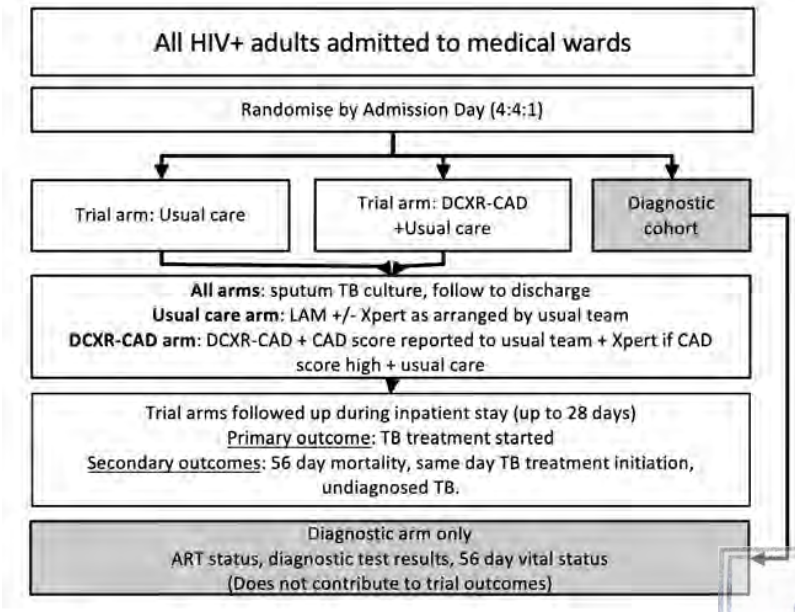
Trial title	Computer Aided Screening for Tuberculosis in Low Resource Environments (CASTLE)
Short title	CASTLE study
Trial Design (methodology)	Single site (Zomba Central Hospital) cluster randomised trial with two trial arms and a third nested observational enhanced diagnostic cohort that will not contribute to trial outcomes (4:4:1 allocation, randomised by admission day). There will be a further 24 non-randomised diagnostic cohort clusters after the conclusion of the randomized trial.
Trial population	HIV infected adult patients requiring admission to medical wards at Zomba Central Hospital. Unit of randomisation will be admission day.
Planned sample size	102 clusters per trial arm (approximately 220 participants per arm). A further 50 clusters in enhanced diagnostic cohort (approximately 100 participants). Total of 254 clusters with approximately 540 participants.
Follow up duration	56 days (eight weeks) from day of recruitment
Recruitment period	January 2020 – March 2022.
Trial intervention	Digital Chest x-ray with Computer Aided Diagnosis (DCXR-CAD) and urine high sensitivity lipoarabinomannan (FujiLAM) screening performed on first day of admission on participants admitted on days assigned to trial intervention arm. Numerical X-ray TB score and interpretation ("Pulmonary TB likely" or "Pulmonary TB not likely"), and FujiLAM results, appended into patient's notes. X-ray imaging available for clinical team review on study computer in order to inform TB treatment decision making. If a participant's CAD score indicates "TB likely", they will have sputum taken for Xpert Mtb/Rif. DCXR-CAD is in addition to usual care in the intervention arm. The control arm is assigned to usual care alone.

	Objective	Outcome Measures / Endpoints
1. Primary	To determine the effect of DCXR-CAD plus FujiLAM plus usual care vs. usual care alone on; 1.1 TB treatment initiations	1.1 Proportion of participants starting TB treatment during course of inpatient stay (censored at 56 days)
2. Secondary	To determine the effect of DCXR-CAD plus FujiLAM plus usual care vs. usual care alone on; 2.1 Mortality (time to event) 2.2 Undiagnosed TB 2.3 Same day TB treatment initation	2.1 Time (in days) to death from any cause, with censoring at 56 days. 2.2 Proportion of participants who are culture positive for *M. tuberculosis* (M.tb) in sputum, who are not started on TB treatment at the time of discharge from hospital or are current inpatients not on TB treatment by the time of culture result being made available. 2.3 Proportion of participants starting TB treatment within 24 from from time of recruitment.
3.Pre-planned analyses.	To determine the effect of DCXR-CAD plus FujiLAM plus usual care vs. usual care alone on; 3.1 Inpatient mortality (proportion of participants dying as inpatients).	3.1 Proportion of participants dying during admission (censored at 56 days) from any cause.

	3.2 Total mortality (proportion experiencing death) 3.2 Proportion of participants with a TB diagnosis that is microbiologically confirmed vs. clinically diagnosed. 3.3 To determine intervention fidelity in this setting. 3.4 To determine diagnostic accuracy of DCXR-CAD in an inpatient, HIV positive setting.	3.2 Proportion of participants dying up to 56 days from any cause. 3.2 Proportion of participants started on TB treatment with (a) microbiologically confirmed TB vs. (b) clinically or radiologically diagnosed TB. 3.3 Proportion of participants randomised to DCXR-CAD plus FujiLAM arm who received DCXR-CAD and had a urine FujiLAM result. 3.4 Sensitivity, specificity, PPV and NPV for CAD score compared to a composite microbiological reference standard and a clinical standard.
4. Other objectives	To describe the range of pathology among people living with HIV requiring admission to hospital (in diagnostic cohort) To describe the prevalence of HIV virological failure in hospital, and HIV viral resistance in people with virological failure.	Descriptive statistics. See section 5.2.

Dissemination of findings	Data will be disseminated to COMREC, local institutions, academic bodies and professional associations within Malawi and internationally (for example, STOP TB partnership). Data will be published in a timely manner in peer reviewed journals. Feedback on results will also be given to Zomba hospital staff and the Department of Medicine, the district TB officers in Zomba district, and the Malawi National HIV programme.

Trial schematic



3. ABBREVIATIONS

ART	Anti-retroviral therapy or treatment.
CrAg	Cryptococcal Antigen
CRF	Case Report Form
CRP	C-reactive protein
CAD	Computer Aided Diagnosis
COM	College of Medicine
COMREC	College of Medicine Research Ethics Committee
(D)CXR	(Digital) Chest x-ray
DCXR-CAD	Digital Chest x-ray with Computer Aided Diagnosis
FujiLAM	Refers to a high sensitivity lipoarabinomannan TB screening assay manufactured by FujiFilm
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH GCP	International Committee Harmonisation Good Clinical Practice
IRIS	Immune Reconstitution Inflammatory Syndrome
LAM	Lipoarabinomannan (a urine based screening test for TB)
LSTHM	London School of Hygiene and Tropical Medicine
LSTM	Liverpool School of Tropical Medicine
NPV	Negative Predictor Value
MLW	Malawi-Liverpool-Wellcome
MTA	Material Transfer Agreement
M. tb	*Mycobacterium tuberculosis*
PJP (or PCP)	*Pneumocystsis jiorevci* pneumonia
PCT	Procalcitonin
PIL	Participant Information Leaflet
PITC	Provider Initiated Counselling and Testing
PLHIV	People living with HIV
PPV	Positive predictor value
(S)AE	(Serious) Adverse Event
STAMP	Refers to a clinical trial of urine LAM among PLHIV (Rapid urine-based screening for tuberculosis in HIV positive patients admitted to hospital in Africa: a pragmatic, multicentre, parallel-group, double-blind, randomised controlled trial, *Gupta Wright* et al, Lancet, 2018)
TB	Tuberculosis
TDM	Therapeutic drug monitoring
WHO	World Health Organisation

4. BACKGROUND AND RATIONALE

4.1. Inpatients living with HIV

Inpatient mortality

People living with HIV who present to hospitals and require admission in WHO AFRO region have an extremely high inpatient mortality rate. A meta-analysis of published African inpatient PLHIV cohorts from 2007 to 2011 showed in hospital mortality of 31%.[1]

Local Malawi data from the STAMP trial cohort of PLHIV admitted to Zomba Central Hospital 2015 – 2017 show overall inpatient mortality of 11.3% and 56-day mortality of 21% in the intervention group with urine LAM screening (by Alere Determine LAM) [reference 2, including unpublished data]. [2]

High TB prevalence

TB prevalence is likely to be high in hospitalized patients living with HIV in Southern and Eastern Africa. Autopsy studies have shown pooled TB prevalence of 43% among nine studies from adults with HIV from WHO AFRO countries. [3] In a study in Cape Town, a third of PLHIV admitted to inpatient wards, regardless of symptoms, had microbiologically confirmed TB when provided with enhanced culture based diagnosis from multiple samples. [4] A meta-analysis of hospitalized PLHIV in WHO AFRO region, relying on routine diagnostics and clinical suspicion, reported that 17% of all admissions were due to TB, making this the leading cause of admission among PLHIV. [5]

People with HIV and TB who are admitted to hospital have a high mortality rate. In the STAMP trial cohort in Zomba, Malawi, mortality at 56 days in people with microbiologically confirmed TB was 31%, compared to 18% among people without diagnosed TB. [2] In a meta-analysis of 160,647 PLHIV admitted to hospital worldwide, 29% admitted those due to TB died prior to discharge from hospital.[5]

Current difficulties with TB diagnosis

TB – particularly when associated with HIV – is difficult to diagnose, with the main problem being low sensitivity of currently-available diagnostic tests.

Nucleic acid amplification tests on sputum using Xpert MTB/Rif are recommended by WHO as the diagnostic tool of choice among PLHIV. Xpert MTB/rif is highly specific (98%) and reasonably sensitive (79% sensitive in PLHIV) compared to sputum culture. [6] However, using Xpert has not been shown to reduce mortality compared to using smear microscopy. [7] Furthermore, inpatients (and especially HIV-positive and seriously ill inpatients) often find it difficult to produce sputum, meaning that diagnostics based on a more readily available specimen would be beneficial. [2,4]

Rapid testing using lateral flow urine tests for lipoarabinomannan (LAM) has been shown to be specific for TB and sensitive for disseminated TB in immunosuppressed people with HIV (LAM diagnostic accuracy). [8] The STAMP trial showed that urine Alere LAM screening led to a non-significant reduction in all-cause mortality at 56 days among inpatients with HIV from 21% to 18%, and a significant mortality reduction in three pre-defined subgroups (those where TB was in differential diagnosis at admission, low CD4 count and low haemoglobin). [2]

However, in a study in Cape Town, urine Alere LAM and sputum Xpert on samples obtained in the first 24 hours of admission still failed to identify nearly 50% of patients who had microbiologically confirmed TB on the basis of extended microbiological sampling across the course of admission. [4]

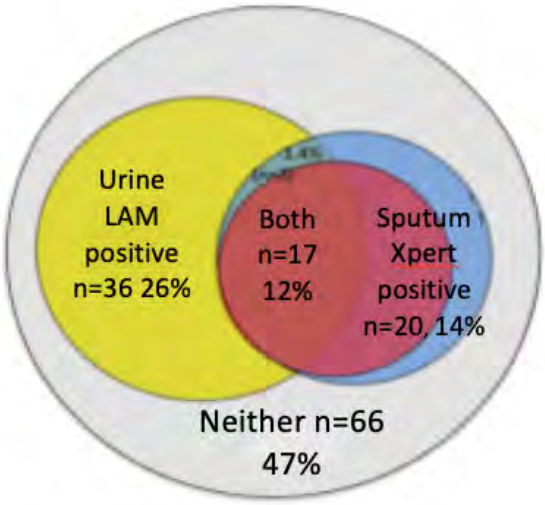


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Figure 1: Venn diagram showing urine LAM (yellow circle) and sputum Xpert MTB/rif positives (blue circle) from samples obtained in 1st 24 hours of admission from 139 PLHIV inpatients with microbiologically confirmed TB (grey circle). Red circle represents sputum smear positive. Reference (4).

High sensitivity LAM

The STAMP trial (and other trials) used a "first generation" LAM assay manufactured by Alere / Abbott (USA). This is the LAM test that has been successfully rolled out across Malawi and other countries.

A new "second generation" high sensitivity LAM assay has recently been developed by FujiFilm corporation (Japan). This FujiLAM has superior sensitivity to the older Alere LAM. A study in South Africa of parallel testing of stored urine samples from hospitalised PLHIV showed that AlereLAM was 42% sensitive whereas FujiLAM was 70% sensitive when compared with a composite microbiological reference standard. [8]

This increased sensitivity means that FujiLAM has the potential to substantially improve the diagnosis of TB and improve patient outcomes even more that the already successful Alere LAM.

FujiLAM has a CE mark as an in vitro diagnostic test (IVD) by the EEA (European Economic Area.

Chest x-ray for TB diagnosis

Chest x-ray is recommended by WHO for TB diagnosis, including for clinical diagnosis of TB if microbiological tests are unavailable or negative. [9] Chest x-ray has high sensitivity for pulmonary TB, even in HIV co-infection, and continues to play an important role in TB diagnosis in high-income settings. Although chest x-ray has been used for many years as a diagnostic tool, widespread implementation in low-resource / high TB prevalence settings has been limited by poor access to high quality equipment and expert radiologists, low specificity (leading to over-diagnosis of TB if chest x-ray alone is used) and high inter-reader variability. [9]

Chest x-ray can be used at several different stages of a TB diagnostic algorithm with diagnostic performance characteristics that depend on the setting. For PLHIV who are unwell and require hospital admission WHO recommends chest x-ray (if available) as part of the initial diagnostic work up. Chest x-ray is further recommended for people who are Xpert MTB/Rif negative or where Xpert MTB/Rif is unavailable as part of further investigations for TB, in conjunction with empiric TB treatment (see algorithm below). [10]

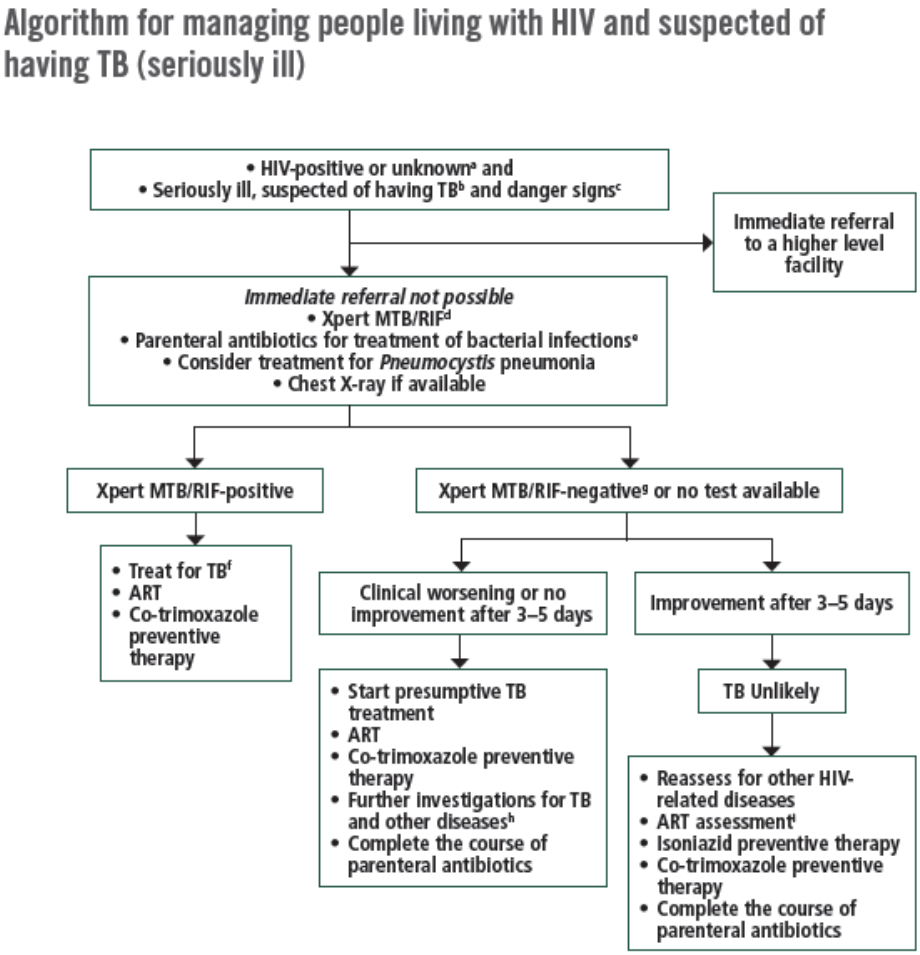

Figure 2. WHO algorithm for PLHIV who are seriously unwell (i.e. requiring hospital admission) and suspected of having TB from 2016 Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection (reference (10)).

