An evidence-based algorithm for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to emergency centres

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Thesis Presented for the Degree of:

DOCTOR OF PHILOSOPHY (EMERGENCY MEDICINE) Division

of Emergency Medicine, University of Cape Town May 2020

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DECLARATION

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Doctor of Philosophy (Emergency Medicine) to the Faculty of Health Sciences, University of Cape Town. It has not been submitted before for any degree or examination at any other university.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publications:

a) Van Hoving DJ, Griesel R, Meintjes G, Takwoingi Y, Maartens G, Ochodo EA. Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals. Cochrane Database of Systematic Reviews 2019, Issue 9. Art. No.: CD012777. DOI: 10.1002/14651858.CD012777.pub2.

b) Van Hoving DJ, Lahri S, Lategan HJ, Nicol MP, Maartens G, Meintjes G. The real-world performance and inter-observer agreement of urine lipoarabinomannan in diagnosing HIV-associated tuberculosis in an emergency center. *JAIDS J Acquir Immune Defic Syndr* 2019; 81(1):e10–4. Doi: 10.1097/QAI.0000000000002002.

c) Van Hoving DJ, Kenge AP, Maartens G, Meintjes G. Point-of-care ultrasound predictors for the diagnosis of tuberculosis in HIV-positive patients presenting to an emergency center. *JAIDS J Acquir Immune Defic Syndr* 2020; 83(4):415-423. doi:10.1097/QAI.0000000000002279

d) Van Hoving DJ, Meintjes G, Maartens G, Kenge AP. A multi-parameter diagnostic clinical decision tree for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre. *Wellcome Open Res*. 2020; 5(4):72. doi:10.12688/wellcomeopenres.15824.1

Signed: Signed by candidate

Daniël J. van Hoving (VHVDAN005)

04 May 2020

ACKNOWLEDGEMENTS

All praise to God who gave me the power to see this through.

I thank my supervisors, Professor Graeme Meintjes, Professor Gary Maartens, and Professor Andre Kengne for their guidance, patience, support and detailed review of the manuscripts and thesis.

I thank Dr Sa'ad Lahri, Dr Hennie Lategan, the clinical staff and the patients at Khayelitsha Hospital where the study was conducted. I thank Rene Goliath (research clinical site coordinator), MK Mpalali and Lindiwe Matebane (research assistants) and the microbiology team from the Division of Medical Microbiology.

I thank Dr Rulan Griesel, Dr Yemisi Takwoingi and Dr Eleanor Ochodo for their assistance and guidance towards the Cochrane Systematic Review.

Lastly, I thank my wife, Sanet, for understanding the many sacrificed hours, and my children (Eben, Hesti, and Sanri) for their unconditional love.

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ABSTRACT

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Title: An evidence-based algorithm for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to emergency centres

Date: 04 May 2020

Background

Tuberculosis remains a prevalent and deadly global disease. Diagnostic delays are partly due to reduced diagnostic performance of tuberculosis tests in HIV-positive people. The use of reliable point-of-care and near-patient diagnostic tests (e.g. urine lipoarabinomannan and point-of-care ultrasound) are increasingly being used and would benefit patients presenting to emergency centres by rapidly diagnosing HIV-associated tuberculosis.

Methods

Two studies were done: i) A systematic (Cochrane) review was done to determine the diagnostic accuracy of abdominal ultrasound for detecting abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals, and ii) A cross-sectional diagnostic study to derive a multi-parameter clinical decision tree, incorporating clinical information, point-of-care ultrasound features, chest x-ray and urine lateral flow lipoarabinomannan.

The cross-sectional study was performed at the emergency centre of Khayelitsha Hospital, a South African district-level hospital in a high HIV-prevalence community, and resulted in three different publications. Consecutive HIV-positive adults presenting with ≥1 WHO tuberculosis symptoms were enrolled over a 16-month period (June 2016 to October 2017). Demographic and clinical information was recorded on a standardized data collection form. Point-of-care ultrasound was performed according to a standardized protocol. Urine lipoarabinomannan assays were done at point-of-care by emergency physicians and repeated in the laboratory. Chest x-rays were reviewed by a single radiologist using a standardized assessment form. The reference standard was a positive tuberculosis culture or Xpert MTB/RIF test on sputum, or appropriate extra-pulmonary samples.

We compared diagnostic accuracy and reproducibility of urine lipoarabinomannan between point-of-care readers and laboratory readers. We determined the diagnostic accuracy of individual point-of-care ultrasound features, performed an external validation of the focused assessment with sonography for HIV/TB (FASH) protocol, and determined independent point-of-care ultrasound predictors of HIV-associated tuberculosis. We derived the decision tree model from multivariable logistic regression models.

Results

Abdominal ultrasound had a pooled sensitivity of 63% (95%CI 43-79; 5 studies, 368 participants; very low-certainty evidence) and a pooled specificity of 68% (95%CI 42-87; 5 studies, 511 participants; very low-certainty evidence) for bacteriologically confirmed tuberculosis.

We screened 556 patients in the cross-sectional study of whom 414 (74.5%) were enrolled. The prevalence of microbiologically confirmed tuberculosis was 41.5% (n=172).

Point-of-care and laboratory-performed urine lipoarabinomannan had similar sensitivity (41.8% vs 42.0%, P=1.0) and specificity (90.5% vs 87.5%, P=0.23). Moderate agreement was found between

point-of-care and laboratory testing (k=0.62), but there was strong agreement between point-of-care readers (k=0.95) and between laboratory readers (k=0.94).

Sensitivity and specificity of ≥1 individual point-of-care ultrasound feature were 73% (95%CI 65-79) and 54% (95%CI 47-60), and of the FASH protocol 71% (95%CI 64-78) and 57% (95%CI 50-63). Independent point-of-care ultrasound predictors identified were intra-abdominal lymphadenopathy of any size (aDOR 3.7; 95%CI 2.0-6.7), ascites (aDOR 3.0; 95%CI 1.5-5.7), and pericardial effusion of any size (aDOR 1.9; 95%CI 1.2-3.0). Two or more independent point-of-care ultrasound predictors had 33% (95%CI 27–41) sensitivity and 91% (95%CI 86-94) specificity.

The best performing model included WHO screening symptoms ≥2, antiretroviral therapy use, urinary lipoarabinomannan, independently predictive point-of-care ultrasound features (ascites, any size pericardial effusion, any size intra-abdominal lymphadenopathy), and chest x-ray (c-statistic 0.82; 95%CI 0.78–0.86). Adding CD4 cell count did not improve the performance of the model. Classification And Regression Tree (CART) analysis positioned urinary lipoarabinomannan as the optimal screening test after WHO symptoms (75% true positive rate, representing 17% of participants).

Conclusion

An evidence-based algorithm for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre was developed. Urinary lipoarabinomannan can be reliably performed at the point-of-care since there was no diagnostic accuracy advantage in laboratory-performed versus point-of-care–performed tests. The role of ultrasound in diagnosing HIV-associated tuberculosis had limitations. The low sensitivity of ultrasound (63% in the systematic review; 73% in the cross-sectional study) and the moderate discrimination (specificity 91%) of the presence of ≥2 independent point-of-care ultrasound predictors indicate that point-of-care ultrasound results should be interpreted in combination with other diagnostic information. The derived decision tree can facilitate the immediate initiation of anti-tuberculosis treatment in about a quarter of patients among whom 75% would have a definitive diagnosis of tuberculosis regardless of CD4 cell count. The 30% false negative rate indicates that the algorithm should not be used to exclude tuberculosis. The performance of the decision tree needs to be further evaluated in settings with a different prevalence of HIV-associated tuberculosis.

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CHAPTER 1

Introduction

Context

Tuberculosis remains a globally prevalent and deadly communicable disease particularly in socio-economically deprived regions of the world.[1] An estimated 10 million people developed tuberculosis during 2018, of which 24% occurred in the World Health Organization (WHO) Africa region and 3% in South Africa.[1]

Co-infection with the Human Immunodeficiency Virus (HIV) worsens the burden significantly. The HIV prevalence among incident tuberculosis cases in the African region and South Africa was 25% and 59%, respectively.[1] South Africa has one of the highest HIV-associated tuberculosis mortality rates at 73 per 100 000 population.[1] The high mortality risk is better understood if one consider that the mortality rate in high tuberculosis burden countries is 4.2 per 100 000 people and globally only 3.3 per 100 000 population.[1]

It is estimated that only 70% of the estimated 10 million incident tuberculosis cases were reported in 2018.[1] The gap in the detection rate could be attributed to either underreporting of confirmed cases or underdiagnoses of tuberculosis cases. Delays in tuberculosis diagnosis relate to the ignorance of health care personnel, the intrinsic properties of tuberculosis diagnostic tests (suboptimal sensitivity), or problems with the actual availability of these tests.[2–5] Co-infection with HIV further increases the diagnostic challenge as HIV-positive patients have more atypical clinical presentations; other opportunistic pulmonary infections with similar presentations; higher rates of smear-negative pulmonary tuberculosis; and higher rates of extrapulmonary tuberculosis.[6–11] HIV infection also alters the performance of diagnostic tests.[12] HIV-positive patients with low CD4 counts often have multi-organ involvement (disseminated tuberculosis) and tuberculosis bacteraemia; putting them at high risk of rapid clinical deterioration and death. [13,14] Any diagnostic delay intuitively results in a delay in initiating therapy, which results in high mortality rate.[13]

Hospital emergency centres are often used as a primary entry point for patients into the health care system. The prevalence of HIV-related cases in South African emergency centres ranges between 25% and 50%,[15,16] while the tuberculosis prevalence is around 15%.[16–18] Patients presenting to emergency centres are often severely ill and prompt management is needed to decrease morbidity and mortality. Rapid diagnosis of HIV-associated tuberculosis can expedite the initiation of anti-tuberculosis treatment. Not only has this the potential to reduce complications, but this is likely to decrease mortality as well.

Rapid diagnosis of HIV-associated tuberculosis in the emergency centre will only be accomplished if point-of-care testing can be successfully implemented. Currently, point-of-care test options are limited. The detection of lipoarabinomannan (LAM) in urine has the potential to be used as a point-of-care test. A lateral flow LAM assay (LF-LAM) strip-test for urine is commercially available,[19] with the

added bonus of having greater diagnostic sensitivity in HIV-positive people than in HIV-negative people.[20] Point-of-care ultrasound can also aid the diagnoses of HIV-associated extrapulmonary tuberculosis; although the predictive value for active tuberculosis varies.[21–23]

No standalone test is sufficiently accurate for diagnosing HIV-associated tuberculosis. An internationally accepted evidence-based algorithm incorporating urinary LAM and point-of-care ultrasound, that can be utilised even in settings where expertise is limited, would be of clinical utility.[24,25]

The studies reported in this thesis aimed to determine the diagnostic accuracy of abdominal ultrasound for detecting HIV-associated disseminated tuberculosis with abdominal involvement, evaluate the diagnostic accuracy of urinary lipoarabinomannan (LF-LAM) in HIV-positive patients presenting to the emergency centre, determine independent point-of-care ultrasound predictors of HIV-associated tuberculosis, and develop a multi-parameter clinical decision tree to rapidly diagnose tuberculosis in HIV-positive patients presenting to the emergency centre.

Setting

The cross-sectional diagnostic study was performed at the emergency centre of Khayelitsha Hospital in Cape Town in the Western Cape Province of South Africa. Khayelitsha Hospital is a 300-bed district level hospital serving a health sub-district with a population of ±500 000, which is predominantly Black African (99%) with high levels of unemployment (38%).[26,27] There is a tremendous burden of disease related to HIV, tuberculosis and interpersonal violence.[15,27] The antenatal HIV prevalence in Khayelitsha is ±34% and the tuberculosis case notification rate ±917/100 000.[28,29] There are seven clinics and one 24-hour community health centre in the hospital's referral area. Khayelitsha Hospital provides inpatient services such as surgical, medical, paediatric and obstetrics. The emergency centre is 30% larger than that of a standard district hospital trauma unit,[26,30] and has a five-bed (including one paediatric cot) dedicated resuscitation area. The emergency centre manages about 30 000 patients per annum and has a 30% admission rate. The HIV-prevalence of patients managed within the resuscitation area is 23%.[15]

Outline of thesis

In **Chapter 2** (Background and literature review) the burden of tuberculosis, diagnostic challenges, and diagnostic options are reviewed. The diagnostic opportunity within the emergency centre for the rapid diagnosis of HIV-associated tuberculosis is also discussed.

Chapter 3 is an extension of the literature review and focuses on the use of abdominal ultrasound in diagnosing abdominal tuberculosis in HIV-positive adults. This was undertaken as a formal Cochrane systematic review and has been published in the *Cochrane Database of Systematic Reviews*.

Chapter 4 describes the real-world performance and inter-observer agreement of urinary lipoarabinomannan (LAM) in HIV-positive patients presenting to the emergency centre. It concludes that urinary LAM is truly a point-of-care test as there was a similar diagnostic performance of point-of-care testing in a real-world emergency care setting compared to laboratory-based readings. It is presented in the form of a manuscript published in *Journal of Acquired Immune Deficiency Syndromes (JAIDS)*.

Point-of-care ultrasound is covered in **Chapter 5**. The study firstly addresses the diagnostic accuracy of individual point-of-care ultrasound features, then externally validated the Focused Abdominal Sonography for HIV/TB (FASH) protocols, before determining independent point-of-care ultrasound predictors of HIV-associated tuberculosis appropriate for use by practitioners in emergency centres. It is presented in the form of a manuscript published in *Journal of Acquired Immune Deficiency Syndromes (JAIDS)*.

The development of a multi-parameter clinical decision tree to rapidly diagnose tuberculosis in HIV-positive patients is presented in **Chapter 6**. It incorporates clinical information, individual point-of-care ultrasound features, and point-of-care performed urinary LAM. It is presented in the form of a manuscript published in *Wellcome Open Research*.

Chapter 7 concludes the thesis' findings in a single discussion. Implications of the research for the field in general are discussed and priorities for future research are identified.

Coherence of the thesis

The coherence of this thesis is underpinned by a central aim (conducting studies that will facilitate the rapid diagnosis of HIV-associated TB in the emergency centre) which was broken down into three distinct objectives (that underpin each of the three studies described in Chapters 4, 5, and 6) . I (Daniël van Hoving) was the first author on all four of the papers included and was the lead investigator on all the studies. All these studies have been undertaken under the joint supervision of Professors Graeme Meintjes, Gary Maartens and Andre Kengne (my PhD supervisors). Dr Eleanor Ochodo was the lead supervisor on the Cochrane systematic review, due to her experience in the specialised field of Diagnostic Test Accuracy reviews. The publications all report on evaluation of diagnostic approaches for the same clinical condition (HIV-associated tuberculosis) and thus contribute to a thematically coherent body of work.

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CHAPTER 2

Background and literature review

Tuberculosis has been described as one of the most lethal diseases in human history.[1] Although it usually affects the lungs (pulmonary tuberculosis), it can also spread to other body sites (extrapulmonary tuberculosis).[2] The causative agent, *Mycobacterium tuberculosis*, was isolated in 1882 by German physician, Robert Koch; [1] yet more than a century later, tuberculosis is still a prevalent and deadly disease.

2.1. Burden of disease

2.1.1. High tuberculosis burden countries

In 1998, the World Health Organization (WHO) compiled a list of high burden countries, in order to demonstrate the scale of the tuberculosis epidemic. Twenty-two countries with the highest absolute number of tuberculosis cases (responsible for 80% of the global total) were identified.[3] Three new lists (tuberculosis, human immunodeficiency virus (HIV)-associated tuberculosis, and multidrug resistant tuberculosis) have been defined for the period 2016–2020 and now contain 30 high tuberculosis burden countries (Table 1). These countries are defined as the top 20 in terms of absolute number of tuberculosis cases (minimum threshold of 10 000 cases per annum) plus an additional 10 countries (that do not already appear in the top 20) with the highest case rates per capita.[2] The 30 high tuberculosis burden countries accounted for 87% of all estimated incident tuberculosis cases worldwide in 2018.[2] Fourteen countries (including South Africa) appear on all three high burden lists (Table 1).[2]

Table 1 High-burden country lists for tuberculosis, HIV-associated tuberculosis and multidrug resistant tuberculosis for the period 2016–2020 (in alphabetical order).[2]

Top 20 countries by estimated absolute number of incident cases		
Tuberculosis [a]	**HIV-associated tuberculosis** [b]	**Multidrug resistant tuberculosis** [b]
Angola	Angola	Bangladesh
Bangladesh	Brazil	China
Brazil	Cameroon	Democratic People's Republic of Korea
China	China	Democratic Republic of the Congo
Democratic People's Republic of Korea	Democratic Republic of the Congo	Ethiopia
Democratic Republic of the Congo	Ethiopia	India
Ethiopia	India	Indonesia
India	Indonesia	Kazakhstan
Indonesia	Kenya	Kenya
Kenya	Lesotho	Mozambique
Mozambique	Malawi	Myanmar
Myanmar	Mozambique	Nigeria
Nigeria	Myanmar	Pakistan
Pakistan	Nigeria	Philippines
Philippines	South Africa	Russian Federation
Russian Federation	Thailand	South Africa
South Africa	Uganda	Thailand
Thailand	United Republic of Tanzania	Ukraine
United Republic of Tanzania	Zambia	Uzbekistan
Viet Nam	Zimbabwe	Viet Nam
Additional 10 countries by estimated incidence rate per 100 000 population (not in top 20)		
Tuberculosis [c]	**HIV-associated tuberculosis** [d]	**Multidrug resistant tuberculosis** [d]
Cambodia	Botswana	Angola
Central African Republic	Central African Republic	Azerbaijan
Congo	Chad	Belarus
Lesotho	Congo	Kyrgyzstan
Liberia	Ghana	Papua New Guinea
Namibia	Guinea-Bissau	Peru
Papua New Guinea	Liberia	Republic of Moldova
Sierra Leone	Namibia	Somalia
Zambia	Papua New Guinea	Tajikistan
Zimbabwe	Swaziland	Zimbabwe

HIV: human immunodeficiency virus

[a] Threshold, >10 000 estimated incident tuberculosis cases per year

[b] Threshold, >1 000 estimated incident cases per year

[c] Minimum number of 10 000 cases per year

[d] Minimum number of 1 000 cases per year

2.1.2. Tuberculosis incidence

The WHO estimated that 10.0 million people (range, 9.0 to 11.1 million) developed tuberculosis during 2018, affecting mainly adults (89%) and males (57%).[2] This is equivalent to 130 cases (range, 118–146) per 100 000 population. The WHO African Region (24%) had the second highest number of estimated cases in 2018, after the WHO South-East Asia Region (44%) and ahead of the WHO Western Pacific Region (18%).[2] South Africa has been labelled as a high tuberculosis burden country since 1998 and has retained that status since.[2,3] The tuberculosis incidence in South Africa in 2018 was estimated to

be 301 000 people, contributing 3% to the global total.[2] South Africa had the sixth highest tuberculosis incidence rate in the world with an estimated 520 cases per 100 000 population (global rate 132 per 100 000 population).[2] Lesotho had the highest estimated tuberculosis incidence rate in 2018 with 611 cases per 100 000 population.[2]

The probability of developing tuberculosis is substantially higher among people living with HIV (PLHIV). Approximately 860 000 (range, 776 000 to 952 000) (8.6%) people diagnosed worldwide with tuberculosis in 2018 were HIV-positive, of these 615 000 (71.5%) were from the WHO African region.[2] The HIV prevalence in incident tuberculosis cases in the African region (25%) was considerably higher than other regions, the closest being Europe (12%) and the Americas (10%).[2] Some African countries reported a HIV-coinfection rate surpassing 50%; reflecting a very high relative risk (19 times) of developing tuberculosis in HIV-positive people compared to the rest of the population.[2] This high burden of TB/HIV co-infection is reflected in South Africa where 59% of tuberculosis cases were HIV infected; the third highest prevalence in the world.[2]

2.1.3. Tuberculosis mortality

Tuberculosis is the leading cause of death from a single infectious agent and the tenth leading cause of death in the world with an estimated 1.2 million deaths (range 1.1 to 1.3 million) in HIV-negative people in 2018.[2]. Additionally, there were about 251 000 deaths (range 223 000 to 281 000) in HIV-positive people.[2] Most of the deaths in HIV-negative individuals occurred in the WHO South-East Asia (51%) and African (32%) regions, whereas deaths in HIV-positive patients occurred predominantly in the WHO Africa region (84%).[2] South Africa registered 63 000 tuberculosis deaths (HIV-negative 21 000, HIV-positive 42 000) in 2018; 4.2% of the global total.[2]

The exact number of tuberculosis deaths among HIV-positive people is difficult to measure since deaths in HIV-positive people are often coded as HIV deaths in the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10), with the contributory cause often not recorded at all.[2,4] Additionally, the diagnosis of tuberculosis might not have been ascertained as HIV co-infection often result in atypical presentations.[5] Autopsy studies show that a high percentage (46%) of adult tuberculosis cases remained undiagnosed pre-mortem.[6]

The WHO African region had the highest HIV-positive tuberculosis mortality rate (20 versus 3.3 globally) per 100 000 people, while South Africa is the country with the third highest rate per 100 000 population (Lesotho 155; Zambia 74; South Africa 73; globally 3.3).[2]

2.2. Global commitments to reduce the tuberculosis burden

Global efforts to reduce the burden of tuberculosis are ongoing, mainly under the lead of the United Nations (UN). The Millennium Development Goals included targets for 2015 to decrease the incidence of tuberculosis (Millennium Development Goal 6c, 'to halt and reverse the incidence of malaria and other major diseases').[7] This target was met as the tuberculosis incidence rate has fallen at an average rate of 1.5% per year since 2000.[7] The other two goals were almost met; the tuberculosis mortality rate decreased by 47% (target: 50% reduction) and the tuberculosis prevalence rate was reduced by 42% (target: 50% reduction).[7] The Sustainable Development Goals followed the Millennium Development Goals in 2016 with new goals, targets and indicators for the period 2016–2030.[8] The main target relating to tuberculosis (Target 3.3 of Sustainable Development Goal 3) are to end the tuberculosis epidemic by 2030.[8]

The WHO subsequently developed and endorsed a new global tuberculosis strategy, the End TB Strategy, for the period 2016–2035.[9] The ambitious goal of the strategy is to end the global tuberculosis endemic, and three indicators with related targets and milestones have been set (Table 2).[2,9] These milestones can only be reached if progress is simultaneously made to improve universal health coverage (Target 3.8 of Sustainable Development Goal 3) and to address broader social and economic determinants influencing the tuberculosis endemic.[2]

Table 2 Indicators, milestones and targets of the End TB Strategy (adapted from the WHO's Global Tuberculosis Report 2019)[2]

Indicators	Milestones		Targets	
	2020	2025	2030[a]	2035
Percentage reduction in the absolute number of tuberculosis deaths (compared with 2015 baseline)	35%	75%	90%	95%
Percentage reduction in the tuberculosis incidence rate (compared with 2015 baseline)	20%	50%	80%	90%
Percentage of tuberculosis-affected households experiencing catastrophic costs due to tuberculosis (level in 2015 unknown)	0%	0%	0%	0%

[a] Linked to the Sustainable Development Goals

The End TB Strategy identified three pillars to accomplish the set targets and milestones presented in Table 2.[2] The three pillars are integrated, patient-centred tuberculosis care and prevention; bold policies and supportive systems; and intensified research and innovation.[2] The ten components of the three pillars are presented in Table 3; of which the early diagnosis of tuberculosis is arguably the most vital component on a clinical level.[9]

Table 3 Pillars and their components of the End TB Strategy[2,9]

Pillar	Component
Integrated, patient-centred care and prevention	Early diagnosis of tuberculosis including universal drug-susceptibility testing, and systematic screening of contacts and high-risk groups
	Treatment of all people with tuberculosis including drug-resistant tuberculosis, and patient support
	Collaborative TB/HIV activities, and management of comorbidities
	Preventive treatment of persons at high risk, and vaccination against tuberculosis
Bold policies and supportive systems	Political commitment with adequate resources for tuberculosis care and prevention
	Engagement of communities, civil society organizations, and public and private care providers
	Universal health coverage policy, and regulatory frameworks for case notification, vital registration, quality and rational use of medicines, and infection control
	Social protection, poverty alleviation and actions on other determinants of tuberculosis
Intensified research and innovation	Discovery, development and rapid uptake of new tools, interventions and strategies
	Research to optimize implementation and impact, and promote innovations

TB: tuberculosis; HIV: human immunodeficiency virus

The global tuberculosis reduction strategy will only be successful if implemented on a national level by each country's government. In 2017, a global ministerial conference on tuberculosis was held in Moscow due to the projections that the Sustainable Development Goals and End TB Strategy targets and milestones might not be reached.[10] This was taken further with commitments by all Member States at the World Health Assembly to accelerate actions to rid the world of tuberculosis.[11] A lot of work has since been done by the WHO including the development of a multi-sectoral accountability framework for tuberculosis, to ensure faster progress towards the set targets and milestones to end the tuberculosis endemic.[2]

2.3. Trends in tuberculosis incidence and mortality

The incidence of tuberculosis in HIV-positive and HIV-negative patients has slowly been declining globally. The rate of decline between 2017 and 2018 was 2.0%; this annual decline needs to increase to 4-5% in order to meet the 20% reduction by 2020 (compared to 2015 baseline) as set by the End TB Strategy (Table 2).[2] A promising trend has been observed with remarkable reductions (4-8% annually) reported in southern Africa (including South Africa). This reduction is likely due to the wider coverage

of antiretroviral therapy (ART).[2] The cumulative reduction from 2015 till 2018 were 12% for the WHO Africa region and are on course to achieve the 2020 milestone.[2]

The global decline in tuberculosis mortality needs to improve. The 11% decline in the total number of tuberculosis deaths since 2015 needs to accelerate to reach the 2020 End TB Strategy milestone of a 35% reduction.[2] The proportion of people with tuberculosis who die from the disease (case fatality ratio (CFR)) needs to decrease to 10% by 2020 (Table 3). The global CFR in 2018 was 15%, but varied substantially between countries. Noticeably, 12 countries among the 30 high tuberculosis burden countries (including South Africa) is on track to reach the 2020 milestone.[2]

The global treatment success rate for tuberculosis in 2017 was 85%, and it is estimated that 48 million deaths were prevented by tuberculosis treatment between 2000 and 2018 in HIV-negative people.[2] In HIV-positive people, the combination of ART and tuberculosis treatment potentially averted 10 million deaths between 2000 and 2018, with a treatment success rate of 75% in 2017.[2]

The early diagnosis and successful treatment (including ART provided alongside tuberculosis treatment for HIV-positive patients) of people with tuberculosis should prevent the majority of tuberculosis - related deaths. Despite the ongoing development of new diagnostic tests, drugs, treatment regimens and vaccines, there are still large and persistent gaps in both the detection and treatment of tuberculosis.[2]

2.4. HIV-associated tuberculosis

A significant interaction between HIV and *M. tuberculosis* exists. HIV co-infection is the single greatest risk factor for developing tuberculosis, and tuberculosis may also cause HIV disease progression by increasing viral replication.[12]

2.4.1. Incidence

PLHIV are at increased risk of active tuberculosis due to a more rapid progression from infection to disease and to the reactivation of latent tuberculosis.[13] In early HIV infection, the risk of tuberculosis increases by 2 to 5-fold and in advanced HIV disease by more than 20-fold.[12] The risk remains increased (around 4-fold) even in patients treated with long term ART.[12] HIV is also a risk factor for multidrug resistant tuberculosis (estimated pooled odds ratio (OR) 1.24, 95% Confidence Interval (CI) 1.04–1.43),[14] extrapulmonary tuberculosis (OR ranging between 1.03 and 16.8),[15] and disseminated tuberculosis.[16]

2.4.2. Extrapulmonary tuberculosis

Extrapulmonary tuberculosis represent about 15% of incident tuberculosis cases,[2] with a clear association with HIV infection.[15] The spread of tuberculosis from the lungs to extrapulmonary sites has

been linked to cell-mediated immunosuppression.[17] Mycobacteria enter the lungs through airborne droplets, after which most bacteria are trapped in alveolar macrophages. T-cell lymphocytes are then activated to generate granulomas around the mycobacteria in order to control the infection. T-cell depletion in HIV infection results in failure to control the infection and dissemination occurs frequently. The more advanced the immunosuppression, the more likely dissemination occurs.

The spread of tuberculosis may be a result of progressive primary infection or from reactivation of a latent focus with subsequent lymphohematogenous spread. Tuberculosis bacilli can spread via the lymphatic system or the blood stream from a primary pulmonary focus of infection. The most vascular organs, such as the liver, spleen, bone marrow and brain, are preferred sites to spread to. These distant foci heal by granulomatous containment, although viable bacilli may still endure in individual lesions. Progressive primary disease at extrapulmonary sites occurs when the foci fails to heal, especially in patients with impaired cell-mediated immunity.

Reactivation of a latent focus may occur at any time after the primary infection and is often related to the waning of specific immune responses. Viable bacilli are disseminated after erosion of the reactivated focus of infection into adjacent lymphatic or blood vessels.

Tuberculosis can spread to any organ, but the most frequent extrapulmonary sites are lymph nodes, the pleura, the central nervous system and bones or joints.[18] In HIV-positive individuals, the pericardium and abdominal organs are also frequently involved.[19]

2.4.3. Disseminated tuberculosis

A distinction should be made between anatomically compartmentalised extrapulmonary tuberculosis and the disseminated disease often seen with advanced HIV infection.[20] Disseminated tuberculosis involves two or more non-contiguous sites and is caused by ongoing lymphohematogenous dissemination;[21,22] resulting in a high prevalence of *M tuberculosis* blood culture positivity in HIV-associated tuberculosis.[23,24]

Disseminated tuberculosis occurs frequently in hospitalised HIV-infected patients,[20,25,26] and is associated with increased morbidity and mortality.[20] A high proportion of these patients require re-hospitalisation within 3 months, and they have a more than 2-fold increased risk of death at 90 days compared to HIV-positive patients with non-disseminated tuberculosis.[20]

The diagnosis of HIV-associated disseminated tuberculosis remains challenging. The reference-standard diagnostic test are mycobacterial blood culture, although urine diagnostics (e.g. lipoarabinomannan assay and Xpert MTB/RIF) have become rapid surrogates.[20]

2.4.4. Clinical manifestations

The clinical disease patterns of tuberculosis is affected by the degree of immunodeficiency.[12] PLHIV with CD4 cell counts above 200 cells/mm^3 present with classical tuberculosis symptoms,[12] however, almost a quarter of HIV-associated tuberculosis in adults can be subclinical (the asymptomatic period during which viable *M. tuberculosis* can be detected in sputum).[27] Adults with HIV-associated tuberculosis often have non-specific clinical presentations, cough less frequently, and more frequently present with extrapulmonary and disseminated disease in the absence of respiratory symptoms and radiological abnormalities.[28] The risk of opportunistic infections (respiratory and non-respiratory) are also increased in HIV-positive patients with tuberculosis.[29]

2.4.5. Mortality

The mortality rate for HIV-associated tuberculosis is high. Almost half (52%) of critically ill HIV-positive patients co-infected with tuberculosis dies within six months.[30] The mortality is strongly associated with the degree of immunosuppression (hazard ratio 4.6, 95% CI 1.6–12.7) with CD4 cell count <50 cells/mm^3),[30] while the initiation of ART greatly reduces the mortality rate.[31]

The high mortality rate can potentially be explained in two ways. Firstly, tuberculosis may be an independent risk factor for progression to death in PLHIV.[32] Secondly, the high mortality reflects the diagnostic challenge of HIV-associated tuberculosis, with post-mortem studies indicating that 46% (95% CI 33–59) of tuberculosis cases remained undiagnosed and therefore untreated at death in PLHIV.[6]

2.5. Diagnostic challenges

A mixture of underreporting of detected cases and underdiagnosis of tuberculosis led to a worldwide gap between the estimated number of incident cases (10 million) and the number of new cases reported (7 million) in 2018.[2]

Delays in tuberculosis diagnosis could relate to the intrinsic properties of tuberculosis diagnostic tests (sensitivity, specificity, etc.), problems with the actual availability of these tests, and the reduced diagnostic performance of tuberculosis tests in HIV co-infection.[33–35] Any diagnostic delay will result in a delay in initiating therapy, which is associated with higher mortality.[36]

The contribution of tuberculosis to HIV deaths is clearly illustrated by autopsy studies, which indicate a very high proportion of tuberculosis in HIV-positive adults (32% to 47%); almost half (46%) of adult tuberculosis cases remained undiagnosed pre-mortem.[6]

In PLHIV, the diagnostic approach to active tuberculosis is similar to that in HIV-negative people. No evidence exists to indicate that the proper initial evaluation of cough in immunocompromised patients is different from that in immunocompetent persons. Tuberculosis should be part of the initial

evaluation of patients who reside in regions with a high prevalence of tuberculosis.[37] On the other hand, in people living with advance immunosuppression, the diagnosis of active tuberculosis depends greatly on clinical suspicion by the physician as the diagnosis is complicated due to more atypical clinical presentations; other opportunistic pulmonary infections with similar presentations; high proportion negative sputum smears;[5] high proportion of inpatients unable to produce sputum (sputum scarce);[38] and high rates of extrapulmonary and disseminated tuberculosis.[39–43] Invasive diagnostic procedures are therefore more often required to establish the diagnosis to avoid under diagnosis and subsequent under treatment of tuberculosis. [44]

2.6. Diagnostic options

The early and accurate diagnosis of tuberculosis has clear advantages. It should reduce the risk of morbidity and mortality, the related socio-economic consequences, and decrease the transmission of tuberculosis.[45]

Typical tuberculosis symptoms might not always be present early in the disease or in immunocompromised people. Therefore, infected people might not seek health care early, or are misdiagnosed when they do attend healthcare facilities. The WHO has subsequently developed guidelines for active case finding to complement passive case finding.[45]

2.6.1. Screening to test for active tuberculosis

Screening is typically defined as testing asymptomatic or apparently healthy people to identify those with a particular disease or at increased risk of a particular disease.[46] The WHO has made recommendations on prioritizing risk groups that should be systematically screened for active tuberculosis (Table 4).[45] All the recommendations were made with low to very low quality of evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria.[47] This review will further only focus briefly on passive screening when symptomatic people present to healthcare facilities.

Table 4 WHO recommendations on prioritizing risk groups for systematically screening of active tuberculosis[45]

Strong recommendations [a]	**Conditional recommendations** [b]
Close contacts (e.g. household)	People in prisons and other penitentiary institutions
HIV-positive patients visiting a healthcare facility	People with an untreated fibrotic chest X-ray lesion
Silica exposed workers (former and current)	People seeking health care and who belong to selected risk groups[c] in settings with tuberculosis prevalence ≥ 0.1% in general population
	Geographically defined subpopulations with extremely high levels of undetected tuberculosis (1% prevalence ≥ 1%)
	Subpopulations that have very poor access to health care

HIV: human immunodeficiency virus

[a] Desirable effects clearly outweigh undesirable effects, and screening judged to be feasible, acceptable and affordable in all settings

[b] Desirable effects of probably outweigh the undesirable effects, but cost effectiveness, feasibility and affordability are uncertain

[c] Risk groups include: Underweight (body mass index <18.5), Gastrectomy or jejunoileal bypass, Diabetes mellitus, Alcohol dependence, Tobacco smoking, Chronic renal failure or haemodialysis, Intravenous drug use, Solid organ transplantation, Old age, Previously treated tuberculosis, Pregnancy.

Symptom-based screen

HIV-positive people attending a healthcare facility should be initially screened by assessing the presence of any one of four clinical symptoms (current cough, fever, weight loss or night sweats).[48] Patients that are symptom free should be offered isoniazid preventive therapy, while patients reporting any one of the symptoms should be further evaluated for active tuberculosis.[48]

The diagnostic performance of the WHO four-symptom screening rule varied significantly with sensitivity ranging between 40% and 96%, and specificity between 5% and 88%.[49–55] The screening rule also perform worse in HIV-positive patients on ART (pooled sensitivity 51%, 95% CI 28-73) than those not on ART (pooled sensitivity 89%, 95% CI 83-94).[56] The pooled specificity decreased from 71% (95% CI 48–86) in people on ART compared to 28% (95% CI 19-40) in ART-naïve people.[56]

C-reactive protein (CRP)

C-reactive protein (CRP) is an acute-phase biomarker, the concentration of which rise in patients with infections such as active tuberculosis. A point-of-care test for CRP is commercially available which is simple and inexpensive. The pooled sensitivity of CRP is 93% (95% CI 88-98), and pooled specificity 60% (95% CI 46-75) using a cut-off point of 10 mg/l in culture positive pulmonary tuberculosis.[57] For screening purposes, sensitivity estimates ranged from 81% to 85% and specificity estimates from 58% to 81%.[57] The estimates of diagnostic accuracy did not differ much by HIV status.[57]

Chest X-ray screening

Chest X-rays have long been used in patients suspected to have tuberculosis, despite being limited by high inter-observer and intra-observer variability in both the pre- and post-HIV era.[58–60] Not only is the diagnostic performance influenced by the reader's expertise, there is no pattern definitively diagnostic of tuberculosis. Radiographic patterns further differ between HIV-negative and HIV-positive patients, and also relate to the degree of immunosuppression.[38,61–64] The diagnostic performance of chest X-ray in HIV-positive patients systematically screened for pulmonary tuberculosis against sputum culture was limited (sensitivity 68%, 95% CI 54–79), specificity 53% (95% CI 45–61), and negative predictive value 83% (95% CI 74–89).[33]

Computer-aided reading of chest X-rays may offer solutions to limited human resources for reading X-rays.[65] Image interpretation is based on machine-learning methods and the computer software analysing X-ray image characteristics. A 2016 systematic review indicated that computer-aided detection software has a moderate to high sensitivity (>85%) that were associated with moderate to poor specificity (23% to 69%).[66] In HIV-associated tuberculosis, a computer-aided algorithm had better accuracy than clinicians (79% versus 65%, $p < 0.001$) and may improve clinician accuracy in the diagnosis of tuberculosis on chest X-ray.[67]

Combined screening: Symptom screen, chest X-ray screen and CD4 cell count

The best performing screening tool for active tuberculosis includes a combination of symptoms, chest X-ray and CD4 cell count stratification. A study on South African gold miners evaluated different screening strategies to exclude tuberculosis;[68] sensitivity increased at the expense of specificity, while the negative predictive value remained similar (Table 5).

Table 5 Diagnostic performance of screening combinations for active tuberculosis (Adapted from Day et al.[68])

Screening criteria	Sensitivity	Specificity	Negative predictive value	People unnecessarily investigated further, N=855 (n)	Tuberculosis cases missed, N=44 (n)
Symptoms					
Night sweats, cough or reported weight loss	59%	76%	97%	209	18
Symptoms + Signs					
Night sweats, cough or measured weight loss	75%	67%	98%	282	11
Symptoms + Signs + Chest X-ray					
Night sweats, cough, measured weight loss or Chest X-ray features compatible with tuberculosis	91%	59%	99%	349	4
Night sweats, cough, measured weight loss or Any chest X-ray abnormality	91%	55%	99%	386	4
Symptoms + Signs + Chest X-ray + CD4 cell count					
Night sweats, cough, measured weight loss, Chest X-ray compatible with tuberculosis or CD4 <200/mm^3	95%	51%	100%	381	2
Night sweats, cough, measured weight loss, Any chest X-ray abnormality or CD4 <200/mm^3	95%	47%	100%	409	2

2.6.2. Laboratory-based diagnosis

Sputum smear microscopy

The first microscopic identification of the tuberculosis bacillus was described by Robert Koch in 1882.[69] Microscopy is still used as the main tuberculosis diagnostic in many places in the world,[70] despite a relative low sensitivity (ranging between 50% and 60%).[71] The sensitivity can be improved with sputum processing methods and the use of fluorescence microscopy.[35,71] The use of light-emitting diode (LED) fluorescence microscopy, has made it more cost-effective.[72]

Smear microscopy is simple and can be performed in basic laboratories. It is a low cost, quick diagnostic test that has high specificity in areas with a high tuberculosis prevalence.[73] The quantitative threshold of smear microscopy (> 5000 bacilli per ml needed to be detected),[74,75] suggests that the most infectious people are detected, but also that a substantial number of tuberculosis cases will be missed.