Currently, countries such as Malawi have low coverage of radiology services, including lack of trained radiologists. For example, in the STAMP trial in Zomba 2015 – 2017 only 23% of PLHIV admitted to hospital had a chest x-ray. [2]

CAD for Chest x-ray interpretation

Computer-assisted detection (CAD) software for chest x-ray interpretation – artificial intelligence algorithms used to classify digital images - is now available, and can be integrated within new digital x-ray units to provide immediate interpretation. [11 12 13 14 15 16 17 18 19]

A recent systematic review of available evidence for one CAD system (CAD4TB, Delft Imaging Systems, Netherlands) showed that sensitivity was as high as reading by radiologists, although

specificity was slightly lower. [12] WHO recommends that *"CAD can be used for TB detection for research, ideally following a protocol that contributes to the required evidence base for guideline development".* [9]

Appendix 1 summarises existing literature on DCXR-CAD for TB diagnosis. There are no completed prospective studies on clinical impact of CAD, although one outpatient prospective study is ongoing in Malawi (PROSPECT study). [20] There are no ongoing or completed studies investigating effectiveness in inpatient populations.

Opportunistic infections, co-morbid conditions other than TB and HIV virological failure

Whilst TB is the leading cause of hospitalization and mortality in people living with HIV, it is clearly not the only cause of mortality.

In general, the epidemiology of prevalent conditions causing hospital admission among PLHIV in Southern and Eastern Africa has been sparsely described. In part this is due to the low availability of laboratory diagnostics in many countries in this region.

With the widespread availability of ART, an increasing proportion of people living with HIV admitted to hospital in Southern and Eastern Africa are immunosuppressed but ART experienced – either current ART users or having interrupted ART use. [21] [22] The WHO guidelines for public health management of advanced HIV states "the evidence supporting the package of interventions for people with advanced disease is derived from studies of ART-naïve patients" and that "further research is required to evaluate the optimal package of interventions to people presenting with treatment failure". [23]

In addition to TB, leading causes of mortality are likely to include sepsis syndromes, bacterial pneumonia, cryptococcal disease and pneumocystis jirovecci pneumonia (PJP), as well as non-infectious causes (for example, heart failure). [1] [24]

The 'ART era' of HIV care in Southern and Eastern Africa is characterized by a high coverage of ART at a population level, but persisting challenges related to treatment interruption or treatment failure. A robust understanding of the epidemiology of prevalent disease among PLHIV admitted to hospital in the 'ART era' is important in order to begin to design a package of interventions to reduce mortality. [23]

Data from STAMP study, also at Zomba Central Hospital in Malawi showed that 32% of PLHIV admitted to Zomba hospital 2015 – 2017 had HIV virological failure (HIV viral load >1000 copies/mL). 82% of people with HIV virological failure had resistance to two or more ART medicines. [25] Since 2017, the Malawi National HIV programme has switched from Efavirenz (from NNRTI class of medicines) to Dolutegravir (integrase inhibitor class) as first line ART. Dolutegravir is reported to have a higher barrier to resistance than NNRTIs. However, in a small study in Chiradzulu, 2 / 3 people with HIV virological failure on dolutegravir-containing ART had dolutegravir resistance mutations. [26]

It is important to guide clinical practice and national guidelines to have an overview of the prevalence of HIV virological failure among PLHIV requiring hospital admission, and the prevalecne of HIV viral resistance following the national switch to dolutegravir-containing ART.

4.2 Study rationale

The need for studies of clinical effectiveness

Although chest x-ray is recommended by the WHO for TB screening and diagnosis in unwell patients in hospital, there is no clear evidence base supporting this recommendation. Empiric TB treatment is common in this setting, and is recommended by WHO in certain circumstances. [10]

Whilst DCXR-CAD for TB diagnosis has similar performance characteristics to a trained human reader, it is not known how systematic chest radiography combined with CAD interpretation will affect clinician's testing practice, rates of TB treatment, and patient outcomes. Therefore a study of clinical impact on patient-important outcomes of this intervention is required.

Were DCXR-CAD shown to be effective in this population, it has the potential to be used at scale in hospitals across Southern and Eastern Africa. Robust and relatively low-cost x-ray units are now available, and published estimated costs of DCXR-CAD are $1.46 per x-ray for TB screening on an outpatient clinic basis (inclusive of cost of x-ray unit over its lifetime, maintenance of x-ray unit, CAD license fee and radiographer time). [19]

Urine LAM screening using Alere LAM has been shown to improve patient outcomes in two trials. There is good evidence that FujiLAM is substantially more sensitive than Alere LAM. However for national TB/HIV programmes and guideline development it is important to know whether use of the more sensitive test leads to an improvement in clinical outcomes. Were FujiLAM shown to be more effective than Alere LAM in this population, it has the potential to replace Alere LAM and be rapidly rolled out by national programmes.

An important factor in the design of the present study is the strategy of investigating **all** patients regardless of whether TB is suspected or not: post-mortem studies show that a substantial burden of TB remains clinically unsuspected and is therefore not empirically treated. [3] We know that symptom screening for TB among PLHIV being admitted to hospital is neither sensitive nor specific. [2 27]

5. RESEARCH QUESTIONS, AIMS AND OBJECTIVES

5.1 Research Questions

- Does implementation of systematic DCXR-CAD plus FujiLAM screening for TB, in combination with usual care diagnostics, in adults living with HIV being admitted to hospital increase the number of adults started on TB treatment compared to usual care alone?

- Is there a suggestion that implementation of systematic DCXR-CAD plus FujiLAM screening for TB, in combination with usual care diagnostics, in adults living with HIV being admitted to hospital reduces mortality by eight weeks, reduces undiagnosed TB or increases the number of people started on TB treatment on same day of admission?

- What are the clinical and microbiological characteristics of adults living with HIV admitted to hospital in a low resource area with a high coverage of dolutegravir based ART?

5.2 Objectives

Objectives	Outcome measures / endpoints
Primary objectives	
1.1 To determine whether the addition of digital chest x-ray with Computer Aided Diagnosis (DCXR-CAD) plus FujiLAM screening for all PLHIV at admission to hospital increases the number of participants starting on TB treatment, compared to usual care.	1.1 Proportion of participants being prescribed TB treatment prior to discharge from hospital (including on the day of discharge), or prior to 56 days (whichever is shorter).
Secondary objective	
2.1. To determine whether the systemic addition of DCXR-CAD plus FujiLAM to all PLHIV at admission to hospital reduces all cause inpatient mortality, compared to usual care.	2.1. Time (in days) to death from any cause, censored at 56 days from recruitment.
2.2. To determine whether the systemic addition of DCXR-CAD plus FujiLAM screening for all PLHIV at admission to hospital reduces the number of participants with undiagnosed sputum-culture positive TB, compared to usual care.	2.2. Proportion of participants with a sputum culture positive for *M. tb* who were not on TB treatment at the earliest timepoint from; • the time of discharge from hospital • 56 days after recruitment into study the time the culture result is reported.
2.3. To determine whether the systemic addition of DCXR-CAD plus FujiLAM screening for all PLHIV at admission to hospital increases the number of people starting TB treatment on the same	2.3. Proportion of all participants who are started on TB treatment within 24 hours from recruitment into study.

day as diagnostic tests.	
Other planned analyses To determine the impact of systematic addition of DCXR-CAD plus FujiLAM on all PLHIV at admission to hospital on;	
3.1 Inpatient mortality.	3.1 Proportion of participants who die prior to discharge from hospital (censored at 56 days).
3.2 56 day mortality (measured as a proportion rather than time to event)	3.2 Proportion of participants who die in 56 days following recruitment.
3.3 The proportion of TB diagnoses that are microbiologically confirmed disease vs. clinically diagnosed.	3.3 Proportions of participants started on TB treatment with (a) a microbiologically confirmed diagnosis of TB and (b) clinically diagnosed TB disease
To ascertain process outcomes;	
3.4 To determine intervention fidelity.	3.4 Proportion of participants randomised to DCXR-CAD arm who have a valid CXR and CAD score recorded.
To assess the diagnostic accuracy of DCXR-CAD in this population;	
3.5 Sensitivity, specificity, positive and negative predictor value compared to (a) a composite microbiological gold standard and (b) a clinical reference standard.	3.5 Sensitivity, specificity, PPV, NPV compared to (a) positive *M. tb* culture from sputum sample or pleural fluid, or positive Xpert MTB/Rif result on sputum or pleural fluid or two or more sputum smears positive for AFBs on microscopy or (b) micrologically confirmed TB **or** a decision to start TB treatment.
Descriptive or exploratory objectives (enhanced diagnostic cohort arm and those being investigated for HIV virological failure)	
4.1 To describe the proportion of partcipants meeting a clinical or clinical / microbiological description for Sepsis Invasive bacterial disease Cryptococcal disease Pneumocystis jirovecci pneumonia Bacterial pneumonia Immune reconstitution inflammatory	4.1 Descriptive analysis. See definitions in appendix 1. Participants can have more than one diagnosis (or none). Stratified by CD4 count category.

syndrome (IRIS) HIV treatment failure	
4.2 To describe the range of values of inflammatory markers and blood TB biomarkers and the relationship between these tests and infectious diagnoses.	4.2 Descriptive analysis.
4.3 To describe the difference in all-cause mortality between the group provided with enhanced diagnostics and those with usual care diagnostics (exploratory outcome).	4.3 Proportion of participants experiencing inpatient mortality in enhanced diagnostic group compared to trial arms.
4.4 To describe the prevelance of HIV virological failure, and ART resistance mutations.	4.4 Descriptive analysis
4.5 To describe the test-retest reliability of urine LAM testing	4.5 Descriptive analysis

6. STUDY DESIGN

A pragmatic, single-centre (Zomba Central Hospital, Malawi) cluster randomised, clinical trial with two trial arms and a third observational enhanced diagnostic arm. The observational diagnostic arm will not contribute to trial outcomes. The unit of randomisation is admissions day (see section 8.1). Clusters (days) will be randomised in a 4:4:1 allocation ratio to one of the two study arms or to the enhanced diagnostic cohort. Neither participants nor research staff interacting with participants will be blinded to study arm, but investigators working with data will remain blinded to allocation as far as possible. All adult patients with HIV being admitted to medical wards who are willing and able to consent are eligible for study inclusion, regardless of presenting symptoms or ART status. Following the conclusion of the randomised trial, people will be recruited in the diagnostic cohort only (no randomization) for a further 24 diagnostic cohort clusters.

Participants randomised to the standard of care arm will receive usual care alone. Usual care includes tests routinely available at Zomba Central Hospital, including (but not limited to) urine Alere LAM and sputum Xpert Mtb/Rif on treating clinician request. COVID-19 testing is available at Zomba hospital to all patients where it is clinically indicated. Participants randomized to the intervention arm will receive usual care plus urine FujiLAM screening plus a DCXR-CAD, with CAD score and FujLAM results appended into their medical notes. If participants have a CAD score above a pre-determined threshold (see section 9.9) the study team will attempt to collect sputum for Xpert Mtb/Rif. Chest X-ray images will be available for clinicans to view on computers in the ward.

Participants in the observational enhanced diagnostic arm will receive an enhanced package of diagnostics as described in section 9.7 and appendix 2. Participants in the trial arms may optionally also ‘opt in’ to provide samples for HIV viral failure investigations.

The usual medical team will be responsible for all clinical management decisions, including the decision to start TB treatment. We will recommend that TB treatment is started is FujiLAM screening is positive.

Outcomes will be ascertained at 56 days following recruitment into study.

7. STUDY POPULATION

7.1. Trial Participants

Adults (aged ≥18 years) living with HIV infection (PLHIV) who require acute admission to medical wards at Zomba Central Hospital and who are willing and able to provide informed consent will be enrolled, regardless of clinical presentation and whether or not TB is clinically suspected.

7.2. Inclusion Criteria

All medical admissions will be screened for eligibility, regardless of the presenting complaint.

Inclusion criteria are;

- Requires acute admission to a hospital medical ward at Zomba Central Hospital for any reason
- Have HIV-infection (existing or new diagnosis, irrespective of ART status)
- Willing and able to give informed consent

7.3. Exclusion Criteria

- Aged <18 years
- Have been admitted to a medical ward for longer than 18 hours
- Taking TB treatment before admission or has received treatment for TB within the preceding 6 months.
- Have already been in the study during an earlier hospital admission.

8. RECRUITMENT, RANDOMISATION AND BLINDING

8.1. Randomisation, allocation and masking of clusters (days)

A randomisation list will be generated in advance with random block size to ensure equal numbers of days are enrolled into each arm. Allocations of clusters will be printed and placed into sequentially numbered opaque sealed envelopes. One envelope will be open each day prior to study recruitment commencing to reveal the allocation for that cluster (day).

None of the Zomba-based research teams, usual care medical team or participants will be blinded to trial arm allocation.

However, in the course of managing data, investigators will remain masked to allocation as far as possible until database lock preceding final analysis. Data monitoring and data cleaning will be done without reference to group allocation. No unblinded interim analysis will be conducted, unless requested by the DSMC. Should the DSMC request an unblinded interim analysis, a statistician from Malawi-Liverpool-Wellcome will run the statistical code to produce unblinded analysis and only the MLW statistician, Prof Fielding (who will represent the trial in a closed DSMC session) and the DSMC will see the unblinded results – the Principal Investigator and other co-investigators will remain blinded.

Following the conclusion of the randomised trial (i.e. once 102 clusters per trial arm have been recruited), randomisation will cease but participants will continue to be recruited into the diagnostic cohort only for a further 24 clusters.

8.2 Screening and recruitment of participants

All adult PLHIV admitted to medical wards (irrespective of clinical presentation and the reason for medical admission) will be referred by health service staff or approached directly by the study team for explanation of the study and assessment of eligibility. All adults of unknown HIV status are offered HIV testing (provider-initiated testing and counseling, PITC) on admission as standard clinical practice. If potential participants report being HIV positive, confirmation will be sought either through confirmation documentation (e.g. health passport or inspection of ART medications) or confirmatory HIV testing offered by the study team.

Information about the study given to patients by study team when inviting for study enrollment screening will be the same regardless of which trial arm is being recruited on that day.

Patients will be screened and offered recruitment to the study until 3pm in the afternoon. The late afternoon cut off is to allow time for study recruitment procedures to take place and to have a FujiLAM urine screen, chest x-ray, CAD score and Xpert (if indicated, and sputum production possible) completed on the day of recruitment.

Patients who attend Zomba in the late afternoon or evening after the cut-off time will not be recruited on that day. However patients who attend in the evening and who are admitted, will be eligible for recruitment on the following day.

Those willing to be screened for eligibility will be assigned a screening ID number and assessed by the study team as per the above criteria. Screening and recruitment logs will be kept for all

potentially eligible patients admitted to each study site and screened patients who either are or are not enrolled, including the reasons for non-enrollment when available.

8.3 Informed Consent

Written informed consent will be sought from participants before any trial-specific procedures are undertaken, with witnessed thumbprint used for participants unable to read and/or write. A copy of the participant information leaflet and signed informed consent will be given to the participant. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

9. INTERVENTIONS, METHODS AND PROCEDURES

9.1 Baseline Assessments and Procedures (trial arms)

Following enrolment the study team will collect information on:

- Age and sex
- Time and date of admission to hospital
- Whether TB was an admitting differential diagnosis
- WHO four symptom TB screening (presence of cough any duration, night sweats, fever or weight loss)
- Whether the participant is currently on ART
- Whether the participant is able to walk unaided
- Address information and mobile phone numbers of the participant and/or designated relatives/next-of-kin.

The study team will be responsible for obtaining, where possible:

- One research sputum specimen (spontaneously expectorated) for mycobacterial culture.
    - If participants are unable to produce a sputum sample initially, they will be instructed on how to do so and left with a sputum specimen container - the research team will periodically attempt again to collect sputum for culture over the course of admission.

If participants choose to opt in to study of HIV virological failure we will also

- Collect information on ART regimen and adherence
- Obtain a 12mL (three teaspoon) blood sample (8mL lithum heparin tube, 4mL purple top tube)

When participants are recruited on intervention days the study team will also arrange, where possible:

- A urine sample (7mL) to be tested with FujiLAM and AlereLAM
- A digital chest x-ray image with CAD score.
- If the CAD score is above a predetermined threshold (determined in a pilot study, see 9.9) then the study team will ask the participant for a sputum sample (in addition to the study sample collected for culture) for Xpert MTB/Rif.
- CAD score and interpretation ("TB likely" vs. "TB not likely") will be appended into participant's notes.

9.2 Baseline assessments and procedures (enhanced diagnostic observational arm)

In addition to sputum samples and chest x-ray (as detailed above in section 9.1), for participants in the enhanced diagnostic arm only, the research clinical officer will be responsible for obtaining, where possible;

- 22mL blood (5mL for blood culture, 6mL in EDTA blood tube, 4mL in a plain tube and 8mL in a lithium heparin tube).

In addition to the baseline questions for the trial arm groups, the study team will also collect information on;

- Presenting complaint
- Systematic review of symptoms
- Vital signs, including SpO2 measurement
- Karnofsky score
- Past or current TB treatment, HIV care, ART duration.
- Validated ART compliance score (where applicable). [28]
- Address information and mobile phone numbers of the participant and/or designated relatives/next-of-kin

9.3 TB Definitions

TB events will be defined as follows for the purpose of this study:

Microbiological diagnosis of TB (confirmed); will have at least two positive Acid Fast Bacilli (AFB) smears or one or more Xpert Mtb/Rif positive or one or more culture positive for *M. tb* on any specimen or a positive urine LAM result. One study sputum culture will be collected per participant, TB results will also be collected from any other samples organised by the routine health services.

Microbiological diagnosis of TB (probable); will have one positive AFB smear from any site.

Microbiological diagnosis of pulmonary TB (confirmed); will have at least one smear-microscopy, Xpert and/or culture positive result(s) on a sputum sample or pleural fluid sample, whether from study sample or routine care sample.

Microbiological diagnosis of pulmonary TB (probable); will have one positive AFB smear from a sputum sample.

Clinical diagnosis of TB; will have a compatible clinical illness and the decision of the responsible clinical team to commence TB treatment in the absence of any positive microbiological tests for TB.

Undiagnosed TB; refers specifically to participants who do not have a microbiological diagnosis of TB made on the basis of study or usual care samples, and have not been empirically started on TB treatment following a clinical diagnosis of TB, and have culture-positive *M. tb* on study sputum culture.

Chest X ray suggestive of TB; will be a CAD score about a pre-determined threshold. The threshold will be determined in the piloting phase (see section 9.9).

Date of TB diagnosis; for microbiological TB diagnoses this will be the date when the positive microbiological test result for TB was communicated to the responsible medical team (eg written in the hospital record), and for clinical diagnoses it will be the date of commencement of TB treatment.

Date of commencement of TB treatment; will be the first date that the participant was recorded in the TB register as having initiated TB treatment.

Time of commencement of TB treatment; will be the time that TB treatment for the specific participant is dispensed from pharmacy to the participant (or their guardian or a ward nurse).

9.4 Definitions of clinical / microbiological diagnoses (enhanced diagnostic arm only)

See appendix 2

9.5 Training for responsible medical team

In addition to training in the trial protocols and procedures, staff responsible for the routine delivery of care for patients who are participants in this trial will be educated with regards to use of chest x-ray for diagnosis of TB in general and the interpretation of CAD scores in particular. This will include information about the sensitivity and specificity of chest x-ray and CAD and estimated setting-specific positive and negative predictive values for TB diagnosis, allowing them to make informed decisions on how to interpret test results.

The study will also provide training on the interpretation of FujiLAM, particularly that it is expected to be more sensitive than Alere LAM.

9.6 Subsequent procedures (trial arms)

Follow-up during hospital admission

The study team will have no role in routine patient investigation, care and management. Participants will remain under the care of their responsible medical teams who will be responsible for all clinical decisions including initiation of TB treatment, ART, co-trimoxazole prophylaxis and treatment for co-morbidities according to local and national guidelines. We will recommend that the usual team commence TB treatment is FujiLAM is positive.

The study team will extract records of during hospital admission by regular review of clinical records and/or discussion with medical team. Information will be recorded in a CRF about events during admission;

- Results of TB tests organized by the routine team
- Results of COVID-19 tests organized by the routine team. (NB. COVID-19 testing is not a study specific activity and study staff will not influence decisions around offering COVID-19 testing)
- Commencement of TB treatment, date and time of starting treatment, reasons for commencement (e.g. microbiological results, chest x-ray or clinical symptoms in the absence of positive microbiological tests).
- Vital status whilst in hospital, including date of death
- Date of discharge and length of hospital stay
- Serious or severe adverse events (see section 11)

Sputum culture results

For participants able to produce sputum, a single sample will be sent for mycobacterial culture and identification at the COM/MLW TB Reference Laboratory in Blantyre, Malawi.

The study team will be informed about positive culture results. If the participant is still an inpatient the report (positive or negative) will be communicated by the study team to the responsible medical team and written in their medical notes. For participants who have been discharged results will be reported to the district TB officer. If a participant has a positive result and was not commenced on

TB treatment the result will also be reported to the participant themselves - the study team will attempt to contact the participant via mobile phone and via relative's mobile phone if necessary.

Follow up at 56 days from recruitment

At 56 days from recruitment, the study team will attempt to contact participant via mobile phone. If they cannot reach the participant by phone they will call telephone numbers of relatives provided by the participant at recruitment. If there is still no response, home tracing will be attempted. The purpose is to ascertain vital status (alive or dead) only, with no other follow up questions. Follow up attempts will start at 56 days from recruitment.

If the participant opted in to have tests for HIV virological failure**, and** had HIV virological failure at recruitment (HIV virus detected) then we will recall them for an in-person visit at 56 days for a further blood sample to see if they have re-supressed their viral load. At this visit we will take a further 12mL blood sample (8mL lithium heparin tube and 4mL purple tube) to repeat tests of HIV viral load and viral resistance.

9.7 Subsequent procedures (enhanced diagnostic observational arm only)

Follow-up during hospital admission

The study team will extract records of TB treatment initiations during hospital admission by regular review of clinical records, the TB register, and discussion with medical team.

Information will be collected about events during admission;

- Any TB tests organized by the routine team, including nature of specimens and results
- Any other laboratory or radiology test organized by the medical team, including results of COVID-19 tests organized by the routine team. (NB. COVID-19 testing is not a study specific activity)
- TB diagnosis, including date of diagnosis.
- Commencement of TB treatment, date of starting treatment, reasons for commencement (e.g. microbiological results, chest x-ray or clinical symptoms in the absence of positive microbiological tests).
- Initiation or administration of ART, including regimen and timing.
- Clinical response to ART to detect any syndrome compatible with IRIS based on ACTG clinical definition (see appendix 2). [29]
- Initiation or administration of co-trimoxazole therapy.
- Initiation of isoniazid preventative therapy,
- Concomitant bacterial infection or prescription of antimicrobials (other than TB treatment).
- Other opportunistic infections based on tests or treatment given.
- Vital status, including time of death.
- Time to discharge and length of hospital stay.
- Serious or severe adverse events (see section 11).

TB specimens and results:

In addition to the sputum for culture at MLW / COM laboratory in Blantyre, a further sputum sample will be submitted to the hospital laboratory for Xpert MTB/RIF testing. Results of Xpert tests will be issued to the responsible medical team as soon as available and communicated to the responsible

medical team to inform decisions regarding TB treatment (anticipated to be within 24-48 hours of admission).

Results from sputum culture will be reported in the same way as participants in the two main trial arms (see above, section 9.6)

Other specimens and results:
Other test results (CD4 count, haemoglobin, HIV viral load if indicated) will be communicated to the responsible medical team as soon as results are available. Where necessary, clinical advice about interpretation of blood culture results and antibiotic sensitivities will be provided with laboratory results.

Some results will be processed in batch (inflammatory markers, TB biomarkers) retrospectively and these results will not be available in a "real time" fashion to influence clinical care. Results for some samples (HIV drug resistance testing and HIV drug levels) will require samples to be transported outside of Malawi, under the terms of a Material Transfer Agreement (MTA). Participants will be asked to give their express consent for this shipping in the ICF.

The routine investigation and management of partcipants according to local protocols will not be altered. Participation in the trial will not prevent the responsible medical team from requesting additional TB (or other) diagnostic tests if clinically indicated.

Follow up at 56 days from recruitment
At eight weeks from recruitment, the study team will contact participant via mobile phone. If they cannot reach the participant by phone they will call numbers of relatives provided by the participant at recruitment. If there is still no response, home tracing will be attempted. The purpose is to ascertain vital status (alive or dead) only with no other follow up questions. Follow up attempts will start at 56 days from recruitment and attempts will continue to be made for up to 14 more days beyond this time.

If the participant had HIV virological failure at recruitment (HIV virus detected) then we will recall them for an in-person visit at 56 days for a further blood sample to see if they have re-supressed their viral load.

9.8 Storage of specimens

No blood samples will be stored from participants in either of the two trial arms unless they opt in to HIV virological failure detection. In this case case plasma samples will be stored as below. For those in intervention arm, urine samples will be stored for assessment of test-retest reliability of urine LAM results. Urine samples will be stored until LAM re-testing completed, up to a maximum of 5 years, and then destroyed.

For participants in the enhanced diagnostic arm or who opt in to HIV virological failure detection, plasma samples and sputum samples will be stored for up to five years for planned studies on TB biomarkers. Participants will be asked to give their express consent for storage of samples for future research on TB diagnostic tests. Urine samples will be stored for assessment of test-retest reliability LAM test results. Urine samples will be stored until LAM re-testing completed, up to a maximum of 5 years, and then destroyed.

9.9 Pilot phase procedures

Prior to trial recruitment, the study will be preceded by a 4-8-week piloting phase to identifying any issues that require resolution, to finalise standard operating procedures, and to ensure data-collection systems are robust. No participants will be randomly allocated to interventions during this period.

Activities to be undertaken during the pilot phase will include:

- Measurement of rates of hospital attendance and prevalence of TB.
- Assessment of timing of participant presentation throughout the working day in order to determine a time cut off for daily recruitment.
- Piloting of study questionnaire completion.
- Piloting of participant physical flow through admission unit and radiography room.
- Piloting of digital chest x-ray and CAD system, including defining a CAD threshold score.
- Piloting of sputum collection procedures.
- Piloting of urine collection procedures
- Piloting of laboratory procedures
- Establishment and refinement of quality control procedures.

During this phase participants will be identified and screened according to the protocol above, but with no randomisation. We anticipate recruiting 40 – 50 participants in the pilot phase.

Participants who consent to be in the study during the pilot phase will undergo a chest x-ray with CAD score and have sputum samples collected for both mycobacterial culture in Blantyre and GeneXpert MTB/Rif locally.

9.10 Study timeline

The timeline proposed for the study is below.

	2019					2020			2021																	2022			
	A	S	O	N	D	J	F	Mar -Jun	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
Ethical approval and permissions	X	X						COVID pause																					
Recruit study team and study staff training.			X	X	X																								
Write SOPs and investigator file		X	X	X																									
Piloting phase						X	X																						
Participant recruitment									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Follow-up participants										X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Database lock and data analysis																												X	X
Write up and disseminate results.																												X	X

10. OUTCOME EVALUATION

10.1 Follow-up procedures and ascertainment of outcomes (trial arms)

Participants will be contacted by the study team by telephone at 56 days to ascertain vital status. If the study team is unable to contact the participant they will contact the next of kin via telephone. If there is no response to several telephone calls on several days, the study team will attempt a home visit.

The usual medical team will arrange follow up (including HIV and ART care) according to usual practice.

10.2 Follow-up procedures and ascertainment of outcomes (enhanced diagnostic observational arm)

Participants enrolled in the enhanced diagnostic observational arm will be contacted by the study team by telephone at 56 days to ascertain vital status. If the study team is unable to contact them, they will contact the next of kin via telephone. If there is no response to several telephone calls on several days, the study team will attempt a home visit

The usual medical team will arrange clinical follow up (including HIV and ART care) according to usual practice.

10.3 Discontinuation/Withdrawal of Participants

Each participant has the right to withdraw from the trial at any time. The reason for withdrawal (if the participant wishes to give a reason) will be recorded in the study termination CRF.

10.4 Definition of End of Trial

The trial will be considered closed following the completion of follow up of the last participant, and once all follow-up and laboratory reports have been received.

11. SAFETY REPORTING

The main study-related procedures in this trial are systematic screening of hospitalised PLHIV for TB using DCXR-CAD and urine FujiLAM, in addition to usual care TB screening and clinical assessment. Usual care TB screening for all PLHIV being admitted to hospital Malawi includes urine Alere LAM and sputum Xpert when requested by clinicians.

It will not be possible to distinguish true-positive CAD results from false-positive CAD results on an individual basis, owing to the lack of a highly sensitive and specific gold-standard confirmatory TB test. However, given the high mortality from undiagnosed TB in this patient group, combined with the high frequency with which empirical TB treatment is used in routine care, and the established good safety profile of standard tuberculosis treatment, the overall potential for harm to participants in the intervention arm is low. WHO recommend chest x-ray in severely-ill PLHIV. TB treatment initiation on clinical grounds alone (with or without chest x-ray evidence of TB) in people who are unwell and may have TB is in keeping with WHO guidance (WHO algorithm in section 4.1, and see also section 14.3).