The shortcomings of smear microscopy is the need for specialized training, its relatively low sensitivity, and that drug susceptibility testing cannot be performed. Microscopy can also not differentiate tuberculous mycobacteria from non-tuberculous mycobacteria or viable from non-viable bacteria.[76] Its performance (especially sensitivity) is also reduced in children, patients with extrapulmonary or disseminated tuberculosis and in HIV-positive individuals.[76] Furthermore, negative and paucibacillary smears are more frequent in HIV-positive people,[41,77] and an increased mortality has been noted in this cohort, most likely resulting from a delay in diagnosis.[78]

The WHO recommended in 2018 that rapid tuberculosis diagnostic tests with the ability to test drug susceptibility should replace microscopy as the initial diagnostic test for tuberculosis.[76]

Culture

Culture is the most accurate option of currently available diagnostic tests with sensitivity of up to 98%.[79] Culture also offer species identification and drug sensitivity and is hence regarded as the ultimate reference standard. However, the proportion of positive cultures varies between clinical specimen types (Table 6).

Table 6 Proportion of cultures positive for detection of M. tuberculosis in different types of clinical specimens (including HIV-positive and HIV-negative patients)[79–82]

Clinical specimen	Positive proportion
Lymph node biopsy	77-90%
Pericardial biopsy	70-94%
Spinal cord biopsy	83%
Sputum	80%
Cerebrospinal fluid	50-70%
Peritoneal biopsy	68%
Urine	< 40%
Blood	39%
Pleural fluid	< 35%
Pericardial fluid	< 30%
Ascitic fluid	< 10%

HIV: human immunodeficiency virus

The growth medium used in cultures can be solid or liquid. The time to detect mycobacterial growth in liquid culture take up to six weeks and up to two months in solid cultures.[79] Liquid cultures are also more sensitive.[79]

Cultured mycobacteria can be detected with the human eye (microscopy) or by machines. The use of automated or semi-automated liquid culture systems have several advantages, including less time to detect substantial amounts of mycobacteria, less technical hands-on time and a lower contamination rate.[83]

A consequence of the long turnaround time is that culture results are often received too late to inform treatment decisions. The need to be performed at central reference laboratories is a further hindrance as samples need to be transported. Not only does this increase the turnaround time, but samples may get lost along the way. In order to use culture to its maximum benefit to diagnose tuberculosis, good systems are required to ensure rapid turn-around time (e.g. sample transport solutions) and timely delivery of information to healthcare facilities or users (e.g. information and communications technology solutions).[84]

In resource-limited settings, culture is selectively used for surveillance of drug sensitivity and to confirm treatment failure and relapse.[41]

HIV-positive patients have a lower bacillary load in their sputum,[85] with more cultures from HIV-positive patients requiring long incubation periods (7-8 weeks) than HIV-negative patients.[77] However, the median time to positivity (3 weeks) was similar and the diagnostic yield was also not associated with the HIV status of the patients.[77]

Blood culture is also used to assist with the diagnosis of tuberculosis in HIV-positive patients. A 2016 systematic review indicated the prevalence of *M. tuberculosis* bacteraemia in HIV-positive adults to be 15.5%.[86] The prevalence increase considerable to 34.8% in patients with severe sepsis.[87] Additional blood cultures further increase the yield for *M. tuberculosis* bacteraemia.[88] A model developed on patients diagnosed with HIV-associated tuberculosis when two blood cultures have been collected before the start of anti-tuberculosis treatment, had a mean predicted probability of *M. tuberculosis* bloodstream infection of 45% in hospitalised patients with one or more WHO danger signs and a CD4 cell count less than 100 /mm^3.[24] A positive blood culture is also a poor prognostic sign with a trend towards increased mortality.[22,89,90]

Xpert MTB/RIF

The Xpert MTB/RIF (Cepheid Inc., Sunnyvale, CA, USA) is a nucleic acid amplification test (NAAT) that uses a polymerase chain reaction (PCR) to amplify the target gene *rpoB*, which is probed with five molecular beacons to detect rifampicin resistance, thus allowing for early diagnosis of tuberculosis and detection of rifampicin resistance. Unprocessed samples are used and results are available within two hours.[91] Very little technical training is required as the only manual step is the addition of a bactericidal buffer; this also minimizes the exposure risk to biohazardous material.

A Cochrane review evaluated the diagnostic accuracy of Xpert MTB/RIF in adult patients presumed to have pulmonary tuberculosis.[34] The pooled sensitivity and specificity of Xpert MTB/RIF to replace smear microscopy as an initial test was 89% and 99%.[34] The pooled sensitivity was 67% as an add-on test following a negative smear microscopy result, with a pooled specificity of 99%.[34] In patients with smear-positive, culture-positive tuberculosis, the pooled sensitivity of Xpert MTB/RIF was 98%.[34] Xpert

MTB/RIF increases tuberculosis detection among culture-positive cases by 23% compared to smear microscopy. However, the diagnostic accuracy of Xpert MTB/RIF is influenced by HIV co-infection, with a 7% decrease in the pooled sensitivity between HIV-positive (79%) and HIV-negative participants (85%).[34] Xpert MTB/RIF also performed well to detect rifampicin resistance with a pooled sensitivity of 95% and pooled specificity of 98%.[34]

Xpert MTB/RIF is also used for the detection of extrapulmonary and disseminated tuberculosis. The results of a Cochrane review where the pooled diagnostic accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis was determined is presented in Table 7.[92] The estimates of Xpert MTB/RIF for disseminated tuberculosis performed on blood ranged from 7% to 56% for sensitivity and 94% to 98% for specificity (no meta-analysis done as only two studies included).[92] The pooled sensitivity and pooled specificity for rifampicin resistance was 95% and 99%.[92]

Table 7 Diagnostic accuracy of Xpert MTB/RIF compared to culture for extrapulmonary tuberculosis.[92]

Clinical specimen	Pooled sensitivity	Pooled specificity
Bone or joint fluid	97%	90%
Bone or joint tissue	92%	82%
Cerebrospinal fluid	71%	98%
Lymph node aspirate	88%	86%
Lymph node tissue	84%	79%
Pericardial fluid	66%	96%
Peritoneal fluid	59%	98%
Pleural fluid	51%	99%
Pleural tissue	31%	97%
Urine	83%	99%

The prevalence of tuberculosis also affects the diagnostic accuracy of Xpert MTB/RIF for most forms of extrapulmonary tuberculosis. Pooled sensitivity was higher in settings with higher tuberculosis prevalence, whereas pooled specificity was lower or similar in lower tuberculosis prevalence settings.[92]

The WHO has recommended the extensive use of Xpert MTB/RIF for the detection of tuberculosis and rifampicin resistance.[93] The recommendations pertaining to adults include:

- The use of Xpert MTB/RIF (instead of microscopy, culture and drug-susceptibility testing) as the initial diagnostic test in patients suspected of having multidrug resistant tuberculosis or HIV-associated tuberculosis (strong recommendation, high-quality evidence).
- The use of Xpert MTB/RIF (instead of microscopy and culture) as the initial diagnostic test in patients suspected of having tuberculosis (conditional recommendation acknowledging resource implications, high-quality evidence).
- The use of Xpert MTB/RIF as a follow-on test to microscopy in patients suspected of having tuberculosis – excluding those at risk of multidrug resistant tuberculosis or HIV-associated

tuberculosis (conditional recommendation acknowledging resource implications, high-quality evidence).

- The use of Xpert MTB/RIF on cerebrospinal fluid (in preference to microscopy and culture) as the initial diagnostic test in patients suspected of having tubercular meningitis (strong recommendation given the urgency of rapid diagnosis, very low-quality evidence).
- The use of Xpert MTB/RIF on specific non-respiratory specimens as a replacement test for microscopy, culture or histopathology in patients suspected of having extrapulmonary tuberculosis (conditional recommendation, very low-quality evidence).

The WHO currently recommends that the Xpert MTB/RIF assay should be used rather than culture as the initial diagnostic test in adults suspected of having HIV-associated pulmonary tuberculosis (strong recommendation, high-quality evidence).[93] Culture (and conventional microscopy) still remain essential for therapy monitoring therapy and for performing drug-susceptibility testing beyond rifampicin.[93]

Xpert MTB/RIF Ultra

The limited sensitivity of Xpert MTB/RIF in detecting pulmonary tuberculosis as well as the limited accuracy of detecting rifampicin resistance, led to the development of the Xpert MTB/RIF Ultra assay (Xpert Ultra). The Xpert Ultra assay incorporates two additional multi-copy amplification targets (IS*6110* and IS*1081*) and a larger DNA reaction chamber making it more sensitive than Xpert MTB/RIF. The accuracy of rifampicin resistance detection was improved by incorporating melting temperature-based analysis instead of real-time PCR.[94]

A large multinational prospective study indicated that Xpert Ultra is more sensitive than Xpert MTB/RIF for detecting culture-positive pulmonary tuberculosis (88% versus 83%), although Xpert MTB/RIF had higher specificity (98% versus 96%).[95] The differences in sensitivity were more substantial in cases with smear-negative pulmonary tuberculosis (Xpert MTB/RIF 46%, Xpert Ultra 63%) and in HIV-positive participants (Xpert MTB/RIF 77%, Xpert Ultra 90%).[95] Xpert Ultra and Xpert MTB/RIF performed similarly for detecting rifampicin resistance; 95% sensitivity in both Xpert Ultra and Xpert MTB/RIF and 98% specificity in both Xpert Ultra and Xpert MTB/RIF.[95]

Xpert Ultra is thus superior to Xpert MTB/RIF for the detection of smear-negative tuberculosis. This informed the 2017 WHO recommendations for the use of Xpert Ultra or Xpert MTB/RIF as the initial diagnostic test for all adults with a clinical suspicion of tuberculosis.[94] The recommendation is also relevant to test for extrapulmonary tuberculosis in selected specimens (cerebrospinal fluid, lymph nodes, and tissue specimens).[94]

Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a molecular assay using a unique temperature-independent DNA amplification technique.[96] TB-LAMP (Eiken Chemical Company Ltd ,Tokyo, Japan) is a commercially available manual assay that can be read with the naked eye under ultraviolet light within an hour.[96] The WHO recommended in 2016 that TB-LAMP can be used as a replacement and/or follow-on test for sputum smear microscopy in adults with signs and symptoms consistent with pulmonary tuberculosis.[96] These recommendations were confirmed by a 2019 systematic review and meta-analysis with sensitivity higher than sputum smear microscopy (pooled sensitivity difference + 13%, 95% CI 5-22) and similar to Xpert MTB/RIF (pooled sensitivity difference − 2.5%, 95% CI -8 to + 3).[97] The specificity of TB-LAMP was similar to both sputum smear microscopy (pooled specificity difference − 1.8%, 95% CI -3.8 to + 0.2) and Xpert MTB/RIF (pooled specificity difference 0.5%, 95% CI -0.9 to + 1.8).[97]

Line probe assay

Line probe assays (LPAs) are rapid molecular diagnostics that are designed to identify *M. tuberculosis* and concurrently detect mutations associated with anti-tuberculosis drug resistance.[98] LPAs detect resistance to other anti-tuberculosis drugs in addition to rifampicin resistance, but it is technically more complex and takes longer than Xpert MTB/RIF.[98] It can be applied to culture isolates or directly to clinical specimens (without the need for isolating the strain first on solid or liquid culture). A meta-analysis of individual studies on the accuracy of LPAs (direct and indirect testing) detecting *M. tuberculosis* indicated a pooled sensitivity of 85% (point estimates ranged from 49% to 100%) and a pooled specificity of 98%.[99] The accuracy for detecting rifampicin resistance (pooled sensitivity 97%, pooled specificity 99%) was better than the detection of isoniazid resistance (pooled sensitivity 89%, pooled specificity 99%).[99] These estimates were determined independent of the type of line probe assay use, the type of testing performed (direct or indirect), or the type of reference standard used.[99]

The WHO recommends the use of commercial molecular LPAs to detect resistance to rifampicin and isoniazid on sputum smear-positive specimens.[99] This recommendation applies to pulmonary and extrapulmonary specimens and to specimens that are culture-positive for *M. tuberculosis*.[99] However, the implementation of LPAs does not eliminate the need for conventional culture-based drug-susceptibility testing of other anti-tuberculosis drugs.[99]

The detection of resistance to anti-tuberculous drugs is very important as people with drug-resistant tuberculosis need to take second-line anti-tuberculous drugs (e.g. Levofloxacin, Moxifloxacin, Bedaquiline, Linezolid).[100] Second-line line probe assays (SL-LPAs) are a group of molecular genetic tests that can rapidly detect the presence of mutations associated with drug resistance to second-line anti-tuberculosis drugs.[100] SL-LPAs detect 86% of patients with fluoroquinolone resistance (by direct testing), 87% of patients with second-line injectable drug (SLID) resistance, and 69% of patients with

extensively drug-resistant tuberculosis.[101] The WHO recommended the use of SL-LPAs as the initial test (instead of phenotypic culture-based drug sensibility testing) to detect resistance to fluoroquinolones and SLIDs in patients with confirmed rifampicin-resistant tuberculosis or multidrug-resistant tuberculosis in 2016.[101]

Antigen detection tests

Tests that detect circulating *M. tuberculosis* antigens in clinical specimens can provide direct evidence of active tuberculosis allowing for immediate initiation of treatment.[102] A major advantage of antigen tests are the potential to be a point-of-care test, since it is easy to operate and results can be available within minutes.[102] Tests using easily obtainable clinical specimens like urine, would be ideal for children, and will avoid the use of more invasive tests.[102]

Antigen detection tests commonly use the sandwich enzyme-linked immunosorbent assay (ELISA) technique, or the lateral-flow immunochromatographic assay.[102] Various antigens have been tested with sensitivity estimates for diagnosing pulmonary tuberculosis ranging between 2% and 100% and for specificity from 33% to 100%.[102] Lipoarabinomannan (LAM) is the antigen target mostly included in these tests.[102]

Lipoarabinomannan (LAM)

LAM is a major and structurally important lipopolysaccharide in the outer cell wall of all mycobacteria.[103] It is released from metabolically active or degrading mycobacterial cells during tuberculosis infection, filtered by the kidney and subsequently detectable in the urine.[104] It is thought that the majority of patients with a positive urine LAM test has renal tuberculosis, and that disseminated tuberculosis without renal involvement occur less frequently.[105] Urine is the specimen in which LAM has the greatest clinical utility, although diagnostic utility has also been studied in sputum,[106] pericardial fluid,[107] cerebrospinal fluid,[108,109] and pleural fluid.[110]

Tests detecting LAM antigen in urine have better diagnostic performance in HIV-positive people than in HIV-negative people.[111] The estimated sensitivity further improves in patients with low CD4 cell counts.[111] Several reasons have been postulated to explain the higher sensitivity of LAM assays in immunosuppressed patients. In the majority of patients it might represents dissemination to the kidney.[105] Increased glomerular permeability may occur due to HIV-related podocyte dysfunction, also resulting in increased levels of LAM in urine.[104] In patients without immune suppression, a lesser degree of antigen-antibody complex formation occurs resulting in less LAM excretion in the urine.[112] Lastly, a greater bacillary burden in HIV-positive patients correlates to the higher sensitivity.[113]

A lateral flow LAM assay (LF-LAM) strip-test for urine, Alere Determine TB LAM Ag assay (AlereLAM; Abbott, Chicago, IL, USA),[114] was the first commercially available LF-LAM assay. A systematic review, including studies published before 11 May 2018,[115] informed the WHO policy on the use of AlereLAM

for the diagnosis of active tuberculosis in HIV-positive patients.[111] The diagnostic accuracy is summarised in Table 8, and indicates suboptimal sensitivity. The use of AlereLAM reduces mortality in patients with advanced HIV disease (pooled risk ratio 0.85 (95% Credible Interval (CrI), 0.76–0.94); absolute effect 35 (95% CrI 14-55) fewer deaths per 1000) despite the suboptimal sensitivity.[111] The WHO recommendations for the use of AlereLAM are described in Table 9.

Table 8 Diagnostic accuracy of the urinary lateral flow lipoarabinomannan assay (Alere Determine TB LAM Ag) in HIV-positive adults.[111]

Setting	**Sensitivity (95% credible interval)**	**Specificity (95% credible interval)**
HIV-positive adults presenting with signs and symptoms of tuberculosis		
Inpatient setting	52% (40% – 64%)	87% (78% – 93%)
Outpatient setting	29% (17% – 47%)	96% (91% – 99%)
All settings	42% (31% – 55%)	91% (85% – 95%)
HIV-positive adults, irrespective of signs and symptoms of tuberculosis		
Inpatient setting	62% (41% – 83%)	84% (48% – 96%)
Outpatient setting	31% (18% – 47%)	95% (87% – 99%)
All settings	35% (22% – 50%)	95% (89% – 98%)
Adults with advanced HIV disease, irrespective of signs and symptoms of tuberculosis		
Inpatient setting, CD4 cell count $\leq$ 200/mm^3	64% (35% – 87%)	82% (67% – 93%)
Outpatient setting, CD4 cell count $\leq$ 200/mm^3	21% (8% – 48%)	96% (89% – 99%)
All settings, CD4 cell count $\leq$ 200/mm^3	26% (9% – 56%)	96% (87% – 98%)
Inpatient setting, CD4 cell count $\leq$ 100/mm^3	57% (33% – 79%)	90% (69% – 97%)
Outpatient setting, CD4 cell count $\leq$ 100/mm^3	40% (20% – 64%)	87% (68% – 94%)
All settings, CD4 cell count $\leq$ 100/mm^3	47% (30% – 64%)	90% (77% – 96%)

HIV: human immunodeficiency virus

Table 9 WHO policy recommendations for the use of the urinary lateral flow lipoarabinomannan assay (Alere Determine TB LAM Ag) to assist the diagnosis of active tuberculosis in HIV-positive adults.[111]

Setting	Recommendation	Strength of recommendation	Certainty of evidence
Inpatient	HIV-positive adults with signs and symptoms of pulmonary and/or extrapulmonary tuberculosis	Strong	Moderate
Inpatient	HIV-positive adults with advanced HIV disease (CD4 cell count <200 cells/mm^3 or WHO clinical stage 3 or 4 at presentation)	Strong	Moderate
Inpatient	HIV-positive adults who are seriously ill (respiratory rate >30/minute, temperature >39 °C, heart rate >120/minute, unable to walk unaided)	Strong	Moderate
Outpatient	HIV-positive adults with signs and symptoms of pulmonary and/or extrapulmonary tuberculosis	Conditional	Low
Outpatient	HIV-positive adults who are seriously ill (respiratory rate >30/minute, temperature >39 °C, heart rate >120/minute, unable to walk unaided)	Conditional	Low
Outpatient	HIV-positive adults with a CD4 cell <100 cells/mm^3, irrespective of signs and symptoms of pulmonary and/or extrapulmonary tuberculosis	Conditional	Low

HIV: human immunodeficiency virus

Another urine-based assay, Fujifilm SILVAMP TB LAM (FujiLAM; Fujifilm, Tokyo, Japan) has been developed, although it is not yet commercially available.[111] It uses a novel technique that allows the detection of lower urinary LAM concentrations.[116] FujiLAM had better sensitivity (70%, 95% CI 53-83) compared to AlereLAM (42%, 95% CI 32-52; difference 28%) using a microbiological reference standard.[117] The estimated specificity of FujiLAM was 91% (95% CI 86-94) compared to 95% (95% CI 88–99) for AlereLAM (difference −4%).[117] FujiLAM detected more cases compared to AlereLAM, regardless of site of disease or type of specimen (Table 10).[118]

Table 10 Diagnostic sensitivity of Fujifilm SILVAMP TB LAM (FujiLAM) and Alere Determine TB LAM Ag (AlereLAM) in HIV-positive adults[118]

Site of tuberculosis	FujiLAM % (95% CI)	AlereLAM % (95% CI)
Both pulmonary and extrapulmonary tuberculosis	91% (87-94)	61% (55-67)
Pulmonary tuberculosis only	60% (51-69)	19% (12-27)
Extrapulmonary tuberculosis only	67% (59 75)	41% (33-49)
Abscess	88% (47-100)	50% (16-84)
Ascites	67% (22-96)	33% (4-78)
Blood	94% (90-97)	70% (64-76)
Cerebrospinal fluid	47% (24-71)	16% (3-40)
Lymph node	75% (43-95)	50% (21-79)
Pleural fluid	68% (55-80)	35% (23-48)
Urine	88% (84-92)	61% (55-66)

CI: Confidence interval

Both these antigen-based tests have the potential to be true point-of-care tests to expedite the diagnosis of HIV-associated tuberculosis.

Serological tests

The detection of antibodies against *M. tuberculosis* in patients' blood was anticipated to be an accessible and easy way to diagnose tuberculosis in resource poor settings.[119] Unfortunately, the diagnostic accuracy for serological tests varies considerably; for pulmonary tuberculosis the sensitivity ranged from 0% to 100% and for specificity between 31% and 100%.[119] Similar heterogeneity was found for extrapulmonary tuberculosis with sensitivity ranging from 0% to 100% and specificity from 59% to 100%.[119] These inconsistent and imprecise findings led to the WHO recommending that serological tests should not be used for the diagnosis of either pulmonary or extrapulmonary tuberculosis.[120]

Histology

Biopsy histology is particularly useful in paucibacillary disease and has a faster turnaround time than culture.[121] The presence of Langhans giant cells, caseation, and granuloma are strong histological evidence of tuberculosis. [122,123] However, the diagnostic yield of histology depends on the size of the specimen as well as the level of immunosuppression. The cellular immune response decreases substantially with CD4 cell counts <100 /mm^3. [124,125] The diagnostic sensitivity for tuberculosis by biopsy differs between tissue samples with lymph nodes 96% (95% CI 88–100),[126] pericardium 64%,[127] and pleura 54% (95% CI 35-73).[128] Non-specific histological findings are present in around 30% of patients with tuberculosis diagnosed in pleural fluid.[129] Skilful interpretation of histology is needed as the differential diagnosis includes other infections (e.g. histoplasmosis, leishmaniasis, syphilis), vasculitis (e.g. Wegener's), non-infectious immunological reactions (e.g. sarcoidosis), neoplasms and other miscellaneous disorders.[122,130]

Histopathology is a reliable method for diagnosis, however it is an invasive procedure, which limits its use.[124]

Cytology

Fine needle aspiration (FNA) cytology is a minimally invasive and cost-effective procedure that is quick to perform.[131] FNA can be used to diagnose pulmonary and extrapulmonary tuberculosis, both superficial (e.g. ulcer, sinus, lymph nodes, breast, thyroid, salivary glands) and deep extrapulmonary sites (e.g. liver, spleen, intestine, pancreas, adrenal glands, prostate, bone).[131] Deep extrapulmonary sites are typically reached via imaging aids like endoscopy.[131] The presence of epithelioid granuloma with or without necrotic material is highly suggestive of mycobacterial lesions.[131] The frequency of cytodiagnosis of tuberculosis varies by organ with lymph nodes having the highest yield (31% - 38%) and the thyroid gland the lowest (0.6% - 1.2%).[131] The frequency of other organs are: lung (5% - 20%), gastrointestinal tract (29%), prostate (6%), salivary gland (6%), and breast (3%). FNA cytology in the

diagnosis of tuberculous lymphadenitis has a sensitivity ranging between 38% and 88% and specificity between 49% and 100%.[126,132–135]

Technical and interpretive errors limits the cytological diagnosis of tuberculosis. Differential diagnoses include nontuberculous mycobacteria, sarcoidosis, leprosy, mycosis, silicone granulomatosis, cancer, granulomatous prostatitis, cat-scratch disease, and fat necrosis.[131]

FNA is also used to collect material for microscopic identification of mycobacteria, culture and molecular biology. The positivity proportion of mycobacterium culture ranges between 21% and 83%.[131]

Biochemistry

Adenosine deaminase

Adenosine deaminase (ADA) is an enzyme that is produced by lymphocytes and plays a role in the immune response to infections. Its accuracy in clinical decision-making is unclear as the diagnostic utility differs greatly through different geographical regions, clinical settings and test result thresholds.

In pleural fluid, sensitivity estimates of ADA for any threshold ranged from 40% to 100% (pooled sensitivity 92% (95% CI 90-93), while specificity estimates ranged between 50% and 100% (pooled specificity 90%, 95% CI 88-91).[136] The pooled sensitivity and specificity are 93% (95% CI 90–95) and 90% (95% CI 87–91) respectively for a threshold of 40±4 IU/L.[136]

In ascitic fluid, an ADA ≥39 IU/L had pooled sensitivity of 100% (95%CI 93-100) and pooled specificity of 97% (95% CI 94-99),[137] while in pericardial fluid (ADA ≥40 IU/L) had pooled sensitivity 88% (95% CI 82-91) and pooled specificity 83% (95% CI 78-88).[138] The sensitivity of ADA (≥5 IU/L) in the diagnosis of tuberculosis in cerebrospinal fluid was 79% (95% CI 75-83) and specificity 91% (95% CI 89-93)[139] A cut-off value of ≥24 IU/l for serum ADA in patients with extrapulmonary tuberculosis patients had 14% sensitivity and 98% specificity, compared to smear positive pulmonary tuberculosis patients (sensitivity 12%, specificity 98%) and smear negative pulmonary tuberculosis patients (sensitivity 6%, specificity 98%).[140]

ADA activity levels can also be elevated in conditions such as cancer (particularly lymphomas), parapneumonic effusions, pulmonary embolism, sarcoidosis, or lupus.[140]

2.6.3. Clinical diagnosis

A definite diagnosis of tuberculosis can only be made with microbiological confirmation. Historically, culture was the only definitive reference standard, but nowadays other microbiological tests (e.g. Xpert MTB/RIF and Xpert Ultra) are also acceptable. However, a substantial number of cases are not bacteriologically confirmed and the diagnosis of tuberculosis is suspected based on a combination of context, clinical symptoms and signs, and various investigations. In 2018, only 55% of global pulmonary

tuberculosis cases were bacteriologically confirmed.[2] This proportion differs between WHO regions; from 41% in the Western Pacific region to 79% in the Americas. In the African region, 65% of cases with pulmonary tuberculosis were bacteriologically confirmed, and 70% in South Africa.[2]

Expanded case definitions

Facilities for microbiological confirmation of tuberculosis are often limited or unavailable in resource-limited settings. In addition, diagnosing active tuberculosis in PLHIV remains an ongoing challenge.[141,142] The use of expanded case definitions and response to anti-tuberculosis treatment has been suggested as an effective way in which to diagnose HIV-associated tuberculosis where microbiological confirmation is lacking.[143] The expanded case definitions developed by expert consensus is presented with its diagnostic accuracy in Table 11.[143] The criteria to determine response to anti-tuberculosis treatment are: Body weight increase ≥5%; Haemoglobin increase ≥1.0 g/dl; C-reactive protein (CRP) levels reduction ≥60%; Karnofsky Performance Score (KPS) increase ≥20 (or ≥10 if baseline score was 80 or 90); and symptom count ratio ≥0.5.[143] The use of two or more criteria enable clinicians to detect 97.5% of participant with confirmed tuberculosis after a 2-month follow-up period.[143]

Trail-of-antibiotics

A 'trial-of-antibiotics' is suggested in many clinical algorithms around the world to address the diagnostic gap of mycobacterial unconfirmed tuberculosis.[144,145] It consists of a course of broad-spectrum antibiotics, with negligible anti-tuberculosis activity, given to symptomatic patients in order to distinguish pulmonary tuberculosis from bacterial lower respiratory tract infections. Patients are considered tuberculosis-negative if they have negative sputum mycobacteriology and have responded to antibiotic treatment, while those who fail to improve are considered to have tuberculosis and are treated as such.[144,145] The WHO had recommended against the use of antibiotics as a diagnostic aid and indicated that antibiotic treatment should be reserved to treat concomitant bacterial infection in HIV-positive adults.[146] The available evidence suggest poor diagnostic performance of 'trial-of-antibiotics' versus mycobacteriology tests with a pooled sensitivity of 67% (95% CI 42–85) and pooled specificity of 73% (95% CI 58–85).[147] This is below internationally defined minimum performance profiles for tuberculosis diagnostics.[148] A randomized clinical trial (ACT-TB study) is currently underway in Malawi to assess the impact of a 'trial-of-antibiotics' on clinical outcomes.[149]

Table 11 Accuracy of expanded case definitions to diagnose active tuberculosis in HIV-positive patients [Adapted and derived from Wilson D, et al. Diagnosing smear-negative tuberculosis using case definitions and treatment response in HIV-infected adults. Int J Tuberc Lung Dis. 2006;10(1):31-38.]

Site	Case definitions	Sensitivity	Specificity
Pulmonary	- Cough for >21 days PLUS - Pulmonary opacification or nodular infiltrate on chest X-ray PLUS - *Pneumocystis jirovecii* (previously *carinii*) pneumonia excluded (using clinical case definition of Centers for Disease Control and Prevention [a]) PLUS - No resolution after treatment with broad-spectrum antibiotic (except in patients with diffuse micro nodular [miliary] infiltrate on chest X-ray, who are started on anti- tuberculosis treatment after cultures are sent)	72%	83%
Lymphadenopathy (Peripheral)	- Significant asymmetrical peripheral nodes (long axis ≥3 cm) PLUS - Fever ≥38°C on two occasions OR - Drenching sweats for >2 weeks	30%	95%
Lymphadenopathy (Visceral)	- Visceral nodes (mediastinal/hilar or abdominal nodes seen on imaging) PLUS - Fever ≥38°C on two occasions OR - Drenching sweats for >2 weeks	63% (mediastinal nodes) 15% (Abdominal nodes)	93% (mediastinal nodes) 98% (Abdominal nodes)
Pleural effusion	- Lymphocytic exudates	16%	93%
Pericardial effusion	- Effusion on ultrasound PLUS - Fever ≥38°C on two occasions OR - Drenching sweats for >2 weeks (aspirate reserved for patients with haemodynamic compromise)	9%	98%
Ascites	- Lymphocytic exudates PLUS - Fever ≥38°C on two occasions OR - Drenching sweats for >2 weeks	6%	100%
Constitutional syndrome	- Wasting (Body Mass Index <18.5 kg/m^2) OR - Documented weight loss of >5% body weight within a month PLUS - Fever ≥38°C on 2 occasions OR - Drenching sweats for >2 weeks	4%	83%

HIV: human immunodeficiency virus

[a] Bilateral interstitial infiltrate; Exertional dyspnoea (onset <3 months); Hypoxia or desaturation ≥5% on effort

Clinical Algorithms

The WHO published recommendations in 2007 to address the diagnostic and treatment challenges of HIV-associated tuberculosis in HIV-prevalent and resource constrained settings.[146] The recommendations related to smear-negative pulmonary tuberculosis and included an algorithm for ambulatory patients and an algorithm for seriously ill patients.[146] The performance of the algorithm for ambulatory patients varied substantially, with sensitivity estimates ranging between 55% and 80%, and specificity between 44% and 79%.[150–152] The algorithm for seriously ill patients resulted in reduced length of hospital stay and reduced mortality.[153–155] The predictive value of the WHO algorithm for seriously ill patients for mortality had a c-statistic of 0.6 (95% CI 0.6–0.7), with being unable to walk unaided the only danger sign that was independently associated with mortality (adjusted OR 2.9, 95% CI 1.6-5.5).[156] The WHO updated the algorithm in 2016, to include the urine LF-LAM assay.[157]

A nurse-led clinical algorithm in South Africa (TB Fast Track) substantially increased the initiation of empirical anti-tuberculosis therapy in patients with advanced HIV disease (CD4 count ≤150 cells per μL).[158] The study was performed in primary health-care centres using point-of-care diagnostic tests (tuberculosis symptoms, body-mass index, point-of-care haemoglobin concentrations, and urine LAM) and should be feasible to implement in resource-limited settings.

2.6.4. Radiological diagnosis

Radiographic imaging plays an important role in the diagnoses of active tuberculosis, and its role has grown.[159] Radiographic studies can be used to diagnose tuberculosis, to determine the extent of the disease, to evaluate response to treatment, and to assist in procedures (e.g. guiding aspirations or biopsies).[159] All radiographic modalities have a role to play and the specific use of a certain imaging modality mainly depends on the patient's clinical profile and whether the modality is accessible and affordable. The conventional chest X-ray remains the initial modality for suspected pulmonary tuberculosis.[159]

Chest X-ray

Chest X-ray has been used for over a century. Chest X-rays are generally obtained at the time of diagnosis; typically, a single posterior-anterior (PA) view is adequate.[160] The sensitivity of the chest X-ray against culture-proven tuberculosis was 53% (95% CI 28-79) and the specificity 67% (95% CI 59-75).[161] The diagnostic performance of chest X-ray is regrettably limited by high inter-observer as well as intra-observer variability, in both the pre- and post HIV era.[58–60] The performance of chest X-ray further depends on the readers' expertise.

There are no radiographic patterns definitively diagnostic of tuberculosis. The disease can present in many ways and can also be mimicked by many conditions. Abnormal radiographic findings should thus prompt further investigations for tuberculosis; however, radiographic findings in HIV-positive people

might be absent despite active disease.[160] Healthcare personnel should thus not exclude tuberculosis on the basis of absent radiographic features.

Typical radiographic features of active tuberculosis used to be lymphadenopathy, consolidation, pleural effusion, and miliary nodules for primary tuberculosis, and apical or upper lung zone consolidations, nodules and cavitation for post-primary (reactivated) tuberculosis.[160] This simplified categorisation has been reconsidered as it has become clear that radiographic appearances of active tuberculosis mainly relates to host factors, particularly immunosuppression.[162]

The presence of mediastinal and hilar lymphadenopathy are strong indicators of active tuberculosis with the differential diagnosis including nontuberculous mycobacterial infection, lymphoma, and metastatic carcinoma.[163] Parenchymal disease is usually depicted as consolidation in a segmental or lobar distribution; the appearance however, is similar to bacterial pneumonia.[164] Pleural effusion is seen in about 25% of active tuberculosis disease, mostly unilateral,[165] whereas miliary disease manifests as diffuse 1–3-mm nodules in a random distribution.[160] Cavitary lesions are a common finding in postprimary tuberculosis (20% – 45% on chest X-ray). The cavitation may be multifocal and is often seen within areas of consolidation.[166] Endobronchial spread manifests radiologically as centrilobular nodules and the tree-in-bud sign; present on computed tomography (CT) of the chest in up to 95% of active tuberculosis cases.[164]

It is often very difficult to distinguish between active and inactive tuberculosis based on radiographic features. Inactive tuberculosis is characterized by stable fibronodular changes for at least 6 months.[166–168] This implies that the radiographic diagnosis of active tuberculosis disease may only be reliably made on the basis of temporal evolution of pulmonary lesions.[169]

Radiographic patterns differ between HIV-negative and HIV-positive patients, and also relate to the degree of immunosuppression.[38,61,170] Features more commonly found in HIV-positive patients are diffuse pulmonary involvement,[77,171,172] lower lung field involvement,[77] miliary pattern,[43,171–174] interstitial pattern,[173,174] and adenopathy.[43,61,171,173–177] HIV-positive patients are also more likely to have normal chest x rays (14%),[74,162,172,178] or atypical patterns.[162,170,179,180]

On the other hand, cavitary lesions,[43,171–177] consolidation,[43,175] atelectasis,[173,174] and pleural effusion[171] are less common in HIV-positive patients. However, some studies found that consolidation [174] and pleural effusion occurred more in HIV-positive patients.[172–174,176] Johnson et al, even indicated that there were no difference in the frequency of atypical radiographic presentations and cavitary disease.[77]

Chest X-ray has a high negative predictive value for active tuberculosis, although an increased false negative rate has been seen in the immunocompromised.[178] This is explained by the higher proportion of normal chest X-rays in HIV-positive patients with active tuberculosis.[74,162,172,178] Furthermore, HIV-

positive patients with pulmonary tuberculosis are also more likely to have negative sputum smear.[5,77,78] This combination (sputum smear negative and normal chest X-ray) has led to an increase in mortality, primarily due to a delay in tuberculosis diagnosis and the subsequent delay to initiate of anti-tuberculosis therapy.[78]

The radiographic appearance of active tuberculosis on chest X-ray is very diverse as seen in the inconsistent findings by different studies done in different settings with different proportions of HIV-positive participants. The paucity of prospective data, especially from HIV-positive patients and from sub-Saharan Africa is a further area of concern.[181] One thing is clear, chest X-rays should not be used in isolation and their diagnostic performance improve if integrated with clinical and laboratory data.[182]

Computed Tomography (CT) Scan

A computed tomography (CT) scan has many advantages over conventional X-rays. It produces a better quality image due to the higher resolution and thus is best utilised to visualise subtle abnormalities and to evaluate soft tissues e.g. brain and abdominal organs. On the other hand, CT scans are expensive, expose patients to a high dose of radiation, and are more time consuming than conventional x-rays.[183] There is also always the risk of an adverse reaction to iodine contrast, as well as the possibility of nephrotoxicity.[159]

The role of CT scan in the diagnosis of tuberculosis is in patients with a normal or inconclusive chest X-ray, to diagnose miliary and extrapulmonary tuberculosis, to determine disease activity and to plan surgical treatment if indicated.[184–187]

The diagnostic accuracy of a CT scan of the chest is better than conventional chest X-rays to diagnose pulmonary tuberculosis. Radiographic findings are better characterised, especially the detection of cavities, lymphadenopathy and miliary disease.[160,187,188] Similar to conventional x-rays, CT features of tuberculosis differ according to the HIV status of patients. Immunocompromised patients have a higher prevalence of atypical lung field involvement, lymphadenopathy, miliary disease, and extrapulmonary manifestations.[43,189,190] HIV-negative patients have a higher prevalence of nodular opacities, consolidation, and cavitation.[43,189,190]

A multi-slice CT scan has become very valuable in evaluating extrapulmonary tuberculosis. It is preferred to conventional barium studies as well as intravenous pyelography.[159] A CT scan of the brain is useful to detect tuberculosis involving the central nervous system.[191] It can detect leptomeningeal tuberculosis as well as its complications (e.g. communicating hydrocephalus, infarctions and/or haemorrhage).[192–194] It can also detect tuberculosis lesions in the brain parenchyma such as a tuberculoma or tuberculous abscesses.[194–198]

CT scan is one of the preferred imaging modalities to investigate possible abdominal tuberculosis.[187] It is very sensitive to detect abdominal lymphadenopathy,[199] but is also used to diagnose tuberculous

peritonitis,[199] gastrointestinal tuberculosis,[199–201] hepatosplenic tuberculosis,[200] genitourinary tuberculosis,[201,202] and tuberculous adrenalitis.[203]

Other areas where CT scans are also used to detect extrapulmonary tuberculosis include the airway (bronchial stenosis),[187,204] pericardium,[205206] neck (scrofula),[187] and musculoskeletal tuberculosis (tuberculous spondylitis).[207,208]

Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) has multi-planar imaging capability and unmatched soft tissue contrast resolution, thus making MRI the modality of choice in evaluating central nervous system tuberculosis.[159,209] Although both CT and MRI can detect leptomeningeal tuberculosis with complications,[210] infarctions are detected earlier with MRI.[211] MRI is also used to demonstrate parenchymal tuberculosis, such as tuberculosis granulomata and miliary tuberculosis [211–213]

The role of MRI has also expanded to other parts of the body (e.g. tuberculous spondylitis),[208,212,214,215] but its widespread use is restricted by high cost, limited availability and slow throughput time.

Ultrasound

Diagnostic medical ultrasound dates back to the early nineteenth century, where it was mainly developed and used in military institutions.[216] Advances in instrumentation improved the quality of obtained images and increased the speed of image acquisition,[217] and has led to ultrasound being recognised as a key diagnostic test.[216]

Ultrasound has substantially expanded the diagnostic capabilities of physicians. It is used in all fields of medicine, in both the adult and paediatric population. Ultrasound is also used in the diagnostic work-up for tuberculosis; historically for extrapulmonary tuberculosis, but recently also for pulmonary tuberculosis. Ultrasound serves as a radiation-free tool for guided aspiration or biopsy in order to obtain specimens for histological, cytological or microbiological proof.[218] It also forms part of expanded case definitions for smear-negative tuberculosis in HIV-positive individuals.[143]

Ultrasound features related to tuberculosis

There are no pathognomonic ultrasound features for tuberculosis and the diagnosis thus requires a high index of suspicion. This review will focus on areas that can be assessed by placing an ultrasound probe on the thoraco-abdominal region of the body. Ultrasound examinations discussed are lung ultrasound, echocardiography, abdominal ultrasound (excluding genitourinary tuberculosis) and the Focused Abdominal Ultrasonography in HIV/TB (FASH) protocol.