A single chest x-ray typically delivers an average effective radiation dose of 0.01mSv, comparable to 10 days of natural background radiation or 30 minutes on a commercial aeroplane flight, and with less than one in a million chance of causing cancer. The potential benefits of chest x-ray as a TB diagnostic tool are likely to outweigh this very small risk, and this is in keeping with current WHO recommendations. [9] [10]

Standard Serious Adverse Event (SAE) reporting will not be possible or appropriate for the reasons outlined above, the trial team will instead investigate and report periodically to the TSC, DSMB and Ethics Committees all instances of

a. Erroneous reporting of laboratory results leading to a participant starting TB therapy in error
b. Breach of confidentiality following TB or HIV diagnosis
c. Needlestick injuries

Deaths will also be systemically recorded as study outcomes and reported to the DMSB and TSC periodically.

12. STATISTICS AND SAMPLE SIZE JUSTIFICATION

12.1 Assumptions

In STAMP the observed morality in Malawi was 11.3% as an inpatient and 22% by 56 days. This is lower than other mortality estimates from cohorts of PLHIV inpatients in the WHO AFRO region. [1,21,22] In our pilot phase the inpatient mortality point estimate was slightly lower (but with a very wide confidence interval).

We observed that 14% of all patients were started on TB treatment in STAMP Malawi arm, (18% of patients in Alere LAM arm and 10% of patients in sputum diagnostics only arm). Of note, in STAMP empiric TB treatment was less common in Malawi (2% of all patients / 11% of all TB treatment initiations in Alere LAM arm) than in South African (9% of all patients / 40% of all TB treatment initiation in Alere LAM arm). [2] In our pilot phase TB treatment initiations were closer to that seen in the usual care arm of STAMP rather than the LAM arm (again, with a very wide confidence interval).

There are no previous studies to provide robust effect size estimates for either of our primary outcomes.

The STAMP trial showed an increase in TB treatment and a non-significant overall reduction in mortality at 56 days when Alere LAM was introduced for TB screening from 21% to 18% (mortality reduction was statistically significant in predefined subgroups of those with CD4 count <100 cells/µL, haemoglobin <8g/dL and where TB was in the differential diagnosis at admission). [2]

We assume a cluster size of 3 - 4 (ie. 3 - 4 people living with HIV admitted per day) based on routine data from Zomba hospital. We assume clusters are relatively similar to each other with a ρ of 0.005. A similar day-of-the-week randomisation design of partner HIV testing among antenatal outpatients in Malawi showed a ρ (0.015). [30]

12.2 Sample size and power (primary outcome)

We assume TB treatment initiation in the control group of 10% (based on data from pilot phase) and our hypothesis is that the DCXR-CAD plus FujiLAM intervention will increase this to 18% (i.e. an absolute risk difference of 8%).

12.3 Sample size and power (secondary outcomes)

We assume 20% of participants in usual care arm will experience death by 56 days (i.e. survival probability 0.8) and hypothesise a hazard ratio of between 0.5 and 0.8.

We assume 1% of participants in usual care arm will start TB treatment within 24 hours (this was not observed at all in our pilot phase) and hypothesise this intervention could increase this to 5%.

We assume 10% of participants have undiagnosed TB – based on the relatively low rate of TB treatment initiation observed in pilot (there are no sputum culture results available from the pilot as of the date of this protocol revision) and hypothesise that this intervention could reduce this to 5%.

As above, for all secondary outcomes we assume cluster size 3 - 4, rho 0.005 and co-efficient variation of cluster size 0.05.

The below table shows the study power and sample size calculations. Appendix 3 contains graphs showing the effect on cluster size and power as assumptions are altered.

Primary outcome (1): Proportion of people started on TB treatment by discharge from hospital									
Cluster size	Proportion started on TB treatment (usual care arm)	Risk difference (increase)	CV cluster size	ρ	1-β	α	Clusters / trial arm	Participants / trial arm	Weeks recruitment (inc. diagnostic cohort clusters)
3	0.1	**0.06**	0.05	0.005	0.8	0.05	166	498	94
3	0.1	**0.08**	0.05	0.005	0.8	0.05	100	300	57
4	0.1	**0.06**	0.05	0.005	0.8	0.05	75	300	43
4	0.1	**0.08**	0.05	0.005	0.8	0.05	126	504	71

Secondary outcome (1): Hazard of death by 56 days							
Cluster size	Survival probability (usual carel arm)	Hazard ratio	CV cluster size	ρ	α	Clusters per trial arm	Power (%)
3	0.2	**0.6**	0.05	0.005	0.05	102	69%
3	0.2	**0.8**	0.05	0.005	0.05	102	21%
4	0.2	**0.6**	0.05	0.005	0.05	102	82%
4	0.2	**0.8**	0.05	0.005	0.05	102	26%
Secondary outcome (2): Proportion of TB treatment initiations within 24 hours from recruitment.							
Cluster size	Proportion all participant on TB treatment 24 hours (usual care arm)	Risk difference (increase)	CV cluster size	ρ	α	Clusters per trial arm	Power (%)
3	0.01	0.05	0.05	0.005	0.05	102	82%
4	0.01	0.05	0.05	0.005	0.05	102	91%
Secondary outcome (3): Prevalance of undiagnosed TB as discharge							
3	0.1	0.05	0.05	0.005	0.05	102	64%
4	0.1	0.05	0.05	0.005	0.05	102	76%

Table 1: Power and sample size calculations under a variety of different effect size, cluster size and cluster similarity values.

We aim to recruit 102 clusters per trial arm, with approximately 306 participants per arm. A further 26 clusters will be recruited in the enhanced diagnostic observational arm, with approximately 78 participants (these clusters do not contribute to trial outcomes). Total study size will therefore be 690 people, recruited in 230 clusters (days).

This will give 81% power with a 5% type 1 error rate to detect an absolute increase of 8% (from a baseline of 10%) TB treatment initiations.

12.4 Data analysis plan

A detailed statistical analysis plan will be written and approved by the DSMC prior to database lock and unblinding. Trial reporting will follow CONSORT Guidelines.

We will report baseline characteristics of randomised participants, stratified by allocated group.

Analysis of the primary and secondary outcomes will be done on an intention to treat basis, with all participants allocated to trial groups included and analysed in the group to which they were randomized (regardless of which intervention was received). Adjustment to statistical estimates will be made (using random effects model) to take into account clustering of outcome by days in this cluster-randomised trial design.

Trial analyses of proportion of patients started on TB treatment (primary outcome) will be compared using risk differences and odds ratios.

Trial analyses of time from randomization to all-cause mortality (secondary outcome) will be compared between intervention arm and usual care arm using survival analysis with Kaplan Meier curves and Cox proportional hazards regression, with the intervention effects summarised by a hazard ratio. Outcomes will be censored at 56 days from recruitment.

Proportions of participants starting TB treatment within 24 hours from recruitment and proportion with undiagnosed TB will be analysed as a secondary endpoints using risk difference and odds ratios.

Descriptive statistics (proportions, means and medians as appropriate) will be used to describe the characteristics of participants and their diagnoses in the diagnostic cohort arm. As a planned analysis the time from randomisation to death will be compared between participants in the enhanced diagnostic cohort arm and the trial intervention arm using survival analysis with Cox proportional hazards; this is an exploratory analysis and the study is not powered for this.

13. DATA MANAGEMENT

13.1 Data Recording and Record Keeping

Electronic case report forms (CRFs) will be completed for each participant as follows;

- Eligibility and screening (collected from participant and medical records)
- Baseline demographic and clinical information (collected from participants where possible, supplemented by information from hospital records)
- Laboratory test results (collected from hospital and laboratory records)
- In-patient hospital follow-up (data collected from hospital records and/or clinical team)
- Hospital discharge or in-patient death (data collected from hospital records and/or clinical team)
- Out-patient follow-up, loss-to-follow-up or outpatient death (data from next-of-kin, relative or official register).

CRFs will be completed electronically on tablets using forms developed in Open Data Kit, preprogrammed with logical data consistency checks. Encrypted data files will be sent via secure internet connection to the study data-hub at the Malawi-Liverpool-Wellcome Trust. Xray images files will be held on secure encrypted password-protected computers and on a secure encrypted server.

The PI will monitor data-completeness and quality and generate lists of data queries on a weekly basis which will be issued to the Zomba-based study team for review, clarification and feedback. All electronic databases will have daily scheduled backups, and a password will be required to gain access to data. The password-protected databases at the trial site will be sent to LSHTM by Secure File Transfer Protocol (SFTP). Only trained staff will be granted access on a need-to-see basis.

Data will be stored using a unique study identifier allocated to each participant at enrolment into the study. Participant name and any other identifying detail will not be included in the main trial data electronic file. In accordance with data protection regulations, identifiers will be deleted entirely from the database as soon as practical to render it fully anonymous.

Paper consent forms will be transported from Zomba to MLW and stored securely.

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

All staff will be trained with respect to data management issues and GCP.

14. MONITORING AND QUALITY ASSURANCE

The trial will be conducted in accordance with the current approved protocol. The investigators will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki, with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996, MRC Guidelines for GCP, and relevant regulations and standard operating procedures (SOPs).

Data quality checks will be inbuilt to the data recording systems. In addition, all data collected will be subject to random sampling for verification of accuracy in relation to source documents. Any problems with data quality will be reported to the PI and appropriate action taken, including increasing frequency of checks.

The London School of Hygiene & Tropical Medicine (LSHTM) will act as the main sponsor for the study and the study may be subject to audit by LSHTM under their remit as sponsor, or assessment by the regulatory authorities, to ensure compliance with protocols, GCP and applicable regulatory requirements.

14.1 Data Safety Monitoring Committee

The Data Safety monitoring committee (DSMC) will monitor progress, advise the PI and investigator team, review safety data and report to the trial funders and sponsor. The DSMC will include an independent chairperson, principal investigator, co-investigators, trial statistician and independent experts. The TMC will be established comprising three independent members. The DSMC will meet prior to trial start and make recommendations concerning adverse events.

14.2 Trial Investigator Team

The investigational team draws on experience of a very strong team of local and international partners with a wealth of clinical experience in diagnosis and management of HIV-associated TB; research experience in clinical evaluation of TB diagnostics; design and contduct of randomized controlled trials; data management and analysis and strong relationships with local, regional and national partners such as TB programmes.

- Dr Rachael Burke (Clinical PhD fellow): Principal Investigator
- Prof Liz Corbett (Professor of Tropical Epidemiology): PhD supervisor and MLW TB / HIV group lead.
- Dr Peter MacPherson (Reader and Wellcome Trust Fellow): PhD supervisor and MLW Public Health Group Lead.
- Dr Ankur Gupta-Wright (Academic Clinical Lecturer): PhD supervisor
- Prof Katherine Fielding (Professor of Medical Statistics & Epidemiology and Director of LSHTM TB Centre): PhD advisor and co-investigator
- Dr Naomi Walker (Liverpool School of Tropical Medicine): PhD advisor and co-investigator
- Dr Marriot Nliwasa (College of Medicine): co-investigator
- Dr Saulos Nyirenda (Zomba Central Hospital): co-investigator
- Dr James Mpunga (National TB Programme): collaborator
- Rose Nyirenda (Department HIV / AIDS): co-investigator
- James Kandulu (Department diagnostics, Ministry of Health): collaborator

14.3. Ethical Considerations and Approvals

The protocol, informed consent form and participant information sheet will be submitted to Research Ethics Committees (REC) of the London School of Hygiene & Tropical Medicine and the College of Medicine Research and Ethics Committee (COMREC).

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Reporting:
The investigators shall submit an annual progress report to the COMREC and LSHTM REC once a year throughout the clinical trial. In addition, an End of Trial notification and final report will be submitted to the REC and host organization.

Expenses and Benefits
All participants are hospital inpatients and will not incur additional transport costs or lost time from economic activities due to participation in the study. [31]

Participants in the enhanced diagnostic arm will be compensated for their time and inconvenience of sample collection. Approximately 30 minutes of time whilst in hospital will be asked of participants to respond medical questionnaire delivered by a research assistant, in addition to screening questions. Participants will be asked to provide 22mL of blood (in a single blood draw) and urine sample and two sputum samples. Participants will be reimbursed equivalent of US$10 in MKw.

Participants in the trial arms will only be required to complete study screening questions and provide very minimal study information (age, sex, 4 TB symptom questions, ever had ART and ascertainment of whether they can stand). A single sputum and a urine sample will be requested, but no other study samples will be collected. Participants admitted on days randomised to the intervention arm will have a DCXR. Participants will be reimbursed the equivalent of US$1 in MKw.

Risks to patients

Risk of harm to participants in this trial is low.

Chest x-ray for all PLHIV who are seriously unwell is already international best practice, and endorsed by WHO, although currently many hospitals in Malawi don't have routine availability of chest x-ray. WHO has recommended that *"Computer aided diagnosis can be used for TB detection for research, ideally following a protocol that contributes to the required evidence base for guideline development."* Therefore, care being provided in the intervention arm is in keeping with international best practice and care in the control arm is in line with national standards and thus risk to participants is low. No drug treatment is mandated as part of the study, and all medicines that participants will likely receive from their usual team as a result of participating in the study are licensed drugs being used for their licensed indications.

FujiLAM is a CE-marked in vitro diagnostic test designed for the purpose of TB diagnosis.

The main potential harms specifically attributable to the intervention arm of the trial relate to more intensive use of chest x-ray as a diagnostic test and unwarranted initiation of empiric TB treatment as a result of a false-positive CAD result.

We anticipate that participants in the intervention arm will benefit from DCXR-CAD screening due to acceleration of TB diagnosis. However, it is possible that such screening may inadvertently be harmful in the following ways:

- Whilst a chest X ray can only provide supportive evidence of TB, rather than microbiological confirmation, more people in the Chest X ray arm may receive empiric TB treatment which may include patients who don't have TB.
- Rapid TB diagnosis may reduce the likelihood that participants receive an empirical course of simple antibiotics as part of the diagnostic work-up and therefore concurrent sepsis, if present, may not be treated
- Rapid TB diagnosis may result in other concurrent pathologies being overlooked.

Risks of inappropriate initiation of empiric TB treatment will be minimised by providing refresher training about chest x-ray for diagnosis of TB and the CAD software, clearly communicating the limitations of radiographic screening approaches. The strong recommendation is to attempt microbiological confirmation (Xpert MTB/Rif) in all people with a chest x-ray flagged as "TB likely".

The risks associated with radiation exposure from a single diagnostic chest X ray are extremely low (equivalent to 30 minute aeroplane flight or 10 days background radiation). As discussed above, chest x-ray in this group of patients is already international best practice and as such there is no radiation exposure for study participants beyond that already recommended by international guidelines.

Participant Confidentiality

Generation or perpetration of stigma is a concern when HIV and TB testing and treatment interventions are being offered, although our previous trials and studies suggest that stigmatisation is relatively uncommon and very rarely results in harm or adverse outcomes.

We will take extensive actions to ensure any potential for stigmatization is removed through ensuring that HIV testing and TB screening activities are undertaken in private areas and confidentiality is maintained. The participants will be identified only by unique study identifier on the main electronic database, with identifiers held in a separate password protected electronic database. The trial will comply with the Data Protection Act, which requires data to be fully anonymised as soon as it is practical to do so.

All documents will be stored securely and only accessible by trial staff and authorised personnel. All staff will be trained in the importance of confidentiality and appropriate data handling.

Any breaches of confidentiality will be systematically reported as an adverse event.