Lung ultrasound

Lung ultrasound can identify parenchymal patterns associated with tuberculosis.[219] Ultrasound features that have been described include consolidations, fibrosis, pleural thickenings, pleural

effusion, subpleural nodules, pneumothorax and miliary patterns.[219–222] The diagnostic accuracy of lung ultrasound was assessed in an Italian study (n=102) where 51 out of the 102 participants had tuberculosis (bacteriologically confirmed or clinically diagnosed).[223] In univariate analysis, apical consolidations (OR 9.65, 95%CI 3.02–30.78), subpleural nodules (OR 5.29, 95%CI 2.27–12.33), superior quadrant consolidations (OR 4.01, 95%CI 1.76–9.14), and multiple consolidations (OR 3.54, 95%CI 1.43–8.78) were significantly associated with tuberculosis.[223] Only apical consolidation (OR 9.67, 95%CI 2.81–33.25) and subpleural nodules (OR 5.30, 95%CI 2.08–13.52) remained significantly associated in a multivariate model (c-statistic 0.799).[223] The diagnostic accuracy of individual ultrasound features and the ultrasound model are presented in Table 12. Twenty-eight patients (27%) were HIV-positive of which 11 had confirmed pulmonary tuberculosis; the study did not include a sub-analysis of HIV-positive patients. Chest X-ray findings in HIV-positive people might be absent despite active disease,[160]and it remains unclear whether lung ultrasound would have adequate diagnostic accuracy for HIV-associated tuberculosis.

Table 12. Diagnostic accuracy of lung ultrasound features in patients with a clinical suspicion of tuberculosis [adapted from Montuori M., Casella F., Casazza G., Franzetti F., Pini P., Invernizzi C., et al. Lung ultrasonography in pulmonary tuberculosis: A pilot study on diagnostic accuracy in a high-risk population. Eur J Intern Med 2019;66:29–34. Table 4, Diagnostic accuracy of LUS signs; p.33. Table 5, Diagnostic accuracy of the model based on LUS signs; p.33.][223]

Ultrasound feature	**Sensitivity (95% Confidence Interval)**	**Specificity (95% Confidence Interval)**
Consolidations	78% (65–89)	35% (22–50)
Subpleural nodules	73% (58–84)	67% (52–79)
Pleural irregularities	73% (58–84)	35% (22–50)
Superior quadrant consolidation	69% (54–81)	65% (50–78)
Apical consolidation	45% (31–60)	92% (81–98)
Multiple consolidations	43% (29–58)	82% (69–92)
Pleural effusion	20% (10–33)	75% (60–86)
Apical consolidation OR subpleural nodules	86% (74–94)	63% (48–76)
Apical consolidation AND subpleural nodules	31% (19–46)	96% (87–100)

Echocardiography

Tuberculous pericarditis is frequently seen in settings with a high HIV prevalence,[224–226] and tuberculosis is the most frequent cause of pericardial disease in Africa regardless of HIV-status.[227] The prevalence of tuberculosis in patients with large clinically significant pericardial effusions in South Africa is 70%;[19] much higher than rest of world (prevalence 10% - 60%).[225] Ultrasound features related to tuberculous pericardial effusion includes large size (>20 mm), cardiac tamponade, thickened pericardium, loculated effusion, and the presence of fibrin strands within the effusion.[228] Although large effusions seems to be related to tuberculosis, pericardial effusion of any size has also been predictive of tuberculosis (OR 2.83, 95% CI 1.62-4.96).[229] The differential diagnosis of pericardial

effusions depends in part on the disease profile of the clinical setting and the patient demographics, but include malignancy, uremia, post-acute myocardial infarction, viral, and chronic idiopathic.[228,230]

Abdominal ultrasound

Tuberculous peritonitis is often seen in association with gastrointestinal tuberculosis.[231] Ascites is a common finding and ultrasound can detect very small quantities of fluid that are clinically unsuspected.[232] Ascites could be free or loculated, and multiple fine mobile fibrin strands or debris may be present.[232,233] The peritoneum can be diffusely thickened (>2 mm) or have an irregular appearance with nodules <5 mm in size.[232] The omentum could be infiltrated resulting in a 'caked' appearance.[232,234] Ultrasound feature related to the small bowel mesentery include nodules (solid/lymph node or cystic/abscess), thickening (>15 mm),[235], loss of normal configuration, or the 'stellate-sign' – fixed loops of bowel with mesentery standing out as spokes.[232] The differential for ultrasound features suggestive of peritoneal tuberculosis are peritoneal carcinomatosis, primary peritoneal mesothelioma, peritonitis, and lymphoma.[232]

Intra-abdominal lymphadenopathy (tuberculous lymphadenitis) is frequently found in abdominal tuberculosis (25% - 93%).[232] Enlarged lymph nodes are even more common in PLHIV,[236] and can be present in isolation or in combination with other ultrasound features.[232] Node involvement reflects the lymphatic drainage of the small bowel and thus include mesenteric, celiac, porta hepatis, and peri-pancreatic lymph nodes.[231] The mesenteric region is most frequently involved in patients with HIV.[236–238] Various ultrasound patterns are found, including increased number of normal size nodes, scattered mildly enlarged nodes, localized cluster of enlarged nodes, or large conglomerated nodal masses.[232,235] Lymph nodes usually have a central hypoechoic area,[232] although calcifications are occasionally present.[233] It is not possible to differentiate tuberculosis from other causes of lymphadenopathy on ultrasound.[239] The differential diagnosis is broad with malignancy (e.g. lymphoma) and other infections (e.g. nontuberculous mycobacteria, fungal, bacterial, sarcoidosis) on top of the list.[231]

Hepatosplenic tuberculosis can present as local or miliary disease. The ultrasound features are non-specific and can be present in several benign or malignant conditions.[232] The liver and spleen can be of normal size or can be enlarged.[232] Single lesions can be hypoechoic, heterogeneous, or a calcified mass, with the differential diagnosis mainly being abscesses and primary or secondary malignant lesions. [232] Multiple ultrasound lesions are usually scattered throughout the organ and can be hypoechoic or hyperechoic (with or without calcification).[232] Fungal infections, pyogenic or amoebic abscesses, Hodgkin's disease, and metastatic carcinoma are frequent differentials to consider.[232]

Tuberculosis involving the pancreas is rare. Related ultrasound features are hypoechoic single lesions, well-defined masses, diffuse pancreatic enlargement, enlarged peri-pancreatic lymph nodes, and small calcifications within the pancreas.[232] The differential diagnoses are malignancies, abscesses, and chronic pancreatitis.[232]

Intestinal tuberculosis most commonly involves the ileocecal region.[236] Uniform and concentric bowel wall thickening, and matted masses (thickened bowel loops, ascites, and regional lymph nodes) are described ultrasound features.[236] Intestinal tuberculosis mimics various conditions including lymphoma, Crohn's disease, amebiasis, and adenocarcinoma.[231]

The prevalence of individual abdominal ultrasound features varies widely (Table 13).[229,231,246–254,235,237,240–245] Many of the studies are limited to descriptive or case control studies,[231,233,248,250–254,235,237,240,241,243,245–247] have a small sample size (n<50),[240,241,243,246,248–253] used a composite or non-robust reference standard,[233,235,249,250,253,237,240–243,245,246,248] or were influenced by incorporation bias.[240,241,245–250]

Table 13. Frequency of individual abdominal ultrasound features relating to tuberculosis

Ultrasound feature	**Frequency**
Abdominal lymph nodes	18% - 100%
Ascites	6% - 100%
Bowel wall thickening	5% - 100%
Biliary tract abnormalities	7%
Dilatation and hypoperistalsis of small bowel loops in areas with mesenteric abnormalities	68%
Gastro-intestinal abnormalities	13%
Hepatic lesions	1% - 7%
Hepatomegaly	3% - 76%
Omental thickening	27%
Pancreatic abnormalities	1%
Pleural effusion	67%
Small bowel mesenteric thickening (>15mm)	11% - 100%
Splenic lesions	13% - 62%
Splenomegaly	4% - 58%
Stellar sign	9%

Evidence of the diagnostic accuracy of individual abdominal ultrasound features is limited and mainly involves HIV-positive patients. A systematic review was performed to determine the diagnostic accuracy of abdominal ultrasound for detecting abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals. The review was published in the Cochrane Database of Systematic Reviews,[255] and forms **Chapter 3** of this thesis.

Focused Abdominal Ultrasonography in HIV/TB (FASH) protocol

The Focused Abdominal Ultrasonography in HIV/TB (FASH) protocol was first described by Heller et al, in 2010.[256] The FASH protocol was modelled on the Focused Assessment with Sonography for Trauma (FAST) protocol and evaluates the patient for the presence of six ultrasound features.[256] These features were selected based on the relative ease to identify the features and consists of pericardial effusion, pleural effusion, ascites, upper abdominal lymph nodes (diameter >15 mm), focal splenic lesions, and focal liver lesions.[256] The first three ultrasound features were grouped as the FASH-basic, while the latter three features, which were deemed more difficult to identify, were included in the FASH-plus.[256,257] The FASH protocol was quickly incorporated into clinical medicine and ultrasound curriculums,[258,259] despite limited evidence supporting its use.

The diagnostic value of the FASH protocol was compared to chest X-ray in a case series of 82 patients with abdominal tuberculosis diagnosed by ultrasound (only a fraction of participants had a microbiologically confirmed diagnosis).[260] In total, 27% of participants had no radiological changes suggestive of pulmonary tuberculosis and would potentially benefit if the FASH protocol was incorporated in diagnostic algorithms.[260]

An Indian study evaluated the FASH protocol in 425 patients of which only 81 (20%) were HIV-positive.[261] A composite reference standard was used to diagnose 285 (67%) patients with tuberculosis. The FASH protocol used did not included ascites, and was deemed positive (≥1 ultrasound feature present) in 118 (41%) patients. HIV-negative patients were as likely as HIV-positive patients to have positive FASH features, although not associated with a diagnosis of tuberculosis. On the other hand, the presence of FASH features in HIV-positive patients was associated with tuberculosis (p=0.004).[261]

A cross-sectional study (n=100) in South Sudan detected positive FASH features in 27% of patients presenting to a voluntary HIV counselling and testing centre. Patients with positive FASH features had lower CD4 cell counts (p=0.003) and were in a more advance WHO HIV stage (p=0.001). A major limitation in the study was the low microbiological confirmation rate of tuberculosis (33%). The FASH protocol was also used as a screening tool, with 12% of FASH-positive patients being asymptomatic.[262]

A Tanzanian study validated the FASH protocol in 191 patients (52% HIV-positive), but included an additional ultrasound feature (ileum wall thickening >4 mm or destructed ileum wall architecture) in the FASH protocol. A composite reference standard was used and patients were followed for 6 months to confirm tuberculosis in 110 (58%) patients. A total of 77 (40%) patients had ≥1 FASH feature present of which 61 (56%) had confirmed tuberculosis. The presence of ≥1 FASH features was associated with confirmed tuberculosis in both univariate (OR 3.11, 95% CI 1.56-6.21) and multivariate analysis (OR 3.33, 95% CI 1.21-9.12).[263]

The FASH protocol has been successfully used via telemedicine,[264,265] and its use in children has been explored.[266,267] Lastly, the FASH protocol has also been used to asses response to anti-tuberculosis treatment. In a small Italian study (n=21), 76% of FASH features resolved after three months of treatment.[268]

Point-of-Care ultrasound

Ultrasound machines and transducers became smaller and lighter over time; subsequently their use has moved to the bedside of critically ill patients.[217] Point-of-Care Ultrasound (PoCUS) refers to the use of ultrasound wherever a patient is being treated. The exact definition varies and synonymous terms include emergency ultrasound, bedside ultrasound, focused ultrasound and clinician-performed ultrasound.[269,270] PoCUS is usually performed, interpreted and integrated into care by clinicians (non-radiologists) at the patients' bedside, often in suboptimal conditions and with time limitations.[269,270] This differs from consultative ultrasound examinations where patients are transported to a department or radiology suite outside the clinical setting after a formal written request for an ultrasound examination.[270] For the purpose of this discussion, PoCUS relates to the use of ultrasound by non-radiologists at the point-of-care.

The utilisation of PoCUS can be roughly divided into five clinical categories:[270]

i) Resuscitative: Use of PoCUS during the resuscitation of an acutely-ill patient,

ii) Diagnostic: Use of PoCUS in a diagnostic imaging capacity,

iii) Symptom or sign-based: Use of PoCUS based upon the patient's symptom or sign (e.g. shocked) – often incorporated in a clinical pathway,

iv) Procedure guidance: Use of PoCUS to assist with a procedure, and

v) Monitoring: Use of PoCUS to monitor the response to the therapeutic management of patients.

Diagnostic PoCUS was first used by non-radiologists in the early 1980's in patients who sustained blunt trauma.[271,272] This evolved into the Focused Assessment with Sonography for Trauma (FAST) examination;[273] a bedside screening tool to help clinicians identify free intrathoracic or intraperitoneal fluid. The evaluation of pneumothoraxes were later included and led to the extended Focused Assessment with Sonography for Trauma (eFAST). A 2019 systematic review (including 75 studies and 24350 participants), indicated that the eFAST examination is useful to rule in the presence of pneumothorax (pooled sensitivity 64%; pooled specificity 99%), pericardial effusion (pooled sensitivity 91%; pooled specificity 94%), and intra-abdominal free fluid (pooled sensitivity 74%; pooled specificity 98%).[274]

PoCUS has subsequently been introduce in multidisciplinary fields, including the FASH-protocol to diagnose HIV-associated tuberculosis as described earlier.

2.7. Testing for tuberculosis in the emergency centre

Hospital emergency centres are often used as a primary entry point for patients into the health care system. In countries with a high tuberculosis burden, many patients presenting to the emergency centre are eventually diagnosed with tuberculosis. The prevalence of pulmonary tuberculosis among patients presenting to the emergency centre at a Brazilian tertiary hospital is 15%,[275] while in South Africa the prevalence of tuberculosis in the emergency centre is between 14% and 17%.[276–278] The high prevalence of tuberculosis in patients presenting to the emergency centre, offers a diagnostic opportunity.

In an emergency centre in Peru, screening all patients who were able to provide one sputum sample for microscopy and culture, substantially increased the detection rate of pulmonary tuberculosis. The number needed to screen was 5.4.[279] Unfortunately, many patients are also missed, especially in lower burden areas. In California, USA, 16% of patients eventually diagnosed with tuberculosis visited the emergency centre in the prior month.[280] These missed diagnoses increased to 26% after 3 months in the USA,[280] and 39% in London, England.[281]

Clinical prediction rules are often used in emergency centres to help clinicians reach a diagnosis or to predict the probability of a specific event occurring.[282] A clinical prediction rule was developed in a South American emergency centre for patients with respiratory symptoms suggestive of pulmonary tuberculosis.[283] The independent predictors of culture-proven pulmonary tuberculosis are presented in Table 14. [283] The c-statistic of the rule was 0.809 (95% CI 0.762–0.856).[283] A score of three or more points had 93% sensitivity and 42% specificity.[283] The incidence of pulmonary tuberculosis in the study setting was 178 per 100 000 population, with an HIV prevalence of only 1%.[283] The data were also used to evaluate 13 previously published clinical prediction rules for pulmonary tuberculosis (Table 15).[284] The number of independent predictors included ranged from 4 to 15, and all included both clinical parameters and radiographic features. Diagnostic estimates varied markedly (sensitivity range 81% - 98%; specificity range 14% - 94%), and only one was derived in a high-prevalence tuberculosis setting with the aim to be used as a diagnostic tool to initiate treatment (the others were derived to detect patients that might need isolation).[284] Point-of-care tests were not included in any of the diagnostic-orientated clinical prediction rules.[284]

Table 14 Clinical prediction rule for culture-proven pulmonary tuberculosis[283]

Independent predictor	Odds Ratio (95% Confidence Interval)	Points
Age (years) <35 35 – 60 ≥61	0.97 (0.96–0.99)	 0 -1 -2
Weight loss	2.79 (1.51–5.18)	+5
Previous pulmonary tuberculosis	0.51 (0.28–0.95)	-3
Miliary pattern on chest X-ray	8.04 (2.79–23.16)	+10
Cavities on chest X-ray	2.54 (1.40–4.62)	+5
Upper lobe infiltrate on chest X-ray	5.64 (3.20–9.93)	+9

Table 15 Clinical prediction rules evaluated by Solari et al.[284]

Author	Country	Independent predictors	Main purpose of clinical prediction rule	Sensitivity	Specificity
Aguilar[285]	USA	- Age - Race - History of active tuberculosis - History of positive tuberculin skin test - History of tuberculosis exposure - Acquired immunodeficiency syndrome - Weight loss (>10% body weight) - Malaise (>1 month) - Sweats (>2 weeks) - Sputum production (> 2 weeks) - Positive tuberculin skin test - White cell count >10000 - White cell count 4000 - 10000 - Positive chest x-ray - Positive thoracic CT scans	Isolation	99%	34%
Bock[286]	USA	- Chest x-ray with upper lobe infiltrate - Chest x-ray with upper lobe cavity - History of having known someone with tuberculosis - Self-reported positive tuberculin skin test - Self-reported isoniazid preventive therapy	Isolation	81%	38%
El-Solh[287]	USA	- Upper zone disease on chest x-ray - History of fever - Weight loss - CD4 cell count	Isolation	100%	48%
Gaeta[288]	USA	- HIV - Injection drug use - Recent positive tuberculin test (≤2 years) - Previous history of pulmonary tuberculosis - Fever	Isolation	96%	14%

		- Haemoptysis - Abnormal pulmonary examination - Chest x-ray consistent with pulmonary tuberculosis			
Moran[289]	USA	- History of tuberculosis - Immigrant - Homeless - History of incarceration - Recent weight loss - Chest x-ray with apical infiltrate - Chest x-ray with a cavitation	Isolation	96%	49%
Mylotte[290]	USA	- Positive acid-fast sputum smear - Localized chest x-ray findings - Residence in a correctional facility - History of weight loss	Isolation	Not given	Not given
Rakoczy[291]	USA	- Chronic symptoms - Upper lobe disease on chest x-ray - Foreign-born status - Immunocompromised state other than human immunodeficiency virus infection	Isolation	97%	42%
Redd[292]	USA	- Abnormal chest x-ray - Fever - Current homeless shelter dweller - Tuberculosis history (history of either positive skin test, active tuberculosis, or tuberculosis exposure).	Isolation	96%	54%
Tattevin[293]	France	- BCG immunization (none or > 10 year ago) - No HIV infection - Homeless - Compatible clinical symptoms - Compatible chest x-ray	Diagnosis	100%	48%
Tessema[294]	Ethiopia	- Weight loss - Fever - Night sweating - Loss of appetite - Chest pain - Chest x-ray likely tuberculosis - Cough - Breathlessness - Haemoptysis	Diagnosis	93%	94%
Wang[295]	Taiwan	- Age < 65 years - Fever - Right upper lung field - Left upper lung field - Consolidation - Cavitation on chest x-ray	Diagnosis	95%	50%
Wisnivsky[296]	USA	- Presence of tuberculosis risk factors or symptoms - A positive tuberculin test result - Fever - Upper-lobe disease on chest x-ray	Isolation	98%	45%

USA: United States of America, CT: Computed Tomography, HIV: human immunodeficiency virus, BCG: Bacilli Calmette-Guerin

Point-of-care testing

Successful point-of-care testing can be defined as the completion of the test-and-treat cycle in one patient encounter.[297] The WHO has made recommendations on the ideal characteristics of a point-of-care test (Table 16).[298] The list of point-of-care (or near-point-of-care) tests include smear microscopy, Xpert MTB/RIF, Xpert MTB/RIF Ultra, GeneXpert OMNI, LF-LAM, and portable digital chest X-ray.[299] PoCUS has been used a point-of-care test, but its exact role in diagnosing tuberculosis are yet to be defined.

Table 16 Ideal characteristic of point-of-care diagnostic test for tuberculosis [Adapted from World Health Organization, High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting, 28-29 April 2014, Geneva, Switzerland][298]

	Ideal test characteristic
1	Ability to detect pulmonary or extrapulmonary tuberculosis
2	Ability to be used in children and adults
3	Ability to be used in HIV-positive or HIV-negative patients
4	Ability to be used in several easily accessible body samples (blood, breath, urine)
5	Ability to be used by health care workers with minimal training
6	Ability to be used in peripheral health facilities or community
7	Robust (withstanding broad ranges of temperature and humidity)
8	Rapid turnaround time (<20 minutes)
9	No or minimal maintenance required
10	Cheap (less than 4 US dollars per test)
11	High sensitivity (similar to that of Xpert MTB/RIF)
12	High specificity (similar to Xpert MTB/RIF)

HIV: human immunodeficiency virus

The implementation of point-of-care tests in the emergency centre setting is ideal, despite the fact that none of the currently available tests meet all the outlined characteristics.[299] LF-LAM is the only true point-of-care test that is feasible for the emergency centre setting. Although the use of point-of-care tests have many advantages (Table 17),[300] care must be taken to ensure that their use positively impacts patient management and flow through the emergency centre.

Table 17 Potential advantages and disadvantages of point-of-care tests[300]

Advantages	**Disadvantages**
Improved quality and efficiency of care	Quality control difficulties
Improved accessibility	Poor or incorrect documentation
Improved patient compliance	Poor connectivity to centralised result portals
Reduce number of patient loss to follow-up	Personnel task shifting (from radiology or laboratory staff to clinical staff)
Earlier initiation of appropriate treatment	Longer patient waiting times
Reduced length of hospital stay	Over-servicing
Reduced complications	Poor regulatory control
Reduced mortality	
Improved patient satisfaction	
Improved clinician satisfaction	

2.8. Empirical therapy for HIV-associated tuberculosis

The diagnostic challenge of HIV-associated tuberculosis together with the high mortality rate has resulted in the common practice of initiation of anti-tuberculosis treatment without bacteriological confirmation (empirical therapy). This approach makes intuitive sense, but there is a growing body of evidence suggesting a lack of mortality benefit in adults with advance immunosuppression receiving empirical tuberculosis treatment directed by a set of pre-specified criteria rather than clinician judgement.

The REMEMBER trial randomised HIV-positive participants with a CD4 count <50 cells per µL to either receive isoniazid preventive therapy or empirical anti-tuberculosis treatment.[301] There was no difference in 6-month mortality between the two groups (absolute risk difference −0·06% (95% CI −3·05 to 2·94).

The STATIS trial also found no difference in mortality at 6 months.[302] The adjusted hazard ratio between HIV-positive adults (CD4 count <100 cells per µL) who received empirical anti-tuberculosis treatment or guided-treatment (based on Xpert MTB/RIF, urine LAM, and chest X-ray) was 0.95 (95% CI 0.63-1.44). There was also no difference in mortality at 12 months (adjusted hazard ratio 0.97 (95% CI 0.67-1.40).[302]

The TB Fast-Track trial further confirmed that empirical anti-tuberculosis treatment for high-risk individuals (HIV-positive with CD4 <150 cells per µL) does not reduce 6-month mortality compared to standard of care (adjusted hazard ratio 0·87 (95% CI 0.61–1.24)).[158]

2.9. Conclusion

Tuberculosis is preventable and curable, yet it remains a deadly communicable disease particularly in socio-economically deprived regions of the world. Despite an ongoing global effort to eradicate tuberculosis, the worldwide case detection rate in 2017 was only an estimated 85%.[2] This is due to a mixture of underreporting of detected cases as well as the underdiagnosis of tuberculosis. The failure to diagnose tuberculosis could relate to the intrinsic properties of tuberculosis diagnostic tests (sensitivity, specificity, etc.), problems with the availability of these tests, and the reduced diagnostic performance of tuberculosis tests in HIV co-infection.[33–35] HIV-positive patients have more atypical clinical presentations and higher rates of smear-negative pulmonary and extrapulmonary tuberculosis. These patients often have multi-organ involvement and tuberculosis bacteraemia; putting them at high risk of rapid clinical deterioration and death.[146]

Patients presenting to emergency centres are often severely ill and prompt management is needed to decrease morbidity and mortality. Rapid diagnosis of tuberculosis can expedite the initiation of anti-

tuberculosis treatment, especially in HIV-positive patients. This has the potential to reduce complications, and is likely to decrease mortality as well. However, the foremost obstacle to rapid tuberculosis treatment initiation in these patients remains lack of rapid accurate diagnostic tests and validated rapid diagnostic algorithms that can be utilised even in settings where expertise is limited.[303,304] No standalone test is sufficiently accurate to diagnose HIV-associated tuberculosis in all patients and an evidence-based algorithm incorporating point-of-care diagnostic tests is needed.

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CHAPTER 3

Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals

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Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals (Review)

Van Hoving DJ, Griesel R, Meintjes G, Takwoingi Y, Maartens G, Ochodo EA

Van Hoving DJ, Griesel R, Meintjes G, Takwoingi Y, Maartens G, Ochodo EA.
Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals.
Cochrane Database of Systematic Reviews 2019, Issue 9. Art. No.: CD012777.
DOI: 10.1002/14651858.CD012777.pub2.

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[Diagnostic Test Accuracy Review]

Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals

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Editorial group: Cochrane Infectious Diseases Group
Publication status and date: New, published in Issue 9, 2019.

Citation: Van Hoving DJ, Griesel R, Meintjes G, Takwoingi Y, Maartens G, Ochodo EA. Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals. *Cochrane Database of Systematic Reviews* 2019, Issue 9. Art. No.: CD012777. DOI: 10.1002/14651858.CD012777.pub2.

ABSTRACT

Background

Accurate diagnosis of tuberculosis in people living with HIV is difficult. HIV-positive individuals have higher rates of extrapulmonary tuberculosis and the diagnosis of tuberculosis is often limited to imaging results. Ultrasound is such an imaging test that is widely used as a diagnostic tool (including point-of-care) in people suspected of having abdominal tuberculosis or disseminated tuberculosis with abdominal involvement.

Objectives

To determine the diagnostic accuracy of abdominal ultrasound for detecting abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals.

To investigate potential sources of heterogeneity in test accuracy, including clinical setting, ultrasound training level, and type of reference standard.

Search methods

We searched for publications in any language up to 4 April 2019 in the following databases: MEDLINE, Embase, BIOSIS, Science Citation Index Expanded (SCI-EXPANDED), Social Sciences Citation Index (SSCI), Conference Proceedings Citation Index- Science (CPCI-S), and also ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform to identify ongoing trials.

Selection criteria

We included cross-sectional, cohort, and diagnostic case-control studies (prospective and retrospective) that compared the result of the index test (abdominal ultrasound) with one of the reference standards. We only included studies that allowed for extraction of numbers of true positives (TPs), true negatives (TNs), false positives (FPs), and false negatives (FNs). Participants were HIV-positive individuals aged

15 years and older. A higher-quality reference standard was the bacteriological confirmation of *Mycobacterium tuberculosis* from any clinical specimen, and a lower-quality reference standard was a clinical diagnosis of tuberculosis without microbiological confirmation. We excluded genitourinary tuberculosis.

Data collection and analysis

For each study, two review authors independently extracted data using a standardized form. We assessed the quality of studies using a tailored Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. We used the bivariate model to estimate pooled sensitivity and specificity. When studies were few we simplified the bivariate model to separate univariate random-effects logistic regression models for sensitivity and specificity. We explored the influence of the type of reference standard on the accuracy estimates by conducting separate analyses for each type of reference standard. We assessed the certainty of the evidence using the GRADE approach.

Main results

We included 11 studies. The risks of bias and concern about applicability were often high or unclear in all domains. We included six studies in the main analyses of any abnormal finding on abdominal ultrasound; five studies reported only individual lesions.

The six studies of any abnormal finding were cross-sectional or cohort studies. Five of these (83%) were conducted in low- or middle-income countries, and one in a high-income country. The proportion of participants on antiretroviral therapy was none (1 study), fewer then 50% (4 studies), more than 50% (1 study), and not reported (5 studies). The first main analysis, studies using a higher-quality reference standard (bacteriological confirmation), had a pooled sensitivity of 63% (95% confidence interval (CI) 43% to 79%; 5 studies, 368 participants; very low-certainty evidence) and a pooled specificity of 68% (95% CI 42% to 87%; 5 studies, 511 participants; very low-certainty evidence). If the results were to be applied to a hypothetical cohort of 1000 people with HIV where 200 (20%) have tuberculosis then:

- About 382 individuals would have an ultrasound result indicating tuberculosis; of these, 256 (67%) would be incorrectly classified as having tuberculosis (false positives).

- Of the 618 individuals with a result indicating that tuberculosis is not present, 74 (12%) would be incorrectly classified as not having tuberculosis (false negatives).

In the second main analysis involving studies using a lower-quality reference standard (clinical diagnosis), the pooled sensitivity was 68% (95% CI 45% to 85%; 4 studies, 195 participants; very low-certainty evidence) and the pooled specificity was 73% (95% CI 41% to 91%; 4 studies, 202 participants; very low-certainty evidence).

Authors' conclusions

In HIV-positive individuals thought to have abdominal tuberculosis or disseminated tuberculosis with abdominal involvement, abdominal ultrasound appears to have 63% sensitivity and 68% specificity when tuberculosis was bacteriologically confirmed. These estimates are based on data that is limited, varied, and low-certainty.

The low sensitivity of abdominal ultrasound means clinicians should not use a negative test result to rule out the disease, but rather consider the result in combination with other diagnostic strategies (including clinical signs, chest x-ray, lateral flow urine lipoarabinomannan assay (LF-LAM), and Xpert MTB/RIF). Research incorporating the test into tuberculosis diagnostic algorithms will help in delineating more precisely its value in diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement.

26 September 2019

Up to date

All studies incorporated from most recent search

All studies identified during the most recent search (4 Apr, 2019) have been incorporated in the review, and one ongoing study identified

PLAIN LANGUAGE SUMMARY

Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in people with HIV

Why is improving tuberculosis diagnosis in people with HIV important?

Diagnosing active tuberculosis in people living with HIV is challenging. People with advanced immunosuppression have high rates of extrapulmonary tuberculosis (tuberculosis outside the lungs).

What is the aim of this review?

The aim of this review is to find out how accurate an ultrasound examination of the abdomen (abdominal ultrasound) is for diagnosing tuberculosis in people with HIV suspected of having tuberculosis in the abdomen or widespread tuberculosis (disseminated tuberculosis) involving the abdomen.

What was studied in the review?

Abdominal ultrasound can be done after other tests (e.g. the chest x-ray did not indicate tuberculosis) or it can be done before other tests in people suspected of having tuberculosis. This review focuses on situations where other tests are not available.

What are the main results in this review?

We found 11 studies, but only six were relevant for the main analyses. The six studies were divided into two groups. In the first group tuberculosis was diagnosed by identifying the organism causing tuberculosis from any specimen (microbiological confirmation). For the second group, tuberculosis was diagnosed when healthcare personnel suspected tuberculosis and started anti-tuberculosis treatment, but without identifying the organism (clinical diagnosis). Three studies provided results for both groups.

The review included five studies (a total of 879 participants) with microbiological confirmation. The results showed that if abdominal ultrasound were to be used in a group of 1000 people with HIV where 200 (20%) have tuberculosis then:

- About 382 individuals would have an ultrasound result indicating tuberculosis; of these, 256 (67%) would be incorrectly classified as having tuberculosis (false positives).

- Of the 618 individuals with a result indicating that tuberculosis is not present, 74 (12%) would be incorrectly classified as not having tuberculosis (false negatives).

How reliable are the results of the studies in this review?

Microbiological confirmation is likely to be a reliable method for deciding whether people really have tuberculosis; clinical diagnosis is likely to be less trustworthy. We found problems in both groups with how studies were conducted. Decreasing the number of false positive results may make abdominal ultrasound appear more accurate than it is. Numbers shown are an average across studies. As estimates from individual studies varied, we cannot be sure that abdominal ultrasound will always produce these results. Not enough people have been studied for us to be confident about the results.

Who do the results of the review apply to?

Studies included in the main analyses were done in Cambodia, India, South Africa, South Sudan, Spain, and Tanzania. Reasons for including people differed between the studies. Four studies used trained radiologists (specialists) or sonographers; two used doctors trained in ultrasound (non-specialists), and two included people without any suspicion of tuberculosis. Across the studies, the percentage of people with a final diagnosis of tuberculosis ranged from 18% to 64%.

What are the implications of this review?

If the test is used to rule in the disease in the absence of other evidence, then, the chance of diagnosing someone with tuberculosis when they actually do not have it is high. Chances of missing a diagnosis of tuberculosis when the test is positive are lower, but a negative test alone is probably insufficient to rule out the disease. These findings should be considered when deciding whether or not to use abdominal ultrasound to test for tuberculosis involving the abdomen and how to interpret the results in the context of other clinical and diagnostic test information.

How up-to-date is this review?

The review authors searched for studies up to 4 April 2019.

SUMMARY OF FINDINGS

Summary of findings 1. Summary of findings for abdominal ultrasound (any abnormality)

Review question: Should abdominal ultrasound be used to diagnose abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals?

Patient or population: HIV-positive individuals

Setting: Healthcare facility

Index test: Abdominal ultrasound

Reference standard: We considered two reference standards. The higher-quality reference standard was bacteriological confirmation of *M tuberculosis* (any clinical specimen including (i) at least one specimen culture positive for *M tuberculosis*, (ii) microscopic identification of acid-fast bacilli on stained sputum smears, lymph node aspirate, or any other specimen; or iii) Xpert MTB/RIF positive). The lower-quality reference standard was clinical diagnosis of TB without microbiological confirmation (including cases diagnosed on the basis of: i) suggestive histology (necrotizing granulomatous inflammation), ii) x-ray abnormalities, iii) extrapulmonary cases without laboratory confirmation, and iv) anti-tuberculosis therapy initiated by a healthcare practitioner for cases with a high suspicion of tuberculosis).

Threshold: Any abnormality found on abdominal ultrasound

Study design: Cross-sectional and cohort

Limitations: A small number of studies and participants were included in the analyses. Risks of bias were generally high in the patient selection domain

Test result	Number of results per 1000 HIV-positive individuals tested (95% CI)			Number of studies	Number of participants	Certainty of the evidence (GRADE)
	Prevalence 10%	Prevalence 20%	Prevalence 40%			
Bacteriological confirmation as reference standard: pooled sensitivity = 63% (95% CI 43% to 79%) and pooled specificity = 68% (95% CI 42% to 87%)						
True positives (participants correctly classified as having tuberculosis)	**63** (43 to 79)	**126** (86 to 158)	**252** (172 to 316)	5	368	⊕⊖⊖⊖ **VERY LOW** [a,b,c,d]
False negatives (participants incorrectly classified as not having tuberculosis)	**37** (21 to 57)	**74** (42 to 114)	**148** (84 to 228)			
True negatives (participants correctly classified as not having tuberculosis)	**612** (378 to 783)	**544** (336 to 696)	**408** (252 to 522)	5	511	⊕⊖⊖⊖ **VERY LOW** [b,c,e,f]

False positives (participants incorrectly classified as having tuberculosis)	**288** (117 to 522)	**256** (104 to 464)	**192** (78 to 348)

Abbreviations: CI: confidence interval
GRADE certainty of evidence (GRADEpro GDT 2015; Schünemann 2016)
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.
The table displays normalized frequencies within a hypothetical cohort of 1000 people at three different tuberculosis prevalences (pre-test probabilities): 10%, 20% and 40%. We selected prevalence values based on the range of prevalence observed across the included studies. We estimated confidence intervals based on those around the point estimates for pooled sensitivity and specificity.
Explanations
[a]Risk of bias: We rated one study at high risk for participant selection since it excluded people unable to produce sputum (Griesel 2019-h). We downgraded the certainty of the evidence by one level.
[b]Indirectness: We deemed three studies to be of high concern for applicability for receiving ultrasound in a tertiary care (referral) centre (Ndege 2019-h; Sculier 2010-h Weber 2018-h). Two studies only included asymptomatic HIV-positive participants (Bobbio 2019-l; Sculier 2010-h). We downgraded the certainty of the evidence by two levels.
[c]Inconsistency: Point estimates were substantially different between studies. We could not explain this variability and we downgraded the certainty of the evidence by one level.
[d]Imprecision:Three studies had a wide 95% CI for true positives and false negatives (Dominguez-Castellano 1998-h; Sculier 2010-h; Weber 2018-h). We downgraded the certainty of the evidence by one level.
[e]Risk of bias: All studies used a higher-quality reference standard. We did not downgrade the certainty of the evidence.
[f]Imprecision: Two studies had a wide 95% CI for true negatives and false positives (Dominguez-Castellano 1998-h; Weber 2018-h). We downgraded the certainty of the evidence by one level.

BACKGROUND

Target condition being diagnosed

Tuberculosis is caused by the bacillus *Mycobacterium tuberculosis.* Although it usually affects the lungs (pulmonary tuberculosis), it can also spread to other body sites (extrapulmonary tuberculosis) (WHO 2018).

An estimated 10 million people were diagnosed with tuberculosis in 2017, and 1.6 million people died from tuberculosis (WHO 2018). Resource-limited countries are the most affected; for example, the African region of the World Health Organization (WHO) had the second highest estimated number of incident cases (2.5 million), but the highest incidence rate (237 versus 133 globally) and mortality rate (HIV-positive: 24 versus 4.0 globally; HIV-negative: 39 versus 17 globally) per 100,000 people (WHO 2018).

The probability of developing tuberculosis is higher among people living with HIV. Approximately 920,000 people diagnosed worldwide with tuberculosis in 2017 were HIV-positive (WHO 2018), with HIV prevalence among incident tuberculosis cases in the African region at 27% (WHO 2018).

The worldwide case detection rate in 2016 was only an estimated 61% (WHO 2017), reflecting a mixture of under-reporting of detected cases and underdiagnosis of tuberculosis. The low detection rate possibly relates to delays in diagnosis, which could be from problems with tuberculosis diagnostic tests (accuracy and availability), the negative influence of HIV infection on the performance of diagnostic tests, and HIV co-infection and the opportunistic conditions that complicate it (Palmieri 2002; Dawson 2010; Padmapriyadarsini 2011; Horne 2019; WHO 2017). Other factors might be weaknesses in health systems and broader social and economic influences (for example, undernourishment, poverty) on the tuberculosis epidemic (WHO 2017). The diagnosis of active tuberculosis in HIV-positive people with advanced immunosuppression is challenging due to more atypical clinical presentations; other opportunistic pulmonary infections with similar presentations; a high proportion of negative sputum smears; and high rates of extrapulmonary tuberculosis (Sharma 2005). This is illustrated by autopsy studies, which indicate a very high proportion of tuberculosis in HIV-positive adults (32% to 47%); almost half (46%) of adult tuberculosis cases remained undiagnosed before death (Gupta 2015).

An estimated 14% of the 6.4 million incident tuberculosis cases in 2017 were extrapulmonary tuberculosis (WHO 2018). In people with HIV-associated tuberculosis, extrapulmonary tuberculosis accounts for up to 50% of all tuberculosis cases (Sharma 2004b; Kingkaew 2009; Namme 2013), and is often disseminated (two or more non-contiguous sites simultaneously infected) (Sharma 2005). Any anatomical site can be involved, but the commonest sites are the lymph nodes, pleura, meninges, and the abdominal cavity (Sharma 2005). Many terms are used in the literature to describe tuberculosis in the abdominal cavity. For the purposes of this Cochrane Review, we use the terms abdominal tuberculosis or disseminated tuberculosis with abdominal involvement, excluding genitourinary tuberculosis. Many abdominal structures can be affected in abdominal tuberculosis or disseminated tuberculosis with abdominal involvement, including involvement of the gastrointestinal tract, peritoneum, omentum, mesentery, intra-abdominal lymph nodes, and solid organs (liver, spleen, pancreas) (Sharma 2004b). People often present with non-specific symptoms and signs, and a high index of suspicion is therefore needed for early diagnosis and timely management. It mimics a large number of medical and surgical conditions, including malignant neoplasms, inflammatory bowel disease, chronic liver disease, and other gastrointestinal infections (Jadvar 1997).