14.4 Possible constraints

We do not envisage any major constraints.

The main possible constraint is slower than expected recruitment. We anticipate recruitment within 13 months, but if recruitment is slower than expected, it is possible to extend duration up to 15 months. The study flow chart, salary budget and clinical trial insurance timeframe have been designed to allow for this extension should it be necessary.

Should there be stock-outs of important items for usual care diagnostics (for example, HIV tests) at Zomba hospital, the trial team would work with the Zomba management committee to help resolve the issue. If necessary, the trial may be able to provide funding to source small quantities of routine care diagnostics if needed to bridge a stock-out. Should there be a prolonged power outage the trial team will work with Zomba management committee to assist to resolve the issue if possible.

Other constraints outside of the control of the investigators include local or regional major incidents, for example related to flooding. As long as Zomba Central Hospital is running a medical inpatient service, the trial should be able to continue. Should a major incident occur such that Zomba hospital was required to cease admitting new medical inpatients the trial would be paused until such time as Zomba hospital can re-commence provision of medical inpatient services.

The COVID-19 pandemic has led to a delay of five months in commencing the trial. We have undertaken safety and risk assessments and are planning to resume study activities in August 2020, with appropriate PPE for staff. We have sought an extension from Wellcome Trust due to this delay.

15. BUDGET AND INSURANCE

Funding

The trial is funded by a Wellcome Trust Clinical PhD fellowship award, held by Dr Rachael Burke.

Insurance

The London School of Hygiene and Tropical Medicine (LSHTM) will act as the main sponsor for the study and holds public liability and clinical trial insurance policies which apply to this study which would operate in the event of any participant suffering harm as a result of their involvement in the research.

Requirements and budget justification

The requirements for the trial (together with their costs) are itemised in the budget below.

Personnel include a research clinical officer and two research assistants (field worker grade) who will be responsible for recruitment of participants, completing questionnaires and ascertaining outcomes (from review of ledgers, hospital records and phonecalls to participants). A contribution to the Zomba radiographer and laboratory technician is also included.

Consumables required include sputum mycobacterial culture (which will be done in College of Medicine laboratories in Blantyre) and a contribution towards routine ministry of health provision of usual care tests (HIV tests, Xpert cartridges, LAM strips, CrAg strips). A license fee for the computer aided diagnosis system is also required. Some more specialist tests (for example blood culture) are also required. Consumables related to collecting samples (needles, blood bottles, sputum pots etc.) are budgeted for.

Transport costs are included for transport between Blantyre and Zomba for samples and the study investigator.

Equipment costs are included for costs of a computer, tablets for electronic data collection, refurbishment of the radiography suite at Zomba and an inverter battery for the X-ray unit and costs of a digital X-ray unit.

There are costs related to clinical trial services, mainly incurred at MLW. This includes data management, printing, clinical research support unit time and health and safety. Clinical trial insurance (from LSHTM) is also required and included in the budget.

The budget also includes costs related to PhD training for the principal investigator (this study is funded by her PhD training fellowship). These include PhD fees, travel to London for PhD upgrading, costs for educational modules and costs related to travelling to scientific conferences.

Other requirements not incurring specific costs include desk space at MLW for the principal investigator and space for the study team at Zomba Central hospital to conduct research activities and to store study equipment.

15. PUBLICATION POLICY

Academic dissemination

Data will be disseminated to COMREC, local institutions, academic bodies and professional associations within Malawi with which the members of the investigational team already have links. Data will also be rapidly made available through presentations at relevant leading international conferences and regional conferences. Data will be published in a timely manner in peer reviewed journals. Findings will be summarised and made readily accessible on the institutional web-sites of the PI (LSHTM) and co-investigators.

Engagement with Policy Makers

Policy-makers both nationally and internationally will be offered the opportunity to be presented with the findings of this study. We will liaise with the National HIV programme around the viral resistance data, especially as this analysis of samples has been designed in conjunction with the National HIV program in order to meet their evidence-for-policy needs. These will include the STOP TB Partnership and the technical working groups of National TB programme and Department of HIV/AIDS of Malawi Ministry of Health. Feedback on results will also be given to Zomba hospital staff and the Department of Medicine and the district TB officers in Zomba district.

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APPENDIX 1: Previous studies of DCXR-CAD

Study	Study design / population	Definitions	Performance of CAD4TB
Muyoyeta et al.	Prospective. Zambia. 391 outpatients in Zambia with cough presenting to a TB clinic "open access point". TB investigations were spot sputum Xpert MTB/Rif and florescence microscopy. 350 patients were able to produce sputum, had valid Xpert results and had per-protocol CxR and included in study. Recruitment June to July 2013 HIV prevalence: 54% TB prevalence (overall): 168 / 350 (48%) Microbiologically confirmed: 97/ 350 (28%) (96 on Xpert, 1 on fluorescence microscopy) Clinically diagnosed: 71 / 350 (20%)	CAD4TB version 1.08 Abnormal CAD: Score > 60 To evaluate CAD, Xpert was used as reference test therefore: TB case: Xpert positive No TB: Xpert negative* ** NB. 71/ (%) cases that are "not TB" in reported specificity and PPV calculation were empirically treated for TB by clinicians.*	Abnormal CAD: 291/350 (83%) Sens: 96/96 (100%) Spec: 59/254 (23%)* PPV: 96/291 (33%) * NPV: 59/59 (100%)
Melendez et al.	Retrospective. UK. 39328 chest x-rays, but 367 had corrupted image data and couldn't be retrieved. 38961 chest x-rays from high risk screening in London. TB microbiologically investigations were as directed by respiratory physicians in a setting where mycobacterial culture is readily available and bronchoscopy available when required. Further radiological investigations (for example, CT chest) were also available on request. Patients recruited between April 2005 and March 2010. HIV prevalence: Not available, likely low. TB prevalence overall: 87 / 38961 (0.2%) Microbiologically confirmed: 61 / 38961 (0.16%) Clinical diagnosis only: 26 / 38961 (0.07%)	CAD4TB version 5 Abnormal CAD: Score > 39.8 * TB case: started on TB treatment on basis of all available information (culture / radiology / history). No TB: not started on TB treatment. * NB. Cut-off not pre-defined but determined as the value that would give 95% sensitivity – intention in this setting is to triage for radiologist reading.	Abnormal CAD: 17,301 / 38961 (44%) Sens: 83/87 (95%) * Spec: 28656 / 38874 (55.7%) PPV: 83 / 17301 (0.48%) NPV: 21656 / 21660 (99.98%) * Cut off chosen to give 95% sensitivity.
Brueninger at al.	Retrospective. Tanzania. Outpatients in Tanzania, referred from neighboring primary care clinics to TB clinic	CAD score cut-off: various cut-offs assessed in paper CAD version: 3.07	<u>CAD score >= 23</u> Abnormal CAD: 333 / 417 (80%)

	for evaluation of possible TB. Retrospective analysis of data from two other studies. Unclear about numbers who were referred but didn't meet the case definition for enrollment in parent studies. 861 patients enrolled in study, assessment of CAD done on subset of 417 patients who were either culture positive or definitely not TB (434 patients who had clinical TB, extra-pulmonary TB, non-tuberculous mycobacteria or were lost to follow up were excluded). Time period of recruitment not stated. HIV prevalence: 379 / 857 (44%) TB prevalence (whole study): 224 / 861 (26%) Microbiologically confirmed pulmonary TB: 194 / 861 (23%) Clinical diagnosis TB: 25 / 861 (2.9%) Extra-pulmonary TB: 5 / 861 (0.6%) TB prevalence (group used to evaluate CAD): Microbiologically confirmed: 194 / 417 (47%)	TB case: Sputum culture positive for *M. tuberculosis* No TB: All smears and culture negative and follow up for 5 months with sustained recovery.	Sens: 184 / 194 (95%) Spec: 74 / 223 (33%) PPV: 184 / 333 (54%) NPV: 74 / 84 (89%) <u>CAD score >=56</u> Abnormal CAD: 234 / 417 (56%) Sens: 165 / 194 (85%) Spec: 154 / 223 (69%) PPV: 165 / 234 (69%) NPV: 154 / 183 (85%)
Rahman et al.	Prospective. Bangladesh. Outpatients at a TB diagnosis referral centre in Dhaka, Bangladesh (walk in patients, those referred from private sector providers, smear negative possible TB from public sector providers) who had cough >2 weeks. 18746 people screened, 18036 symptomatic, 95% produced sputum for Xpert. Total population 17066. HIV prevalence not measured, estimated <1%. TB prevalence; 2623 / 17134 (15%)	CAD4TB version 3.07 CAD score cut-off; 63 was selected to have same sensitivity as radiologist. TB case: Xpert positive No TB: Xpert negative.	<u>CAD score >=63</u> Abnormal CAD: 10912 / 17066 (64%) Sens: 2374/2623 (91%) Spec: 5905 / 14443 (41%) PPV: 2374 / 10912 (22%) NPV: 5905/6154 (96%)

	All Xpert positive, not stated if any empiric TB diagnoses made by clinicians.		
Zaidi et al.	Prospective. TB access point in Pakistan with patients who either self-presented or were referred by a private provider (community health workers also did symptom screening at private provider clinics to highlight TB possibility). 6845 patients presented to TB clinic, 6090 had a valid Xpert result. HIV prevalence; not stated. TB prevalence: 925 / 6090 (15%)	CAD4TB version 3.07 CAD score cut off: >60 TB case: Xpert positive No TB: Xpert negative	Abnormal CAD: 4360/6090 (72%) Sens: 892/925 (97%) Spec: 1697 / 5165 (33%) PPV: 892/4360 (20%) NPV: 1697/1730 (98%)
Phillipsen et al.	Retrospective data. South Africa. Data from TB-NEAT trial. All self-referred patients with presumptive TB, presenting to clinic in Cape Town. 419 patients recruited, 6 patients missing x-ray, 3 patients software war unable to make a determination, 22 had no conclusive Xpert or culture tests. 388 patients included in study. HIV prevalence; 128/388 (33%) TB prevalence; 71/388 (18%)	CAD4TB version 3.07 CAD score cut off: >60 TB case: Culture positive sputum No TB: Culture negative sputum * 6 cases were Xpert positive / culture negative but counted as "no TB" in this analysis.	Abnormal CAD: 233/388 (60%) Sens: 63/71 (89%) Spec: 170 / 317 (54%) PPV: 63 / 233 (27%) NPV: 147/155 (95%) *NB. Performance characteristics of CAD4TB alone are not directly in paper, but possible to ascertain based on information that is in paper.*

APPENDIX 2: Clinical and clinical / microbiological definitions

Sepsis [32]	Clinical suspicion of infection (defined as decision to start antibiotics) plus a q-SOFA score >=2. qSOFA score is calculated as; Hypotension (SBP ≥100mmHg), altered mental state (GCS < 15), tachypnoea (Respiratory rate ≥ 22) [One point for each].
Invasive bacterial disease (proven)	Blood culture positive for any bacterial organism. Excluding blood cultures positive for coagulase negative staphylococci, viridans group streptococcal spp., rothia spp., corynebacterium, micrococcus spp., bacillus spp. or any other blood culture isolate thought likely to represent contamination.
Invasive bacterial disease (suspected) [33]	Documented fever or patient gives history of fever **AND** PCT > 0.5
Bacterial pneumonia	(A) History of >=2 of cough, fever, shortness of breath, sputum production **AND** evidence of consolidation on chest x-ray.
Cryptococcal disease	Positive serum cryptococcal antigen
PCP / PJP [34,35]	SpO2 < 92% **AND** evidence of diffuse infiltrates on chest x-ray **AND** no evidence of bacterial pneumonic changes on chest x-ray.
Malaria	Positive malaria film **AND** presence of documented fever or patient gives history of fever
IRIS [29]	IRIS events will be defined according to the Aids Clinical Trials Group consensus definitions (2009) (Aids Clinical Trial Group, 2009). An event will be judged to be compatible with IRIS if the following criteria are met; Initiation, reintroduction or change in antiretroviral therapy/regimen or therapy for opportunistic infections (OI). and Symptoms and/or signs that are consistent with an infectious or inflammatory condition. and These symptoms and/or signs cannot be explained by a newly acquired infection, the expected clinical course of a previously recognized infectious agent, or the side effects of medications. Note that as per the Aids Clinical Trials Group guidelines there is no requirement to demonstrate CD4 count improvement or viral load reduction as all clinical events will be judged less than 4 weeks following initiation or change of antiretroviral.
HIV treatment failure	Reports current ART use **AND** has detectable HIV viral load.
COVID-19 disease	A positive PCR test for SARS-CoV2 (regardless of symptoms)

	OR A positive lateral flow rapid diagnostic test (RDT) **PLUS** symptoms and signs (including radiology findings) of COVID-19.

Appendix B: CASTLE Statistical Analysis Plan

The CASTLE study

Computer Aided Screening for Tuberculosis in Low Resource Environments

Statistical Analysis Plan

Version: 1.0
Date: 2022-04-25

1. Administrative Information

Title: Statistical Analysis Plan for Computer Aided Screening for Tuberculosis in Low Resource Environments (CASTLE) trial.

Trial registration: NCT04545164

SAP version: V1.0 2022-04-25

Protocol version: V7.0 2021-11-13

SAP revision history	
V0.9 2021-05-25	Initial version
V1.0 2022-04-25	Updated version. Updated reference to protocol version Edited a sentence about "CONSORT" flow chart to state this would summarise clusters as well as individuals. Added a clause at end of outcome definition to make clear that people discovered to be ineligible after recruitment won't be included in ITT analysis (consistent with protocol and rest of SAP). Added a comment about p values for interaction being reported for subgroups if journal prefers. Added comment about fitting a multi-level model if possible (to estimate ICC and compare to model with robust standard errors). Added a comment that both R and stata will be used. Added appendix of results tables and figures to be reported

Roles and responsibilities			
Principal Investigator / PhD student	Dr Rachael Burke	London School of Hygiene & Tropical Medicine	Rachael.burke@lsthm.ac.uk +44 77295058417
PhD supervisor / co-investigator	Prof Liz Corbett	London School of Hygiene & Tropical Medicine	Liz.corbett@lshtm.ac.uk + 265 999981439
PhD supervisor / co-investigator	Dr Peter MacPherson	Liverpool School of Tropical Medicine	peter.macpherson@lstmed.ac.uk +265 99 717 6230
PhD supervisor / co-investigator	Dr Ankur Gupta-Wright	London School of Hygiene & Tropical Medicine	Ankur.gupta-wright@lshtm.ac.uk
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Co-investigator	Dr Naomi Walker	London School of Hygiene & Tropical Medicine	Naomi.walker@lshtm.ac.uk
Sponsor	LSHTM Research Governance Office.	London School of Hygiene & Tropical Medicine. Keppel Street, London. WC1E 7HT.	rgio@lshtm.ac.uk
Data Safety and Monitoring Committee	Prof Graeme Meintjes, Prof Joe Jarvis, Dr Euphemia Sibanda, Prof Mavuto Mukaka	University of Cape Town, London School of Hygiene &Tropical Medicine, Liverpool School of Tropical Medicine, Mahidol-Oxford Tropical Medicine Research Unit (respectively)	

2. Introduction

Background and rationale:

The CASTLE study is a randomised trial to assess impact of digital chest X-ray with computer aided diagnosis (DCXR-CAD) on TB treatment initiation and inpatient mortality among people living with HIV admitted to hospital. Please refer to the study protocol for further information.