Index test(s)

Many HIV-positive people with low CD4 counts have abdominal tuberculosis or disseminated tuberculosis with abdominal involvement. As sputum smears are frequently negative in HIV-associated tuberculosis, it is common clinical practice, supported by WHO guidelines, to reach a tuberculosis diagnosis on the basis of imaging results and clinical case definitions (Wilson 2006; WHO 2016). Ultrasound is such an imaging test that can be used as a diagnostic tool (Heller 2010a; Heller 2010b; Patel 2011; Giordani 2013; Sharma 2017), although the only WHO recommendation refers to the use of ultrasound to diagnose pericardial effusions (WHO 2006). Ultrasound uses sound waves to produce images of structures and organs within the body, and has traditionally been performed by trained specialists in dedicated radiology departments. However, the numerous advantages of ultrasound (e.g. rapidly performed, portable, non-invasive, repeatable, etc.) have led to many physicians in different specialties adopting ultrasound (Adhikari 2014). The use of ultrasound by trained medical professionals (non-radiologists) is particularly relevant in resource-limited settings. Computed tomography (CT) or magnetic resonance imaging (MRI) is expensive, mostly only available in tertiary-level settings, and require specially-trained personnel to perform and report these examinations. Many low-income and middle-income countries have a high tuberculosis burden (WHO 2018), but without widespread access to specialists and tertiary-level imaging. However, ultrasound machines are mostly accessible and their use by non-radiologists would be of great value.

Abdominal ultrasound (an ultrasound examination evaluating the abdominal cavity) may be useful in HIV-positive people with suspected abdominal tuberculosis or disseminated tuberculosis with abdominal involvement. Ultrasound techniques to diagnosis HIV-associated tuberculosis are easily learned by non-radiologists and quick to perform (less than 10 minutes) (Heller 2010a). The ultrasound findings are non-specific, and various other diseases may present with the same features. For example, intra-abdominal lymphadenopathy can be due to other infections (for example, cryptococcosis, histoplasmosis); lymphomas (non-Hodgkin's lymphoma and Hodgkin's lymphoma); and Kaposi's sarcoma (Martin-Bates 1993).

Clinical pathway

Any structure or organ in the abdominal cavity (for example, gastrointestinal tract, pancreatobiliary system, peritoneum, and lymph nodes) can be affected by tuberculosis disease. The presentation varies considerably and depends on the specific organ involved (Sharma 2017); other diseases are also often mimicked (Sharma 2004a). Common presenting symptoms are abdominal pain, anorexia, bowel disturbances, fever, and weight loss. The clinical examination often reveals abdominal tenderness, ascites, and solid organ enlargement (for example, hepatomegaly, splenomegaly, or hepatosplenomegaly) (Ibrahim 2005; Mandal 2011; Sharma 2017).

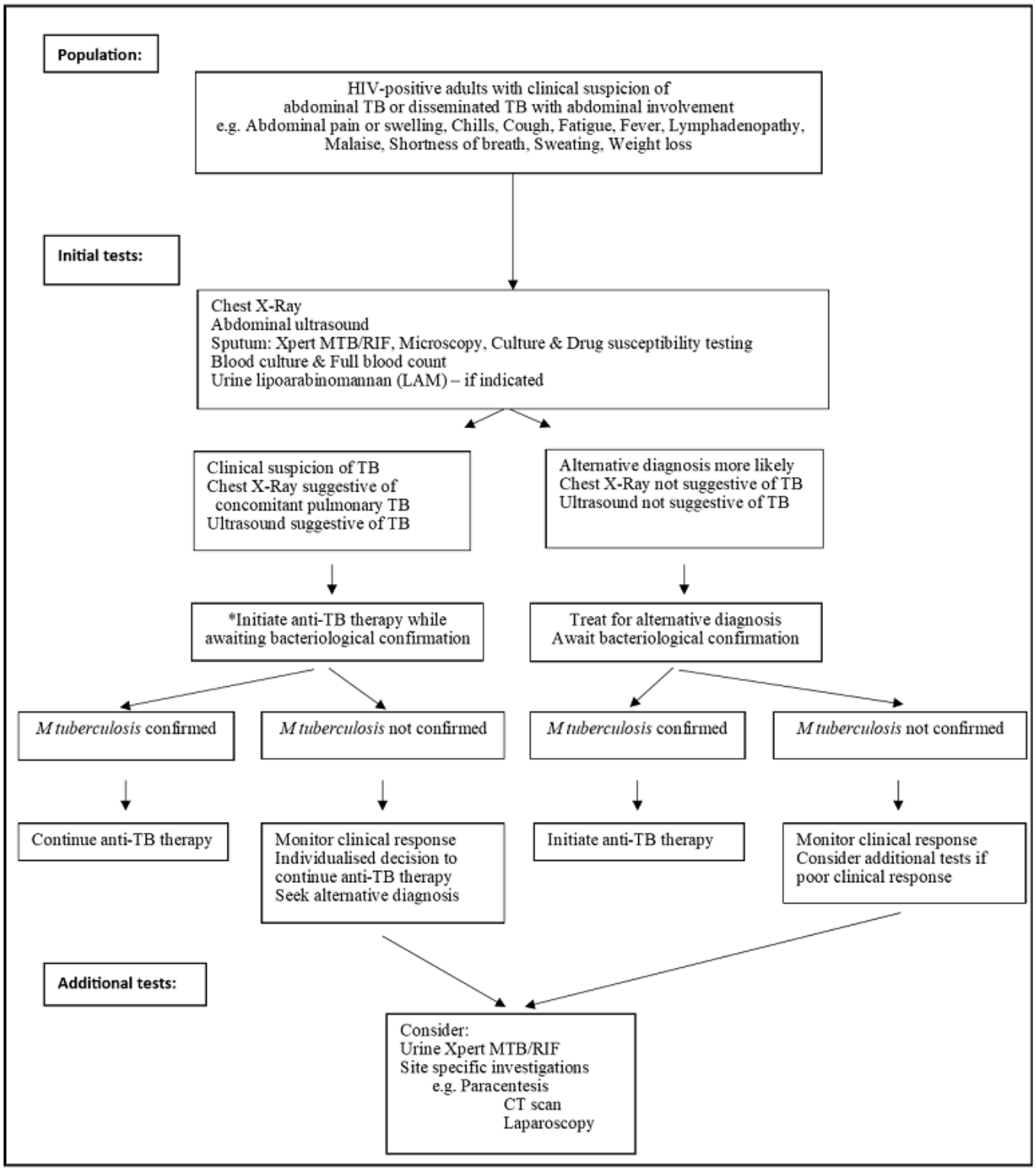
Essential diagnostic tests for individuals who are suspected of having abdominal tuberculosis or disseminated tuberculosis with abdominal involvement include a chest x-ray, sputum evaluation (if able to produce) for bacteriological confirmation of tuberculosis disease (smear or culture or Xpert MTB/RIF), and blood cultures (WHO 2013b). Urine specimens remain a convenient clinical sample for the diagnosis of tuberculosis. Although conventional tuberculosis diagnostics applied to urine specimens have limited clinical utility, the use of urinary lipoarabinomannan (LAM) has been recommended by the WHO in HIV-positive adults with advanced immunosuppression (CD4 cell count of 100 cells/µL or less) or in HIV-positive adults who are seriously ill (respiratory rate above 30/min, temperature above 39 °C, heart rate above 120/min and unable to walk unaided), regardless of their CD4 cell count (WHO 2015; Shah 2016). These tests are usually done in the primary care setting and higher.

Abdominal ultrasound has become part of the initial diagnostic work-up in adults living with HIV where abdominal tuberculosis or disseminated tuberculosis with abdominal involvement is suspected (especially in those with a low CD4 count), despite the lack of robust evidence of validity from large studies (NICE 2016). The diagnostic pathway might vary in different settings if there are ultrasound findings suggestive of tuberculosis. In resource-limited settings this might be enough evidence to initiate anti-tuberculosis treatment, but in high-resource settings it would prompt site-specific investigations which could include CT scan, paracentesis, laparoscopy, fine needle aspiration, or stool examination.

A presumptive diagnosis of abdominal tuberculosis or disseminated tuberculosis with abdominal involvement can be made in the setting of known active pulmonary tuberculosis, although fewer than half of chest radiographs are compatible with active or healed tuberculosis (Chow 2002). However, data are lacking in HIV-positive individuals.

WHO recommends immediate initiation of anti-tuberculosis therapy in people living with HIV who have clinical features of disseminated tuberculosis (WHO 2016). Bacteriological confirmation of tuberculosis from any specimen remains important, but treatment should not be delayed until results become available (Figure 1). People started on anti-tuberculosis therapy without bacteriological confirmation should be assessed after one month to evaluate the clinical response to treatment. They should be re-assessed and an alternative diagnosis sought if there is no clinical improvement.

Figure 1. Diagnostic workup of HIV-positive individuals with suspected abdominal tuberculosis or disseminated tuberculosis with abdominal involvement



HIV: Human Immunodeficiency Virus; TB: Tuberculosis

* In high resource settings, this would most likely prompt additional site-specific investigations (additional tests) and not immediate initiation of treatment

Role of index test(s)

Abdominal ultrasound is often combined with existing tests such as chest x-ray, haemoglobin, etc. to reach a diagnosis of abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in clinical practice. However, all the existing tests that could inform a confirmed diagnosis may not always be available.

Alternative test(s)

Ascitic fluid analysis suggestive of abdominal tuberculosis or disseminated tuberculosis with abdominal involvement includes a leukocyte count of 150 to 4000 cells/mL, which consists predominantly of lymphocytes (Sharma 2004a; Sanai 2005). The ascitic fluid is usually an exudate with the protein content greater than 30 g/L and the serum-ascites albumin gradient (SAAG) less than 11 g/L (Sharma 2004a; Sanai 2005). Adenosine deaminase activity (ADA) of ascitic fluid (> 39 IU/L) is also suggestive of abdominal tuberculosis (Riquelme 2006), while the ascites to blood glucose ratio is usually less than 0.96 (Wilkins 1984). Acid-fast bacilli (AFB) smear and culture of ascitic fluid also have disappointingly low yields (Chow 2003), while Xpert MTB/RIF for peritoneal tuberculosis using peritoneal fluid has a pooled sensitivity of 59% (credible interval (CrI) 45 to 74) and a pooled specificity of 98% (CrI 96 to 99) (Kohli 2018).

Different imaging modalities can be useful to diagnose abdominal tuberculosis or disseminated tuberculosis with abdominal involvement. Abdominal x-rays are of very limited value, but can assist with the diagnosis of intestinal obstruction and perforation (Debi 2014). CT features include thickening of the peritoneum, omentum, and bowel wall; lymph nodes (especially if these have hypodense centres due to caseous necrosis); and ascites with strands, debris, and fine septations (Sharma 2004a; Lee 2012). The excellent soft tissue resolution and multiplanar acquisition of MRI have resulted in it being used to evaluate solid organs and lymphadenopathy (Joshi 2014). However, CT and especially MRI are expensive and access is very limited in resource-limited settings. Barium studies may be useful for intrinsic bowel abnormalities such as strictures, fistulae, and erosions (Sharma 2004a; Debi 2014).

Colonoscopy with biopsy is a useful non-operative diagnostic procedure to obtain material for histology and culture (Kim 1998). Mucosal nodules and transverse ulcers in the bowel are very suggestive of tuberculosis, with definitive results obtained from tissue sent for polymerase chain reaction (PCR), Ziehl-Neelsen stain, and culture (Kim 1998; Sharma 2004a). Laparoscopy is useful in two ways: (i) it allows visual inspection of the peritoneum; and (ii) it permits specimens for histology, AFB stain, and culture to be obtained. However, imaging modalities as described above provide a safer, less invasive and less expensive alternative, but may be less specific since they are unable to provide a definitive microbiological diagnosis (Sanai 2005).

Most studies relating to the diagnosis of tuberculosis were done in HIV-negative people and the true diagnostic accuracy of the above tests in those living with HIV remains uncertain. Expanded clinical case definitions were developed to diagnose smear-negative tuberculosis in HIV-positive people living in resource-limited settings (Wilson 2006), including abdominal tuberculosis or disseminated tuberculosis with abdominal involvement (Wilson 2006; WHO 2016). For example, a person presenting with symptoms and signs suggestive of abdominal tuberculosis or disseminated tuberculosis with abdominal involvement can be started on anti-tuberculosis treatment if the ascitic fluid consists of a lymphocytic exudate along with either a fever of 38 °C or more on two occasions or drenching sweats for more than two weeks (Wilson 2006). In this study, the positive predictive value for abdominal lymph nodes diagnosed by ultrasound was 94% (Wilson 2006). Augmented by the use of objective criteria to monitor response to treatment within the first eight weeks, this approach has reasonable diagnostic accuracy (Wilson 2006).

Rationale

Multiple studies of various quality and designs have looked at the use of abdominal ultrasound as a diagnostic tool for abdominal tuberculosis or disseminated tuberculosis with abdominal involvement, with varying sensitivity, specificity, and predictive values for diagnosing tuberculosis (Monill-Serra 1997-l; Mugala 2006; Sinkala 2009-l; Sculier 2010-h; Patel 2011). Abdominal ultrasound may be used alone, in combination with existing tests (chest radiograph, full blood count), or as an add-on following negative results from existing tests (smear microscopy, sputum Xpert MTB/RIF, sputum culture, chest radiograph). The role of abdominal ultrasound as an add-on test is an important clinical question because it may reflect the way that abdominal ultrasound is used in practice, especially in resource-limited settings. However, after a scoping search, we did not find any studies that have evaluated the accuracy of ultrasound as an add-on test or in combination with other tests.

OBJECTIVES

To determine the diagnostic accuracy of abdominal ultrasound for detecting abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals.

Secondary objectives

To investigate potential sources of heterogeneity in test accuracy, including clinical setting, ultrasound training level, and type of reference standard.

METHODS

Criteria for considering studies for this review

Types of studies

We included cross-sectional, cohort, or diagnostic case-control studies (prospective and retrospective) that compared the result of the index test (abdominal ultrasound) with one of the reference standards (see Reference standards). Case-control studies may overestimate sensitivity and specificity, but we include them because we anticipated identifying few relevant studies. We only included studies in which the study authors reported the numbers of true positives (TPs), true negatives (TNs), false positives (FPs), and false negatives (FNs), or where we were able to derive the data from reported statistics. We also wrote to all study authors where data were missing. We excluded descriptive studies (for example, case series).

Participants

We included all HIV-positive individuals (aged 15 years and older) with a clinical suspicion of abdominal tuberculosis or disseminated tuberculosis with abdominal involvement (excluding genitourinary tuberculosis), who were investigated using an abdominal ul-

trasound examination. We also considered studies that included confirmed cases of abdominal tuberculosis and controls. We did not place any restrictions on setting. Although abdominal ultrasound can be used to evaluate children, microbiological confirmation of tuberculosis is far more difficult than in adults, and so we excluded children where possible.

Index tests

We included studies that evaluated the accuracy of abdominal ultrasound. We did not place any restrictions on the type of ultrasound machine used or the qualification of the person performing the ultrasound, but recorded these data. A positive result was an ultrasound scan with abnormal findings suggestive of abdominal tuberculosis or disseminated tuberculosis with abdominal involvement, including, but not limited to, free abdominal fluid, abdominal lymph nodes, hepatic lesions, and splenic lesions. A negative result was an ultrasound scan with no abnormal findings.

Target conditions

Active disease due to *M tuberculosis* – either abdominal tuberculosis or disseminated tuberculosis with abdominal involvement.

Reference standards

We used a hierarchy of reference standards. The reference standard diagnosis typically relates to microbiological confirmation (microscopy or culture), although histopathological characteristics strongly support a diagnosis of active tuberculosis in clinically and epidemiologically appropriate settings. Xpert MTB/RIF assay (an automated nucleic acid amplification test) can also identify *M tuberculosis.* A clinical diagnosis of tuberculosis is sometimes used in the absence of confirmative tests, for example, probable tuberculosis can be defined as the clinical picture of tuberculosis without objective diagnostic tuberculosis criteria and treated for tuberculosis by the attending physician. Although this approach is clinically useful, it is very subjective as it relies on the clinical gestalt of the treating physician. We therefore viewed it as a lower-quality reference standard.

The primary (higher-quality) reference standard was bacteriological confirmation of any clinical specimen including (i) at least one specimen culture positive for *M tuberculosis*, (ii) microscopic identification of AFB on stained sputum smears, lymph node aspirate, or any other specimen; or iii) Xpert MTB/RIF positive (WHO 2013a). We considered a positive result on any of these tests as a positive result for the microbiological (higher-quality) reference standard and a tuberculosis case, since not all of the tests might have been performed or might have a positive result. The reference standard for culture was either solid or liquid culture for *M tuberculosis* complex (Lawn 2011). The sensitivity of smear microscopy can be increased by examining more than one sample, using fluorescence microscopy, and using physical and chemical sputum processing techniques including centrifugation, sedimentation, and bleach (Steingart 2006a; Steingart 2006b). We therefore included studies that used any of these techniques.

The secondary (lower-quality) reference standard was clinical diagnosis of tuberculosis without microbiological confirmation. A clinically diagnosed tuberculosis case is one that has been diagnosed with active tuberculosis by a healthcare practitioner and where anti-tuberculosis therapy has subsequently been initiated. This definition lacks bacteriological confirmation but includes cases diagnosed on the basis of suggestive histology (necrotizing granulomatous inflammation), x-ray abnormalities, and extrapulmonary cases without laboratory confirmation (WHO 2017). Using clinical diagnosis as a reference standard could potentially bias test accuracy because abdominal ultrasound is often used to inform the clinical decision to treat for tuberculosis (incorporation bias). We included these studies, as incorporation bias had a small effect in diagnostic accuracy estimates (Rutjes 2006), and we used an adapted version of the revised tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2).

Search methods for identification of studies

Electronic searches

Vittoria Lutje (VL), the Information Specialist for the Cochrane Infectious Diseases Group (CIDG), performed literature searches up to 4 April 2019, without language restrictions. She searched MEDLINE (PubMed, 1946 to 4 April 2019); Embase (Ovid, 1947 to 4 April 2019); Biosis (Web of Science, 1926 to 4 April 2019); Science Citation Index Expanded (SCI-EXPANDED), Social Sciences Citation Index (SSCI), both 1900 to 4 April 2019, and Conference Proceedings Citation Index- Science (CPCI-S), 1990 to 4 April 2019, (all three in the Web of Science). She also searched ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform (ICTRP; apps.who.int/trialsearch/) for trials in progress. The search terms and strategy are reported in Appendix 1.

Searching other resources

We examined the reference lists of relevant reviews and studies; and searched websites of the WHO, the Stop TB Partnership, and the National Institute of Allergy and Infectious Diseases (NIAID). We also performed forward citation searching of relevant articles using the PubMed ‘related articles' feature, Google Scholar, and ISI citation indices. We also contacted study authors for additional information if we deemed it necessary.

Data collection and analysis

Selection of studies

Two review authors (DJvH and RG) independently judged study eligibility by examining the title and abstract of each article identified by the literature search and excluded obviously irrelevant studies. We obtained the full-text article if either review author considered the abstract to be potentially eligible. The two review authors independently assessed each full-text article against the predefined inclusion and exclusion criteria, as stated in the ‘Criteria for considering studies for this review' section. The two review authors resolved any disagreements by discussion. If the review authors could not reach consensus, a third review author (GrM) made the final decision. We maintained a list of all articles excluded after full-text assessment and their reasons for exclusion in the ‘Characteristics of excluded studies' table. The study selection process is also illustrated using a PRISMA flow diagram.

Data extraction and management

We developed a standardized data extraction form before two review authors (DJvH and RG) independently extracted data. The extracted data were:

1. Details of study: first author, publication year, journal, study design, inclusion/exclusion criteria

2. Characteristics of study population: age, gender, estimated tuberculosis prevalence in study setting; estimated HIV prevalence in study setting, antiretroviral therapy (ART) status
3. Reference standard: bacteriological, clinical
4. Index test: general (abdominal ultrasound normal or abnormal), specific (individual findings on ultrasound), training level of person performing the ultrasound, additional tests (and their results)
5. Details of outcome: number of indeterminate, missing or unavailable test results, number of TP, TN, FP, and FN results

We resolved any discrepancies in data extraction by discussion, and a third review author (GrM) had the final say.

Assessment of methodological quality

We used the revised tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) to assess the risks of bias and applicability of included studies (Whiting 2011). We tailored the tool to the context of the review, as shown in Appendix 2. Two review authors (DJvH and RG) independently assessed methodological quality using the tailored QUADAS-2 tool. We resolved any disagreements through consensus or by consulting a third review author (EAO). We present the results in graphs, text, and the 'Characteristics of included studies' table.

Statistical analysis and data synthesis

In our primary meta-analyses, we used the individual participant as the unit of analysis (that is, any abnormal finding versus none) and not individual ultrasound findings. Clinically, it is also useful to know the accuracy of individual ultrasound findings, as it is plausible that some findings are better indicators of tuberculosis than others. We therefore determined the accuracy of individual ultrasound findings in secondary analyses.

We only included studies that reported test thresholds to enable us to construct 2 x 2 tables and also to select an appropriate method of meta-analysis. Studies used different criteria to determine the positivity of ultrasound. For example, studies may define an ultrasound scan as positive based on the presence of any abnormal abdominal finding including (but not limited to) organ enlargement, the presence or number of hepatic or splenic lesions, or the presence or size of abdominal nodes. For the primary analysis we thus defined the threshold as the presence or absence of any abnormal lesion. In order to produce clinically meaningful results, we conducted two separate sets of primary meta-analyses by estimating the pooled sensitivity and specificity for each type of reference standard (higher quality and lower quality).

For the secondary analyses (individual lesion as unit of analysis), we did not estimate the pooled sensitivity and specificity because some studies did not report thresholds and those that did used different thresholds. We only report the range of sensitivity and specificity.

We used the number of TPs, FPs, FNs, and TNs to construct 2 x 2 tables using the criteria specified in the studies. We plotted the estimates of sensitivity and specificity from the included studies on forest plots using Review Manager 5 software (Review Manager 2014).

We used the bivariate model (Chu 2006) to estimate pooled sensitivity and specificity at common thresholds. We fitted the models using the xtmelogit command in Stata version 15.0 (StataCorp, College Station, TX, USA).

Investigations of heterogeneity

Potential sources of heterogeneity included the type of reference standard (higher quality versus lower quality), clinical setting (any setting versus tertiary/referral hospital), and ultrasound training level (radiologist versus non-radiologist). We stratified the primary analysis by the type of reference standard. Due to the small number of included studies and sample sizes we did not investigate other sources of heterogeneity.

Sensitivity analyses

We did not perform sensitivity analyses because of the small number of included studies.

Assessment of reporting bias

We did not carry out a formal assessment of publication bias.

Assessment of the certainty of the evidence

We used the GRADE approach (Schünemann 2016) and GRADEpro Guideline Development Tool (GDT) software (GRADEpro GDT 2015) to assess the certainty of the evidence (also called the quality of the evidence). We rated the certainty of the evidence as either high (not downgraded), moderate (downgraded by one level), low (downgraded by two levels), or very low (downgraded by more than two levels) for five domains: risk of bias, indirectness, inconsistency, imprecision, and publication bias. For each domain, the certainty of evidence started as high if there were high-quality observational studies (cross-sectional or cohort studies) that enrolled participants with diagnostic uncertainty. We used our judgement to classify the reason for downgrading as either serious (downgraded by one level) or very serious (downgraded by two levels).

Two review authors (DJvH and RG) discussed judgements and applied GRADE in the following way.

Risk of bias: we used the tailored QUADAS-2 to assess risks of bias.

Indirectness: we used the tailored QUADAS-2 for concerns of applicability and evaluated the studies for important differences between the populations studied (for example, age) and the setting. We made judgements on whether the differences were sufficient to lower our certainty in the results.

Inconsistency: we downgraded the certainty of the evidence for unexplained inconsistency in sensitivity and specificity estimates.

Imprecision: we considered a point estimate to be substantially different if it would alter a clinical decision. We considered the width of the CI, and whether a different clinical decision would be made if the lower or upper boundary of the CI represented the truth. We also made judgements on the imprecision of projected ranges for TP, FN, TN, and FP for a given prevalence of tuberculosis.

Publication bias: as recommended, we did not downgrade the certainty of evidence for publication bias for the following reasons (Schünemann in press). We did not detect studies done for-profit interest. Included studies had small sample sizes and accuracy estimates were low and imprecise. We did an extensive search in electronic databases and grey literature and did not identify completed studies that were unpublished. We only identified one on-

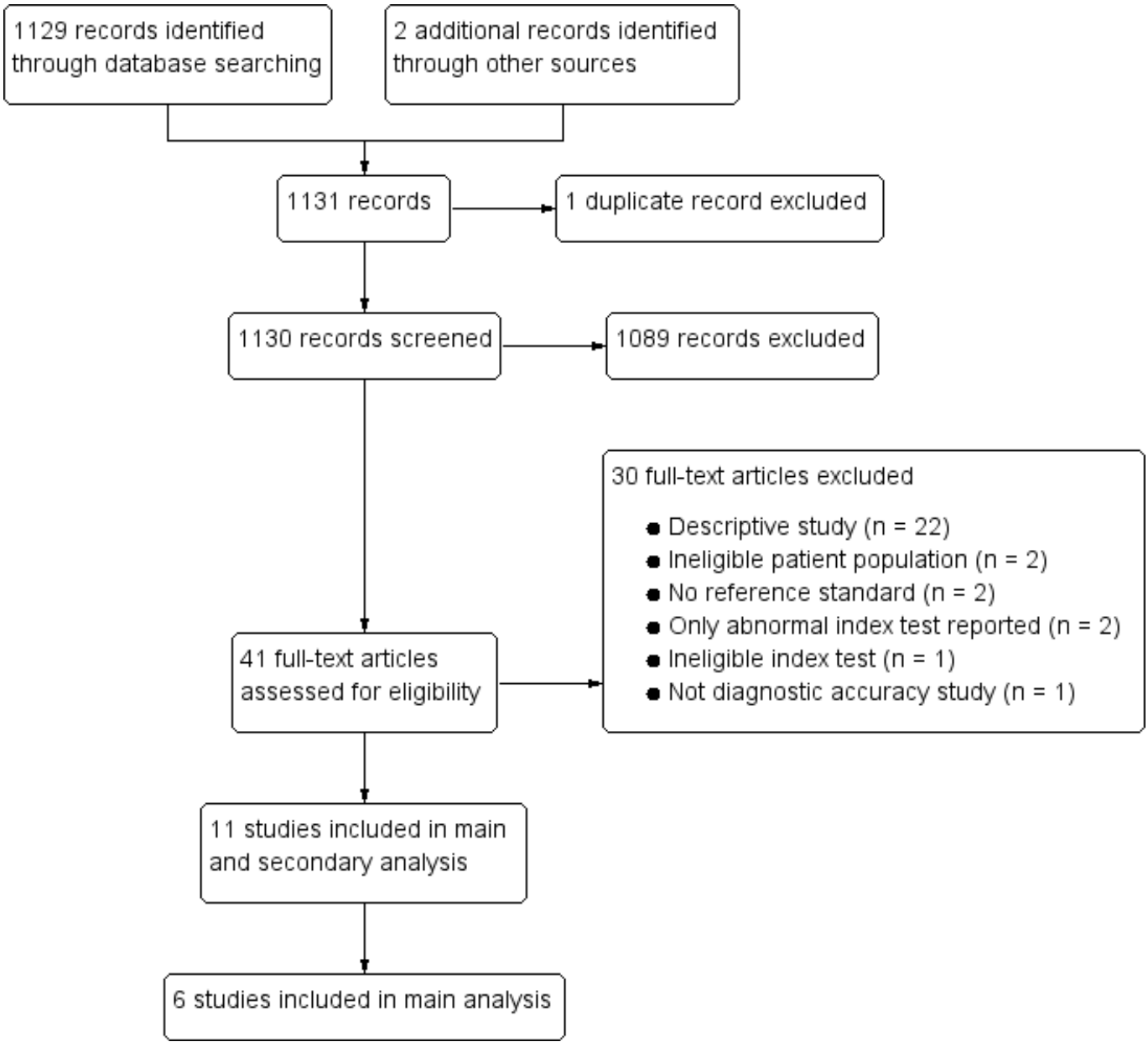
going study, the results of which are not yet registered in the Pan African Clinical Trials Registry (Trial ID: PACTR201712002829221) (PACTR201712002829221).

RESULTS

Results of the search

Our search yielded 1129 records. We identified two additional studies through contact with experts. After we removed one duplicate, we had 1130 records. We excluded 1089 records based on a review of title, abstract, or both. We retrieved 41 full-text articles and excluded 30 studies for the following reasons: descriptive study (22 studies); ineligible participant population (2 studies); no reference standard reported (2 studies); ineligible index test evaluated (1 study); only abnormal index test reported (2 studies); and not a diagnostic accuracy study (1 study). We therefore include 11 unique studies in this review (Barreiros 2008-h; Bobbio 2019-l; Dominguez-Castellano 1998-h; Griesel 2019-h; Kaneria 2009-l; Monill-Serra 1997-l; Ndege 2019-h; O'Keefe 1998-h; Sculier 2010-h; Sinkala 2009-l; Weber 2018-h). We listed the excluded studies and reasons for their exclusion in the Characteristics of excluded studies section. Figure 2 shows the flow of studies through the screening process.

Figure 2. Study flow diagram.



Three studies were conducted in low-income countries, three in lower-middle-income countries, two in upper-middle-income countries, and three in high-income countries. We noted poor reporting on the estimated prevalence of tuberculosis and HIV in

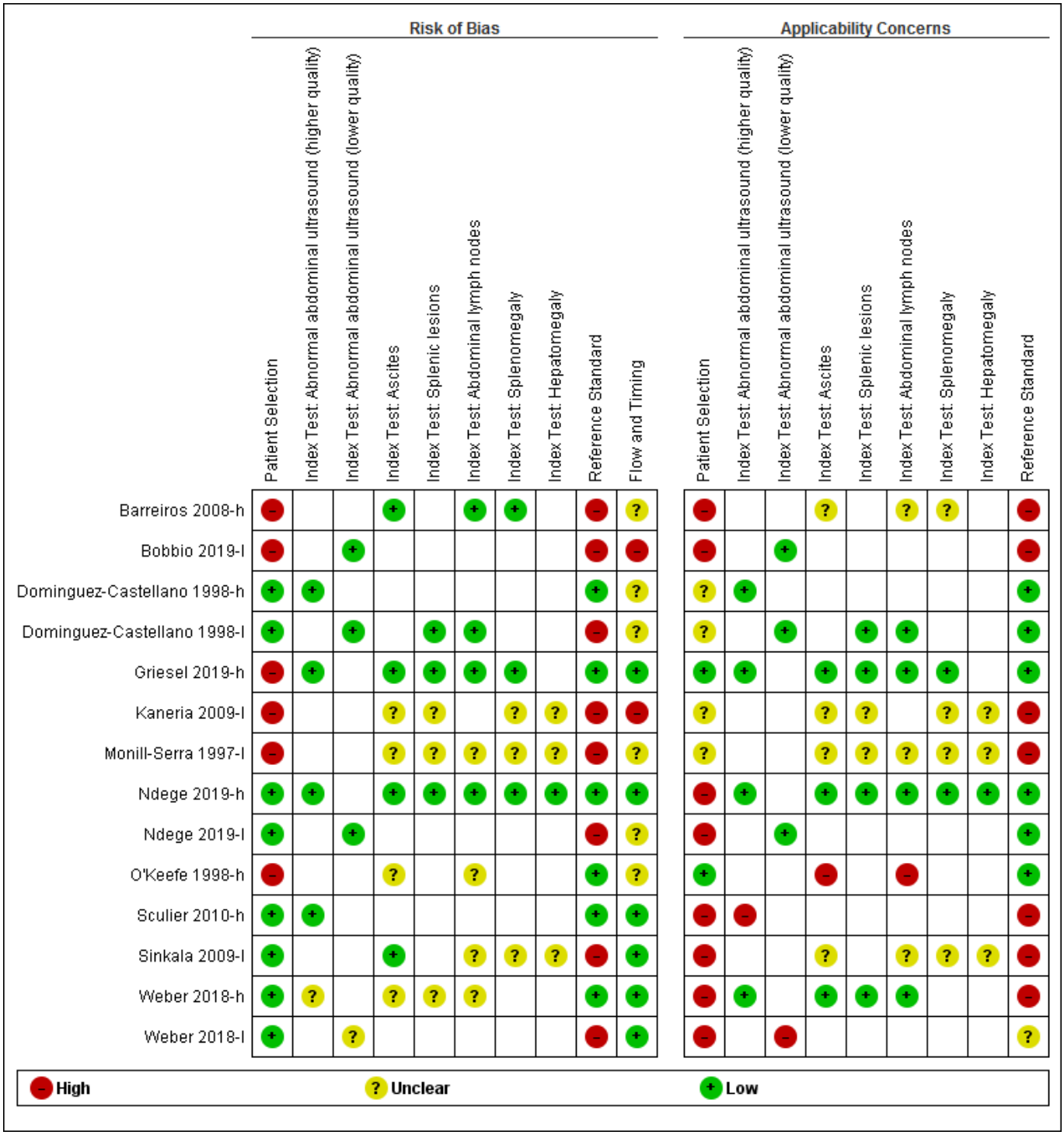
study setting, qualification of sonographer and setting in which ultrasound was performed. Studies used different criteria to determine the positivity of ultrasound (see Characteristics of included studies section). Key findings of included studies are presented in Table 1 and Table 2.

We contacted the authors of all 11 studies, of whom five responded. We received unpublished data from four studies (Weber 2018-h; Bobbio 2019-l; Griesel 2019-h; Ndege 2019-h), and one study clarified the qualification of the sonographer (O'Keefe 1998-h).

Methodological quality of included studies

We present the results of the methodological assessment of the 11 studies in Figure 3. The results are reported below separately for studies included in the primary analyses (any abnormal finding) and those included in the secondary analyses (individual lesions). Studies that used a higher-quality reference standard are indicated with the suffix 'h' and studies that used a lower-quality reference standard are indicated with the suffix 'l'.

Figure 3. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study. Suffix (h) indicates higher quality reference standard; suffix (l) indicates lower quality reference standard.



Studies of any abnormal finding included in primary analyses

Six studies with a higher-level reference standard contributed data (Figure 3). One study was considered to be at high risk of bias in the patient selection domain since it excluded people unable to produce sputum (Griesel 2019-h). Concerns about applicability (i.e. are there concerns that the included participants do not match the review question?) were deemed high in four studies, since they included asymptomatic people (Sculier 2010-h; Bobbio 2019-l) or were conducted in a referral or tertiary setting (Sculier 2010-h; Weber 2018-h; Bobbio 2019-l; Ndege 2019-h). One study was deemed of unclear concern as the setting in which the ultrasound was done was not reported (Dominguez-Castellano 1998-h). In the index test

domain, we considered one study to be at unclear risk of bias because, although the study did specify thresholds for positivity, the test was sometimes interpreted with knowledge of the results of the reference standard (Weber 2018-h). We considered the conduct and interpretation of the index test to be of high concern for applicability in one study where the ultrasound was performed by a trained radiologist (Sculier 2010-h). In the reference standard domain, all studies used a higher-quality reference standard (microbiological confirmation). We regarded two studies as being of high concern for applicability, as neither study speciated mycobacteria isolated in culture (Sculier 2010-h; Weber 2018-h). For the flow and timing domain, we considered one study to be at unclear risk of bias because the study did not report the interval between the index test and the reference standard, and it was unclear if all participants received the same reference standard (Dominguez-Castellano 1998-h).

For the main analyses (abnormal versus normal ultrasound examination), four studies with a lower-level reference standard contributed data (Figure 3). We considered one study to be at high risk of bias in the patient selection domain because it did not enrol participants consecutively or randomly (Bobbio 2019-l). Concerns about applicability (i.e. are there concerns that the included participants do not match the review question?) were deemed high in three studies since they included asymptomatic participants (Bobbio 2019-l), or the study was conducted in a referral or tertiary setting (Weber 2018-l; Bobbio 2019-l; Ndege 2019-l). We rated one study at unclear concern as the setting in which the ultrasound was done was not reported (Dominguez-Castellano 1998-l). In the index test domain, we considered one study to be at unclear risk of bias because the index test was sometimes interpreted with knowledge of the results of the reference standard (Weber 2018-l). In the reference standard domain, we considered all studies to be at high risk of bias because the studies included a lower-quality reference standard (clinical diagnosis) (Dominguez-Castellano 1998-l; Weber 2018-l; Bobbio 2019-l; Ndege 2019-l). We rated one study at unclear concern for applicability since it is unclear whether all clinically diagnosed participants improved on anti-tuberculosis treatment (Weber 2018-l). In terms of the flow and timing domain, we considered one study to be at unclear risk of bias because the study did not report the interval between the index test and the reference standard, and it was unclear if all participants received the same reference standard (Dominguez-Castellano 1998-l). We judged one study to be at high risk of bias because not all participants received a reference standard and not all participants received the same reference standard (Bobbio 2019-l).

Studies of individual lesions included in secondary analyses

Nine studies contributed data (Figure 3). In the patient selection domain, we deemed five studies (56%) to be at high risk of bias because: i) three studies used a case-control design (Monill-Serra 1997-l; Barreiros 2008-h Kaneria 2009-l); ii) one study excluded patients with a CD4 cell count of 200 or more (O'Keefe 1998-h); and iii) one study excluded patients unable to produce sputum (Griesel 2019-h). For applicability, we judged four studies (44%) to be at high concern since one study included HIV-negative participants (Barreiros 2008-h), and the ultrasound examination was performed in a tertiary or referral centre in three studies (Sinkala 2009-l; Weber 2018-h; Ndege 2019-h). We rated three studies at unclear concern as the setting in which the ultrasound was done was not reported (Monill-Serra 1997-l; Dominguez-Castellano 1998-l; Kaneria 2009-l). In the index test domain we judged five studies (56%) to be at unclear risk of bias because four studies did not specify (or it was unclear) whether index test results were interpreted without knowledge of the results of the reference standard (Monill-Serra 1997-l; O'Keefe 1998-h; Kaneria 2009-l; Weber 2018-h), and three studies did not report prespecified thresholds (O'Keefe 1998-h; Kaneria 2009-l; Sinkala 2009-l). We considered the conduct and interpretation of the index test to be of high concern for applicability in one study where the ultrasound was performed by a trained radiologist (O'Keefe 1998-h); we rated four studies at unclear concern since we were not able to make a decision on the qualification of the person performing the index tests (Monill-Serra 1997-l; Barreiros 2008-h; Kaneria 2009-l; Sinkala 2009-l). Five studies (56%) used a lower-quality reference standard and were deemed at high risk of bias in the reference standard domain (Monill-Serra 1997-l; Dominguez-Castellano 1998-l; Barreiros 2008-h; Kaneria 2009-l; Sinkala 2009-l). We rated five studies at high concern for applicability for the reference standard since mycobacteria isolated in culture were not speciated (Monill-Serra 1997-l; Barreiros 2008-h; Kaneria 2009-l; Sinkala 2009-l; Weber 2018-h). For the flow and timing domain, we considered one study to be at high risk of bias because not all participants received a reference standard and not all participants received the same reference standard (Kaneria 2009-l). Four studies were deemed to be at unclear risk of bias since: i) three studies did not report the interval between the index test and the reference standard, and it was unclear if all participants received the same reference standard (Monill-Serra 1997-l; Dominguez-Castellano 1998-l; Barreiros 2008-h); and ii) one study did not report the interval between the index test and the reference standard, and not all participants received the same reference standard (O'Keefe 1998-h).

Findings

For the diagnostic accuracy of abdominal ultrasound (main and secondary analyses), the 11 studies included 1319 participants. The median number of participants in the studies was 100 (interquartile range (IQR) 58 to 134). The proportion of tuberculosis cases in the non-case-control studies ranged from 17.5% (Sculier 2010-h) to 71.0% (Sinkala 2009-l), median 40.6% (IQR 27.5 to 53.7). Table 1 present key characteristics for each of the 11 studies. Three studies used a case-control design (Monill-Serra 1997-l; Barreiros 2008-h; Kaneria 2009-l) and eight studies used cross-sectional or cohort design (Dominguez-Castellano 1998-h; O'Keefe 1998-h; Sinkala 2009-l; Sculier 2010-h; Weber 2018-h; Bobbio 2019-l; Griesel 2019-h; Ndege 2019-h). Eight studies (73%) were conducted in low-income or middle-income countries, while the remaining three studies were conducted in high-income countries. Results of the primary and secondary analyses are summarized in Table 3.

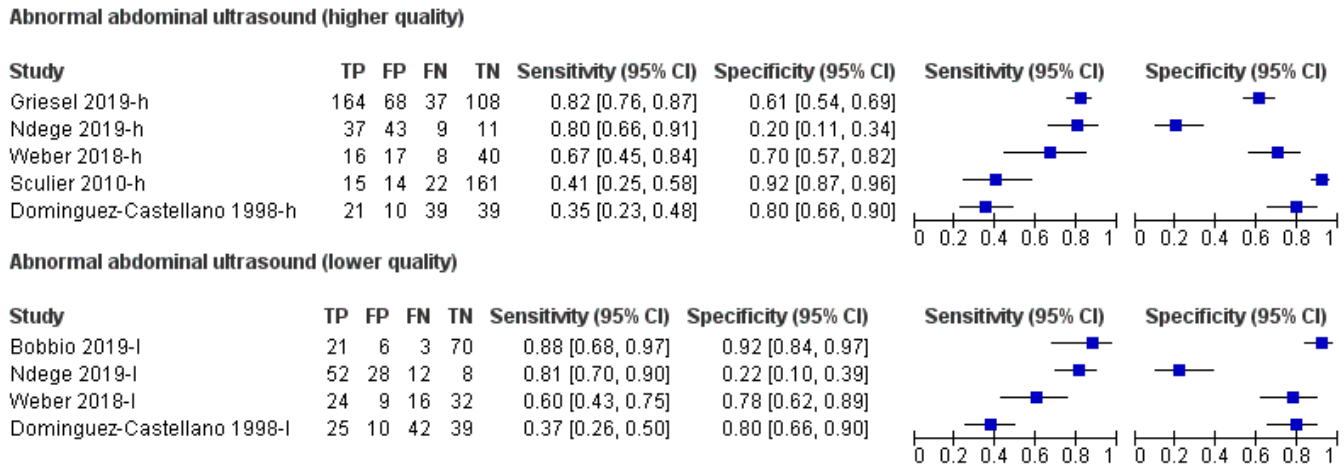
I. Any abnormal abdominal ultrasound finding for tuberculosis detection

We included six of the 11 studies in the primary analyses (Dominguez-Castellano 1998-h; Dominguez-Castellano 1998-l; Sculier 2010-h; Weber 2018-h; Weber 2018-l; Bobbio 2019-l; Griesel 2019-h; Ndege 2019-h; Ndege 2019-l); three studies provided data for each type of reference standard.

Five studies (879 participants) used a higher-quality reference standard (Dominguez-Castellano 1998-h; Sculier 2010-h; Weber 2018-h; Griesel 2019-h; Ndege 2019-h). Study estimates of sensitivity and specificity ranged from 35% to 82% and from 20% to 92%. The

pooled sensitivity and specificity were 63% (95% CI 43% to 79%) and 68% (95% CI 42% to 87%), respectively (Figure 4).

Figure 4. Forest plot of abdominal ultrasound for detecting abdominal TB or disseminated TB with abdominal involvement. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Suffix (h) indicates higher quality reference standard; suffix (l) indicates lower quality reference standard.



Four studies (397 participants) used a lower-quality reference standard (Dominguez-Castellano 1998-l; Weber 2018-l; Bobbio 2019-l; Ndege 2019-l). Sensitivity estimates ranged from 37% to 88% and specificity estimates ranged from 22% to 92% (Figure 4). The pooled sensitivity and specificity were 68% (95% CI 45% to 85%) and 73% (95% CI 41% to 91%), respectively.

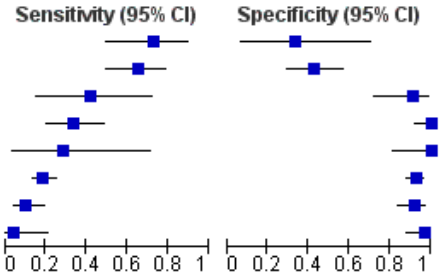
II. Splenic lesions on abdominal ultrasound for tuberculosis detection

We included six studies involving 916 participants, of whom 477 had tuberculosis (Monill-Serra 1997-l; Dominguez-Castellano 1998-l; Kaneria 2009-l; Weber 2018-h; Griesel 2019-h; Ndege 2019-h). Sensitivity estimates were very heterogeneous and ranged from 13% to 62%. Specificity estimates were less heterogeneous and ranged from 86% to 100% (Figure 5).

Figure 5. Forest plot of individual findings on ultrasound for detecting abdominal TB or disseminated TB with abdominal involvement. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Suffix (h) indicates higher quality reference standard; suffix (l) indicates lower quality reference standard.

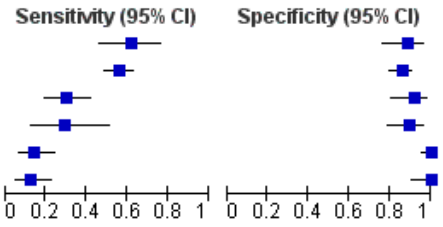
Ascites

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Sinkala 2009-l	16	6	6	3	0.73 [0.50, 0.89]	0.33 [0.07, 0.70]
Ndege 2019-h	30	31	16	23	0.65 [0.50, 0.79]	0.43 [0.29, 0.57]
O'Keefe 1998-h	5	2	7	21	0.42 [0.15, 0.72]	0.91 [0.72, 0.99]
Kaneria 2009-l	15	0	30	45	0.33 [0.20, 0.49]	1.00 [0.92, 1.00]
Barreiros 2008-h	2	0	5	18	0.29 [0.04, 0.71]	1.00 [0.81, 1.00]
Griesel 2019-h	38	13	163	163	0.19 [0.14, 0.25]	0.93 [0.88, 0.96]
Monill-Serra 1997-l	8	6	68	70	0.11 [0.05, 0.20]	0.92 [0.84, 0.97]
Weber 2018-h	1	2	23	55	0.04 [0.00, 0.21]	0.96 [0.88, 1.00]



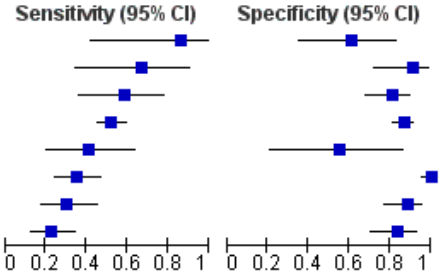
Splenic lesions

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Kaneria 2009-l	28	5	17	40	0.62 [0.47, 0.76]	0.89 [0.76, 0.96]
Griesel 2019-h	113	25	88	151	0.56 [0.49, 0.63]	0.86 [0.80, 0.91]
Dominguez-Castellano 1998-l	20	4	47	45	0.30 [0.19, 0.42]	0.92 [0.80, 0.98]
Weber 2018-h	7	6	17	51	0.29 [0.13, 0.51]	0.89 [0.78, 0.96]
Monill-Serra 1997-l	11	0	65	76	0.14 [0.07, 0.24]	1.00 [0.95, 1.00]
Ndege 2019-h	8	0	56	36	0.13 [0.06, 0.23]	1.00 [0.90, 1.00]



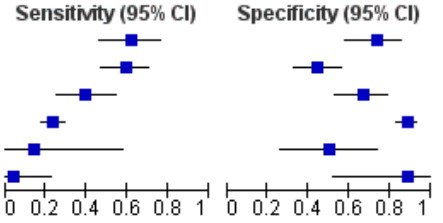
Abdominal lymph nodes

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Barreiros 2008-h	6	7	1	11	0.86 [0.42, 1.00]	0.61 [0.36, 0.83]
O'Keefe 1998-h	8	2	4	21	0.67 [0.35, 0.90]	0.91 [0.72, 0.99]
Weber 2018-h	14	11	10	46	0.58 [0.37, 0.78]	0.81 [0.68, 0.90]
Griesel 2019-h	105	23	96	153	0.52 [0.45, 0.59]	0.87 [0.81, 0.92]
Sinkala 2009-l	9	4	13	5	0.41 [0.21, 0.64]	0.56 [0.21, 0.86]
Monill-Serra 1997-l	27	0	49	76	0.36 [0.25, 0.47]	1.00 [0.95, 1.00]
Ndege 2019-h	14	6	32	48	0.30 [0.18, 0.46]	0.89 [0.77, 0.96]
Dominguez-Castellano 1998-l	15	8	52	41	0.22 [0.13, 0.34]	0.84 [0.70, 0.93]



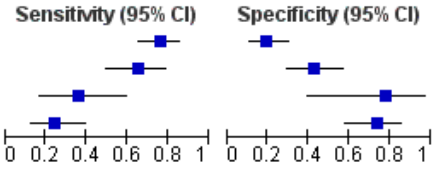
Splenomegaly

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Kaneria 2009-l	28	12	17	33	0.62 [0.47, 0.76]	0.73 [0.58, 0.85]
Monill-Serra 1997-l	45	42	31	34	0.59 [0.47, 0.70]	0.45 [0.33, 0.57]
Ndege 2019-h	18	18	28	36	0.39 [0.25, 0.55]	0.67 [0.53, 0.79]
Griesel 2019-h	47	20	154	156	0.23 [0.18, 0.30]	0.89 [0.83, 0.93]
Barreiros 2008-h	1	9	6	9	0.14 [0.00, 0.58]	0.50 [0.26, 0.74]
Sinkala 2009-l	1	1	21	8	0.05 [0.00, 0.23]	0.89 [0.52, 1.00]



Hepatomegaly

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Monill-Serra 1997-l	58	61	18	15	0.76 [0.65, 0.85]	0.20 [0.11, 0.30]
Ndege 2019-h	30	31	16	23	0.65 [0.50, 0.79]	0.43 [0.29, 0.57]
Sinkala 2009-l	8	2	14	7	0.36 [0.17, 0.59]	0.78 [0.40, 0.97]
Kaneria 2009-l	11	12	34	33	0.24 [0.13, 0.40]	0.73 [0.58, 0.85]



III. Intra-abdominal lymph nodes on abdominal ultrasound for tuberculosis detection

Eight studies involving 917 participants (included 455 tuberculosis cases) reported on intra-abdominal lymph nodes on abdominal ultrasound (Monill-Serra 1997-l; Dominguez-Castellano 1998-l; O'Keefe 1998-h; Barreiros 2008-h; Sinkala 2009-l; Weber 2018-h; Griesel 2019-h; Ndege 2019-h). The sensitivities ranged from 22% to 86% and specificities from 56% to 100% (Figure 5).

IV. Ascites on abdominal ultrasound for tuberculosis detection

We included eight studies involving 891 participants, of whom 433 had tuberculosis (Monill-Serra 1997-l; O'Keefe 1998-h; Barreiros 2008-h; Kaneria 2009-l; Sinkala 2009-l; Weber 2018-h; Griesel 2019-h; Ndege 2019-h). Sensitivity and specificity estimates were very heterogeneous and ranged from 4% to 73% and from 33% to 100% respectively (Figure 5).

V. Splenomegaly

Six studies (775 participants, 397 tuberculosis cases) reported splenomegaly (Monill-Serra 1997-l; Barreiros 2008-h; Kaneria 2009-l; Sinkala 2009-l; Griesel 2019-h; Ndege 2019-h). Estimates were very heterogeneous and ranged from 5% to 62% for sensitivity and 45% to 89% for specificity (Figure 5).

VI. Hepatomegaly

Four studies (373 participants, of whom 189 had tuberculosis) were included for hepatomegaly. The sensitivity ranged from 24% to 76% and specificity from 20% to 78% (Figure 5).

Investigations of heterogeneity

We did not investigate heterogeneity, due to limited data.

DISCUSSION

This systematic review of the diagnostic accuracy of abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals summarizes the current literature and includes 11 studies. Six studies reported on abdominal ultrasound with any abnormal finding, and nine studies reported on individual ultrasound findings. Studies were conducted in low-, middle- and high-income countries. Five studies were performed in referral or tertiary-level healthcare facilities, and in four studies the ultrasound examinations were performed by radiologists.

Summary of main results

We have summarized the main results in Summary of findings 1. An abdominal ultrasound with any abnormal finding had a pooled sensitivity of 63% (95% CI 43% to 79%) and a pooled specificity of 68% (95% CI 42% to 87%) when bacteriological confirmation was used as the (higher-quality) reference standard. The pooled sensitivity was 68% (95% CI 45% to 85%) and the pooled specificity was 73% (95% CI 41% to 91%) when the reference standard was clinical diagnosis without microbiological confirmation (lower-quality reference standard).

The sensitivity of abdominal ultrasound is of concern, due to the high chance of missing tuberculosis cases (high false negative rate). This means that HIV-positive individuals who have tuberculosis may be wrongly classified as not having tuberculosis, with a delay in initiating appropriate treatment. Ultrasound examination is operator-dependent and subjective, with the possibility of missing subtle signs. Ultrasound also evaluates anatomical changes, and abnormalities might not occur in individuals with advanced immunosuppression.

The effect of the type of reference standard used is reflected in the improvement in both the sensitivity and specificity in the lower-quality reference standard group. The primary concern with a lower-quality reference standard (clinical diagnosis) is that clinicians may overdiagnose tuberculosis for fear of missing or delaying a diagnosis that could result in excess morbidity and mortality, particularly among HIV-postive adults. This would result in an overestimation of the diagnostic accuracy of abdominal ultrasound, as fewer false positive and negative results would occur. In addition, in studies where abdominal ultrasound is part of the reference standard, incorporation bias would further result in an overestimation of diagnostic accuracy.

The estimates of sensitivity for the primary and secondary analyses were low and very heterogeneous. This means that a negative abdominal ultrasound should not be used to rule out abdominal tuberculosis or disseminated tuberculosis with abdominal involvement.

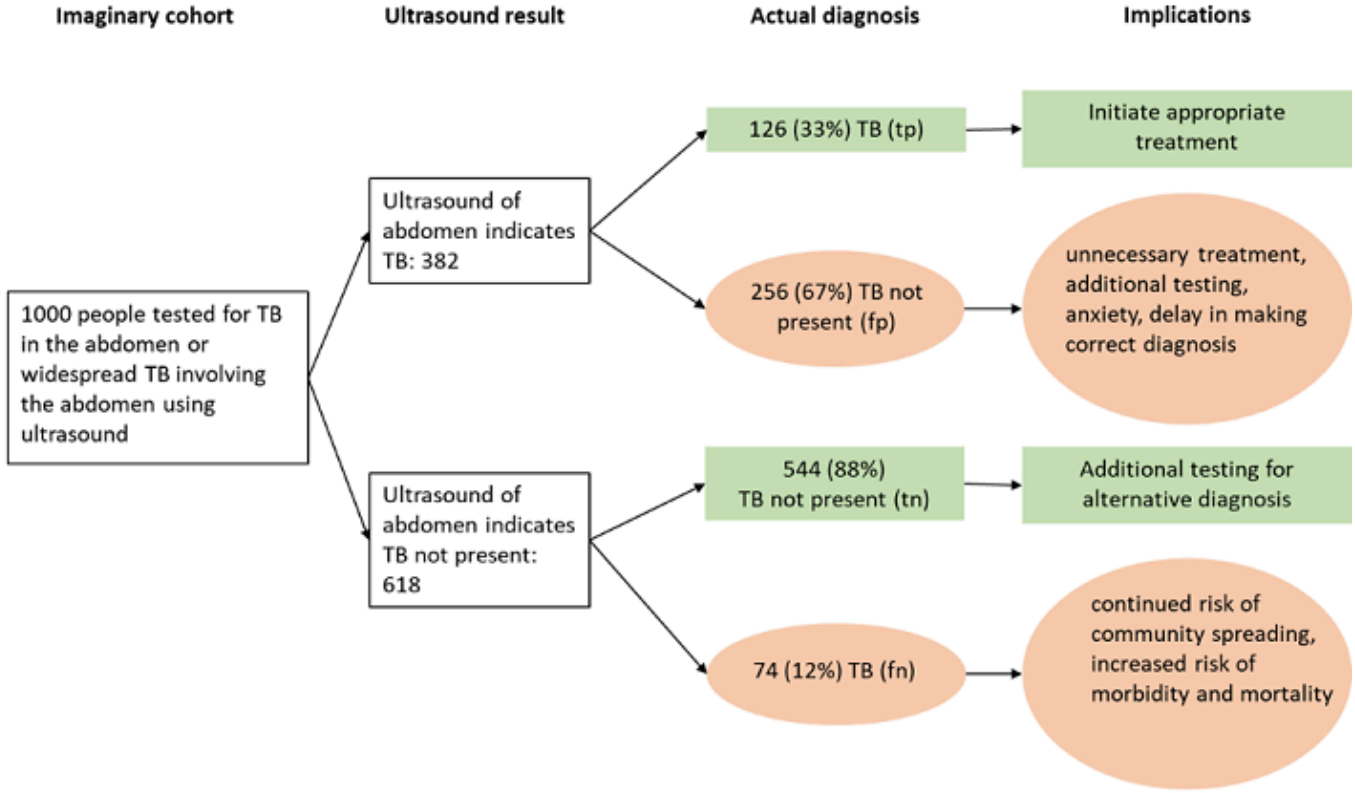
Specificity estimates were very heterogeneous, especially for hepatomegaly and splenomegaly.

Application of the main meta-analytic findings to a hypothetical cohort

The main findings of the review were illustrated by applying the results to a hypothetical cohort of 1000 HIV-positive individuals thought to have tuberculosis. We presented different scenarios where the tuberculosis prevalence varies from 10% to 20% to 40%. The consequences of false positive results are probably unnecessary initiation of treatment, additional testing with subsequent morbidity, patient anxiety, and possible delay in further diagnostic evaluation. The consequences of false negative results are the continued risk of community transmission of tuberculosis and an increased risk of patient morbidity and mortality.

If the pooled estimates (from using a higher-quality reference standard) for an abdominal ultrasound with any abnormal finding are applied to a hypothetical cohort of 1000 HIV-positive individuals where 100 (10%) of them actually have tuberculosis, abdominal ultrasound would be expected to miss 37 tuberculosis cases and falsely diagnose 288 people as tuberculosis cases (Summary of findings 1). For a prevalence of 20% (200 tuberculosis cases), 74 tuberculosis cases will be missed and 256 people will be falsely diagnosed as having tuberculosis (Figure 6) while for a prevalence of 40% (400 tuberculosis cases), 148 tuberculosis cases will be missed and 192 people will be falsely diagnosed as having tuberculosis (Summary of findings 1).

Figure 6. Flow diagram summarizing the main results in hypothetical cohort with TB prevalence 20%



tp: true positive – test is positive (indicates TB) and patient has TB
fp: false positive – test is positive (indicates TB) but patient does not have TB
tn: true negative – test is negative (indicates TB not present) and patient dies not have TB
Fn: false negative – test is negative (indicates TB not present) but patient has TB

Strengths and weaknesses of the review

The findings in this review are based on comprehensive literature searches, strict inclusion criteria, and standardized data extraction. The search included studies published in all languages and we corresponded with study authors to obtain additional and unpublished data. However, as diagnostic accuracy studies are poorly indexed, we acknowledge that we may have missed some studies despite the comprehensive search.

The main limitations of the review were the small number of studies and participants included in the analyses. The results were very heterogeneous with a high false negative rate, and should therefore be interpreted with caution. The high risks of bias in the patient selection domain and the reference standard domain further weaken our confidence in the results. A further limitation in the reference standard was the use of microscopic identification of acid-fast bacilli on stained sputum smears. Although smear positivity has high specificity in high tuberculosis prevalence settings, it is not a perfect reference standard as smear will also detect non-tuberculous mycobacteria, which are found in a higher proportion in low-prevalence tuberculosis settings.

Applicability of findings to the review question

We had high concern about the applicability of the included studies to our review question. We foresee that in clinical practice abdominal ultrasound to diagnose abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals would be most beneficial when performed by non-radiologists in non-tertiary endemic settings. Most studies were performed in tertiary settings with trained radiologists or sonographers performing the ultrasound examination, and it is possible that the accuracy of abdominal ultrasound may be lower when performed in a different setting or by less experienced users. The predictive values of any diagnostic test are influenced by disease prevalence, so the inclusion of studies performed in low tuberculosis-burden countries would have decreased the positive predictive value of abdominal ultrasound. Two studies included HIV-positive participants without a clinical suspicion of tuberculosis. In these studies, abdominal ultrasound has been used as a screening test and not a diagnostic test. This will further affect the diagnostic accuracy of abdominal ultrasound and increase the risk of inappropriate additional testing and initiation of anti-tuberculous treatment. Studies were carried out under research conditions, and it is possible that the diagnostic accuracy of abdominal ultrasound might be lower in routine practice.

AUTHORS' CONCLUSIONS

Implications for practice

Abdominal ultrasound had a sensitivity of 63% among HIV-positive individuals suspected of having abdominal tuberculosis or dissem-

inated tuberculosis with abdominal involvement. The high false negative rate suggests that ultrasound cannot be relied on alone for the diagnosis of tuberculosis. The specificity of 68% of any abnormal finding on abdominal ultrasound further indicates that care must be taken to not use abdominal ultrasound alone to rule in tuberculosis, as the false positive rate is high. The presence of individual findings such as ascites, splenic lesions and intra-abdominal lymphadenopathy had a higher specificity as evidenced by the range of study estimates, and, if proven in large prospective studies, might be a useful indicator for tuberculosis involving the abdomen. In light of our review findings, the intended role for ultrasound is to be used with other tests, such as lateral flow urine lipoarabinomannan assay (LF-LAM), chest x-ray and Xpert MTB/RIF or Xpert Ultra, to confirm the diagnosis of abdominal tuberculosis or disseminated tuberculosis with abdominal involvement.

Implications for research

Future studies that evaluate the diagnostic accuracy of abdominal ultrasound in HIV-positive people should use a robust reference standard with speciation to ensure that tuberculosis is correctly diagnosed. Larger, prospective, well-designed studies that recruit a representative sample of participants are also needed. The role of abdominal ultrasound in addition to existing diagnostic strategies (e.g. chest x-ray, LF-LAM, Xpert MTB/RIF) needs to be evaluated, as well as its incorporation into tuberculosis diagnostic algorithms.

ACKNOWLEDGEMENTS

We acknowledge Vittoria Lutje and Joy Oliver for their assistance with the MEDLINE draft search strategy. Dr Karen R Steingart was the academic editor for this review.

Eleanor A Ochodo is supported by a grant from the Wellcome Trust Foundation (grant number: 109939). The Wellcome Trust has no role in the design, conduct, and interpretation of this review.

Graeme Meintjes is supported by the Wellcome Trust (098316), the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant No 64787), NRF incentive funding (UID: 85858) and the South African Medical Research Council through its TB and HIV Collaborating Centres Programme with funds received from the National Department of Health (RFA# SAMRC-RFA-CC: TB/HIV/AIDS-01-2014).

Yemisi Takwoingi is supported by the UK National Institute for Health Research (NIHR) through a postdoctoral fellowship award (PDF-2017-10-059) and the NIHR Birmingham Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

The editorial base of the Cochrane Infectious Diseases Group is funded by UK aid from the UK government for the benefit of low- and middle-income countries (project number 300342-104). The views expressed do not necessarily reflect the UK government's official policies.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Barreiros 2008-h

Study characteristics	
Patient sampling	Case-control design

Barreiros 2008-h *(Continued)*

Patient characteristics and setting	Country: Germany Setting: Not reported High tuberculosis burden country: No High HIV-associated tuberculosis burden country: No Sample size: 7 cases (of these 3 HIV-negative); 18 controls (of these 9 HIV-negative) Median age (range): Cases 41 (27 - 66); Controls 36 (21 - 69) Gender proportion (M:F): Cases 3:4; Controls 11:7 Proportion on antiretroviral therapy (ART): Not reported
Index tests	Sonographer qualification: Not reported Threshold(s): • Thickened bowel wall: > 5 mm; • Intramural abscess: thickened hypervascular bowel wall > 8 mm with non-vascularized, oval-shaped, intramural mass-like lesions; • Extramural abscess: Circumscribed hypoechoic or echo-free fluid collections > 10 mm next to fistula; • Lymph nodes: Longitudinal diameter > 20 mm; • Splenomegaly: > 13.5 cm
Target condition and reference standard(s)	Target condition: Intestinal tuberculosis Confirmation of active tuberculosis: "…based on clinical, endoscopic, histologic, radiologic and operative findings including microbiology (in all) and polymerase chain reaction (PCR) (in 5 patients) of biopsies taken during endoscopy."
Flow and timing	
Comparative	
Notes	Second control group of healthy persons not included 4 cases and 9 controls were HIV-positive Cases had pulmonary tuberculosis only (randomly selected) Reference standard results not delineated

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	No		

Barreiros 2008-h *(Continued)*

Did the study avoid inappropriate exclusions?	Yes		
		High	**High**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Unclear**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Unclear**
DOMAIN 2: Index Test Splenomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Unclear**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Was incorporation bias avoided?	Yes		
		High	**High**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Unclear		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Unclear	

Bobbio 2019-l

Study characteristics	
Patient sampling	Cross-sectional design
Patient characteristics and setting	Country: South Sudan Setting: Referral hospital High tuberculosis burden country: No High HIV-associated tuberculosis burden country: No Sample size: 100 Median age (range): Not available (only categories available) Gender proportion (M:F): 48:52 Proportion on antiretroviral therapy (ART): 3%
Index tests	Sonographer qualification: Clinician trained in ultrasound Threshold(s): At least one of • Pericardial effusion; • Periportal/para-aortic lymph nodes (> 1.5 cm in diameter); • Focal splenic lesions; • Pleural effusion or consolidation of lung; • Ascites without alternative explanation; • Focal liver lesion
Target condition and reference standard(s)	Target condition: Disseminated tuberculosis Confirmation of active tuberculosis: Acid-fast bacilli sputum smears, ultrasound, clinical diagnosis
Flow and timing	
Comparative	
Notes	

Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		High	**High**

Bobbio 2019-l *(Continued)*

DOMAIN 2: Index Test Abnormal abdominal ultrasound (lower quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Was incorporation bias avoided?	No		
		High	**High**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	No		
		High	

Dominguez-Castellano 1998-h

Study characteristics	
Patient sampling	Prospective cross-sectional
Patient characteristics and setting	Country: Spain Setting: Not reported High tuberculosis burden country: No High HIV-associated tuberculosis burden country: No Sample size:116 Age: 31.56 ± 4.68 years (mean ± SD) Gender proportion: Not reported Proportion on antiretroviral therapy (ART): Not reported
Index tests	Sonographer qualification: “Medical sonographer”

Dominguez-Castellano 1998-h *(Continued)*

	Threshold(s): • Multiple splenic focal lesions: hypoechoic, < 10 mm diameter, poorly-defined / irregular borders, homogeneous distribution; • Abdominal adenopathy: hypo or isoechoic, between 1 and 3 cm, around hepatic hilum, spleen, aorta or celiac trunk; • Hypo or hyperechoic focal liver lesions
Target condition and reference standard(s)	Target condition: Pulmonary tuberculosis, Extra-pulmonary tuberculosis and disseminated tuberculosis (with or without abdominal involvement) Confirmation of active tuberculosis: Smear microscopy, Lowenstein culture
Flow and timing	
Comparative	
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		Low	**Unclear**
DOMAIN 2: Index Test Abnormal abdominal ultrasound (higher quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Yes		
		Low	**Low**

Dominguez-Castellano 1998-h *(Continued)*

DOMAIN 4: Flow and Timing	
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Unclear
Were all patients included in the analysis?	Yes
Did all patients received a reference standard?	Yes
	Unclear

Dominguez-Castellano 1998-l

Study characteristics	
Patient sampling	Prospective cross-sectional
Patient characteristics and setting	Country: Spain Setting: Not reported High tuberculosis burden country: No High HIV-associated tuberculosis burden country: No Sample size:116 Age: 31.56 ± 4.68 years (mean ± SD) Gender proportion: Not reported Proportion on antiretroviral therapy (ART): Not reported
Index tests	Sonographer qualification: “Medical sonographer” Threshold(s): • Multiple splenic focal lesions: hypoechoic, < 10 mm diameter, poorly-defined / irregular borders, homogeneous distribution; • Abdominal adenopathy: hypo or isoechoic, between 1 cm and 3 cm, around hepatic hilum, spleen, aorta or celiac trunk; • Hypo or hyperechoic focal liver lesions
Target condition and reference standard(s)	Target condition: Pulmonary tuberculosis, extra-pulmonary tuberculosis and disseminated tuberculosis (with or without abdominal involvement) Confirmation of active tuberculosis: Compatible with clinical and radiography findings with improvement to anti-tuberculosis treatment
Flow and timing	

Dominguez-Castellano 1998-I *(Continued)*

Comparative
Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		Low	**Unclear**
DOMAIN 2: Index Test Abnormal abdominal ultrasound (lower quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Splenic lesions			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Was incorporation bias avoided?	Unclear		

Dominguez-Castellano 1998-l *(Continued)*

		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Unclear		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Unclear	

Griesel 2019-h

Study characteristics	
Patient sampling	Prospective cross-sectional
Patient characteristics and setting	Country: South Africa Setting: Secondary-level hospitals High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes Sample size: 377 Age: Median (IQR) tuberculosis cases: 35 (30 - 41); Non-tuberculosis controls: 36 (30 - 42) Gender proportion (M:F) tuberculosis cases: 64:137; Non-tuberculosis controls: 64:112 Proportion on antiretroviral therapy (ART): tuberculosis cases: 59/201 (29%); Non-tuberculosis controls: 61/176 (35%)
Index tests	Sonographer qualification: Trained sonographers Threshold(s): • Lymph nodes (long-axis length: any and ≥ 10 mm in diameter); • Splenic hypoechoic lesions; • Spleen enlargement ≥ 110 mm; • Any one of abdominal, pleural, or pericardial effusions
Target condition and reference standard(s)	Target condition: Tuberculosis Confirmation of active tuberculosis: Positive culture for *M tuberculosis*

Griesel 2019-h *(Continued)*

Flow and timing
Comparative
Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
		High	**Low**
DOMAIN 2: Index Test Abnormal abdominal ultrasound (higher quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Splenic lesions			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**

Griesel 2019-h *(Continued)*

DOMAIN 2: Index Test Splenomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Yes		
		Low	**Low**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Low	

Kaneria 2009-l

Study characteristics	
Patient sampling	Case-control
Patient characteristics and setting	Country: India Setting: Not reported High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes Sample size: 90 Age: Mean (range) Cases: Male 36.4 (24 - 60), Female 33.41 (25 - 60); Controls: Male 39.46 (24 - 60), Female 38.71 (25 - 61) Gender proportion: M:F Cases: 31:14; Controls: 30:15

Kaneria 2009-l *(Continued)*

	Proportion on antiretroviral therapy (ART): Cases: 7/45 (15.6%); Controls: 15/30 (50%)
Index tests	Sonographer qualification: Not reported Threshold(s): Not reported
Target condition and reference standard(s)	Target condition: Pulmonary tuberculosis, extra-pulmonary tuberculosis and disseminated tuberculosis (with or without abdominal involvement) Confirmation of active tuberculosis: Microscopic identification of AFB and compatible clinical findings
Flow and timing	
Comparative	
Notes	

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
		High	**Unclear**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	No		
		Unclear	**Unclear**
DOMAIN 2: Index Test Splenic lesions			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	No		
		Unclear	**Unclear**
DOMAIN 2: Index Test Splenomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		

Kaneria 2009-l *(Continued)*

If a threshold was used, was it pre-specified?	No		
		Unclear	**Unclear**
DOMAIN 2: Index Test Hepatomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	No		
		Unclear	**Unclear**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Was incorporation bias avoided?	Unclear		
		High	**High**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	No		
		High	

Monill-Serra 1997-l

Study characteristics	
Patient sampling	Case-control
Patient characteristics and setting	Country: Spain Setting: Not reported High tuberculosis burden country: No High HIV-associated tuberculosis burden country: No Sample size: 152 Age: Cases: Mean 30; Range 20 - 49; Controls: Not reported

Monill-Serra 1997-I *(Continued)*

	Gender proportion: M:F Cases: 56:20; Controls: Not reported Proportion on antiretroviral therapy (ART): Not reported
Index tests	Sonographer qualification: Not reported Threshold(s): • Lymph nodes > 1.5 cm; • Splenomegaly long axis > 12 cm or subjective impression; • Hypoechoic splenic lesions 0.5 cm to 1.0 cm (Not prespecified)
Target condition and reference standard(s)	Target condition: Disseminated tuberculosis (with or without abdominal involvement) Confirmation of active tuberculosis: Microbiological (culture) or histopathological examination
Flow and timing	
Comparative	
Notes	Controls were HIV-positive with no associated neoplastic illness or opportunistic infection

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	No		
		High	**Unclear**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Unclear**
DOMAIN 2: Index Test Splenic lesions			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		

Monill-Serra 1997-l *(Continued)*

If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Unclear**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Unclear**
DOMAIN 2: Index Test Splenomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Unclear**
DOMAIN 2: Index Test Hepatomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Unclear**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Yes		
		High	**High**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Unclear		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Unclear	

Ndege 2019-h

Study characteristics	
Patient sampling	Prospective cohort
Patient characteristics and setting	Country: Tanzania Setting: Referral hospital High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes Sample size: 100 (original study size including HIV-negative n = 191) Age: Median 38 years; IQR 32 - 44 years Gender proportion: M:F 47:53 Proportion on antiretroviral therapy (ART): 56%
Index tests	Sonographer qualification: Board-certified sonographers Threshold(s): • Original FASH: pleural or pericardial effusion, ascites, abdominal lymph nodes > 1.5 cm, hypoechogenic lesions in the liver or spleen, ileum wall thickening > 4 mm or destructed ileum wall architecture; • Splenomegaly > 140 mm in long axis; • Hepatomegaly ≥ 2 cm below costal margin; • Pleural or pericardial fibrin strands in presence of effusion
Target condition and reference standard(s)	Confirmed tuberculosis was defined as ≥ 1 positive microbiological result from any site confirmed by Xpert MTB/RIF assay and/or bacteriologic culture (growth of *M tuberculosis*) in sputum, pleural fluid, ascites, cerebrospinal fluid, urine or lymph node aspirate
Flow and timing	
Comparative	
Notes	

Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		

Ndege 2019-h *(Continued)*

Did the study avoid inappropriate exclusions?	Yes		
		Low	**High**
DOMAIN 2: Index Test Abnormal abdominal ultrasound (higher quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Splenic lesions			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Splenomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 2: Index Test Hepatomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		

Ndege 2019-h *(Continued)*

		Low	**Low**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Yes		
		Low	**Low**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Low	

Ndege 2019-l

Study characteristics	
Patient sampling	Prospective cohort
Patient characteristics and setting	Country: Tanzania Setting: Referral hospital High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes Sample size: 100 (original study size including HIV-negative n = 191) Age: Median 38 years; IQR 32 - 44 years Gender proportion: M:F 47:53 Proportion on antiretroviral therapy (ART): 56%
Index tests	Sonographer qualification: Board-certified sonographers Threshold(s): • Original FASH: pleural or pericardial effusion, ascites, abdominal lymph nodes > 1.5 cm, hypoechogenic lesions in the liver or spleen, ileum wall thickening > 4 mm or destructed ileum wall architecture; • Splenomegaly > 140 mm in long axis; • Hepatomegaly ≥ 2 cm below costal margin;

Ndege 2019-I *(Continued)*

	• Pleural or pericardial fibrin strands in presence of effusion
Target condition and reference standard(s)	Confirmed tuberculosis was defined as ≥ 1 positive microbiological result from any site confirmed by Xpert MTB/RIF assay and/or bacteriologic culture (growth of *M tuberculosis*) in sputum, pleural fluid, ascites, cerebrospinal fluid, urine or lymph node aspirate. In addition, the identification of acid-fast bacilli in sputum by another health centre, or adenosine deaminase (ADA) ≥ 40 U/ml in pleural fluid, ≥ 35 U/ml in pericardial fluid and ≥ 30 U/ml in ascitic fluid were accepted as microbiological confirmation. Probable tuberculosis was defined as negative microbiological tests in a participant in whom anti-tuberculosis therapy (prescribed based on clinical suspicion or on chest x-ray) in the absence of an alternative diagnosis led to a resolution of clinical signs and symptoms, radiographic and sonographic signs, and to an increase in body weight documented 2 months after start of anti-tuberculosis treatment
Flow and timing	
Comparative	
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		Low	**High**
DOMAIN 2: Index Test Abnormal abdominal ultrasound (lower quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**Low**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Unclear		

Ndege 2019-l *(Continued)*

		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Unclear	

O'Keefe 1998-h

Study characteristics	
Patient sampling	Prospective cross-sectional
Patient characteristics and setting	Country: South Africa Setting: Non-tertiary setting High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes Sample size: 35 (original study size n = 44) Age: Mean 32.9; Range 18.4 - 53.3 Gender proportion: M:F 26:18 Proportion on antiretroviral therapy (ART): 0/44 (0%)
Index tests	Sonographer qualification: Radiologist Threshold(s): Not reported
Target condition and reference standard(s)	Target condition: Disseminated tuberculosis with abdominal involvement) Confirmation of active tuberculosis: Microbiological (culture) or postmortem evidence
Flow and timing	
Comparative	
Notes	Only 35/44 had ultrasound examination
Methodological quality	

O'Keefe 1998-h *(Continued)*

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
		High	**Low**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	No		
		Unclear	**High**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	No		
		Unclear	**High**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Yes		
		Low	**Low**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Unclear	

Sculier 2010-h

Study characteristics	
Patient sampling	Prospective cross-sectional
Patient characteristics and setting	Country: Cambodia Setting: “not-for-profit referral hospital” High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: No Sample size: 212 Age: Median (IQR) 34 (29 - 41.5) years (included participants < 18 years) Gender proportion: M 40%, F 60% Proportion on antiretroviral therapy (ART): Not reported
Index tests	Sonographer qualification: “Trained radiologist” Threshold(s): • Any lymph nodes ≥ 1.2 cm; • Ascites; • Hepatomegaly; • Splenomegaly; • Hepatic or splenic hypoechoic lesions with or without organ enlargement
Target condition and reference standard(s)	Target condition: Disseminated tuberculosis (with or without abdominal involvement) Confirmation of active tuberculosis: Culture
Flow and timing	
Comparative	
Notes	Substudy

Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		Low	**High**

Sculier 2010-h *(Continued)*

DOMAIN 2: Index Test Abnormal abdominal ultrasound (higher quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	**High**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Yes		
		Low	**High**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Low	

Sinkala 2009-l

Study characteristics	
Patient sampling	Prospective cross-sectional
Patient characteristics and setting	Country: Zambia Setting: “secondary and tertiary care hospital” High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes Sample size: 31 Age: Mean (SD) All: 33.4 (8.3) years (in text: mean 33.1 range 18 - 54); tuberculosis: 30.7 (6.9); No tuberculosis: 39.8 (8) Gender proportion: M:F All: 8:23; tuberculosis: 7:15; No tuberculosis: 1:8 Proportion on antiretroviral therapy (ART): Not reported

Sinkala 2009-I *(Continued)*

Index tests	Sonographer qualification: Not reported Threshold(s): Not reported
Target condition and reference standard(s)	Target condition: Abdominal tuberculosis Confirmation of active tuberculosis: "…definitive diagnosis of tuberculosis was made by demonstration of *M tuberculosis* infection via positive bacteriological culture and/or granulomatous inflammation on histopathological examination with positive Ziehl-Neelsen (ZN) staining on microscopy. A presumptive diagnosis of tuberculosis was made when granulomatous inflammation was seen on microscopy, or when visual inspection on laparoscopy was consistent with tuberculosis and the patient's clinical response to anti-tuberculous treatment was good. Laparoscopic features felt to be consistent with tuberculosis for the purpose of making a presumptive diagnosis were the presence of tubercles, fibro adhesive peritonitis, or caseating lymphadenopathy."
Flow and timing	
Comparative	
Notes	Ultrasound used as part of inclusion and exclusion criteria (selection bias)

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		Low	**High**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	No		
		Low	**Unclear**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	No		
		Unclear	**Unclear**

Sinkala 2009-l *(Continued)*

DOMAIN 2: Index Test Splenomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	No		
		Unclear	**Unclear**
DOMAIN 2: Index Test Hepatomegaly			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	No		
		Unclear	**Unclear**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Was incorporation bias avoided?	Yes		
		High	**High**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Low	

Weber 2018-h

Study characteristics	
Patient sampling	Prospective controlled cohort
Patient characteristics and setting	Country: India Setting: Tertiary setting High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes

Weber 2018-h *(Continued)*

	Sample size: 81 (original study size including HIV-negative n = 425) Age: Overall median (IQR) 43 (31.5 - 55); HIV only 43 (38 - 48) (included participants < 18 years) Gender proportion: Overall: M 328/425 (77%); HIV-positive M 56/81 (69%) Proportion on antiretroviral therapy (ART): 29/81 (35.8%)
Index tests	Sonographer qualification: Clinician trained in the study's ultrasound protocol but without formal ultrasound training Threshold(s): • FASH: at least 1 of pericardial or pleural effusion, focal liver or splenic lesions, or abdominal lymphadenopathy; • Pericardial effusion: qualitative assessment; • Focal liver lesions: Size 2 mm to 15 mm; multiple in appearance; • Focal splenic lesions: Size 2 mm to 15 mm; multiple in appearance; • Abdominal lymphadenopathy: Max diameter at least 15 mm
Target condition and reference standard(s)	Target condition: Pulmonary tuberculosis and extra-pulmonary tuberculosis Confirmation of active tuberculosis: "…'confirmed tuberculosis' (i.e., positive fluorescent microscopy, polymerase chain reaction, or tuberculosis culture)…"
Flow and timing	
Comparative	
Notes	Includes patients ≥ 16 years "…therapeutic and diagnostic management was fully the responsibility of the attending hospital doctor." Additional info received from authors

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		Low	**High**
DOMAIN 2: Index Test Abnormal abdominal ultrasound (higher quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	No		

Weber 2018-h *(Continued)*

If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Low**
DOMAIN 2: Index Test Ascites			
Were the index test results interpreted without knowledge of the results of the reference standard?	No		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Low**
DOMAIN 2: Index Test Splenic lesions			
Were the index test results interpreted without knowledge of the results of the reference standard?	No		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Low**
DOMAIN 2: Index Test Abdominal lymph nodes			
Were the index test results interpreted without knowledge of the results of the reference standard?	No		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**Low**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Was incorporation bias avoided?	Yes		
		Low	**High**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Low	

Weber 2018-I

Study characteristics	
Patient sampling	Prospective controlled cohort
Patient characteristics and setting	Country: India Setting: Tertiary setting High tuberculosis burden country: Yes High HIV-associated tuberculosis burden country: Yes Sample size: 81 (original study size including HIV-negative n = 425) Age: Overall median (IQR) 43 (31.5 - 55); HIV only 43 (38 - 48) (included participants < 18 years) Gender proportion: Overall: M 328/425 (77%); HIV-positive M 56/81 (69%) Proportion on antiretroviral therapy (ART): 29/81 (35.8%)
Index tests	Sonographer qualification: Clinician trained in the study's ultrasound protocol but without formal ultrasound training Threshold(s): • FASH: at least 1 of pericardial or pleural effusion, focal liver or splenic lesions, or abdominal lymphadenopathy; • Pericardial effusion: qualitative assessment; • Focal liver lesions: Size 2 mm to 15 mm; multiple in appearance; • Focal splenic lesions: Size 2 mm to 15 mm; multiple in appearance; • Abdominal lymphadenopathy: Max diameter at least 15 mm
Target condition and reference standard(s)	Target condition: Pulmonary tuberculosis and extra-pulmonary tuberculosis Confirmation of active tuberculosis: "…'clinical tuberculosis' (no microbiological confirmation, but clinical tuberculosis diagnosis and tuberculosis treatment initiated)…"
Flow and timing	
Comparative	
Notes	Includes patients ≥ 16 years "…therapeutic and diagnostic management was fully the responsibility of the attending hospital doctor." Additional info received from authors

Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		

Weber 2018-l *(Continued)*

Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
		Low	**High**
DOMAIN 2: Index Test Abnormal abdominal ultrasound (lower quality)			
Were the index test results interpreted without knowledge of the results of the reference standard?	No		
If a threshold was used, was it pre-specified?	Yes		
		Unclear	**High**
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Was incorporation bias avoided?	No		
		High	**Unclear**
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Did all patients received a reference standard?	Yes		
		Low	

Suffix (h) indicates higher-quality reference standard; suffix (l) indicates lower-quality reference standard

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Abiri 1985	Descriptive study
Agarwal 2010	No reference standard
Akinkuolie 2008	Descriptive study

Study	Reason for exclusion
Aubry 1994	Descriptive study
Barthwal 2005	Descriptive study
Batra 2000	Descriptive study
Chen 2009	Descriptive study
Clarke 2007	Descriptive study
Emby 2002	Descriptive study
Feng 2016	Ineligible index test
Giordani 2013	Descriptive study
Heller 2010a	Descriptive study
Heller 2013	Descriptive study
Heller 2017	Descriptive study
Ibrahim 2005	Descriptive study
Jain 1995	Ineligible patient population
Kedar 1994	Descriptive study
Landoni 2002	Descriptive study
Ouedraogo 2016	Only abnormal index test reported
Patel 2011	Only abnormal index test reported
Porcel-Martin 1998	Descriptive study
Sheikh 1999	Descriptive study
Solomon 1998	Not a diagnostic accuracy study
Soriano 1991	Descriptive study
Spalgais 2013	Descriptive study
Spalgais 2017	No reference standard
Tarantino 2003	Descriptive study
Tarantino 2004	Descriptive study
Tshibwabwa 2000	Ineligible patient population
Wafai 2017	Descriptive study

Characteristics of ongoing studies *[ordered by study ID]*

PACTR201712002829221

Trial name or title	Ultrasound in managing tuberculosis: A randomized controlled two-center study
Target condition and reference standard(s)	Target condition: Extrapulmonary tuberculosis Reference standard: Not stipulated
Index and comparator tests	Index test: eFASH (extended focused assessment with sonography for HIV and tuberculosis) and a management algorithm Comparator group: Standard of care (Management according to the decision of the treating physician)
Starting date	September 2018
Contact information	mrohacek@ihi.or.tz
Notes	

DATA

Presented below are all the data for all of the tests entered into the review.