This document outlines how data will be analysed.

3. Study Methods

Trial design

CASTLE is a cluster randomised trial with two trial arms. Clusters are day of admission to hospital.

Arm 1 **Usual Care alone**	Any TB diagnostic tests standardly available at Zomba central hospital, on treating clinician request. Includes sputum Xpert, urine AlereLAM and conventional plain film CxR, if requested by usual care team.
Arm 2 **Interventions (DCXR-CAD + FujiLAM + usual care)**	FujiLAM + AlereLAM + DCXR-CAD for everyone (regardless of symptoms). Study team members will arrange sputum Xpert if CAD score >=60. This is in addition to usual care (as above)

CASTLE has a third arm which is a nested observational diagnostic cohort ("diagnostic cohort"). People admitted on days assigned to diagnostic cohort arm do not contribute to trial outcomes.

The overall allocation ratio is 4:4:1 to usual care : interventions : diagnostic cohort. Accordingly, there is equal allocation (4:4) to the two trial arms.

Randomisation

Randomisation codes were generated randomly using block randomisation with varying randomly assigned block sizes (blocks could be 9, 18 or 27 days).

R statistical software with package "RandomizerR" was used to generate sequences using the following code:

```
a <- rpbrPar(300,rb=c(9,18,27), K=3, ratio=c(4,4,1), groups=c("usual","CXR+LAM","cohort"))
b <- genSeq(a,50,220188) # includes a seed
c <- getRandList(b)
d <- t(c)
```

Fifty separate sequences were generated using above code (by RMB) and exported to a spreadsheet. A second academic researcher (not affiliated with the CASTLE study) chose one of the fifty sequences and co-ordinated the printing of allocation codes and putting each cluster (day) allocation into sequentially numbered, sealed, opaque envelopes.

Each morning, the CASTLE trial team open the next sealed envelope in the sequence to reveal the allocation for that day.

Sample size

We plan to recruit 102 clusters per trial arm.

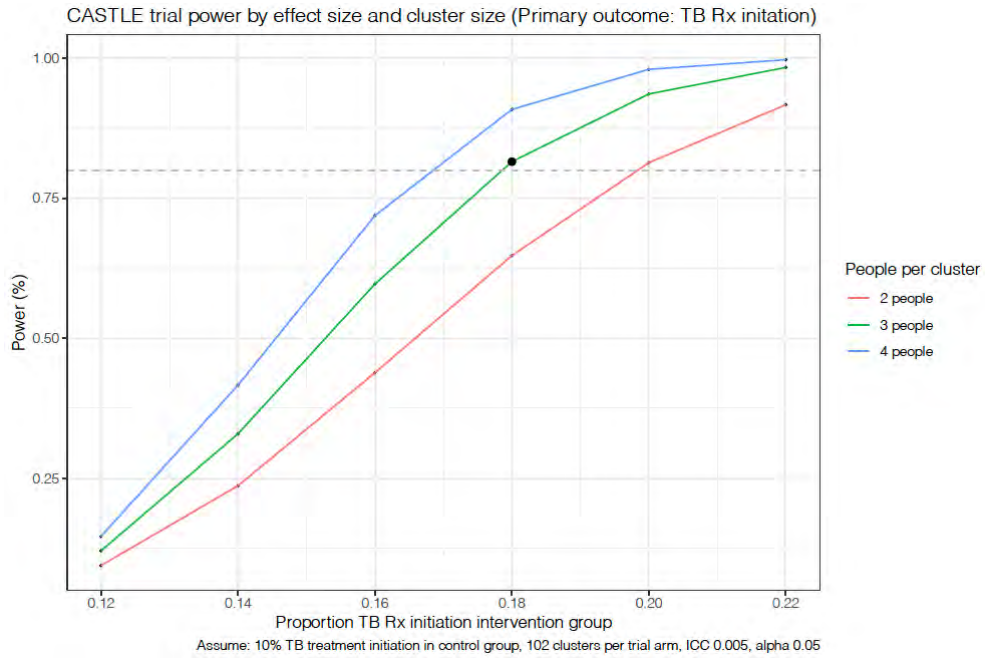
Sample size was calculated for primary outcome (TB treatment initiation). We assumed 10% people in usual care arm would initiate TB treatment by the time of hospital discharge, and 18% of people in intervention arm would initiate TB treatment. We assumed clusters with three participants per cluster and minimal clustering of outcome (ICC of 0.005).

Sample size for primary outcome was calculated in R using 'clusterPower' package

library(clusterPower)

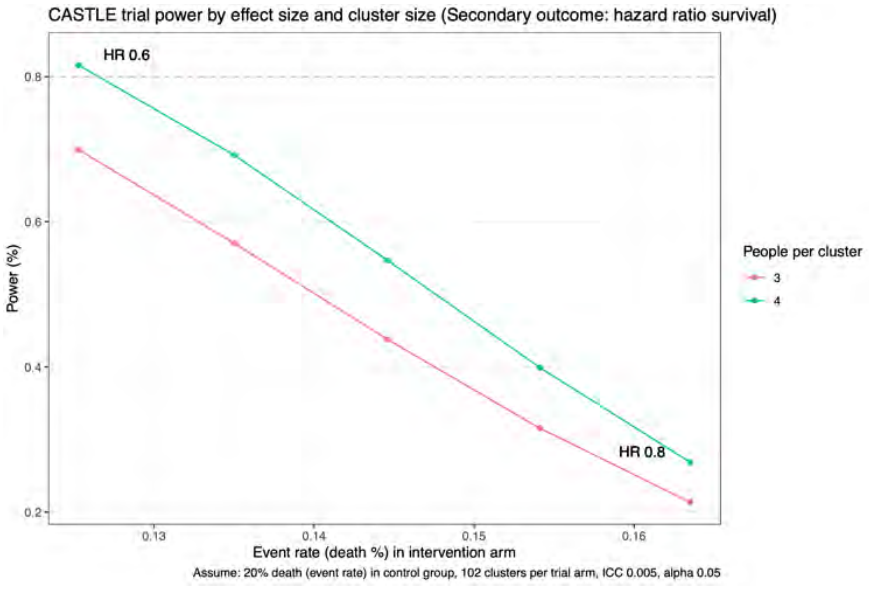
p_int <- c(0.12,0.14,0.16,0.18,0.20,0.22)

pwr_3 <- crtpwr.2prop(alpha = 0.05, power = NA, m = 102, n = 3, cv = 0.1, p1 = p_int, p2 = 0.1, icc = 0.005, pooled = FALSE, p1inc = TRUE)



Sample size for secondary outcome was calculated in stata using below command (assuming 80% survival by 56 days):

power logrank 0.8, k1(102) m1(3,4) hratio(0.6,0.65,0.7,0.75,0.8) rho(0.005) cvcluster(0.05)



If there were three participants per cluster, K 0.005 and TB treatment initiations 10% in usual care and 18% in intervention arm, then with 102 clusters per arm we will have 81% power to detect a difference between arms at least that large.

For secondary (survival) outcome, if there are four people per cluster and a hazard ratio of 0.6 (ICC 0.005 and survival probability 80% in usual care group) then with 102 clusters per arm we could have 81.6% power to detect an effect at least that large. With three people per cluster the power drops to 69.9%.

Framework

The hypothesis for CASTLE is that DCXR-CAD plus FujiLAM plus AlereLAM plus usual care is superior to usual care alone.

Statistical interim analyses and stopping guidance

There are no planned interim analyses.

Timing of final analysis

Final analysis will be conducted when the final participant has completed 56 days from time of enrolment.

Timing of outcome assessments

The primary outcome (TB treatment initiation) will be assessed at the time the participant is discharged from hospital (including TB treatment started on the day of discharge), participant dies or at 56 days from enrolment, whichever is the earlier.

The secondary outcomes are: TB treatment initiation within 24 hours from enrolment in trial, measured up to 24 hours from enrolment; undiagnosed TB at the time of discharge from hospital, measured at the time participant is discharged from hospital or dies or 56 days from enrolment whichever is earlier; and time-to-death, measured up to 56 days from enrolment.

4. Statistical Principles

Confidence intervals and *p* values

All applicable statistical tests (p values) will be two-sided. All confidence intervals will be 95% and will be two sided.

There is a single primary outcome and three secondary outcomes. No adjustments for multiplicity are planned.

Adherence and protocol deviations

Adherence to intervention is defined as receiving a dCXR and urine LAM test (for those in intervention arm). Analysis is intention-to-treat and is not affected by adherence to intervention.

Protocol deviations are defined in LSHTM-SOP-012-02 (LSHTM Research Governance and Integrity Office, 2019-11-28). Major and minor protocol deviations and any protocol violations will be summarised in an appendix to main trial paper.

Analysis populations

CASTLE will be analysed on an intention to treat basis.

The analysis population will include all randomised participants according to the intervention their cluster was randomised to receive, regardless of whether the intervention was received or not. Participants enrolled into a randomised cluster who were subsequently found to be ineligible for the CASTLE trial will be removed from analysis.

Should there be a major protocol deviation / violation involving large numbers of participants not receiving the intervention to which their cluster was assigned, we would consider a per protocol additional analysis.

5. Trial Population

Screening data

The following summaries will be presented for all screened potential participants:

Number of days recruiting, number of potential participants screened, number of screened potential participants not recruited and the reason for non-recruitment.

Eligibility

Eligibility for CASTLE is detailed in the protocol.

Inclusion:	Adult (age >= 18 years), admitted to medical ward at Zomba Central Hospital (for any reason), HIV positive (new diagnosis or known diagnosis)
Exclusion:	Unable or unwilling to consent, already on TB treatment or has received TB treatment in past six months, admitted for longer than 18 hours at the time of start of assessment for CASTLE enrolment.

Recruitment

A "CONSORT" diagram will be used to summarise the number of clusters randomised, and people screened, eligible, consented, randomised, receiving their allocated intervention and withdrawing / lost to follow up.

Withdrawal / follow up

Should any participant withdraw from the study, this will be described, including the level of consent withdrawal (i.e. consent withdrawal for any or all of intervention, for follow up or for data retention). The reasons for withdrawal (where stated by participant) will be captured.

For participants who are lost to follow up by 56 days, they will be censored from follow up at the last time they were observed alive by the study team (this will usually be date of discharge from hospital) or the last time a reliable witness (for example, a friend or family member) reports observing the person alive.

Baseline participant characteristics

Baseline participant characteristics for categorical variables will be summarised by frequencies and percentages. Continuous data (age, CD4 count) will be summarised by median and interquartile

range. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

The primary analysis will be unadjusted for baseline characteristics. If there is a major imbalance in baseline characteristics, we would consider a secondary sensitivity analysis adjusted for the imbalanced variable.

6. Analysis

Outcome definitions

	Definition	Timing	Notes
Primary outcome			
1. Proportion of people starting TB treatment	Participant started on TB treatment. Numerator is all those starting TB treatment and denominator is all those enrolled, excluding anyone found to be ineligible after enrolment..	From time of enrolment in CASTLE trial to end of the day in which a participant was discharged from hospital, or time participant died, or 56 days from enrolment in CASTLE whichever is sooner.	TB treatment as recorded in Zomba Central hospital TB register (paper-based ledger). Every participant's name checked against TB register within a week of discharge. Includes people started on TB treatment even if TB treatment subsequently stopped. Includes TB treatment started on the same day as discharge from hospital (up to midnight on the day of discharge).
Secondary outcomes			
2. Mortality (time to event)	Death from any cause	From time of enrolment in CASTLE trial to 56 days from enrolment. For those who are not reached at or after 56 days, their time in trial will be censored at the time of death or time they were last known to be alive by a reliable witness.	Includes in hospital deaths and deaths after discharge from hospital up to 56 days from enrolment. Deaths reported by relatives, mainly phonecall based tracing and home visits if unable to contact by phone. If a person dies on the same day as enrolment they will be assigned 0.5 of a day survival time.
3. Proportion starting same day TB treatment.	TB treatment started within 24 hours of enrolment in CASTLE trial.	Measured from time of enrolment in CASTLE for further 24 hours.	Time of starting TB treatment is time that guardian picks up TB medicines from TB office at Zomba Central Hospital (or ward nurse picks up medicines if no guardian). Time of picking up TB medications determined by observation of study team and guardian / relative / patient / nurse report (as time is not recorded in paper ledger, only date).
4. Proportion with undiagnosed TB.	Sputum culture at mycobacterial lab in Blantyre grows *M. tuberculosis complex* organisms **and** participant did not start TB treatment at or before time of discharge from hospital or death or 56 days from enrolment (whichever is sooner).	TB treatment initiation as defined in primary outcome.	People whose sputum grows non-tuberculous mycobacteria are not included as having undiagnosed TB. People who start TB treatment within time frame defined in primary outcome, but who subsequently interrupt TB treat are not treated as having undiagnosed TB. Denominator is all people in CASTLE trial, not just those who produce sputum.
Other outcomes / measurements			

Inpatient mortality	Death from any cause	From time of enrolment until time of hospital discharge or 56 days from enrolment, whichever is sooner.	Determined through observation, notes review, asking healthcare staff and the ward paper ledger.
56 day mortality (measured as a proportion)	Death from any cause	From time of enrolment in CASTLE trial to 56 days from enrolment. Analysed as binary data.	In hospital deaths determined as above, deaths after discharge from hospital ascertained from phone-call with nominated next of kin.
Microbiologically confirmed TB.	Proportion of all TB diagnoses that are microbiologically confirmed.	Refers to TB diagnoses made before discharge from hospital or before 56 days from enrolment, whichever is sooner.	Microbiologically confirmed means at least two positive Acid Fast Bacilli (AFB) smears or one or more Xpert Mtb/Rif positive or one or more culture positive for M. tb on any specimen or a positive urine LAM result. Includes samples collected by CASTLE team and those by usual care team.
Intervention fidelity	Proportion of people recruited in intervention clusters who have a CxR with CAD score and urine LAM result recorded	We will record any instance where intervention not delivered on the same day as enrolment.	Reasons for not receiving interventions will also be detailed.

Analysis methods

	Metric to be reported	Method of calculation
Primary outcome		
1. TB treatment initiation	Risk ratio of TB treatment initiation by allocation arm. We will also calculate and report absolute risk difference.	Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio. Absolute risk difference will be obtained from binomal GLM with identity link function.

Secondary outcomes		
2. Mortality	Hazard ratio for death (time to event)	Cox regression using robust standard errors to account for clustering.
3. Same day TB treatment initiation	Risk ratio of TB treatment initiation within 24 hours from enrolment by allocation arm.	Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio,
4. Undiagnosed TB	Risk ratio of undiagnosed TB by allocation arm.	Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.

Other outcomes		
5. Inpatient mortality	Risk of death prior to discharge from hospital.	Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.
6. 56 day mortality (measured as a proportion)	Risk of death within 56 days from enrolment (i.e. analysed as a proportion rather than time to event)	Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.
7. Microbiologically confirmed TB.	Proportion of TB	Numerator is those with microbiologically confirmed TB and denominator is all who had TB diagnosed.
8. Intervention fidelity	Proportion	Numerator is all those who received DCXR with valid CAD score and a FujiLAM urine result, denominator is all those recruited in clusters allocated to intervention arm.