Table Tests. Data tables by test

Test	No. of studies	No. of participants
1 Abnormal abdominal ultrasound (higher quality)	5	879
2 Abnormal abdominal ultrasound (lower quality)	4	397
3 Ascites	8	891
4 Splenic lesions	6	916
5 Abdominal lymph nodes	8	917
6 Splenomegaly	6	775
7 Hepatomegaly	4	373

Test 1. Abnormal abdominal ultrasound (higher quality).

Test 2. Abnormal abdominal ultrasound (lower quality).

Test 3. Ascites.

Test 4. Splenic lesions.

Test 5. Abdominal lymph nodes.

Test 6. Splenomegaly.

Test 7. Hepatomegaly.

ADDITIONAL TABLES

Table 1. Key findings of included studies

Author (publication year)	Study design	Country	Clinical setting	Target condition definition	Qualification of person performing index test	Sample size	Tuberculosis proportion in study
Barreiros 2008-h	Case-control	Germany	Not reported	Gastro-intestinal tuberculosis	Not reported	25[a] (7 cases, 18 pulmonary tuberculosis controls)	-
Bobbio 2019-l	Cross-sectional	South Sudan	Referral hospital	Extra-pulmonary tuberculosis	Trained non-radiologist	100	24%
Dominguez-Castellano 1998-h; Dominguez-Castellano 1998-l	Cross-sectional	Spain	Not reported	Extra-pulmonary tuberculosis	Sonographer	116	55% (higher) 58% (lower)
Griesel 2019-h	Cross-sectional	South Africa	Non-tertiary hospital	Culture-positive tuberculosis	Sonographer	377	53%
Kaneria 2009-l	Case-control	India	Not reported	Pulmonary tuberculosis, extra-pulmonary tuberculosis, disseminated tuberculosis	Not reported	90 (45 cases, 45 HIV-positive controls without any pathology)	-
Monill-Serra 1997-l	Case-control	Spain	Not reported	Disseminated tuberculosis	Not reported	152 (76 cases, 76 HIV-positive controls without any pathology)	-
Ndege 2019-h; Ndege 2019-l	Cohort	Tanzania	Referral hospital	Pulmonary tuberculosis, extra-pulmonary tuberculosis, disseminated tuberculosis	Board-certified sonographers	100 (191 original study sample)	46% (higher) 64% (lower)
O'Keefe 1998-h	Cross-sectional	South Africa	Non-tertiary hospital	Disseminated tuberculosis	Radiologist	35 (44 original study sample)	34%
Sculier 2010-h	Cross-sectional	Cambodia	Referral hospital	Disseminated tuberculosis	Radiologist	212	18%
Sinkala 2009-l	Cross-sectional	Zambia	Tertiary hospital	Abdominal tuberculosis	Not reported	31	71%
Weber 2018-h; Weber 2018-l	Cohort	India	Tertiary hospital	Disseminated tuberculosis	Trained non-radiologist	81 (425 original study sample)	30% (higher)

Table 1. Key findings of included studies *(Continued)*

						49% (lower)	

[a]Includes five HIV-negative participants.
Suffix (h) indicates higher-quality reference standard; suffix (l) indicates lower-quality reference standard.

Table 2. Indext test threshold and reference standard of included studies

Author (publication year)	Index test variable included (threshold)	Reference standard quality and definition
Barreiros 2008-h	Ascites (any) Lymphadenopathy (abdominal and perihepatic nodes with longitudinal diameter > 20 mm) Splenomegaly (> 135 mm)	Lower: Clinical, endoscopic, histologic, radiologic and operative findings including microbiology and polymerase chain reaction of biopsies taken during endoscopy
Bobbio 2019-l	Any abnormality (Presence of ≥ 1: i) pericardial effusion, ii) periportal/para-aortic lymph nodes (> 15 mm diameter), iii) focal splenic lesions, iv) pleural effusion or consolidation of the lung, v) ascites without alternative explanation)	Lower: Sputum microscopy OR clinical reasons OR Focused Assessment with Sonography in HIV-associated tuberculosis (FASH)
Dominguez-Castellano 1998-h; Dominguez-Castellano 1998-l	Any abnormality (presence of ≥ 1: i) multiple hypoechoic splenic lesions (< 10 mm), ii) any abdominal adenopathy, iii) hypo- or hyperechoic liver lesions)	Higher: Microscopy OR culture Lower: Microscopy OR culture OR clinical or radiographic indications and response to treatment
Griesel 2019-h	Any abnormality (presence of ≥ 1: i) abdominal lymph nodes (any size), ii) splenic hypoechoic lesions, iii) splenomegaly (≥ 110 mm), iv) any one of abdominal, pleural, or pericardial effusions) Ascites (any) Lymphadenopathy (any size) Splenic lesions (hypoechoic) Splenomegaly (≥ 110 mm)	Higher: Positive culture for *M tuberculosis* from any site
Kaneria 2009-l	Ascites (any) Hepatomegaly (not defined) Lymphadenopathy (diameter > 15 mm) Splenic lesions (multiple, hypoechoic, 5 mm to 10 mm diameter) Splenomegaly (not defined)	Lower: Lymphocytic predominance and elevated adenosine deaminase (ADA) levels in pleural or ascitic fluid OR granulomatous lymphadenitis and acid-fast bacilli in lymph node OR sputum microscopy
Monill-Serra 1997-l	Ascites (any) Hepatomegaly (not defined) Lymphadenopathy (> 15 mm diameter) Splenic lesions (hypoechoic nodes) Splenomegaly (long axis > 120 mm or subjective impression)	Lower: Blood culture positive for *M tuberculosis* OR medullary bone or liver biopsy with granulomatous inflammation or culture positive for *M tuberculosis* OR microbiological or histopathological confirmation in ≥ 2 non-contiguous extra-pulmonary sites
Ndege 2019-h; Ndege 2019-l	Any abnormality (presence of ≥ 1: i) pleural or pericardial effusion, ii) ascites, iii) abdominal lymph nodes > 15 mm, iv) hypoechogenic lesions in the liver or spleen, v) ileum wall thickening > 4 mm or destructed ileum wall architecture) Ascites (any) Hepatomegaly (not defined)	Higher: Xpert MTB/RIF assay and/or bacteriologic culture (growth of *M tuberculosis*) Lower: Positive Xpert MTB/RIF assay and/or bacteriologic culture (growth of *M tuberculosis*) OR acid-fast bacilli in sputum OR raised adenosine deaminase (ADA) levels in pleural, pericardial or ascitic fluid OR negative microbiological

Table 2. Indext test threshold and reference standard of included studies *(Continued)*

	Lymphadenopathy (> 15 mm diameter) Splenomegaly (not defined)	tests and improvement 2 months after start of anti-tuberculosis treatment
O'Keefe 1998-h	Ascites (any) Lymphadenopathy (not defined)	Higher: Positive mycobacterial blood or bone marrow cultures OR positive mycobacterial cultures from 2 or more other sites OR post mortem evidence
Sculier 2010-h	Any abnormality (presence of ≥ 1: i) any lymph nodes ≥ 12 mm, ii) ascites, iii) hepatomegaly, iv) splenomegaly, v) hepatic or splenic hypoechoic lesions with or without organ enlargement)	Higher: Positive culture for *M tuberculosis* from any site
Sinkala 2009-l	Ascites (any) Hepatomegaly (not defined) Lymphadenopathy (not defined) Splenomegaly (not defined)	Lower: Positive bacteriological culture OR granulomatous inflammation with positive Ziehl-Neelsen (ZN) staining on microscopy OR granulomatous inflammation on microscopy OR visual inspection on laparoscopy consistent with tuberculosis (presence of tubercles, fibro-adhesive peritonitis, or caseating lymphadenopathy) and favourable response to anti-tuberculous treatment
Weber 2018-h; Weber 2018-l	Any abnormality (presence of ≥ 1: i) pericardial or pleural effusion, ii) focal liver or splenic lesions, iii) abdominal lymphadenopathy) Ascites (any) Hepatomegaly (not defined) Lymphadenopathy (≥ 15 mm diameter) Splenic lesions (multiple, hypoechoic, 2 mm to 5 mm diameter)	Higher: Positive fluorescent microscopy, polymerase chain reaction, or tuberculosis culture Lower: Microbiological confirmation (fluorescent microscopy, polymerase chain reaction, culture) OR clinical diagnosis and anti-tuberculous treatment initiated

Suffix (h) indicates higher quality reference standard; suffix (l) indicates lower quality reference standard

Table 3. Summary estimates of sensitivity and specificity for any abnormality and individual abdominal ultrasound findings

Abdominal ultrasound finding	Number of studies	Number of participants (tuberculosis-cases)	Pooled sensitivity (95% CI) %	Pooled specificity (95% CI) %	Range of sensitivity %	Range of specificity %
Any abnormality (higher-quality reference standard)	5	879 (368)	63 (43 to 79)	68 (72 to 87)	35 to 82	20 to 92
Any abnormality (lower-quality reference standard)	4	397 (149)	68 (45 to 85)	73 (41 to 91)	37 to 88	22 to 92
Splenic lesions	6	916 (477)	Not calculated	Not calculated	13 to 62	86 to 100

Table 3. Summary estimates of sensitivity and specificity for any abnormality and individual abdominal ultrasound findings *(Continued)*

Intra-abdominal lymph nodes	8	917 (455)	Not calculated	Not calculated	22 to 86	56 to 100
Ascites	8	891 (433)	Not calculated	Not calculated	4 to 73	33 to 100
Splenomegaly	6	775 (397	Not calculated	Not calculated	5 to 62	45 to 89
Hepatomegaly	4	373 (189)	Not calculated	Not calculated	24 to 76	20 to 78

APPENDICES

Appendix 1. Search strategy

Ovid MEDLINE® Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE® <1946 to Present>

1 extrapulmonary tuberculosis.mp.

2 Peritonitis, Tuberculous/ or Tuberculosis, Gastrointestinal/ or Tuberculosis, Hepatic/

3 abdominal tuberculosis.mp.

4 Tuberculosis, Hepatic/ or liver tuberculosis.mp. or gastric tuberculosis.mp. or intestinal tuberculosis.mp.

5 Tuberculosis, Miliary/

6 disseminated tuberculosis.mp.

7 1 or 2 or 3 or 4 or 5 or 6

8 HIV infection.mp. or HIV Infections/

9 exp HIV/

10 human immunodeficiency virus.mp.

11 Acquired Immunodeficiency Syndrome/ or acquired immunodeficiency syndrome.mp.

12 (acquired immun* and deficiency syndrome).mp.

13 ((HIV* adj2 (people or person* or patient*)) or PLHIV).mp.

14 8 or 9 or 10 or 11 or 12 or 13

15 7 and 14

16 Radiography, Abdominal/

17 X-Ray Diffraction/ or x-ray*.mp.

18 (ultrasound or barium).mp.

19 Tomography, X-Ray Computed/

20 (comput* adj2 tomograph*).mp.

21 Magnetic Resonance Imaging/

22 (MRI or CAT).mp.

23 Ultrasonography/ or ultrasonograph*.mp.

24 Bacteriological Techniques/ or Sputum/ or sputum specimen.mp.

25 liquid culture system.mp.

26 Xpert MTB*.mp.

27 Genotype MTBDR*.mp.

28 (lipoarabinomannan or LAM or LF-LAM).mp.

29 QuantiFERON-TB-Gold.mp. or Tuberculin Test/ or tuberculin.mp.

30 Diagnostic Imaging/ or Point-of-Care Systems/

31 (Laparotomy or laparoscopy or fine needle aspiration).mp.

32 CD4 Lymphocyte Count/

33 Ascites/diagnosis or Ascites/microbiology or Paracentesis/ or Laparoscopy/

34 colonoscopy.mp. or Colonoscopy/

35 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34

36 15 and 35

Embase 1947-Present, updated daily

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1 tuberculosis.mp. or tuberculosis/

2 (Abdominal or gastroenteric or gastrointestinal or intestinal or hepatic or liver or splenic).mp.

3 1 and 2

4 abdominal tuberculosis/

5 miliary tuberculosis/

6 HIV infection.mp. or Human immunodeficiency virus infection/

7 human immunodeficiency virus.mp.

8 acquired immune deficiency syndrome/

9 ((HIV* adj2 (people or person* or patient*)) or PLHIV).mp.

10 6 or 7 or 8 or 9

11 abdominal ultrasound.mp.

12 X ray/ or radiography/ or X ray*.mp.

13 (ultrasound or barium).mp.

14 (comput* adj2 tomograph*).mp.

15 Magnetic Resonance.mp. or nuclear magnetic resonance/

16 Ultrasonography.mp. or echography/

17 sputum analysis/ or sputum cytodiagnosis/ or sputum culture/

18 microbiological examination/

19 liquid culture/

20 (Xpert MTB* or Genotype MTBDR*).mp.

21 (lipoarabinomannan or LAM or LF-LAM).mp.

22 (QuantiFERON-TB-Gold or Tuberculin Test).mp.

23 "point of care system"/

24 (Laparotomy or laparoscopy).mp

25 ascites fluid analysis/ or ascites/ or ascites fluid cytology/

26 colonoscopy/

27 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26

28 3 and 10 and 27

BIOSIS Previews

You searched for: **TOPIC:** ("liver tuberculosis" or "gastric tuberculosis" or "intestinal tuberculosis" or "abdominal tuberculosis".) *AND* **TOPIC:** (HIV or AIDS or "acquired immunodeficiency syndrome") *AND* **TOPIC:** (ultrasound or ultrasonography or scan or "Magnetic Resonance Imaging" or MRI or tomography)

Timespan: All years. **Indexes:** BIOSIS Previews.

Web of Science Core Collection

You searched for: **TOPIC:** ("liver tuberculosis" or "gastric tuberculosis" or "intestinal tuberculosis" or "abdominal tuberculosis") *AND* **TOPIC:** (HIV or AIDS or "acquired immunodeficiency syndrome") *AND* **TOPIC:** (ultrasound or ultrasonography or scan or "Magnetic Resonance Imaging" or MRI or tomography)

Timespan: All years. **Indexes:** SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH

ClinicalTrials.gov and WHO ICTRP

tuberculosis and ultrasound, tuberculosis and ultrasonography, tuberculosis and MRI

Appendix 2. QUADAS-2 tool tailored to the context of the review

Domain	Patient selection	Index test	Reference standard	Flow and timing
Description	**Methods of patient selection**	**How index test was conducted and reported**	**How reference standard was conducted and reported**	**Describe patients that did not receive and time interval between index test or reference standard**
Signalling questions (yes, no, or unclear)	Consecutive or random sample of patients? • Yes if the study reported consecutive enrolment or random sampling of patients. • No if patients were purposefully selected, for example based on previous test results	Index test results interpreted without knowledge of the results of reference standard? • Yes if it is apparent that ultrasound (and test combinations) results were interpreted without knowledge of reference standard results. • No if results of ultrasound (and test combinations) were interpreted with knowledge of the reference standard results.	Reference standard likely to correctly classify the target condition? • Yes if the higher quality reference standard was used (that is, culture, microscopic identification of acid-fast bacilli, or Xpert MTB/RIF).	Was there an appropriate interval between index test and reference standard? • Yes if abdominal ultrasound and the

(Continued)

(other tests or reference standard). • Unclear if the study did not explicitly state consecutive enrolment or random sampling, and it was unclear how patients were sampled.	• Unclear if insufficient information on how ultrasound (and test combinations) was interpreted.	• No if the lower quality reference standard was used (that is, not coupled with any mentioned in higher quality reference). • Unclear if insufficient information on the reference standard(s) used.	reference standard(s) (samples taken or clinical diagnosis made) were performed at the same time or if the time interval is less than one week. • No if the time period between ultrasound and the reference standard is more than one week. • Unclear if insufficient or no information on the time interval.
Was a case-control design avoided? • Yes if a case-control design was not used. • No if patients with known disease (cases) and patients without the disease (controls) were clearly enrolled (such that participants are unrepresentative of the spectrum of patients seen in clinical practice). • Unclear if the study design used was not clearly reported.	Pre-specified threshold used? • Yes if the study states the use of one, pre-specified, cut-off value, for example, "abdominal lymph nodes greater than 10 mm in the shortest diameter were deemed as a positive result". • No if multiple cut-off values were evaluated and an optimal one (based on maximising test accuracy) was subsequently chosen. • Unclear if a cut-off was used but was not reported, or only one cut-off value was reported, but was not explicitly pre-specified in the study.	Reference standard results interpreted without knowledge of the results of index test? • Yes if results of the reference standard are interpreted without knowledge of ultrasound results. However, the clinical reference standard may incorporate ultrasound. • No if results of the reference standard were interpreted with knowledge of ultrasound results • Unclear if there is insufficient information on whether or not the reference standard results were interpreted	Did all patients receive a reference standard? • Yes if all participants received a reference standard. • No if one or more participants did not receive a reference standard. • Unclear if there is insufficient information to de-

(Continued)

	with knowledge of ultrasound results	termine whether or not all patients received a reference standard.
Did the study avoid inappropriate exclusions? • Yes if no patients were excluded after inclusion in the study. • No if specific populations were excluded (for example, pregnant patients, elderly), or patients with high CD4 counts were excluded because of low clinical suspicion of TB. • Unclear if unreported or insufficient information given to make a decision.	Was incorporation bias avoided (inclusion of index test as part of the reference standard)? • Yes if abdominal ultrasound was not used as part of the reference standard. • No if abdominal ultrasound formed part of the reference standard. • Unclear if insufficient information given to make a decision.	Did all patients receive the same reference standard? • Yes if study participants received the same reference standard (regardless of ultrasound result). • No if participants did not receive the same reference standard. • Unclear if there is insufficient information to determine whether or not all patients received the same reference standard.
		Were all patients included in the analysis? • Yes if all participants recruited into the study were in-

(Continued)

				cluded in the analysis. • No if some participants recruited into the study were excluded in the analysis. • Unclear if unreported or insufficient information given to make a decision.
Risk of bias[a](high, low, or unclear)	Could the selection of patients have introduced bias?	Could the conduct or interpretation of the index test have introduced bias?	Could the reference standard, its conduct, or its interpretation has introduced bias?	Could the patient flow have introduced bias?
Applicability concerns (high, low, or unclear)	Are there concerns that the included patients do not match the review question? • High if participants received ultrasound in a tertiary care (referral) centre or if asymptomatic HIV-positive participants included. • Low if participants received ultrasound in any setting, or if HIV-positive individuals with presumptive abdominal tuberculosis or disseminated tuberculosis with abdominal involvement included. • Unclear if insufficient information to make a decision.	Are there concerns that the index test, its conduct, or interpretation differs from the review question? • High if, for example, specially trained radiologists performed the ultrasound. • Low if non-radiologists performed the ultrasounds. • Unclear if insufficient information to make a decision.	Are there concerns that the target condition as defined by the reference standard does not match the review question? • High if studies did not speciate mycobacteria isolated in culture or clinically diagnosed TB cases were not followed up to evaluate treatment response. • Low if studies did speciate mycobacteria isolated in culture or clinically diagnosed TB cases improved on anti-TB therapy. • Unclear if insufficient information to make a decision.	Not applicable

Abbreviations: TB: tuberculosis

[a]**Grading criteria for 'Risk of bias' assessment**

- If all signalling questions for a domain are answered 'yes' then we will judge the risk of bias to be 'low'.
- If any signalling question is answered 'no' this will flag the potential for bias and we will judge risk of bias with a senior review author.

- If all signalling questions or most of them were answered 'no', then we will judge the risk of bias as 'high'.
- We will assign the 'unclear' category when the study authors report insufficient data to permit a judgment.

CONTRIBUTIONS OF AUTHORS

Daniël J van Hoving and Eleanor A Ochodo wrote the protocol with input from Yemisi Takwoingi, Rulan Griesel, Graeme Meintjes, and Gary Maartens. Daniël J van Hoving and Rulan Griesel reviewed articles for inclusion and extracted data. Discrepancies were resolved by Graeme Meintjes. Eleanor A Ochodo analysed the data with input from Yemisi Takwoingi. Daniël J van Hoving and Eleanor A Ochodo interpreted the analyses and drafted the manuscript. Graeme Meintjes, Gary Maartens and Yemisi Takwoingi provided critical revisions to the manuscript. All review authors read and approved the final manuscript draft.

DECLARATIONS OF INTEREST

Daniël J van Hoving has no conflicts of interest to declare.

Graeme Meintjes has no conflicts of interest to declare.

Yemisi Takwoingi has no conflicts of interest to declare.

Rulan Griesel has no conflicts of interest to declare.

Gary Maartens has no conflicts of interest to declare.

Eleanor A Ochodo has no conflicts of interest to declare.

SOURCES OF SUPPORT

Internal sources

- South African Medical Research Council, South Africa.
- Liverpool School of Tropical Medicine, UK.

External sources

- Department for International Development, UK.

  Project number 300342-104
- Wellcome Trust Foundation, UK.

  Grant: 109939
- Wellcome Trust Foundation, UK.

  Grant: 098316
- South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation, South Africa.

  Grant: 64787
- National Research Foundation incentive funding, South Africa.

  UID: 85858
- TB and HIV Collaborating Centres Programme, South African Medical Research Council, South Africa.

  RFA# SAMRC-RFA-CC: TB/HIV/AIDS-01-2014

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We amended the protocol title from *Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive adults* to *Abdominal ultrasound for diagnosing abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals*.

Our review differed from the Cochrane protocol in several ways (Van Hoving 2017). In the protocol we stated a secondary objective to determine the diagnostic accuracy of combinations of abdominal ultrasound and existing tests (chest radiograph, full blood count) for detecting abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals. However, we could not find any study that evaluated abdominal ultrasound as an add-on test or in combination with other tests, and we therefore did not report on this.

The MEDION database is not active anymore and has not been searched.

In the protocol, we stated that we would have one primary meta-analysis at individual patient level. However we decided to have two sets of primary meta-analyses; one with 2 x 2 tables generated with a higher-quality reference standard and the other with a lower-quality reference standard. As stated in the analysis section, some studies produced two data points (with higher-quality and lower-quality reference standards). Because we did not want to lose information by only selecting one data point for each study and also to produce meaningful results, we present two sets of meta-analyses. We used Stata instead of SAS for all analyses.

Due to insufficient data we did not investigate all potential sources of heterogeneity as stated in the protocol (including clinical setting, and ultrasound training level).

We defined adults in the protocol as participants aged 18 years or older. Two studies included participants under 18 years (older than 15 years) (Sculier 2010-h; Weber 2018-h; Weber 2018-l). We included the studies as i) the number of paediatric cases was low, ii) many countries manage 15-year-old patients as adults, and iii) the results would be valuable for policy making. However, we have downgraded the certainty of the evidence for applicability concerns due to indirectness.

We judged publication bias using three criteria: for-profit interest, only studies detected that produce precise estimates of high accuracy despite small sample size, and knowledge about studies that were conducted but are not published.

APPENDIX TO CHAPTER 3

The potential clinical role of abdominal ultrasound and the implication for practice

The accuracy of abdominal ultrasound refers to its ability to correctly identify patients with abdominal tuberculosis or disseminated tuberculosis with abdominal involvement. The diagnostic accuracy (estimates of sensitivity and specificity) need to be put in context, depending on the role of the abdominal ultrasound in the clinical pathway. Abdominal ultrasound can be considered as a screening (triage) test where it is applied to unselected patients to identify whom require confirmatory testing for tuberculosis (e.g. Xpert MTB/RIF). In these situations, it is preferable to include a test where the sensitivity is maximised in order to be able to exclude patients with a negative test (i.e. low false-negative rate). Abdominal ultrasound had a pooled sensitivity of 63% (95%CI 43% to 79%) in studies using a higher-quality reference standard (bacteriological confirmation). This is considerably lower than the WHO's target product profile for a triage tool for tuberculosis that suggests a minimum requirement of 90% sensitivity,[1] and thus the use of abdominal ultrasound as a screening test for abdominal tuberculosis or disseminated tuberculosis with abdominal involvement is not currently supported.

Abdominal ultrasound can also be used in addition to existing tests (add-on). In this situation abdominal ultrasound will be used in a more select patient population and might fulfil the role of a secondary screening test; for example using abdominal ultrasound in symptomatic patients who had a negative urinary LAM test to identify which patients require more specific confirmatory testing. It can also guide physicians to make empiric treatment decisions while the results of the more specific confirmatory tests are pending. As a secondary screening test, the emphasis of diagnostic accuracy estimates will shift to a higher specificity (i.e. low false-positive rate). The WHO's target product profile for a triage tool for tuberculosis suggests a minimum specificity of 70%.[1] Abdominal ultrasound had a pooled specificity of 68% (95% CI 42% to 87%) in studies using a higher-quality reference standard (bacteriological confirmation), but it was not used as secondary screening test and further studies are needed before recommendations can be made.

Variation in diagnostic thresholds will further influence the clinical role of abdominal ultrasound. The threshold for a positive abdominal ultrasound finding may differ and can be maximised to either sensitivity or specificity. If abdominal ultrasound is to be used as a screening test, then the threshold trade off needs to enhance the sensitivity in order to safely exclude those without abdominal tuberculosis or disseminated tuberculosis with abdominal involvement (i.e., a high true-negative rate). On the other hand, the selected threshold can maximise specificity if abdominal ultrasound is used as

a secondary screening test. The emphasis here is to ensure that those with a positive abdominal ultrasound finding actually have abdominal tuberculosis or disseminated tuberculosis with abdominal involvement (i.e., a high true-positive rate).

Sensitivity analyses: studies with case-control design excluded

Diagnostic case-control studies are prone to spectrum bias, which can potentially lead to an overestimate of sensitivity and specificity. Three studies with a case-control design were included, of which none were included in the primary analysis. The three diagnostic case-control studies were part of the nine studies included in the secondary analysis (evaluating individual abdominal ultrasound lesions). Results of the primary and secondary analyses are summarized in Table 3. A formal sensitivity analysis was not performed due to the small number of included studies, but the results of the primary and secondary analyses where studies with a case-control design were excluded are presented in Table 3b.

Table 3b. Summary estimates of sensitivity and specificity for any abnormality and individual abdominal ultrasound findings (diagnostic case-control studies excluded).

Abdominal ultrasound finding	**Number of studies**	**Number of participants (tuberculosis cases)**	**Pooled sensitivity (95% CI) %**	**Pooled specificity (95% CI) %**	**Range of sensitivity %**	**Range of specificity %**
Any abnormality (higher-quality reference standard)	5	879 (368)	63 (43 to 79)	68 (72 to 87)	35 to 82	20 to 92
Any abnormality (lower-quality reference standard)	4	397 (149)	68 (45 to 85)	73 (41 to 91)	37 to 88	22 to 92
Splenic lesions	4	674 (356)	Not calculated	Not calculated	13 to 56	86 to 100
Intra-abdominal lymph nodes	6	740 (372)	Not calculated	Not calculated	22 to 67	56 to 91
Ascites	5	624 (305)	Not calculated	Not calculated	4 to 73	33 to 96
Splenomegaly	3	508 (369)	Not calculated	Not calculated	5 to 39	67 to 89
Hepatomegaly	2	131 (68)	Not calculated	Not calculated	36 to 65	43 to 78

References

1. World Health Organization. Meeting report: High-priority target product profiles for new tuberculosis diagnostics. Geneva: World Health Organization; 2014. Available from: http://www.who.int/tb/publications/tpp_report/en/

CHAPTER 4

Real-World Performance and Interobserver Agreement of Urine Lipoarabinomannan in Diagnosing HIV-Associated Tuberculosis in an Emergency Centre

Real-World Performance and Interobserver Agreement of Urine Lipoarabinomannan in Diagnosing HIV-Associated Tuberculosis in an Emergency Center

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Background: The urine lipoarabinomannan (LAM) lateral flow assay is a point-of-care test to diagnose HIV-associated tuberculosis (TB). We assessed the performance of urine LAM in HIV-positive patients presenting to the emergency center and evaluated the interobserver agreement between emergency center physicians and laboratory technologists.

Setting: A cross-sectional diagnostic study was performed at the emergency center of a district hospital in a high HIV-prevalence community in South Africa.

Methods: Consecutive HIV-positive adults presenting with ≥1 WHO TB symptom were enrolled over a 16-month period. A urine LAM test was performed at point-of-care by an emergency physician and interpreted independently by 2 physicians. A second test was performed in the laboratory and interpreted independently by 2 laboratory technologists. The reference standard was a positive TB culture or Xpert MTB/RIF test on sputum or appropriate extrapulmonary samples. We compared diagnostic accuracy and reproducibility of urine LAM between point-of-care readers and laboratory readers.

Results: One thousand three hundred eighty-eight samples (median, 3 samples/participant) were sent for TB microbiology tests in 411 participants; 170 had confirmed TB (41.4%). Point-of-care and laboratory-performed urine LAM had similar sensitivity (41.8% vs 42.0%, $P = 1.0$) and specificity (90.5% vs 87.5%, $P = 0.23$). Moderate agreement was found between point-of-care and laboratory testing ($\kappa = 0.62$), but there was strong agreement between point-of-care readers ($\kappa = 0.95$) and between laboratory readers ($\kappa = 0.94$). Positive percent agreement between point-of-care and laboratory readers was 68% and negative percent agreement 92%.

Conclusion: There is no diagnostic accuracy advantage in laboratory-performed versus point-of-care–performed urine LAM tests in emergency care centers in high-burden settings.

Key Words: tuberculosis, lipoarabinomannan, point-of-care, HIV, agreement, emergency

(*J Acquir Immune Defic Syndr* 2019;81:e10–e14)

INTRODUCTION

Diagnosing active tuberculosis (TB) in HIV-positive patients with advanced immunosuppression is challenging.[1] Patients with HIV-associated TB needing emergency care are at high risk of mortality, exacerbated by delay in starting TB treatment. Autopsy studies of HIV-positive adults reported that 46% TB cases were undiagnosed antemortem.[2] Rapid, simple, inexpensive, and accurate diagnostic tests for TB in HIV-positive patients are thus needed.

Assays that detect lipoarabinomannan (LAM) in urine are recommended by the WHO for use in HIV-positive adults who are seriously ill or have advanced immunosuppression

Received for publication December 18, 2018; accepted February 4, 2019.
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G. Meintjes was supported by the Wellcome Trust (098316 and 203135/Z/16/Z), the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant No 64787), NRF incentive funding (UID: 85858), and the South African Medical Research Council through its TB and HIV Collaborating Centres Programme with funds received from the National Department of Health (RFA# SAMRC-RFA-CC: TB/HIV/AIDS-01-2014). G. Maartens was supported by the NRF incentive funding (UID: 85810). The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of this report.

The opinions, findings, and conclusions expressed in this manuscript reflect those of the authors alone. The remaining authors have no funding or conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).
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(CD4 cell count ≤ 100 cells/μL).[3] Although the sensitivity of the current assay is suboptimal, urinary LAM performs better in patients at high risk of mortality,[4,5] where its use can decrease mortality.[6,7]

The development of a low-cost, rapid, lateral flow assay has made urine LAM a useful point-of-care test.[8] However, most studies to date performed the LAM lateral flow assay in a laboratory,[4] and none was performed in an emergency care setting. We assessed the performance of urine LAM in HIV-positive patients presenting to the emergency center and evaluated the interobserver agreement between emergency center physicians and laboratory technologists.

METHODS

Design and Setting

A cross-sectional diagnostic study was performed at the emergency center of Khayelitsha Hospital in Cape Town, South Africa, which is a 300-bed district-level hospital with an emergency center that manages about 30,000 patients per annum and has a 30% admission rate. A recent study found that the prevalence of HIV infection was 23% in severely ill patients managed in the emergency center.[9] The enrollment period was June 2016 through October 2017.

Population

All HIV-positive adults with a WHO TB symptom who were evaluated in the emergency center of Khayelitsha Hospital were eligible. Inclusion criteria were as follows: age ≥18 years; HIV-positive (clinical records or laboratory confirmation); having at least 1 TB symptom (cough of any duration, fever, drenching night sweats, or weight loss); and informed consent. Exclusion criteria were as follows: having taken anti-TB treatment within 3 months before enrollment; presenting to the emergency center more than 24 hours before screening; pregnancy; trauma, gynecological or psychiatric-related presentation; and main clinical presentation of meningitis syndrome or new focal neurology.

All participants provided written informed consent, and the study was approved by the Human Research Ethics Committee of the University of Cape Town and the Western Cape Provincial Health Research Committee.

Procedures and Samples

Consecutive patients admitted to the emergency center were screened for eligibility from Monday to Thursday. Demographic and clinical information were recorded on a standardized data collection form. Microbiologic tests to confirm TB included Xpert MTB/RIF (urine and sputum) and liquid mycobacterial culture of sputum and blood. Urine, blood, and sputum samples were obtained from all patients whenever possible. Urine was collected in sterile single-use disposable containers. Patients who could not produce a spontaneously voided urine sample were catheterized. Urine samples were tested for the presence of LAM on site and then the residual urine was transported on ice to reach the laboratory within 6 hours of collection. Two sputum samples were collected; sputum induction with hypertonic saline in an ultrasound nebulizer was performed when a spontaneous sputum could not be produced. On-site urine LAM test results were made available to managing clinicians as soon as they became available. Samples collected as part of the routine standard of care by hospital clinicians (nonstudy) were not duplicated, and appropriate extrapulmonary samples were included as part of the reference standard.

Fresh urine samples were tested for the presence of LAM (Alere Determine TB LAM Ag test; Alere Inc., Waltham, MA). A drop of urine (±60 μL) was applied to the sample pad on the test strip. The test was performed once and independently read at different times by 2 clinicians between 25 and 120 minutes later, under ambient hospital lighting conditions. One clinician (D.J.V.H.) was the primary reader for all the tests, while the most senior clinician on duty in the emergency center on the day of enrollment performed the second read. Strict adherence to the suggested time frame for reading the strips was impossible because both clinicians still had clinical duties that sometimes delayed the reading of the test. However, reading was never performed before the recommended minimum time. The second reader was blinded to the primary reader's interpretation and to clinical data and other results. The lowest reading of the 2 readers was taken as the final point-of-care result. A separate urine LAM test was also performed in the laboratory according to the manufacturer's recommendations.[8] Two trained laboratory technologists independently recorded the result, and the lowest result was taken as the final laboratory result. The laboratory readers were blinded to each other's results and to clinical status and microbiological test results. The final LAM result was deemed positive at the grade 1 cutoff.

CD4 counts were not repeated if performed within 3 months before enrollment. Study microbiology tests were performed by the research microbiology laboratory at the University of Cape Town. Microbiology samples were processed with standardized protocols in an accredited laboratory. Sputum and concentrated urine samples were tested using the Xpert MTB/RIF assay (GX4) (Cepheid Inc., Sunnyvale, CA),[10] and sputum was also cultured in liquid media (MGIT; Becton Dickson, Sparks, MD). BACTEC MYCO/F Lytic blood culture bottles (Becton Dickson) were filled with at least 5 mL of blood and incubated for up to 6 weeks. The MTBDRplus assay (Hain Lifescience, Nehren, Germany) was used to identify cultured isolates as *Mycobacterium tuberculosis* complex.

Case Definition

A confirmed TB case was defined as the detection of *M. tuberculosis* complex by Xpert MTB/RIF and/or culture on any specimen from any anatomical site obtained during hospital admission (including any sample collected by clinical staff) and up to 6 weeks after hospital discharge. Tests performed after discharge from the hospital were identified using the National Health laboratory Service TrakCare Lab Webview—a web viewer providing access to all National Health laboratory Service results within South Africa.

STATISTICAL METHODS

Statistical Analysis

Summary statistics were used to describe the variables. Comparisons were performed using the *t*-test or Mann–Whitney test when comparing means or medians or the χ^2-test when comparing proportions. Diagnostic test characteristics [with 95% confidence intervals (CI)] were calculated using standard formulas. Sensitivity and specificity were compared with the McNemar test, which was applied separately to participants with confirmed TB (sensitivity comparison) and those without TB (specificity comparison).[11] Reproducibility was measured using κ statistics with 95% CI and was interpreted as previously described.[12] Statistical analyses were performed using MedCalc for Windows, version 18.5 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2018) and SPSS Statistics for Windows, Version 25.0 (IBM Corp, Released 2017, Armonk, NY).

RESULTS

A total of 556 patients were screened, 427 (76.8%) of whom were enrolled; 16 patients were excluded from the analysis (see Figure, Supplemental Digital Content, http://links.lww.com/QAI/B286 which illustrates the flow of study participants). In total, 1388 samples from the 411 evaluated participants (median per participant 3; range 1–9) were sent for microbiological testing. Fifteen participants (3.6%) had only 1 Xpert MTB/RIF, 11 (2.7%) had only 1 culture, and 385 (93.7%) had at least 1 Xpert MTB/RIF plus at least 1 culture performed. TB was confirmed in 170 (41.4%) participants (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B286 for the results of confirmatory tests). The characteristics of study participants with and without confirmed TB are summarized in supplementary material (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B286 for demographic and clinical characteristics of the study population). Sixty-three (26.1%) participants without confirmed TB were started on TB treatment of which 7 were based on a positive point-of-care LAM test alone (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B286 for reasons why TB treatment was initiated in relation to LAM results).

Two laboratory results were excluded [no test performed n = 1 (TB confirmed); invalid result n = 1 (TB not confirmed)]. The result of the second reader of the laboratory tests was not completed for 1 participant, and the reading of the single reader was taken as the final result. The point-of-care urine LAM test was positive in 94/411 (22.9%) participants, compared with 101/409 (24.6%) of the laboratory-performed tests ($P = 0.54$). No point-of-care result was inconclusive, whereas 10 of the laboratory results were; these results were regarded as negative. Point-of-care and laboratory-performed urine LAM had similar sensitivity ($P = 1.0$) and specificity ($P = 0.23$) (Table 1).

Strong agreement was found between the 2 point-of-care readers (κ = 0.95 [95% CI: 0.92 to 0.99]) and between the 2 laboratory readers [κ = 0.94 (95% CI: 0.91 to 0.98)] for both groups but only moderate agreement between point-of-care and laboratory testing [κ = 0.62 (95% CI: 0.53 to 0.71)] (Table 2). The positive agreement between point-of-care and laboratory readings was 69/101 (68.3%) and the negative agreement was 283/308 (91.9%) (Table 2). Eight of the 11 participants with inconclusive laboratory readings had confirmed TB; 6 of these 8 had a positive point-of-care reading. In cases with differences in grade reading, most of the disagreements (55/90, 61.1%) were between negative and grade 1 cutoffs (see Tables, Supplemental Digital Content, http://links.lww.com/QAI/B286 for the level of agreement relating to difference in grades).

DISCUSSION

Our findings suggest that the urine LAM test can provide results in busy emergency care settings that are similar to those provided with laboratory testing. The modest sensitivity (42% overall) of urine LAM among HIV-positive patients presenting to the emergency center is similar to that described previously[4] and indicates that it should not be used as a rule-out test. High interobserver agreements were found both between readers at the point-of-care (κ = 0.95) and the research laboratory (κ = 0.94), but only a moderate level of agreement (κ = 0.62) was found between point-of-care readers and laboratory readers. However, despite the lower level of agreement between point-of-care and laboratory readers, the diagnostic accuracy of urine LAM was similar

TABLE 1. Comparison of Diagnostic Accuracy of LAM Between Point-of-Care and Laboratory Testing on Urine Samples

	Point-of-Care*	Laboratory†
Sensitivity, % (95% CI)		
Overall	41.8 (34.3 to 49.6)	42.0 (34.5 to 50.0)
CD4 ≤ 100 cells/μL	55.1 (45.2 to 64.8)	57.6 (47.6 to 67.1)
Specificity, % (95% CI)		
Overall	90.5 (86.0 to 93.9)	87.5 (82.6 to 91.4)
CD4 ≤ 100 cells/μL	86.5 (78.7 to 92.2)	83.8 (75.6 to 90.1)
Positive predictive value, % (95% CI)		
Overall	75.5 (66.8 to 82.6)	70.3 (61.8 to 77.6)
CD4 ≤ 100 cells/μL	79.7 (70.5 to 86.7)	77.2 (68.3 to 84.2)
Negative predictive value, % (95% CI)		
Overall	68.8 (65.8 to 71.6)	68.2 (65.1 to 71.1)
CD4 ≤ 100 cells/μL	66.7 (61.6 to 71.4)	67.4 (62.0 to 72.4)
Positive likelihood ratio, (95% CI)		
Overall	4.4 (2.9 to 6.7)	3.4 (2.3 to 4.9)
CD4 ≤ 100 cells/μL	4.1 (2.8 to 6.7)	3.6 (2.3 to 5.6)
Negative likelihood ratio, (95% CI)		
Overall	0.6 (0.6 to 0.7)	0.7 (0.6 to 0.8)
CD4 ≤ 100 cells/μL	0.5 (0.4 to 0.7)	0.5 (0.4 to 0.6)

Inconclusive tests (n = 10) were regarded as negative (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B286, where inconclusive results were excluded).

*Overall n = 411, CD4 ≤ 100 cells/μL n = 218.

†Overall n = 409 [missing (n = 1) and invalid (n = 1) results excluded], CD4 ≤ 100 cells/μL n = 218.

TABLE 2. Level of Agreement of LAM Readings (Positive or Negative) Between Point-of-Care Reading and Laboratory Reading

	Laboratory Reading, n (%)		
	Negative*	Positive†	
Point-of-care reading, n (%)			
Negative	*283 (69.2)*	32 (7.8)	315 (77)
Positive†	25 (6.1)	*69 (16.9)*	94 (23)
	308 (75.3)	101 (24.7)	409 (100)

Urine test not performed by laboratory (n = 1) and invalid laboratory result (n = 1) excluded (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B286, where inconclusive results were excluded); italicized values represent agreement.
*Includes inconclusive (n = 10) results.
†Positive: Urine LAM grade 1 cutoff or higher.

between clinicians and laboratory technologists. Most (61%) of the disagreements between the laboratory and the point-of-care readings occurred at the grade 1 cutoff point, which may have significant clinical implications because this cutoff is used for making decisions on whether to treat for TB.

The diagnostic performance of urine LAM in our busy emergency care setting is comparable with that in other settings.[4] Unlike most other studies, clinicians performing and reading the test strips were not dedicated study staff and had to fit the study-related processes in between their normal clinical duties. Despite there often being delays in reading the test strips, the diagnostic accuracy of point-of-care readings was similar to that of laboratory-performed readings.

The high interobserver agreement between laboratory personnel and between point-of-care clinicians was similar to previous studies.[13–16] There are 3 possible explanations for the lower interobserver agreement between the laboratory and point-of-care readings. First, urine samples should be tested immediately because there might be a decrease in LAM concentrations after 2 hours.[17] Urine was transported on ice to reach the laboratory within 6 hours; 43 (11%) of laboratory readings had a lower score than point-of-care readings, whereas 47 (12%) point-of-care readings had a lower score than the respective laboratory reading, which makes this explanation unlikely. Second, the interpretation of the reference card requires a subjective discrimination between the intensity of the color bands.[18] Subjectivity might explain differences between individual readers but would not explain differences between groups of readers. Instead, there may have been a shared approach between point-of-care readers and a shared approach between laboratory readers, but the approach itself might have differed because the point-of-care group trained together but separately from the laboratory group. Finally, the discrepancy could just relate to errors made by both groups but in different patients.

Uninterpretable readings were more common than previously described. Up to 2% of tests have been reported as uninterpretable, although some studies had no invalid or uninterpretable results.[4] Only the laboratory readers recorded uninterpretable readings and all by the same individual reader. It is thus possible that more strict criteria for defining uninterpretable readings were applied.

The diagnostic performance of urine LAM as a point-of-care test in a real-world emergency care setting was similar to that of laboratory reading. Urinary LAM is thus truly a point-of-care test.

ACKNOWLEDGMENTS

The authors acknowledge the valuable contribution of Rene Goliath (research clinical site coordinator, Institute of Infectious Disease and Molecular Medicine, University of Cape Town) and the microbiology team from the Department of Clinical Laboratory Sciences (Division of Medical Microbiology, University of Cape Town).

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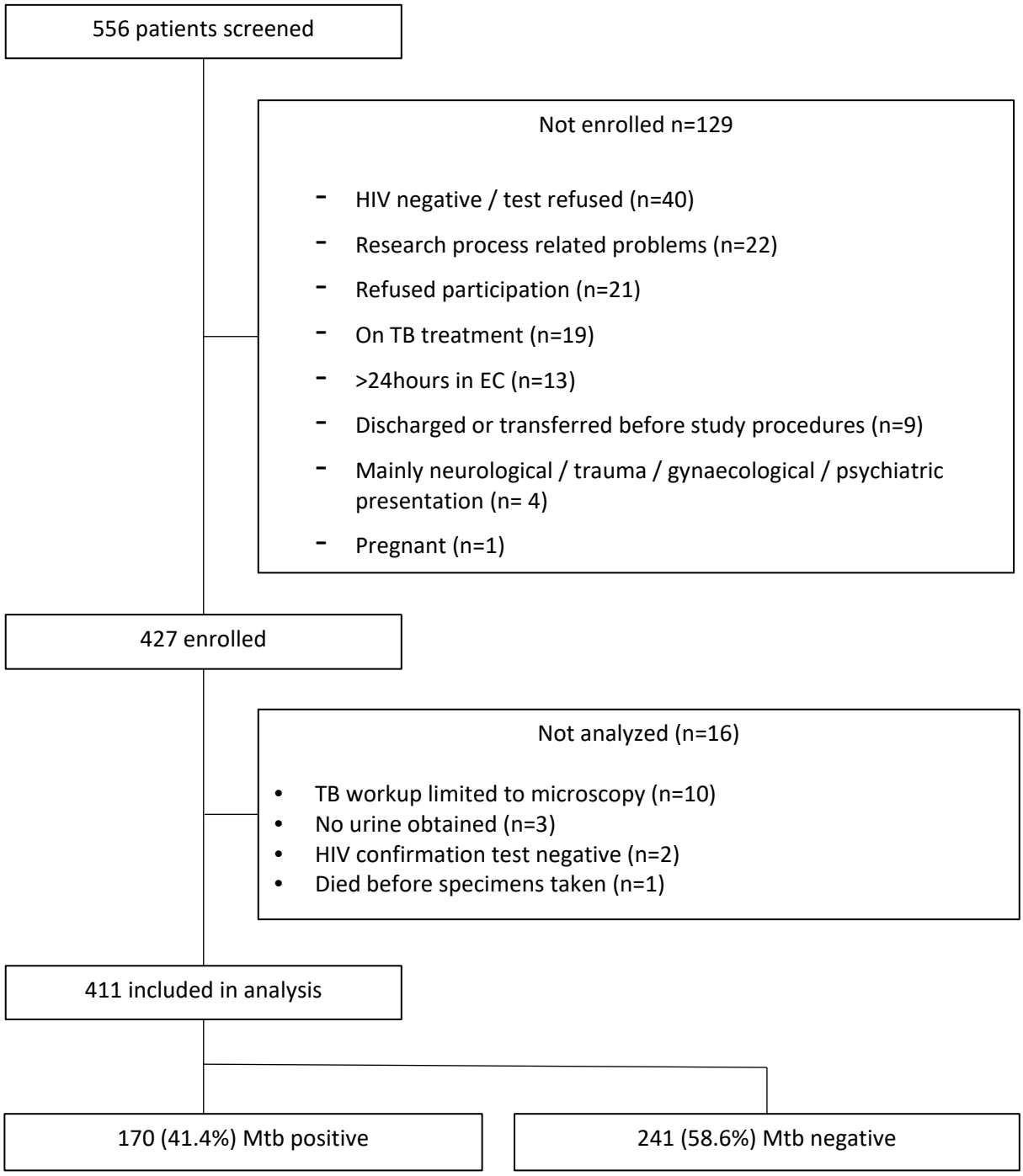
Supplemental Digital Content 1

Figure. Study flow diagram



Supplemental Digital Content 2

Table. Results of confirmatory testing in participants with confirmed TB

Number of participants with confirmed TB	Xpert test positive[a]	Only Xpert test positive	Xpert and culture (any site) tests positive	Xpert and blood culture tests positive	Only culture test positive (any site)	Only blood culture test positive	Culture test positive (any site)	Culture test positive (blood)
All (n=170)	125	32	93	44	45	19	138	63
Laboratory LAM positive (n=71)	54	12	42	28	18	9	59	37
Point-of-care LAM positive (n=71)	55	12	43	29	16	10	59	39

a: Only sputum positive n=61, Sputum and extra-pulmonary samples positive n=27, Only extra-pulmonary samples positive n=37

Supplemental Digital Content 3

Table. Demographic and clinical characteristics of study population

Characteristics at enrollment, n (%) unless otherwise specified	n	All (n = 411)	Mtb* confirmed (n = 170)	Mtb not confirmed (n = 241)	p-value†
Demographics					
Age (years) (Median (IQR))	411	35 (30-43)	35 (25-42)	36 (31-44)	0.13
Male	411	171 (41.6%)	71 (41.8%)	100 (41.5%)	0.96
Clinical history					
Current cough of any duration	411	350 (85.2%)	147 (86.5%)	203 (84.2%)	0.53
Weight loss within last month	411	352 (85.6%)	154 (90.6%)	198 (82.2%)	0.02†
Night sweats within last month	411	218 (53.0%)	102 (60.0%)	116 (48.1%)	0.02†
Fever within last month	411	200 (48.7%)	75 (44.1%)	125 (51.9%)	0.12
Previous TB (any time)	409	214 (52.3%)	71 (41.8%)	143 (59.8%)	<0.01†
Previous TB ≤ 2 years	409	75 (18.3%)	27 (15.9%)	48 (20.1%)	0.28
Antiretroviral therapy naïve	411	123 (29.9%)	58 (34.1%)	65 (27.0%)	0.12
Currently on antiretroviral therapy	411	194 (47.2%)	71 (41.8%)	123 (51.0%)	0.06
Defaulted antiretroviral therapy	411	88 (21.4%)	41 (24.1%)	47 (19.5%)	0.26
Unknown if on antiretroviral therapy	411	6 (1.5%)	0 (0.0%)	6 (2.5%)	0.44
First line antiretroviral regime	411	162 (39.4%)	53 (31.2%)	109 (45.2%)	<0.01†
Second line antiretroviral regime	411	47 (11.4%)	13 (7.6%)	34 (14.1%)	0.04†
Unknown antiretroviral regime	411	79 (19.2%)	46 (27.1%)	33 (13.7%)	<0.01†
Clinical findings					
Height (centimeter) (Median (IQR‡))	379	163 (157-169)	163 (157-167)	163 (157-170)	0.25
Weight (kilogram) (Median (IQR))	380	54 (46-65)	53 (46-61)	55 (47-67)	0.13
Body Mass Index (kg/m^2) (Median (IQR))	379	20 (17-24)	20 (17-24)	20 (18-25)	0.38
Underweight (Body Mass Index < 18.5 kg/m^2)	379	136 (35.9%)	60 (37.7%)	76 (34.5%)	0.52
Temperature (centigrade) (Median (IQR))	408	36.8 (36.1-37.8)	36.8 (36.1-38.0)	36.7 (36.2-37.8)	0.63
Systolic blood pressure (mmHg) (Median (IQR))	411	111 (98-125)	111 (99-123)	111 (97-127)	0.81
Diastolic blood pressure (mmHg) (Median (IQR))	411	71 (61-81)	72 (60-80)	71 (61-82)	0.98
Heart rate (beats per minute) (Mean ± SD)	411	122 ± 22	126 ± 23	119 ± 21	0.01†
Respiratory rate (breaths per minute) (Median (IQR))	408	24 (19-30)	24 (18-30)	24 (20-32)	0.11
Oxygen saturation level (Median (IQR))	403	97 (94-99)	98 (95-99)	96 (93-99)	0.08
Hemoglobin (g/dl) (Mean ± SD§)	407	9.7 ± 2.6	9.0 ± 2.4	10.3 ± 2.7	<0.01†
Mean corpuscular volume (fL¶) (Mean ± SD)	404	86.7 ± 9.0	84.8 ± 8.1	87.9 ± 9.3	<0.01†
White blood cell count (x 10^9/l) (Median (IQR))	409	8.4 (5.8-13.6)	7.5 (5.4-12.6)	9.1 (6.3-14.7)	<0.01†
Platelet count (Median (IQR))	407	283 (195-391)	272 (199-383)	288 (186-402)	0.51
CD4 cell count (cells/µL) (Median (IQR))	405	87 (30-218)	65 (23-154)	121 (37-268)	<0.01†

*Mtb = Mycobacterium tuberculosis; †Indicates statistical significance; ‡IQR= Interquartile range; §SD = Standard Deviation; ¶Femtoliter

Normality were assessed visually (using histograms and Q-Q plots) and with the Shapiro-Wilks test. Homogeneity of variance were determined with Levene's test. For continuous data, the Mann-Whitney U test (non-parametric distribution) or the Independent t-test (parametric distribution) were used. Pearson's chi-squared test or Fischer's exact test were used for categorical data. A significance level of .05 was applied.

Supplemental Digital Content 4

Table. Reasons for initiating TB treatment in participants without confirmed TB (n=63) in relation to point-of-care LAM results

Point-of-care LAM positive[a] (n = 16)[b]		Point-of-care LAM negative (n = 47)[c]	
Reason for initiating TB treatment	**n**	**Reason for initiating TB treatment**	**n**
Only LAM positive	7	Suggestive abdominal ultrasound	20
Suggestive chest x-Ray	4	Suggestive chest X-Ray	8
Suggestive abdominal ultrasound	3	Suggestive abdominal ultrasound & suggestive chest X-Ray	6
Raised adenosine deaminase in effusion fluid[d]	1	Suggestive lumbar puncture or suggestive computer tomography of the brain	4
Caseous necrosis on microscopic examination	1	Not improving on antimicrobial agents	4
		Raised adenosine deaminase in effusion fluid[d]	3
		Suggestive computer tomography of the abdomen	1
		Suggestive chest x-ray & raised adenosine deaminase in effusion fluid[d]	1

a: Positive LAM defined as grade 1 or higher
b: Laboratory LAM negative n=6
c: Laboratory LAM positive n=6
d: Defined as adenosine deaminase > 30 U/L

Supplemental Digital Content 5

Table. Comparison of diagnostic accuracy of lipoarabinomannan (LAM) between point-of-care and laboratory testing on urine samples (missing, invalid and inconclusive results excluded)

	Point-of-Care*	Laboratory†
Sensitivity, % (95%CI‡)		
Overall	41.8 (34.3 to 49. 6)	44.1 (36.3 to 52.1)
CD4 ≤ 100 cells/µL	55.1 (45.2 to 64.8)	59.8 (49.6 to 69.4)
Specificity, % (95%CI)		
Overall	90.5 (86.0 to 93.9)	87.4 (82.5 to 91.3)
CD4 ≤ 100 cells/µL	86.5 (78.7 to 92.2)	83.5 (75.2 to 89.9)
Positive Predictive Value, % (95%CI)		
Overall	75.5 (66.8 to 82.6)	70.3 (61.9 to 77.5)
CD4 ≤ 100 cells/µL	79.7 (70.5 to 86.7)	77.2 (68.3 to 84.2)
Negative Predictive Value, % (95%CI)		
Overall	68.8 (65.8 to 71.6)	69.8 (66.7 to 72.8)
CD4 ≤ 100 cells/µL	66.7 (61.6 to 71.4)	68.9 (63.3 to 74.0)
Positive Likelihood Ratio, (95%CI)		
Overall	4.4 (2.9 to 6.7)	3.5 (2.4 to 5.1)
CD4 ≤ 100 cells/µL	4.1 (2.8 to 6.7)	3.6 (2.3 to 5.7)
Negative Likelihood Ratio, (95%CI)		
Overall	0.6 (0.6 to 0.7)	0.6 (0.6 to 0.7)
CD4 ≤ 100 cells/µL	0.5 (0.4 to 0.7)	0.5 (0.4 to 0.6)

*Overall n=411, CD4≤100 cells/µL n =218;

†Overall n=399, CD4≤100 cells/µL n=211;

‡Confidence interval;

Excluded results: missing (n=1), inconclusive (n=10), and invalid (n=1)

Supplemental Digital Content 6

Table. Level of agreement of lipoarabinomannan (LAM) readings (positive or negative) between point-of-care reading and laboratory reading (missing, invalid, and inconclusive results excluded)

	Laboratory reading, n(%)			
Point-of-care reading, n(%)	**Negative**	**Positive***	**Inconclusive**	
Negative	*275 (67.1)*	32 (7.8)	8 (2.0)	**315 (77)**
Positive	23 (5.6)	*69 (16.8)*	2 (0.5)	**94 (23)**
Inconclusive *	0 (0.0)	0 (0.0)	*0 (0.0)*	**0 (0.0)**
	298 (72.7)	**101 (24.6)**	**10 (2.4)**	**409 (100)**

*Urine lipoarabinomannan (LAM) grade-1 cut off or higher

Urine not done by laboratory (n=1) and invalid laboratory results (n=1) excluded;

Shaded areas represent agreement

Supplemental Digital Content 7

Table. Level of agreement of lipoarabinomannan (LAM) readings between point-of-care reading and laboratory reading on urine samples relating to difference in grades (inconclusive results regarded as negative)

Point-of-care reading, n(%)	Laboratory reading, n(%)					
	0*	1+	2+	3+	4+	
0	283 (69.2)	32 (7.8)	0 (0.0)	0 (0.0)	0 (0.0)	**315 (77.0)**
1+	23 (5.6)	24 (5.9)	7 (1.7)	3 (0.7)	0 (0.0)	**57 (13.9)**
2+	2 (0.5)	6 (1.5)	4 (1.0)	4 (1.0)	0 (0.0)	**16 (3.9)**
3+	0 (0.0)	4 (1.0)	7 (1.7)	7 (1.7)	1 (0.2)	**19 (4.6)**
4+	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.2)	**2 (0.5)**
	308 (75.3)	**66 (16.1)**	**19 (4.6)**	**14 (3.4)**	**2 (0.5)**	**409 (100)**

*Includes inconclusive (n=10) results

Urine not done by laboratory (n=1) and invalid laboratory result (n=1) excluded;

Shaded areas represent agreement

Table. Level of agreement of lipoarabinomannan (LAM) readings between point-of-care reading and laboratory reading on urine samples relating to difference in grades (missing, invalid, and inconclusive results excluded)

Point-of-care reading, n(%)	Laboratory reading, n(%)						
	0	1+	2+	3+	4+	Inconclusive*	
0	275 (67.1)	32 (7.8)	0 (0.0)	0 (0.0)	0 (0.0)	8 (2.0)	**315 (77.0)**
1+	21 (5.1)	24 (5.9)	7 (1.7)	3 (0.7)	0 (0.0)	2 (0.5)	**57 (13.9)**
2+	2 (0.5)	6 (1.5)	4 (1.0)	4 (1.0)	0 (0.0)	0 (0.0)	**16 (3.9)**
3+	0 (0.0)	4 (1.0)	7 (1.7)	7 (1.7)	1 (0.2)	0 (0.0)	**19 (4.6)**
4+	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	**2 (0.5)**
Inconclusive*	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**0 (0.0)**
	298 (72.9)	**66 (16.1)**	**19 (4.6)**	**14 (3.4)**	**2 (0.5)**	**10 (2.4)**	**409 (100)**

Urine not done by laboratory (n=1) and invalid (n=1) results excluded;

Shaded areas represent agreement

APPENDIX TO CHAPTER 4

Limitations

The primary clinician reader (D.J.V.H.) had access to participants' clinical presentation while all other readers did not; this could have influence the interpretation of the urine LAM test. Ideally all test interpreters need to be blinded to prior information to prevent overestimating the sensitivity and specificity of the test. This was not possible due to limited resources and various measures were put in place to limit bias. These include using a standardised test procedure and an independent second reading of the same test strip. The second reader was blinded to the primary reader's interpretation and to clinical data and other results. The lowest reading of the two readers was also taken as the final point-of-care result to limit overestimation. Strong agreement was found between the two point-of-care clinician readers ($\kappa = 0.95$ [95% CI: 0.92 to 0.99]), and we are satisfied that the diagnostic accuracy of the point-of-care performed urine LAM test are not falsely increased.

CHAPTER 5

Point-of-Care Ultrasound Predictors for the Diagnosis of Tuberculosis in HIV-Positive Patients Presenting to an Emergency Centre

Point-of-Care Ultrasound Predictors for the Diagnosis of Tuberculosis in HIV-Positive Patients Presenting to an Emergency Center

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Background: The performance of point-of-care ultrasound (PoCUS) to diagnose HIV-associated tuberculosis has not been evaluated in large prospective studies. We determined the diagnostic accuracy of individual PoCUS features, performed an external validation of the focused assessment with sonography for HIV/TB (FASH) protocol, and determined independent PoCUS predictors of HIV-associated tuberculosis appropriate for use by emergency center practitioners.

Setting: A cross-sectional diagnostic study was performed at the emergency center of Khayelitsha Hospital (Cape Town, South Africa).

Methods: HIV-positive adults with the suspicion of having tuberculosis were prospectively enrolled. PoCUS was performed according to a standardized protocol. Reference standard was the detection of *Mycobacterium tuberculosis* using Xpert MTB/RIF or culture.

Results: We enrolled 414 participants: 243 female, median age 36 years, median CD4 cell count 86/mm^3, and 172 (42%) had tuberculosis. Sensitivity and specificity were $\geq$1 individual PoCUS feature [73% (95% CI: 65 to 79), 54% (95% CI: 47 to 60)], FASH protocol [71% (95% CI: 64 to 78), 57% (95% CI: 50 to 63)]. Independent PoCUS predictors identified were intra-abdominal lymphadenopathy of any size (aDOR 3.7 (95% CI: 2.0 to 6.7)], ascites [aDOR 3.0 (95% CI: 1.5 to 5.7)], and pericardial effusion of any size [aDOR 1.9 (95% CI: 1.2 to 3.0)]. The c-statistic for the derivation model was 0.680 (95% CI: 0.631 to 0.729), compared with 0.630 (95% CI: 0.576 to 0.684) of the FASH protocol. Two or more independent PoCUS predictors had 91% (95% CI: 86 to 94) specificity.

Conclusion: The presence of 2 or more independent PoCUS predictors (intra-abdominal lymphadenopathy, ascites, and pericardial effusion) had moderate discrimination for HIV-associated tuberculosis in patients presenting to the emergency center.

Key Words: HIV, tuberculosis, diagnosis, prediction, ultrasound, emergency center

(*J Acquir Immune Defic Syndr* 2020;83:415–423)

Received for publication July 16, 2019; accepted December 9, 2019.

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G. Meintjes was supported by the Wellcome Trust (098316 and 203135/Z/16/Z), the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant No 64787), NRF incentive funding (UID: 85858) and the South African Medical Research Council through its TB and HIV Collaborating Centres Programme with funds received from the National Department of Health (RFA# SAMRC-RFA-CC: TB/HIV/AIDS-01-2014). G. Maartens was supported by NRF incentive funding (UID: 85810). D.J.v.H. received a Mindray ultrasound machine from the RCA division of Ascendis Medical to use in a part of the study. The remaining authors have no funding or conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The opinions, findings, and conclusions expressed in this article reflect those of the authors alone. For the remaining authors, none were declared.

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INTRODUCTION

Tuberculosis is the leading cause of death in adults infected with the HIV globally.[1] Some of these deaths could be prevented with early diagnosis and treatment. Diagnosing tuberculosis in HIV-positive patients is challenging because they have atypical clinical presentations, higher rates of smear-negative pulmonary and extrapulmonary tuberculosis, and often cannot produce sputum.[2–5]

Ultrasound can identify features associated with extrapulmonary tuberculosis in HIV-positive patients[6] and has been included in the WHO diagnostic algorithm for seriously ill HIV-positive patients with a positive tuberculosis symptom screen.[7] Point-of-care ultrasound (PoCUS) has been used to improve the diagnoses of pericardial, pleural, and abdominal

tuberculosis in HIV-positive patients.[8–11] The focused abdominal sonography for HIV/TB (FASH) protocols are easily learned and quick to perform.[12] The FASH basic evaluates 3 easily recognized variables: pericardial effusion, pleural effusion, and ascites.[13] The 3 additional FASH plus features are more difficult to identify upper abdominal lymph nodes (>15 mm in diameter), focal hypoechoic splenic, and focal hypoechoic liver lesions.[13] The FASH combined (or just FASH protocol) evaluates all 6 of the features of the FASH basic and FASH plus.[13]

Most studies evaluating the diagnostic performance of abdominal ultrasound are retrospective,[14,15] have a small sample size (≤100 participants),[8,16–18] used a case–control design,[19–21] or used reference standards that were not robust.[10,18,22,23] Several studies were also limited by incorporation bias (index test incorporated in composite reference standard),[8,14,15,17] which overestimates the diagnostic accuracy of the test.[24] A systematic review evaluating abdominal ultrasound in bacteriologically confirmed HIV-associated tuberculosis reported pooled sensitivity of 63% and pooled specificity of 68%, and reported very low-quality evidence—only one study evaluating PoCUS met the inclusion criteria for the review.[25] There are no large prospective studies evaluating the diagnostic accuracy of PoCUS to diagnose tuberculosis in HIV-positive adults using a robust reference standard. We conducted a prospective cross-sectional study to determine the diagnostic accuracy of PoCUS for HIV-associated tuberculosis using a microbiological reference standard that included systematic tuberculosis testing of multiple clinical samples. Our first objective was to determine the diagnostic accuracy of individual PoCUS features. Our second objective was to perform an external validation of the FASH protocols using FASH-specific thresholds. Our third objective was to determine independent PoCUS predictors of HIV-associated tuberculosis appropriate for use by practitioners in emergency centers.

METHODS

Study Participants

We conducted a cross-sectional diagnostic accuracy study with prospective data collection in the emergency center of Khayelitsha Hospital, which is a public sector district hospital in a densely populated partially informal settlement in Cape Town, South Africa. Khayelitsha has a HIV prevalence of 27%[26] and an annual tuberculosis notification rate of 917 per 100,000 persons.[27] The emergency center manages ±30,000 patients per annum with an admission rate around 30%. The HIV prevalence of patients managed in the resuscitation unit is 23%.[28]

Consecutive patients presenting to the emergency center were screened on Monday to Thursday from June 2016 through October 2017. HIV-positive adults (≥18 years) presenting with any one of the WHO tuberculosis symptoms (cough of any duration, fever, drenching night sweats, or weight loss) were deemed suspicious of having tuberculosis[29] and were eligible for inclusion. HIV status was determined through clinical records or by laboratory confirmation using a rapid test algorithm. Exclusion criteria were antituberculosis treatment within the previous 3 months, pregnancy, presented to the emergency center >24 hours before being screened, a main clinical presentation of meningitis syndrome or new focal neurology, or patients presenting with primarily trauma, gynecological, or psychiatric conditions. Data from this cohort on urine lipoarabinomannan diagnostic performance were previously published.[30]

Written informed consent was obtained from all participants using a two-phase consent process. Severely ill participants were provided with a short one-page consent form indicating what extra tests would be performed, and that these would be used to facilitate diagnosis of tuberculosis and for research purposes. Full consent was obtained once patients had recovered and agreed to participate. The study was approved by the Human Research Ethics Committee of the University of Cape Town.

Test Methods

PoCUS was performed by a single emergency physician (the first author, with adequate training and credentials)[31] according to a standardized protocol. PoCUS was performed and interpreted in real time in the emergency center directly after consent was taken and before any specimens were collected. At the time of the PoCUS, the physician performing it had access to the clinical information but not to results from the reference standard. PoCUS was performed using either a Mindray M5 ultrasound system with a 3C5s (2.5–6.5 MHz) convex probe and a 7L4s (5.0–10 MHz) linear probe (Mindray DS USA, Inc, Mahwah, NJ) or a NanoMaxx ultrasound system with a L38n (10-5 MHz) linear array probe and a C60n (5-2 MHz) curved array probe (SonoSite Inc, Bothell, WA).

The PoCUS examination assessed for pericardial and pleural effusions, focal splenic and hepatic lesions, ascites, and intra-abdominal lymph nodes. Focal splenic and hepatic lesions were deemed positive if hypoechoic or hyperechoic lesions of any size or number were present.[32] The maximum diameter was noted. The presence of intra-abdominal lymph nodes were assessed in the periportal, para-aortic, splenic (hilar), and mesenteric areas. The maximum diameter was documented (irrespective of axis). Two thresholds for pericardial effusion (any size and ≥5 mm) were used to determine whether minimal sized effusions, with <5 mm corresponding to <100 mL of fluid,[33] should be used as the cutoff value for positivity. Different thresholds for intra-abdominal lymph nodes (any size, ≥5, ≥10, ≥15 mm) were considered because the literature differs regarding the optimal threshold to be used.[10,13,34,35]

FASH-specific thresholds were used to determine diagnostic accuracy of the FASH protocols.[13] FASH basic was deemed positive if any of pericardial effusion, ascites, or pleural effusion were present.[13] FASH plus was positive if upper abdominal lymph nodes (≥15 mm in diameter), focal hypoechoic splenic lesions, or focal hypoechoic liver lesions were present.[13] FASH combined (or just FASH protocol) was positive if any feature of the FASH basic or the FASH plus was present.

The reference standard was the detection of *Mycobacterium tuberculosis* from Xpert MTB/RIF or culture.[13] Sterile single-use disposable containers were used for urine collection; participants were catheterized if needed. Test results were made available to managing clinicians as soon as they became available. Any result from other specimens from any anatomical site obtained as part of the routine standard of care by hospital or clinic clinicians (nonstudy) were included if they were taken during admission or within 6 weeks after hospital discharge.

The National Health Laboratory Service performed all the tests. The Xpert MTB/RIF assay (GX4) (Cepheid Inc, Sunnyvale, CA) was used to test sputum specimens and concentrated urine samples.[36] Sputum specimens were cultured in mycobacterial growth indicator tubes (MGIT; Becton Dickson, Sparks, MD). BACTEC MYCO/F Lytic blood culture bottles (Becton Dickson) were filled with at least 5 mL of blood and incubated for up to 6 weeks. The MTBDRplus assay (Hain Lifescience, Nehren, Germany) was used to identify culture isolates as *M. tuberculosis* complex. CD4 cell counts were performed on admission unless performed within 3 months before enrolment. Laboratory personnel were blinded to PoCUS results.

Statistical Analysis

The sample size was determined with the aim of including more than the recommended 10 candidate predictors (including interaction terms) from multivariable logistic regression analyses.[37] The tuberculosis prevalence in HIV-positive patients in the emergency center is around 25%,[28] and a sample size of 400 HIV-positive participants was deemed adequate to include 100 tuberculosis cases.

Summary statistics were used to describe the variables. Normal distribution of quantitative variables was assessed visually (using histograms and Q-Q plots) and for statistical significance (Shapiro–Wilk test). Homogeneity of variance was determined with the Levene test. Comparisons were performed using the *t* test or Mann–Whitney *U* test when comparing means or medians, respectively. The Pearson χ^2-test or Fisher exact test were used for comparing proportions. Suboptimal PoCUS views, where the presence or absence of specific PoCUS features could not be determined, were included as feature not present. Analyses for the diagnostic performance of individual PoCUS features and to externally validate the FASH protocols were performed using MedCalc for Windows, version 18.5 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2018) and SPSS Statistics for Windows, Version 25.0 (IBM Corp. Released 2017. Armonk, NY: IBM Corp) (see Supplemental Digital Content, http://links.lww.com/QAI/B419, which describes the methods used for calculating the confidence intervals for the diagnostic characteristics).

The odds ratio [with 95% confidence intervals (CIs)] was used as a measure of association of individual variables with prevalent tuberculosis. Univariable analyses were done to identify predictors of tuberculosis using a 10% significance level. The a priori selected predictors were: pericardial effusion (any size), pericardial effusion (≥5 mm), any pleural effusion, ascites, any splenic lesion, intra-abdominal lymph node (any size), intra-abdominal lymph node (≥5 mm), intra-abdominal lymph node (≥10 mm), and any liver lesion. The statistical significant PoCUS feature with the lowest positive threshold was included, where different thresholds for positivity was used (eg, intra-abdominal lymph node). Multivariable logistic regressions were then used to determine which of the predetermined predictors independently contributed to predicting tuberculosis. The overall model development followed the stepwise approach described by Collett.[38] The model's calibration performance was assessed by a calibration plot and the Hosmer–Lemeshow test. Model discrimination was assessed with a *c*-statistic.[39] Internal validation was performed using 2000 bootstrap resamples with replacement (with each sample being of the same size as the original sample).[40] The mean of the area under the receiver-operator characteristic curve (AUC) (and 95% CI) from applying the 2000 bootstrap models to the original sample was used as the measure of internal validation. Analyses were performed using SPSS Statistics for Windows, Version 25.0 (IBM Corp Released 2017, Armonk, NY: IBM Corp), R statistical software version 3.4.3 (November 30, 2017) [The R Foundation for Statistical Computing Platform], and SAS software (Copyright 2018 SAS Institute Inc SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc, Cary, NC).

RESULTS

Participant Characteristics

We screened 556 patients, 414 (74.5%) of whom were included in the final data set. Reasons for exclusion are shown in Figure 1. We obtained 1390 samples [median of 3 samples per participant (25–75 percentile 2–4 samples)] for detection of *M. tuberculosis* during admission, of which 1005 (72.3%) were obtained within 24 hours (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B419, which describe the clinical samples sent for mycobacterial testing). At least 2 samples from at least 2 different anatomic sites were obtained in 350 (84.5%) participants. Forty-five participants not diagnosed with tuberculosis during admission were investigated for tuberculosis within 3 months of hospital discharge; only one participant had a positive test (culture) on a sputum sample. Tuberculosis was microbiologically confirmed in 172 participants (41.5%) (only Xpert MTB/RIF positive n = 32, 18.6%; only culture positive n = 47, 27.3%; both Xpert MTB/RIF and culture positive n = 93, 54.1%).