Notes on analysis methods

Notes on table

- If GLM with log-binomial link function fails to converge then we will use an alternative method to calculate risk ratio, or report an odds ratio.
- We will also attempt to fit a mutli-level model, using cluster as a random effect and intervention arm as a fixed effect. This will be used to determine amount of clustering by calculating an Intra-class correlation coefficient (ICC). The primary analysis is the GLM with robust standard errors (as in table above), if there is an important difference in estimates between the two methods we will report both.
- If a multi-level model won't fit (for example, because clusters are too small), this will be noted. In this instance we will be unable to report a measure of the amount of clustering of outcome.

Sensitivity analyses

If there are large numbers (>5%) of participants who do not receive their allocated intervention, then we will perform a sensitivity analysis on a per-protocol basis, where outcomes are based on intervention actually received rather than intervention each cluster was allocated to.

Subgroup analyses

For the primary outcome (TB treatment initiation) and for time-to-death outcome, we will analyse the intervention effect (RR and RD) on outcomes by subgroups of those with and without TB in the differential diagnosis at admission ("TB suspects") and by CD4 count. CD4 count measurement isn't a study specific activity, but where it is measured by hospital we will capture data, and we will analyses outcomes in subgroups CD4 =<100 cells/mm^3, CD4 > 100 cells/mm^3 and CD4 not measured. Where a person has more than one CD4 count measured in hospital, the CD4 count closest to admission will be used. P values for interaction will be reported if this is consistent with guidance from editors and reviewers of the journal the report is submitted to.

Missing data

No imputation will be performed for missing data. There will be no missing data for day recruited or cluster (i.e. arm) allocation. If an outcome is missing then it will be reported as such and not imputed.

No adjustment by baseline variables is planned, therefore missing data other than randomisation arm and outcome does not affect analysis. There is no imputation for missing covariates at baseline.

For the mortality secondary outcome (time to event), participants will be censored at the time they were last known to be alive.

Additional analyses

The diagnostic cohort (which does not contribute to trial outcomes) will be analysed mainly using descriptive statistics.

Within trial arms, as an exploratory analysis, we will compare risk of death by 56 days from enrolment among those with HIV virological failure and those without HIV virologic failure. This will be in a subset of participants who have HIV viral load measured (a convenience sample based on when protocol amendment to collect HIV viral load and time of day of admission so that lab staff are available to process samples).

It is envisaged that data from participants in the diagnostic cohort are reported in a separate report, and not the main trial paper.

Harms

Adverse events are defined in study protocol.

Statistical software

The analysis will be carried out using both R statistical software (v. 4.0.3) and Stata (v.15). In R, packages 'lme4', 'sandwich', 'lmtest', 'survival' and 'survminer' will be used.

Appendix

Appendix including suggested "dummy" tables and figures to present trial results is below. The exact formatting (and whether in main paper or an appendix) to be determined according to requirements of journal.

The appendix also includes suggested dummy tables and figures describing diagnostic test results and how TB diagnoses were made. We may revise presentation of the TB diagnostic visualisations

(but not the formal trial results) once we analyse data in order to best present the data for clarity and understanding.

7. References

Reich NG, Myers JA, Obeng D, Milstone AM, Perl TM (2012) Empirical Power and Sample Size Calculations for Cluster-Randomized and Cluster-Randomized Crossover Studies. PLoS ONE 7(4): e35564. https://doi.org/10.1371/journal.pone.0035564

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Richard J. Hayes, Lawrence H. Moulton. Cluster Randomised Trials (Chapman & Hall/CRC Biostatistics Series) 2nd Edition.

Harrell FE. Introduction. In: Harrell Jr Frank E, editor. Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis. Springer International Publishing; 2015

Zeileis A, Hothorn T (2002). "Diagnostic Checking in Regression Relationships." *R News*, 2(3), 7–10. https://CRAN.R-project.org/doc/Rnews/. **lmtest package.**

Zeileis A (2006). "Object-Oriented Computation of Sandwich Estimators." *Journal of Statistical Software*, 16(9), 1–16. doi: 10.18637/jss.v016.i09. **Sandwich package.**

Therneau T (2021). *A Package for Survival Analysis in R*. R package version 3.2-10, https://CRAN.R-project.org/package=survival. **Survival package**

Pantelli, N. LSHTM-SOP-012-02 Protocol / trial violations and deviations. LSHTM Research Governance and Integrity Office, 2019-11-28.

In addition, at the time of writing these were the versions of following relevant trial documents;

CASTLE protocol: v7.0 2021-11-13
Data Management Plan: v1.0 2019-10-30
Data and Safety Monitoring Committee charter: v1.0 2020-08-27

Appendix C: CASTLE Data Safety Monitoring Committee Charter

The CASTLE study

Computer Aided Screening for Tuberculosis in Low Resource Environments

Document: Data Monitoring and Safety Committee Charter

Version: 1.0

Date last updated: 2019-08-27

1 Introduction

1.1 Trial name

Computer Aided Screening for Tuberculosis in Resource Limited Environments (CASTLE)

1.2 Trial registration

The study will be registered with ISRCTN before commencement.

1.3 Ethics reference number

The CASLTE study has received ethical review as follows;

LSHTM Research Ethics Committee. Reference 17799.

University of Malawi College of Medicine Research and Ethics Committee (COMREC). Reference: P.08/19/2772.

1.4 Sponsor and Funder

The London School of Hygiene and Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance and Integrity Office:

London School of Hygiene and Tropical Medicine
Keppel Street
Lonwon WC1E 7HT
+44 2079272626
rgio@lshtm.ac.uk

The funder is the Wellcome Trust, via a Wellcome Trust Clinical PhD Fellowship awarded to Dr Rachael Burke.

1.5 Investigators

A full list of investigators is listed in the protocol. The principal investigator is Dr Rachael Burke (a PhD candidate). Her PhD supervisors and co-investigators are Prof Liz Corbett and Dr Peter MacPherson. Prof Katherine Fielding is a co-investigator.

1.6 Scope of charter

The purpose of this document is to describe the roles and responsibilities of the Trial Monitoring Committee for the CASTLE trial, including the timing of meetings, methods of providing information, frequency and format of meetings, statistical issues and relationships with other committees.

2 Trial summary (please also refer to trial protocol)

2.1.1 Background

Despite the widespread availablity of ART at community level, people living with HIV (PLHIV) who require admission to hospital in WHO AFRO region are frequently very immunosuppressed and have

a high mortality. Undiagnosed TB is likely to be a major contributor to mortality. Suboptimal TB diagnostics contribute to the problem.

Digital chest X-ray with computer aided diagnosis (DCXR-CAD) is a promising new technology to assist with X-ray interpretation, particularly in a setting where skilled radiologists are not routinely available. Fujifilm SILVAMP TB LAM (FujiLAM) is a CE-marked high sensitivity urine LAM test. Retrospective studies on stored samples have shown superior sensitivity of FujiLAM compared to the older urine LAM test manufactured by Alere / Abbott.

Our trial hypothesis is that admission screen for TB among adults living with HIV using DCXR-CAD and FujiLAM plus usual care can increase the number of people starting on TB treatment and reduce inpatient mortality, compared to usual care alone.

A more detailed summary of scientific background with references is available in the protocol.

2.1.2 Summary

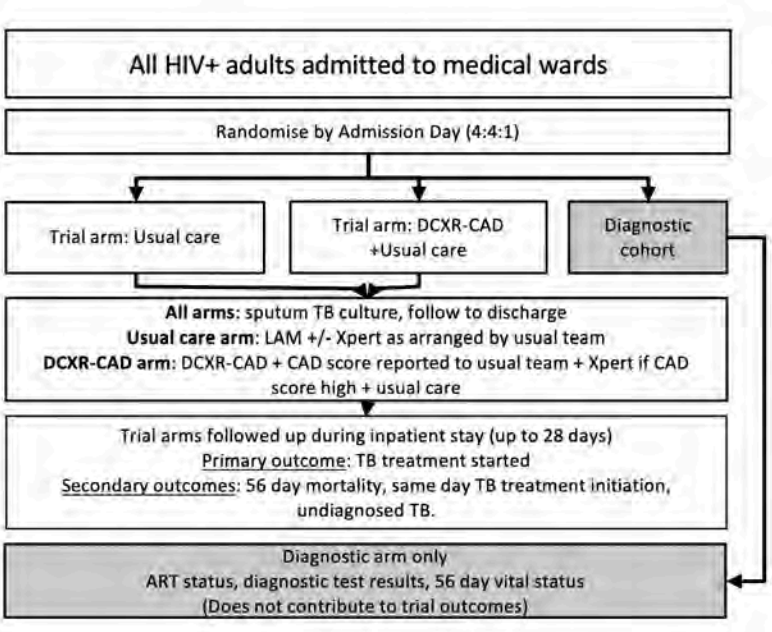
Trial title	Computer Aided Screening for Tuberculosis in Low Resource Environments (CASTLE)
Short title	CASTLE study
Trial Design (methodology)	Single site (Zomba Central Hospital) cluster randomised trial with two trial arms and a third nested observational enhanced diagnostic cohort that will not contribute to trial outcomes (4:4:1 allocation, randomised by admission day).
Trial population	HIV infected adult patients requiring admission to medical wards at Zomba Central Hospital. Unit of randomisation will be admission day.
Planned sample size	102 clusters per trial arm (approximately 306 participants). A further 26 clusters in enhanced diagnostic cohort (approximately 78 participants). Total of 230 clusters with approximately 690 participants..
Follow up duration	56 days (eight weeks) from day of recruitment
Recruitment period	January 2020 – March 2021.
Trial intervention	Digital Chest x-ray with Computer Aided Diagnosis (DCXR-CAD) and urine high sensitivity lipoarabinomannan (FujiLAM) screening performed on first day of admission on participants admitted on days assigned to trial intervention arm. Numerical X-ray TB score and interpretation ("Pulmonary TB likely" or "Pulmonary TB not likely"), and FujiLAM results, appended into patient's notes. X-ray imaging available for clinical team review on study computer in order to inform TB treatment decision making. If a participant's CAD score indicates "TB likely", they will have sputum taken for Xpert Mtb/Rif. DCXR-CAD is in addition to usual care in the intervention arm. The control arm is assigned to usual care alone.

2.1.3 Objectives and outcomes

	Objective	Outcome Measures / Endpoints
1. Primary	To determine the effect of DCXR-CAD plus FujiLAM plus usual care vs. usual care alone on; 1.1 TB treatment initiations	1.1 Proportion of participants starting TB treatment during course of inpatient stay (censored at 56 days)
2. Secondary	To determine the effect of DCXR-CAD plus FujiLAM plus usual care vs. usual care alone on; 2.1 Mortality (time to event) 2.2 Undiagnosed TB	2.1 Time (in days) to death from any cause, with censoring at 56 days. 2.2 Proportion of participants who are culture positive for *M. tuberculosis* (M.tb) in sputum, who are not started on TB treatment at the time of discharge

	2.3 Same day TB treatment initation	from hospital or are current inpatients not on TB treatment by the time of culture result being made available. 2.3 Proportion of participants starting TB treatment within 24 from from time of recruitment.
3.Pre-planned analyses.	To determine the effect of DCXR-CAD plus FujiLAM plus usual care vs. usual care alone on; 3.1 Inpatient mortality (proportion of participants dying as inpatients). 3.2 Total mortality (proportion experiencing death) 3.3 Proportion of participants with a TB diagnosis that is microbiologically confirmed vs. clinically diagnosed. 3.4 To determine intervention fidelity in this setting. 3.4 To determine diagnostic accuracy of DCXR-CAD in an inpatient, HIV positive setting.	 3.1 Proportion of participants dying during admission (censored at 56 days) from any cause. 3.2 Proportion of participants dying up to 56 days from any cause. 3.3 Proportion of participants started on TB treatment with (a) microbiologically confirmed TB vs. (b) clinically or radiologically diagnosed TB. 3.4 Proportion of participants randomised to DCXR-CAD plus FujiLAM arm who received DCXR-CAD and had a urine FujiLAM result. 3.4 Sensitivity, specificity, PPV and NPV for CAD score compared to a composite microbiological reference standard and a clinical standard.
4. Diagnostic cohort	To describe the range of pathology among people living with HIV requiring admission to hospital	Descriptive statistics.

2.1.3.1 Trial schematic



3 Roles and responsibilities

3.1 Aims of the DSMC committee

The aim of the DSMC is to protect the safety of study participants, to assist and advise the Principal Investigator (Dr Rachael Burke, a PhD student), co-investigators (including Prof Liz Corbett and Dr Peter MacPherson, her PhD supervisors) and collaborators so as to protect the validity and credibility of the trial, and to monitor the overall conduct of the clinical trial. The DSMC will also provide advice through its chairperson to the sponsor of the trial.

The DSMC will be a single body which will take on the joint roles of a traditional Trial Steering Committee and a Data Safety and Monitoring Board.

A full list of co-investigators and collaborators is listed on the CASTLE study protocol.

3.2 Terms of reference

The DSMC will

1) Receive and review the progress and accruing trial data (by default, blinded data only but the independent members may request to review unblinded data in a closed session);
2) Advise the principal investigator and co-investigators on the conduct of the trial;
3) Review and comment on the statistical analysis plan.

3.3 Specific roles of the DSMC

1) Provide expert oversight of the trial
2) Monitor recruitment rates and encourage investigators to develop strategies to deal with any recruitment problems
3) Assess the impact and relevance of any accumulating external evidence
4) Advise on protocol modifications relevant to data aspects as suggested by investigators or sponsors (eg. to inclusion criteria, trial endpoints, or sample size)
5) Review amendments to the protocol, where appropriate
6) Review the statistical analysis plan
7) Encourage the timely reporting of trial results
8) Monitor adverse events (see protocol for specific adverse events which will be reported)
9) Maintain confidentiality of all trial information that is not already in the public domain

4 Before the trial starts

The DSMC membership will review and provide comments on the trial protocol, and hold their first meeting before commencement of randomisation of participants. The objective of the first meeting is to discuss the protocol including adverse event reporting and analysis plans with the principal investigator and co-investigators.

The first meeting will also involve planning future meetings and reviewing what the contents of the data report should be (dummy tables in annex 3 and 4).

5 Composition

The members of the DSMC for the CASTLE trial are:

1) Prof Joe Jarvis, London School of Hygiene and Tropical Medicine

2) Prof Graeme Meintjes, University of Cape Town

3) Dr Euphemia Sibanda, Liverpool School of Tropical Medicine

4) Dr Mavuto Mukaka, Mahidol-Oxford Tropical Medicine Research Unit

The DSMC membership includes statistical and clinical expertise. Prof Jarvis will be the chairperson.

As this trial is her PhD project, the Principal Investigator will act as the facilitator of the DSMC. She will be responsible for arranging meetings of the DSMC, coordinating reports, producing and circulating minutes and action points. The PI / facilitator will be the central point for all DSMC communications between the DSMC and other bodies, will be copied into all correspondence between DSMC members and will be kept aware of trial issues as they arise.

DSMC members will not be asked to formally sign a contract but should formally register their agreement to join the group by confirming (1) that they agree to be a member of the DSMC and (2) that they agree with the contents of this Charter. Any potential competing interests should be declared at the same time. Members should complete and return the form in Annexes 1 or 2.

Additional observers may be in attendance through (parts of) the DSMC meetings in order to provide input on behalf of the trial's Sponsor/Funder or to provide specific relevant expertise.

6 Preparation of reports to the DSMC

The PI will prepare the open report.

It is anticipated that all committee meetings will be open. The first meeting will be before the commencement of the trial, thus a closed portion to this meeting is unnecessary.

By default, the interim meeting(s) during the course of the trial will also be open. However, at the committee's request, a closed session will be convened consisting only of the independent members of the committee and a statistician representative from the trial (Prof Fielding). At this closed session unblinded data could be presented and discussed.

If the independent members of the DSMC request to view the unblinded data in a closed session then the PI will write the statistical analysis code which will be used for the data analysis for the closed report. She will not run the data analysis for the closed report so as to remain blinded. The statistical code will be run and the report will be compiled by an independent statistician from Malawi Liverpool Wellcome Clinical Research Programme.

7 Relationships

Trial management is described in the protocol.

The DSMC has an advisory role for the trial.

DSMC members will not be paid for their services.

DSMC members are asked to disclose information about any competing interests.

Formally, the DSMC reports to the sponsor (the London School of Hygiene and Tropical Medicine) and their meeting reports will be available to the sponsor. Excepting major issues, we envisage the comments from the DSMC will be taken forward for action by the principal investigator and/or her supervisors / co-investigators.

8 Organisation of DSMC meetings

DSMC meetings will be conducted once prior to commencement of trial, once during the trial (after 6 – 9 months) and once at the conclusion of the trial (12-15 months). Should issues arise during the course of the trial, the DSMC will meet more frequently – either at the request of the PI or co-investigators or at the request of the independent members of the committee.

The meetings will be conducted by teleconference.

By default, the meeting will have only an open session. However, should the DSMC request a closed session to review unblinded data then a meeting with a closed session will be convened.

Attendance by the study investigational team (with the exception of Prof Fielding) will be restricted to the open session – i.e. if there were to be a closed session this would be for will be for independent DSMC members and Prof Fielding only.

9 Trial documentation and procedures to ensure confidentiality and proper communication

An outline of the intended content of material to be available in open sessions will be prepared and agreed at the first DSMC meeting.

The DSMC will receive the reports at least 1 week before any meetings.

The PI will take minutes of the open session. Should a closed session be convened Prof Fielding could make brief notes, alternatively - if requested - the PI would arrange for an administrator to take minutes.

The DSMC will report its recommendations in a written report, which will be copied to the sponsor. It is anticipated that the actions from meeting will be taken forward by the principal investigator.

Where the independent members of the DSMC have met in a closed session, they will present their recommendations to the rest of the DSMC (ie. the principal investigator and co-investigators) and these will be included in the written report.

10 Decision making

The DSMC may make recommendations such as:

1) No action needed, trial continues as planned
2) Modifying target recruitment, or pre-analysis follow-up, based on any change to the assumptions underlying the original trial sample size calculation (but not on any emerging differences)
3) Sanctioning and/or proposing protocol changes

4) Early stopping due, for example, to clear benefit or harm of a treatment, futility or external evidence

Every effort should be made to achieve consensus. The role of the Chair is to summarise discussions and encourage consensus.

It is important that the implications (e.g. ethical, statistical, practical, financial) for the trial be considered before any decision is made.

10.1 When is the DSMC quorate for decision-making?

At least two independent members of the DSMC should be present, plus the PI.

The PI (who is also the DSMC facilitator) will, ahead of a planned meeting, coordinate with all DSMC members and identify an agreeable meeting date. Two independent members unless otherwise agreed) can still run a DSMC meeting. If the DSMC is considering recommending major action after such a meeting the chair should talk with the absent members as soon after the meeting as possible to check if they agree. If they do not, a further teleconference should be arranged with the full DSMC. If the report is circulated before the meeting, DSMC members who will not be able to attend the meeting may pass comments to the DSMC Chair for consideration during the discussions.

If a member does not attend a meeting, it will be ensured that the member is available for the next meeting. If a member does not attend a second meeting, they will be asked if they wish to remain part of the DSMC. If a member does not attend a third meeting, they will cease to be a DSMC member.

11 Reporting

11.1 To whom will the DSMC report their recommendations/decisions, and in what form?

The DSMC will report their decisions to the PI and co-investigators who will be responsible for implementing any actions resulting.

The DSMC report will be copied to the sponsor as a matter of course. Should major action be recommended, this will be highlighted to the sponsor.

11.2 Whether minutes of the meeting be made and, if so, by whom and where they will be kept

Notes of key points and actions will be made by the PI (who also acts as the DSMC facilitator). This will include details of whether potential competing interests have changed for any attendees since the previous meeting. The draft minutes will be initially circulated for comment to those DSMC members who were present at the meeting. The DSMC Chair will sign off the final version of minutes or notes.

11.3 What will be done if there is disagreement within the DSMC (particularly between the investigators and the independent members) ?

The DSMC is the oversight body for the trial and combines the traditional roles of TSC and DSMB into one committee.

Should there be disagreement within the DSMC, the committee would first try to resolve the issue internally by consensus, under the guidance of the independent chairperson.

In exceptional circumstances, if the matter could not be resolved by consensus, the DSMC chair may contact the trial sponsor who may convene a meeting chaired by a senior member of LSHTM or an external expert who is not directly involved with the trial. Depending on the reason for the disagreement confidential data and/or data by trial and may have to be revealed to all or some of those attending such a meeting: this would be minimised where possible.

12 After the trial

DSMC members will be named and their affiliations listed in trial report, unless they explicitly request otherwise. A brief summary of the timings and conclusions of DSMC meetings may also be included in this report.

13 Annex 1: Trial Monitoring Committee members register of their assent

I, **_____** agree

1) to be on the CASTLE Trial DSMC committee
2) with the contents of the CASTLE Trial DSMC Charter
3) to keep CASTLE Trial DSMC data reports and meeting outputs confidential

Signature: _____ Date: _____

14 Annex 2: Suggested competing interests form

Potential competing interests of DSMC

Possible competing interest(s) should be disclosed.

Potential competing interests

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the sponsor
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial
- Hands-on participation in the trial
- Involvement in the running of the trial (for independent members only, this conflict is not relevant to members who are on the DSMC by virtue of their involvement with running the trial)
- Intellectual conflict eg. strong prior belief in the trial's intervention arm
- Involvement in regulatory issues relevant to the trial procedures
- Investment (financial or intellectual) in competing products
- Involvement in the publication

Please complete the following section and return to the Principle Investigator.

☐ **No,** I have no competing interests to declare

☐ **Yes,** I have competing interests to declare (please detail below)

Please provide details of any competing interests:

Name: _____

Signed: _____ Date: _____

15 Annex 3: Data for open session

Data will not indicate study arm and the following will be presented

- Enrolment and accrual

Study week (4 clusters per week)	Number potential participants screened	Number enrolled (total in 4 clusters for that week)	Running total clusters and participants

- Participant retention

Number of participants reached 56 day follow up	Number reached to ascertain outcome	Comments on strategies to reach participants

- Any participants withdrawn?

- Participant baseline characteristics

Characteristics	Median (IQR) or proportion
Age	
Sex	
On ART or not	
TB suspected at admission?	

- In hospital deaths observed

n in hospital deaths	n people discharged from hospital alive (or alive in hospital and >56 days from enrollment)	n admitted to hospital

- Deaths by 56 days

n community deaths by 56 days from enrolment	n people known to be alive at 56 days	n participants not yet reached 56 days from enrolment	n participants >56 days from

			enrolment but not reached

- TB treatment initiations

n TB treatment initiations	**N participants**

- TB sputum *culture* results (NB. Does not include TB tests (eg. Xpert) done locally)

	N participants
N participants	
Number (%) sputum samples produced and received in lab	
Number (%) culture positive	
Number (%) culture negative	
Number (%) culture ongoing	
Number (%) contaminated / otherwise unavailable results.	

- Adverse events

	Number of events
NB. Inpatient death reported as a trial outcome (see above)	
Error TB results reporting leading to participant starting TB treatment in error	
Breach of confidentiality following TB or HIV diagnosis	
Needlestick injuries	

Further details about circumstances of adverse events (if required)

- Have the investigators become aware of any participants who were diagnosed with TB, but were later discovered to have a diagnosis other than TB? If details of cases to be outlined below.

Participants who were diagnosed with TB initially, but later discovered to have a non-TB diagnosis

16 Annex 4: Data for closed session

Part 1: Aggregate data presented with statistical comparisons (for determining safety)

- TB treatment initiation by study arm

Arm 1 (usual care)	Arm 2 (DCXR-CAD and FujiLAM intervention)	Arm 3 (diagnostic cohort)	Arm 1vs arm 2 95% CI, p-value	Arm 1 vs arm 3 95% CI, p-value

- Mortality by study arm

Arm 1 (usual care)	Arm 2 (DCXR-CAD and FujiLAM intervention)	Arm 3 (diagnostic cohort)	Arm 1vs arm 2 95% CI, p-value	Arm 1 vs arm 3 95% CI, p-value

- LTFU (withdrawl) by study arm

Arm 1 (usual care)	Arm 2 (DCXR-CAD and FujiLAM intervention)	Arm 3 (diagnostic cohort)	Arm 1vs arm 2 95% CI, p-value	Arm 1 vs arm 3 95% CI, p-value

Part 2: Within intervention arm comparisons of diagnostic performance

	CAD score below threshold	CAD score above threshold
All		
Sputum produced for Xpert (study team)		
Xpert positive (study Xpert)		
TB treatment initiation (n/N)		
% TB treatment initiations that are empiric (without microbiological confirmation)		

	AlereLAM positive	AlereLAM negative	
FujiLAM positive			
FujiLAM negative			
			N = Total with urine sample and valid LAM results

Those who are FujiLAM positive and AlereLAM negative (N=)		
Study sputum culture		
	Not done	
	Positive	
	Negative	
CAD score		
	Not done	
	Above threshold	
	Below threshold	
Study Xpert		
	Not done (NB. Not mandated if CAD score below threshold)	
	Positive	
	Negative	
TB treatment initiation		
	Yes	
	No	

Appendix D: CASTLE trial report CONSORT checklist

Table 1: CONSORT 2010 checklist of information to include when reporting a cluster randomised trial

Section/Topic	Item No	Standard Checklist item	Extension for cluster designs	Page No *
Title and abstract				
	1a	Identification as a randomised trial in the title	Identification as a cluster randomised trial in the title	Title page (p1)
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)[1,2]	See table 2	Abstract (p2)
Introduction				
Background and objectives	2a	Scientific background and explanation of rationale	Rationale for using a cluster design	p.4
	2b	Specific objectives or hypotheses	Whether objectives pertain to the cluster level, the individual participant level or both	p.3
Methods				
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Definition of cluster and description of how the design features apply to the clusters	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons		p.3-4
Participants	4a	Eligibility criteria for participants	Eligibility criteria for clusters	p.3 (clusters), p.3/4 (individuals)
	4b	Settings and locations where the data were collected		p.4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when	Whether interventions pertain to the cluster level, the individual participant level or both	p.4

		they were actually administered		
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Whether outcome measures pertain to the cluster level, the individual participant level or both	p.5
	6b	Any changes to trial outcomes after the trial commenced, with reasons		NA (no changes)
Sample size	7a	How sample size was determined	Method of calculation, number of clusters(s) (and whether equal or unequal cluster sizes are assumed), cluster size, a coefficient of intracluster correlation (ICC or *k*), and an indication of its uncertainty	p.5
	7b	When applicable, explanation of any interim analyses and stopping guidelines		NA
Randomisation:				
Sequence generation	8a	Method used to generate the random allocation sequence		p.4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Details of stratification or matching if used	p.4
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Specification that allocation was based on clusters rather than individuals and whether allocation concealment (if any) was at the cluster level, the individual participant level or both	p.4
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Replace by 10a, 10b and 10c	p.4

	10a		Who generated the random allocation sequence, who enrolled clusters, and who assigned clusters to interventions	p.4
	10b		Mechanism by which individual participants were included in clusters for the purposes of the trial (such as complete enumeration, random sampling)	p.4
	10c		From whom consent was sought (representatives of the cluster, or individual cluster members, or both), and whether consent was sought before or after randomisation	p.4
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how		p.4 (which states there was no blinding)
	11b	If relevant, description of the similarity of interventions		NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	How clustering was taken into account	p. 5
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses		p.5
Results				
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	For each group, the numbers of clusters that were randomly assigned, received intended treatment, and were analysed for the primary outcome	Fig 1, p.7
	13b	For each group, losses and exclusions after randomisation, together with reasons	For each group, losses and exclusions for both clusters and individual cluster members	Fig 1, p.7

Recruitment	14a	Dates defining the periods of recruitment and follow-up		p.6
	14b	Why the trial ended or was stopped		p.6
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Baseline characteristics for the individual and cluster levels as applicable for each group	p.8 Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	For each group, number of clusters included in each analysis	p. 8 (and figure 1 and table 2)
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Results at the individual or cluster level as applicable and a coefficient of intracluster correlation (ICC or k) for each primary outcome	p.8
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended		Table 2
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory		Table 2
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms[3])		NA
Discussion				
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses		p. 14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Generalisability to clusters and/or individual participants (as relevant)	Discussion in general (about

			high hospital mortality)
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	p. 12
Other information			
Registration	23	Registration number and name of trial registry	p.6
Protocol	24	Where the full trial protocol can be accessed, if available	p.6
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	p.6

** Note: page numbers optional depending on journal requirements*

Table 2: Extension of CONSORT for abstracts[1,2] to reports of cluster randomised trials

Item	Standard Checklist item	Extension for cluster trials
Title	Identification of study as randomised	**Identification of study as cluster randomised**
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)	
Methods		
Participants	Eligibility criteria for participants and the settings where the data were collected	**Eligibility criteria for clusters**
Interventions	Interventions intended for each group	
Objective	Specific objective or hypothesis	**Whether objective or hypothesis pertains to the cluster level, the individual participant level or both**
Outcome	Clearly defined primary outcome for this report	**Whether the primary outcome pertains to the cluster level, the individual participant level or both**
Randomization	How participants were allocated to interventions	**How clusters were allocated to interventions**
Blinding (masking)	Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment	
Results		
Numbers randomized	Number of participants randomized to each group	**Number of clusters randomized to each group**
Recruitment	Trial status[1]	
Numbers analysed	Number of participants analysed in each group	**Number of clusters analysed in each group**
Outcome	For the primary outcome, a result for each group and the estimated effect size and its precision	**Results at the cluster or individual participant level as applicable for each primary outcome**
Harms	Important adverse events or side effects	
Conclusions	General interpretation of the results	
Trial registration	Registration number and name of trial register	
Funding	Source of funding	

[1] Relevant to Conference Abstracts

REFERENCES

1 Hopewell S, Clarke M, Moher D, Wager E, Middleton P, Altman DG, et al. CONSORT for reporting randomised trials in journal and conference abstracts. *Lancet* 2008, 371:281-283

2 Hopewell S, Clarke M, Moher D, Wager E, Middleton P, Altman DG at al (2008) CONSORT for reporting randomized controlled trials in journal and conference abstracts: explanation and elaboration. *PLoS Med* 5(1): e20

3 Ioannidis JP, Evans SJ, Gotzsche PC, O'Neill RT, Altman DG, Schulz K, Moher D. Better reporting of harms in randomized trials: an extension of the CONSORT statement. *Ann Intern Med* 2004; 141(10):781-788.