Baseline demographic and clinical characteristics of participants with and without confirmed tuberculosis are presented in Table 1. Respiratory system related diagnoses were the most frequent alternative diagnoses in those without confirmed tuberculosis (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B419 for alternative diagnoses in participants without microbiologically confirmed tuberculosis); 63 participants without microbiologically confirmed tuberculosis were empirically started on antituberculosis therapy (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B419, which present reasons for clinical

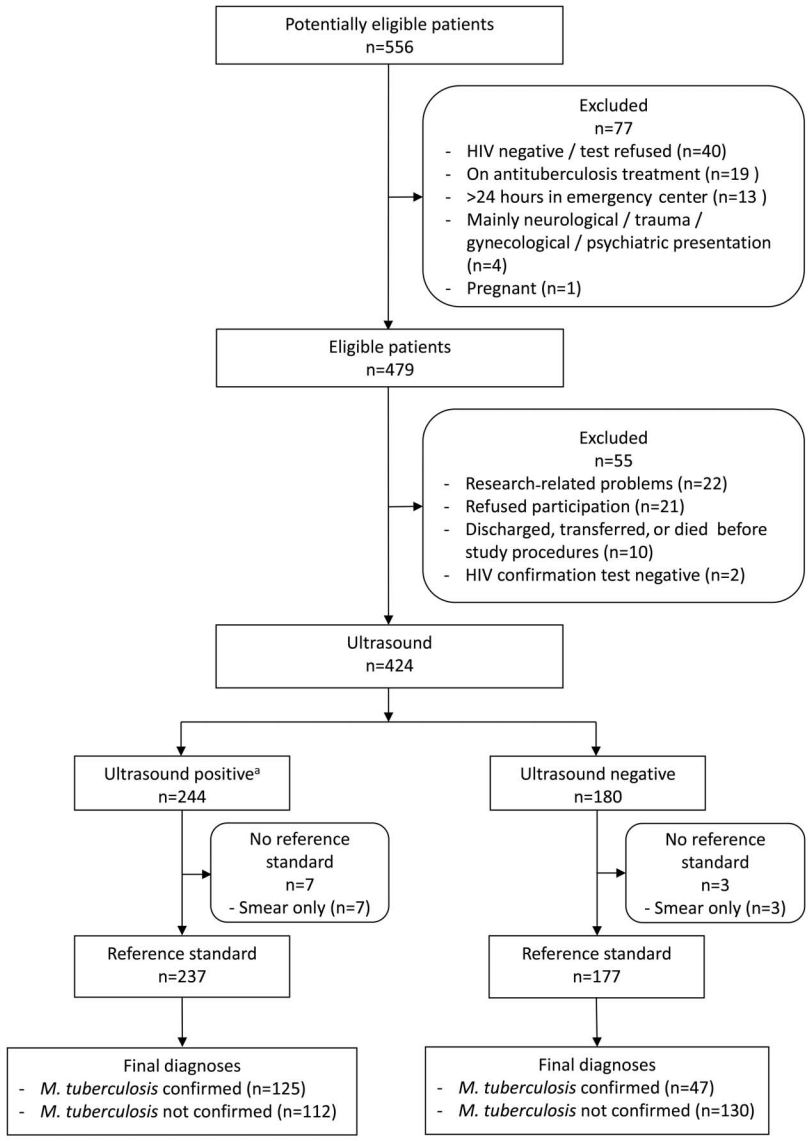

FIGURE 1. Flow diagram of study participants.

diagnosis of tuberculosis without microbiological confirmation). The all cause in-hospital mortality was 7.2% (n = 30), with no statistical difference between those with or without confirmed tuberculosis (15/172 versus 15/242; $P = 0.33$).

Diagnostic Accuracy of Individual PoCUS Features

The sensitivity and specificity of ≥1 individual PoCUS feature was 73% (95% CI: 65% to 79%) and 54% (95% CI: 47% to 60%) (Table 2) (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B419, which lists the number of true positives, false positives, true negatives, and false negatives for each individual PoCUS feature). In participants with a CD4 cell count ≤100/mm^3, the sensitivity increased to 82% (95% CI: 74% to 89%) and the specificity to 55% (95% CI: 46% to 65%) (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B419, which describes the diagnostic accuracy of PoCUS in participants with CD4 cell count ≤100/mm^3).

External Validation of FASH-Protocols

The FASH-combined protocol (any abnormal FASH-specific feature) had a sensitivity of 71% (95% CI: 64% to 78%) and specificity of 57% (95% CI: 50% to 63%) (Table 3). Sensitivity increased to 80% (95% CI: 71% to 87%) and specificity was 56% (95% CI: 47% to 66%) in participants with CD4 cell counts ≤100/mm^3 (Table 3). The *c*-statistic for the FASH-basic was 0.609 (95% CI: 0.554 to 0.664), for the FASH-plus 0.598 (95% CI: 0.542 to 0.654) and for the FASH-combined 0.630 (95% CI: 0.576 to 0.684).

TABLE 1. Baseline Demographics and Clinical Characteristics of Study Population

Characteristics at Enrolment	N	All (N = 414) (n (%) Unless Otherwise Specified)	*M. tuberculosis* Confirmed (N = 172) (n (%) Unless Otherwise Specified)	*M. tuberculosis* Not Confirmed (N = 242) (n (%) Unless Otherwise Specified)	*P*
Demographics					
Age (yrs) [median (Q_1–Q_3*)]	414	36 (30–43)	35 (30–42)	36 (31–44)	0.12
Gender: male	414	171 (41.3)	71 (41.3)	100 (41.3)	0.99
Clinical history					
Current cough of any duration	414	352 (85.0)	148 (86.0)	204 (84.3)	0.62
Weight loss within last month	414	355 (85.7)	156 (90.7)	199 (82.2)	0.02
Night sweats within last month	414	218 (52.7)	102 (59.3)	116 (47.9)	0.02
Fever within last month	414	200 (48.3)	75 (43.6)	125 (51.7)	0.11
Previous tuberculosis (any time)	413	218 (52.8)	73 (42.4)	145 (60.2)	<0.01
Previous tuberculosis ≤ 2 yrs	218	76 (34.9)	29 (39.7)	47 (32.4)	0.29
Antiretroviral therapy naïve	414	125 (30.2)	64 (37.2)	61 (25.2)	0.13
Currently on antiretroviral therapy	414	195 (47.1)	62 (36.0)	133 (55.0)	0.07
Defaulted antiretroviral therapy	414	87 (21.0)	44 (25.6)	43 (17.8)	0.28
Unknown if on antiretroviral therapy	414	7 (1.7)	2 (1.2)	5 (2.1)	0.44
First-line antiretroviral regimen	195	120 (61.5)	40 (64.5)	80 (60.2)	0.04
Second-line antiretroviral regimen	195	42 (21.5)	11 (17.7)	31 (23.3)	0.46
Unknown antiretroviral regimen	195	33 (16.9)	11 (17.7)	22 (16.5)	0.84
Clinical findings					
Weight (kilogram) [median (Q_1–Q_3)]	383	54 (46–65)	53 (46–61)	55 (47–67)	0.15
Body mass index (kg/m^2) [median (Q_1–Q_3)]	382	20 (17–24)	20 (17–24)	20 (18–25)	0.41
Underweight (body mass index < 18.5 kg/m^2)	382	138 (33.3)	61 (35.5)	77 (31.8)	0.54
Temperature (centigrade) (mean ± SD)	411	36.8 (36.1–37.8)	36.8 (36.1–38)	36.8 (36.2–37.8)	0.68
Systolic blood pressure (mm Hg) [median (Q_1–Q_3)]	414	111 (98–125)	112 (99–124)	110 (97–127)	0.94
Diastolic blood pressure (mm Hg) [median (Q_1–Q_3)]	414	71 (61–81)	72 (60–80)	71 (61–82)	0.91
Heart rate (beats per minute) (mean ± SD)	414	122 ± 22	127 ± 23	119 ± 21	<0.01
Respiratory rate (breaths per minute) [median (Q_1–Q_3)]	411	24 (19–30)	24 (18–30)	24 (20–32)	0.15
Oxygen saturation level [median (Q_1–Q_3)]	406	97 (94–99)	97 (95–99)	96 (93–99)	0.11
Hemoglobin (g/dL) (mean ± SD)	410	9.7 ± 2.7	9.0 ± 2.4	10.2 ± 2.7	<0.01
Mean corpuscular volume (fL) (mean ± SD)	407	86.6 ± 8.9	84.8 ± 8.1	87.9 ± 9.3	<0.01
White blood cell count (× 10^9/L) [median (Q_1–Q_3)]	412	8.3 (5.8–13.6)	7.4 (5.4–12.6)	9.1 (6.2–14.7)	<0.01
Platelet count [median (Q_1–Q_3)]	410	284 (195–390)	272 (199–383)	288 (187–400)	0.46
CD4 cell count (/mm^3) [median (Q_1–Q_3)]	408	86 (30–218)	65 (23–155)	121 (37–266)	<0.01

*Q_1–Q_3 = 25th–75th percentile.

Independent PoCUS Predictors

The most significant independent predictors of tuberculosis were any intra-abdominal lymph node [adjusted diagnostic odds ratio (aDOR) 3.7 (95% CI: 2.0 to 6.7)], ascites [aDOR 3.0 (95% CI: 1.5 to 5.7)], and any pericardial effusion [aDOR 1.9 (95% CI: 1.2 to 3.0)] (Table 4). The presence of any 2 independent PoCUS predictors had 91% (95% CI: 86% to 94%) specificity and a positive likelihood ratio of 3.7 (95% CI: 2.4 to 5.8) (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B419, which describe the diagnostic accuracy of independent PoCUS predictors for the diagnosis of HIV-associated tuberculosis). Good agreement between the occurrence of tuberculosis estimated by the derived model and the frequency of tuberculosis observed in the study population was indicated in the calibration curve and by the Hosmer–Lemeshow test (χ^2 = 0.210; P = 0.90). The *c*-statistic of the model was 0.680 (95% CI: 0.631 to 0.729). Good stability of the model was indicated with bootstrap internal validation (*c*-statistic 0.679, 95% CI: 0.668 to 0.680), with an optimism estimate of 0.003 (95% CI −0.052 to 0.059) (see Figure, Supplemental Digital Content, http://links.lww.com/QAI/B419, which presents validation plots for the assessment of variables included in the multivariable logistic regression model aimed for the diagnosis of HIV-associated tuberculosis).

DISCUSSION

This is the first large prospective study to assess the diagnostic accuracy of PoCUS for diagnosing HIV-associated tuberculosis using a robust microbiologic reference standard. A

TABLE 2. Diagnostic Accuracy of Individual Point-Of-Care Ultrasound Features for Diagnosing Tuberculosis in HIV-Positive Participants

	n*	DOR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Individual ultrasound feature				
Intra-abdominal nodes (any location, any size)	70†	4.9 (2.8 to 8.8)	30% (23% to 37%)	92% (88% to 95%)
Intra-abdominal nodes (any location, ≥5 mm)	69†	4.8 (2.7 to 8.5)	29% (22% to 36%)	92% (88% to 95%)
Intra-abdominal nodes (any location, ≥10 mm)	39†	4.1 (2.0 to 8.5)	16% (11% to 23%	95% (91% to 98%)
Intra-abdominal nodes (any location, ≥15 mm)	8†	2.4 (0.6 to 10.1)	3% (1% to 7%)	99% (97% to 100%)
Ascites	54	4.0 (2.2 to 7.5)	22% (16% to 29%)	93% (89% to 96%)
Splenic lesions (hypoechoic, any size)	94‡	3.9 (2.4 to 6.4)	37% (29% to 44%)	87% (82% to 91%)
Splenic lesions (any)	104‡	3.0 (1.9 to 4.7)	37% (30% to 45%)	83% (78% to 88%)
Pericardial effusion (≥5 mm)	106§	2.7 (1.7 to 4.2)	37% (29% to 44%)	82% (77% to 87%)
Pericardial effusion (any size)	141§	2.6 (1.7 to 3.9)	47% (39% to 54%)	75% (69% to 80%)
Pleural effusion (any)	72	1.5 (0.9 to 2.5)	21% (15% to 28%)	85% (80% to 89%)
Hepatic lesions	1	0.5 (0.0 to 11.5)	0% (0% to 2%)	100% (98% to 100%)
Combination of individual ultrasound features‖				
≥ 1 positive feature	237	3.1 (2.0 to 4.7)	73% (65% to 79%)	54% (47% to 60%)
≥ 2 positive features	123	4.2 (2.7 to 6.6)	47% (39% to 55%)	83% (77% to 87%)
≥ 3 positive features	58	4.6 (2.5 to 8.4)	24% (18% to 32%)	93% (89% to 96%)
≥ 4 positive features	19	8.2 (2.3 to 28.5)	9% (5% to 15%)	99% (96% to 100%)

	PPV (95% CI)	NPV (95% CI)	LR (+) (95% CI)	LR (−) (95% CI)
Individual ultrasound feature				
Intra-abdominal nodes (any location, any size)	73% (62% to 81%)	65% (62% to 67%)	3.8 (2.3 to 6.2)	0.8 (0.7 to 0.9)
Intra-abdominal nodes (any location, ≥5 mm)	72% (62% to 81%)	65% (62% to 67%)	3.7 (2.3 to 6.1)	0.8 (0.7 to 0.9)
Intra-abdominal nodes (any location, ≥10 mm)	72% (57% to 83%)	62% (60% to 63%)	3.6 (1.8 to 7.0)	0.9 (0.8 to 0.9)
Intra-abdominal nodes (any location, ≥15 mm)	63% (29% to 87%)	59% (58% to 60%)	2.3 (0.6 to 9.7)	1.0 (1.0 to 1.0)
Ascites	70% (58% to 80%)	63% (61% to 65%)	3.3 (1.9 to 5.8)	0.8 (0.8 to 0.9)
Splenic lesions (hypoechoic, any size)	67% (58% to 75%)	66% (63% to 69%)	2.9 (2.0 to 4.2)	0.7 (0.6 to 0.8)
Splenic lesions (any)	62% (53% to 69%)	65% (62% to 68%)	2.2 (1.5 to 3.1)	0.8 (0.7 to 0.9)
Pericardial effusion (≥5 mm)	59% (51% to 67%)	65% (62% to 67%)	2.1 (1.5 to 2.9)	0.8 (0.7 to 0.9)
Pericardial effusion (any size)	57% (50% to 63%)	66% (63% to 70%)	1.8 (1.4 to 2.4)	0.7 (0.6 to 0.8)
Pleural effusion (any)	50% (40% to 60%)	60% (58% to 62%)	1.4 (0.9 to 2.1)	0.9 (0.9 to 1.0)
Hepatic lesions	0%	58% (58% to 59%)	0	1.0 (1.0 to 1.0)
Combination of individual ultrasound features‖				
≥ 1 positive feature	53% (49% to 57%)	73% (68% to 78%)	1.6 (1.3 to 1.9)	0.5 (0.4 to 0.7
≥ 2 positive features	66% (58% to 73%)	69% (65% to 72%)	2.7 (2.0 to 3.7)	0.6 (0.6 to 0.8)
≥ 3 positive features	72% (60% to 82%)	63% (61% to 66%)	3.7 (2.1 to 6.3)	0.8 (0.7 to 0.9)
≥ 4 positive features	84% (61% to 95%)	61% (59% to 62%)	7.5 (2.2 to 25.4)	0.9 (0.9 to 1.0)

*Suboptimal views included as negative feature (see Table, Supplemental Digital Content, http://links.lww.com/QAI/B419, where suboptimal views were excluded).
†Number of suboptimal views included as negative feature = 46.
‡Number of suboptimal views included as negative feature = 8.
§Number of suboptimal views included as negative feature = 3.
‖Any one of intra-abdominal lymph nodes (any location, any size), ascites, any splenic lesion, pericardial effusion (any size), any pleural effusion, and any hepatic lesion.
DOR, diagnostic odds ratio (ultrasound feature present versus absent); LR (+), likelihood ratio for positive test; LR (−), likelihood ratio for negative test; PPV, positive predictive value; NPV, negative predictive value.

further strength of our study was that tuberculosis testing for defining the reference standard was performed on multiple samples. Individual PoCUS features had poor sensitivity but moderate specificity, in keeping with published literature. FASH protocols performed similarly to the individual PoCUS features, with improved sensitivity in participants with CD4 cell counts $\leq 100/mm^3$. Independent PoCUS predictors of tuberculosis were ascites, any intra-abdominal lymph node(s), and any pericardial effusion. The derived model had moderate discrimination and good calibration. We suggest that the presence of 2 or more of the independent PoCUS features (specificity 91%, positive likelihood ratio 3.7) would be appropriate to initiate treatment for tuberculosis in high-burden settings while awaiting results of microbiological tests.

The sensitivity of at least one individual positive feature on PoCUS for HIV-associated tuberculosis was 73%, whereas the specificity of at least 2 abnormalities present was 83% (Table 2). These findings are comparable with other studies assessing formal ultrasound and not PoCUS.[23,35]

The FASH protocols were developed based on clinical intuition, and their diagnostic accuracy was not reported.[12,13] A spatial external validation (same investigators, different

TABLE 3. Diagnostic Accuracy of the Focused Assessment With Sonography for HIV/TB (FASH) Protocols for Diagnosing Tuberculosis in HIV-Positive Participants

	n	DOR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR (+) (95% CI)	LR (−) (95% CI)
All participants (n = 414)								
FASH basic*	199	2.5 (1.7 to 3.7)	61% (53% to 68%)	61% (55% to 67%)	53% (48% to 58%)	69% (64% to 73%)	1.6 (1.3 to 1.9)	0.6 (0.5 to 0.8)
FASH plus†	99	3.5 (2.2 to 5.6)	37% (30% to 45%)	86% (80% to 90%)	65% (56% to 72%)	66% (63% to 69%)	2.6 (1.8 to 3.7)	0.7 (0.7 to 0.8)
FASH combined‡	227	3.2 (2.1 to 4.8)	71% (64% to 78%)	57% (50% to 63%)	54% (49% to 58%)	73% (68% to 78%)	1.6 (1.4 to 1.9)	0.5 (0.4 to 0.7)
CD4 cell count $\leq$ 100/mm^3 (n = 220)								
FASH basic*	111	2.9 (1.7 to 5.1)	64% (54% to 73%)	63% (53% to 71%)	62% (55% to 68%)	64% (57% to 71%)	1.7 (1.3 to 2.3)	0.6 (0.4 to 0.8)
FASH plus†	77	4.7 (2.5 to 8.6)	52% (42% to 62%)	81% (73% to 88%)	73% (64% to 80%)	64% (59% to 68%)	2.8 (1.8 to 4.2)	0.6 (0.5 to 0.7)
FASH combined‡	135	5.0 (2.8 to 9.1)	80% (71% to 87%)	56% (47% to 66%)	64% (58% to 69%)	74% (66% to 81%)	1.8 (1.4 to 2.3)	0.4 (0.2 to 0.5)

*Any of one of pericardial effusion (any size), ascites, or pleural effusion detected.
†Any of one of upper abdominal lymph nodes (≥15 mm in diameter), focal hypoechoic splenic lesions, or focal hypoechoic liver lesions detected.
‡Any positive feature as part of FASH basic or FASH plus.
DOR, diagnostic odds ratio (ultrasound feature present versus absent); LR (+), likelihood ratio for positive test; LR (−), likelihood ratio for negative test; PPV, positive predictive value; NPV, negative predictive value.

setting) was performed in India[22] of 81 HIV-positive participants, and the reference standard included both confirmed and possible tuberculosis. The FASH protocol tested differed from the original,[12,13] with any versus multiple focal splenic and liver lesions, and ascites was excluded. The 60% sensitivity and 78% specificity (calculated from presented data) is different from our findings (sensitivity 71%, specificity 57%). A full external validation (different investigators, different setting) was performed in Tanzania but included HIV-negative participants, their reference standard included a probable tuberculosis group without microbiological confirmation,[41] and they included an additional ultrasound variable (ileum wall thickening >4 mm or destroyed architecture); they reported sensitivity and specificity of 56% and 61%, respectively.

The sensitivity of individual PoCUS features and the FASH-protocols improved in participants with CD4 cell count ≤100/mm^3, whereas specificity was similar. These findings are similar to other studies where low CD4 cell counts were associated with extrapulmonary tuberculosis and more extensive abdominal involvement.[14–16,18,34] Ultrasound features are thus more likely to be present in patients with more advanced immunosuppression.

We identified 4 PoCUS predictors (lowest positive threshold) with significant univariable association with tuberculosis (any pericardial effusion, ascites, any splenic lesion, and any intra-abdominal lymphadenopathy). Our results can be compared with ultrasound predictors that were significantly associated with tuberculosis in 4 other studies: multiple splenic lesions (2–15 mm) and abdominal lymphadenopathy

TABLE 4. Point-Of-Care Ultrasound Predictors of Microbiologically Confirmed HIV-Associated Tuberculosis

Predictor Variable	B	Standard Error	Wald *P*	Odds Ratio (95% CI)	−2 Log Likelihood
Univariable association					
Ascites	1.388	0.317	0.000	4.01 (2.15 to 7.46)	540.923
Intra-abdominal lymph nodes (any size)*	1.599	0.292	0.000	4.95 (2.08 to 5.06)	528.036
Intra-abdominal lymph nodes (≥5 mm)*	1.571	0.292	0.000	4.81 (2.71 to 8.53)	529.482
Intra-abdominal lymph nodes (≥10 mm)*	1.407	0.371	0.000	4.08 (1.97 to 8.46)	545.892
Pericardial effusion (any size)†	0.948	0.213	0.000	2.58 (1.70 to 3.92)	541.806
Pericardial effusion (≥5 mm)†	0.984	0.231	0.000	2.68 (1.70 to 4.21)	543.443
Pleural effusion (any)	0.415	0.260	0.111	1.52 (0.91 to 2.52)	559.497
Any splenic lesion (any size)‡	1.096	0.234	0.000	2.99 (1.89 to 4.74)	539.380
Multivariable Association§	**B**	**Standard Error**	**Wald *P***	**Adjusted Odds Ratio (95% CI)**	**−2 Log Likelihood**
Intra-abdominal lymph nodes (any size)*	1.313	0.304	0.000	3.7 (2.0–6.7)	506.150
Ascites	1.086	0.334	0.001	3.0 (1.5–5.7)	
Pericardial effusion (any size)†	0.642	0.228	0.005	1.9 (1.2–3.0)	
Constant	−0.927	0.140	0.000		

*Number of suboptimal views included as negative feature = 46.
†Number of suboptimal views included as negative feature = 3.
‡Number of suboptimal views included as negative feature = 8.
§Ultrasound predictors with the lowest positive threshold were included, where different thresholds for positivity were used.

(>15 mm) in an Indian study [22]; pleural effusion, abdominal lymph nodes (>15 mm), and hepatomegaly in a Tanzanian study [41]; lymph nodes (≥10 mm), hypoechoic splenic lesions, splenomegaly (≥110 mm), and abdominal/pleural/pericardial effusion in a South African study [34]; and splenic lesions, ascites, pericardial effusion, and lymphadenopathy (>10 mm) in another South African study.[10] Three of these studies used a less robust composite reference standard than in our study,[10,22,41] while 2 also included HIV-negative or HIV-unknown participants.[10,41]

In our study ascites, pericardial effusion and any size intra-abdominal lymphadenopathy were independent predictors of tuberculosis after multivariable logistic regression. Independent predictors in other studies were multiple intra-abdominal lymph nodes (≥12 mm),[35] abdominal lymph nodes (≥10 mm),[34] hypoechoic splenic lesions,[34] and abdominal/pleural/pericardial effusion.[34] Both these studies used a robust reference standard, and formal ultrasound examinations were performed in radiology departments.

Our study has limitations. First, PoCUS might not be applied to all participants in real-life scenarios as patients with a clear nontuberculosis diagnosis after the clinical examination (eg, pneumonia) will be managed accordingly. Second, PoCUS examinations were performed by a single, experienced operator. A second reviewer and or sonographer would have enhanced the generalizability of the features. The main strength of our study is the robust microbiologic reference standard applied. It was a pragmatic study as PoCUS was performed under routine conditions experienced in the emergency center.

Our study suggests that ultrasound for the diagnosis of HIV-associated tuberculosis can be used at the point of care. PoCUS is limited by moderate sensitivity, and care must be taken to not rule tuberculosis out after a negative PoCUS examination. The presence of 2 or more independent PoCUS predictors suggests that PoCUS can be used as a rule-in tuberculosis test in emergency centers in high-burden settings. Further research needs to be performed to externally validate the findings, particularly to assess its performance in settings with different prevalences of tuberculosis and alternative diagnoses. Finally, the impact on outcomes of integrating PoCUS into tuberculosis diagnostic algorithms needs to be assessed.

CONCLUSION

We identified independent PoCUS predictors (intra-abdominal lymphadenopathy, ascites, and pericardial effusion) for diagnosing tuberculosis in HIV-positive patients presenting to the emergency center. Although the presence of at least 2 independent PoCUS predictors had better specificity than the FASH protocols, it only had moderate discrimination. Further refinement and possible inclusion of other clinical features should be investigated. These findings could contribute to the development of a clinically orientated point-of-care algorithm to expedite the diagnosis of tuberculosis and initiation of antituberculosis therapy in acute care settings in regions with a high burden of HIV-associated tuberculosis.

ACKNOWLEDGMENTS

The authors acknowledge the valuable contribution of Rene Goliath (research clinical site coordinator, Institute of Infectious Disease & Molecular Medicine, University of Cape Town), the staff of the National Health Laboratory Service (NHLS), and the staff and administration of the Western Cape Department of Health.

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Supplemental Digital Content 1

Methods used for calculating the confidence intervals for the diagnostic characteristics

The estimates and confidence intervals were calculated using MedCalc for Windows, version 18.5 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2018). They stipulate the methods used as follows:

- “Confidence intervals for sensitivity, specificity and accuracy are "exact" Clopper-Pearson confidence intervals.”
- “Confidence intervals for the likelihood ratios are calculated using the "Log method" as given on page 109 of Altman et al. 2000.” [Altman DG, Machin D, Bryant TN, Gardner MJ (Eds) (2000) Statistics with confidence, 2nd ed. BMJ Books.]
- “Confidence intervals for the predictive values are the standard logit confidence intervals given by Mercaldo et al. 2007.” [Mercaldo ND, Lau KF, Zhou XH (2007) Confidence intervals for predictive values with an emphasis to case-control studies. Statistics in Medicine 26:2170-2183.]
- “The odds ratio (OR), its standard error and 95% confidence interval are calculated according to Altman, 1991.” [Altman DG (1991) Practical statistics for medical research. London: Chapman and Hall.]

The above can be accessed at **https://www.medcalc.org/calc/diagnostic_test.php** and **https://www.medcalc.org/calc/odds_ratio.php**.

Supplemental Digital Content 2

Table. Total clinical samples sent for mycobacterial testing (includes 'research' and 'routine' samples)

Sample	Samples obtained within 24 hours						Total samples obtained during admission					
	No. (%) patients producing ≥ 1 sample	**Total no. samples**	**Total no. Xpert tests done**	**Total no. cultures done**	**No. positive culture and Xpert tests (%)**	**No. (%) TB patients with ≥1 positive culture or Xpert test**	**No. (%) patients producing ≥ 1 sample**	**Total no. samples**	**Total no. Xpert tests done**	**Total no. cultures done**	**No. positive culture and Xpert tests (%)**	**No. (%) TB patients with ≥1 positive culture or Xpert test**
Urine	266 (69.5)	278	277	1	57 (20.5)	55 (37.2)	312 (75.4)	328	322	6	64 (19.5)	62 (36.0)
Sputum[a]	227 (59.3)	349	289	232	202 (38.8)	88 (59.5)	291 (70.3)	581	466	441	291 (32.2)	112 (65.1)
Blood	312 (81.5)	343	0	343	66 (19.2)	61 (41.2)	345 (83.3)	404	0	404	72 (17.8)	64 (37.2)
Fine needle aspirate (FNA)	4 (1.0)	4	2	4	5 (83.3)	3 (2.0)	10 (2.4)	12	5	6	9 (81.8)	5 (2.9)
Cerebrospinal fluid (CSF)	13 (3.4)	13	12	4	0 (0.0)	0 (0.0)	31 (7.5)	32	27	13	0 (0.0)	0 (0.0)
Pleural fluid	15 (3.9)	16	3	14	8 (47.1)	8 (5.4)	24 (5.8)	27	4	26	13 (44.8)	12 7.0)
Pericardial fluid	0 (0)	0	0	0	0 (0.0)	0 (0.0)	2 (0.5)	2	0	2	2 (100)	2 (1.2)
Ascitic fluid	1 (0.3)	1	0	1	1 (100)	1 (0.7)	2 (0.5)	2	0	2	1 (50.0)	1 (0.6)
Other (swab, tracheal aspirate)	1 (0.3)	1	1	1	0 (0.0)	0 (0.0)	2 (0.5)	2	1	2	0 (0.0)	0 (0.0)
	383 (92.5)	**1005**	**584**	**600**	**339 (28.6)**	**148 (86.0)**	**414 (100)**	**1390**	**825**	**902**	**452 (26.2)**	**172 (100)**

[a]Culture and Xpert MTB/RIF done on sputum taken on the same day were counted as two samples

Supplemental Digital Content 3

Table. Distribution of alternative diagnoses in participants without microbiologically confirmed tuberculosis

Alternative diagnoses (alphabetically)	**n (%)**
Appendicitis	2 (0.8)
Bronchiectasis	6 (2.5)
Bronchitis	1 (0.4)
Congestive cardiac failure	2 (0.8)
Gastro-enteritis (acute & chronic)	13 (5.4)
Clinical diagnoses of tuberculosis	63 (26.0)
Chronic Lymphocytic Leukaemia (CLL)	1 (0.4)
Colon carcinoma	1 (0.4)
Constipation	1 (0.4)
Chronic Obstructive Pulmonary Disease (COPD) exacerbation	1 (0.4)
Lower Respiratory Tract Infection / Pneumonia	96 (39.7)
Cor Pulmonale	1 (0.4)
Delirium	2 (0.8)
Duodenitis	1 (0.4)
Dysentery	3 (1.2)
E. Coli bacteraemia	1 (0.4)
Empyema	2 (0.8)
Gallstones	2 (0.8)
Human Immunodeficiency Virus (HIV) wasting syndrome	3 (1.2)
Interstitial lung disease	1 (0.4)
Kaposi sarcoma	2 (0.8)
Liver carcinoma	1 (0.4)
Lung abscess	1 (0.4)
Meningitis	1 (0.4)
Non-tuberculous mycobacterial infection (disseminated)	1 (0.4)
Pneumocystis pneumonia	11 (4.5)
Pelvic inflammatory disease	1 (0.4)
Progressive multifocal leukoencephalopathy	1 (0.4)
Scleroderma	1 (0.4)
Renal failure (acute & chronic)	7 (2.9)
Thrombotic thrombocytopenic purpura	1 (0.4)
Undifferentiated abdominal pain	3 (1.2)
Unknown diagnosis	5 (2.1)
Urosepsis	2 (0.8)
Vitamin B12 deficiency	1 (0.4)
	242 (100)

Supplemental Digital Content 4

Table. Reason for clinical diagnosis of tuberculosis without microbiological confirmation

Diagnostic test	**n**
Suggestive formal abdominal ultrasound done in radiology department	19
Suggestive chest X-ray	9
Positive urine lipoarabinomannan (LAM)	7
Suggestive formal abdominal ultrasound and suggestive chest X-ray	6
Not improving on empiric antibiotics	4
Raised adenosine deaminase (ADA) in effusion fluid (pleural or ascitic)	4
Cerebrospinal fluid suggestive of tuberculous meningitis (TBM)	4
Suggestive chest X-ray and positive urine LAM	3
Suggestive formal abdominal ultrasound and positive urine LAM	2
Psoas abscess on formal ultrasound	2
Caseous necrosis on biopsy (histology)	1
Suggestive computer tomography (CT) scan of abdomen	1
Suggestive chest X-ray and raised ADA in effusion fluid	1
Total	**63**

Supplemental Digital Content 5

Table. Number of true positives, false positives, true negatives, and false negatives for each individual point-of-care ultrasound feature

	Sub-optimal views included as negative								Sub-optimal views excluded						
All participants (N=414)															
Ultrasound feature	**TP**	**FN**	**TN**	**FP**	**N**	**Sn**	**Sp**		**TP**	**FN**	**TN**	**FP**	**N**	**Sn**	**Sp**
Pericardial effusion (any)	80	92	181	61	414	47%	75%		80	91	179	61	411	47%	75%
Pericardial effusion (≥ 5mm)	63	109	199	43	414	37%	82%		63	108	197	43	411	37%	82%
Splenic lesions (hypoechoic)	63	109	211	31	414	37%	87%		63	107	205	31	406	37%	87%
Splenic lesions (any)	64	108	202	40	414	37%	83%		64	106	196	40	406	38%	83%
Intra-abdominal nodes (any size)	51	121	223	19	414	30%	92%		51	101	197	19	368	34%	91%
Intra-abdominal nodes (≥ 5mm)	50	122	223	19	414	29%	92%		50	102	197	19	368	33%	91%
Intra-abdominal nodes (≥ 10mm)	28	144	231	11	414	16%	95%		28	124	205	11	368	18%	95%
Intra-abdominal nodes (≥ 15mm)	5	167	239	3	414	3%	99%		5	147	213	3	368	3%	99%
Pleural effusion	36	136	206	36	414	21%	85%		36	136	206	36	414	21%	85%
Ascites	38	134	226	16	414	22%	93%		38	134	226	16	414	22%	93%
Hepatic lesions (any)	0	172	241	1	414	0%	100%		0	172	241	1	414	0%	100%
≥ 1 positive feature	125	47	130	112	414	73%	54%		125	47	130	112	414	73%	54%
≥ 2 positive features	81	91	200	42	414	47%	83%		81	91	200	42	414	47%	83%
≥ 3 positive features	42	130	226	16	414	24%	93%		42	130	226	16	414	24%	93%
≥ 4 positive features	16	156	239	3	414	9%	99%		16	156	239	3	414	9%	99%
Participants with CD4 cell count ≤ 100mm^3 (n=220)															
	TP	**FN**	**TN**	**FP**	**N**	**Sn**	**Sp**		**TP**	**FN**	**TN**	**FP**	**N**	**Sn**	**Sp**
Pericardial effusion	52	56	84	28	220	48%	75%		52	55	82	28	217	49%	75%
Pericardial effusion (≥ 5mm)	43	65	94	18	220	40%	84%		43	64	92	18	217	40%	84%
Splenic lesions (hypoechoic)	55	53	92	20	220	51%	82%		55	53	89	20	217	51%	82%
Splenic lesions (any)	56	52	89	23	220	52%	79%		56	52	86	23	217	52%	79%
Intra-abdominal nodes (any size)	43	65	100	12	220	40%	89%		43	56	86	12	197	43%	88%
Intra-abdominal nodes (≥ 5mm)	42	66	100	12	220	39%	89%		42	57	86	12	197	42%	88%
Intra-abdominal nodes (≥ 10mm)	27	81	107	5	220	25%	96%		27	72	93	5	197	27%	95%
Intra-abdominal nodes (≥ 15mm)	5	103	111	1	220	5%	99%		5	94	97	1	197	5%	99%
Pleural effusions	21	87	101	11	220	19%	90%		21	87	101	11	220	19%	90%
Ascites	29	79	103	9	220	27%	92%		29	79	103	9	220	27%	92%
Hepatic lesions (any)	0	108	112	0	220	0%	100%		0	108	112	0	220	0%	100%
≥ 1 positive feature	89	19	62	50	220	82%	55%		89	19	62	50	220	82%	55%
≥ 2 positive features	60	48	90	22	220	56%	80%		60	48	90	22	220	56%	80%
≥ 3 positive features	33	75	103	9	220	31%	92%		33	75	103	9	220	31%	92%
≥ 4 positive features	14	94	110	2	220	13%	98%		14	94	110	2	220	13%	98%

Abbreviations: TP, True positives; FP, False positives; TN, True negatives; FN, False negatives; N, Total; Sn, Sensitivity; Sp, Specificity

Supplemental Digital Content 6

Table. Diagnostic accuracy of individual point-of-care ultrasound features for diagnosing tuberculosis in HIV-positive participants with CD4 cell count ≤100/mm^3 (n=220)

	n	DOR (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	LR (+) (95%CI)	LR (-) (95%CI)
Individual ultrasound feature								
Intra-abdominal nodes (any location; ≥ 10mm)	32[a]	**7.0** (2.6 - 19.0)	**27%** (19% - 37%)	**95%** (88% - 98%)	**84%** (68% - 93%)	**56%** (53% - 60%)	**5.4** (2.2 - 13.3)	**0.8** (0.7 - 0.9)
Intra-abdominal nodes (any location; any size)	55[a]	**5.5** (2.7 - 11.3)	**40%** (31% - 50%)	**89%** (82% - 94%)	**78%** (67% - 87%)	**61%** (57% - 65%)	**3.7** (2.1 - 6.7)	**0.7** (0.6 - 0.8)
Intra-abdominal nodes (any location; ≥ 15mm)	6[a]	**5.4** (0.6 – 46.9)	**5%** (2%- 10%)	**99%** (95% - 100%)	**83%** (37% - 98%)	**52%** (51% - 53%)	**5.2** (0.6 – 43.7)	**1.0** (0.9 – 1.0)
Intra-abdominal nodes (any location; ≥ 5mm)	54[a]	**5.3** (2.6 – 10.8)	**39%** (30% - 49%)	**89%** (82% - 94%)	**78%** (66% - 86%)	**60%** (56% - 64%)	**3.6** (2.0 – 6.5)	**0.7** (0.6 - 0.8)
Splenic lesions (hypoechoic; any size)	75[b]	**4.6** (2.5 - 8.5)	**51%** (41% - 61%)	**82%** (73% - 88%)	**73%** (64% - 81%)	**63%** (58% - 67%)	**2.8** (1.8 - 4.3)	**0.6** (0.5 - 0.7)
Ascites	38	**4.2** (1.9 - 9.4)	**27%** (19% - 36%)	**92%** (8**5% - 96%**)	**76%** (62% - 87%)	**57%** (53% - 60%)	**3.3** (1.7 - 6.7)	**0.8** (0.7 - 0.9)
Splenic lesions (any)	79[b]	**4.0** (2.2 - 7.3)	**49%** (39% - 58%)	**75%** (65% - 82%)	**65%** (56% - 73%)	**60%** (55% - 65%)	**1.9** (1.3 - 2.8)	**0.7** (0.6 - 0.9)
Pericardial effusion (≥ 5mm)	61[c]	**3.4** (1.8 - 6.5)	**40%** (31% - 50%)	**84%** (76% - 90%)	**71%** (60% - 80%)	**59%** (55% - 63%)	**2.5** (1.5 - 4.0)	**0.7** (0.6 - 0.9)
Pericardial effusion (any)	80[c]	**2.8** (1.6 - 4.9)	**52%** (42% - 62%)	**79%** (70% - 86%)	**71%** (62% - 79%)	**62%** (57% - 67%)	**2.5** (1.6 - 3.7)	**0.6** (0.5 - 0.8)
Pleural effusion (any)	32	**2.2** (1.0 – 4.9)	**19%** (12% - 28%)	**90%** (83%- 95%)	**66%** (49% - 79%)	**54%** (51% - 56%)	**2.0** (1.0 – 3.9)	**0.9** (0.8 - 1.0)
Hepatic lesions (any)	0	-	-	-	-	-	-	-
Combination of individual ultrasound features[d]								
≥ 1 positive feature	139	**5.8** (3.1 - 10.8)	**82%** (74% - 89%)	**55%** (46% - 65%)	**64%** (59% - 69%)	**77%** (68% - 84%)	**1.9** (1.5 - 2.3)	**0.3** (0.2 - 0.5)
≥ 2 positive features	82	**5.1** (2.8 - 9.3)	**56%** (46% - 65%)	**80%** (72% - 87%)	**73** (64% - 80%)	**65%** (60% - 70%)	**2.8** (1.9 - 4.3)	**0.6** (0.4 - 0.7)
≥ 3 positive features	42	**5.0** 2.3 - 11.1)	**31%** (22% - 40%)	**92%** (85% - 96%)	**78** (65% - 88%)	**58%** (55% - 61%)	**3.8** (1.9 - 7.6)	**0.8** (0.7 - 0.9)
≥ 4 positive features	16	**8.2** (1.8 – 37.0)	**13%** (7% - 21%)	**98%** (94% - 100%)	**88** (62% - 97%)	**54%** (52% - 56%)	**7.3** (1.7 - 31.2)	**0.9** (0.8 - 1.0)

Abbreviations: DOR, Diagnostic Odds Ratio (ultrasound feature present versus absent); CI, Confidence interval; PPV, Positive predictive value; NPV, Negative predictive value; LR(+), Likelihood ratio for positive test; LR(-), Likelihood ratio for negative test;

[a]Number of sub-optimal views included as negative feature = 23

[b]Number of sub-optimal views included as negative feature = 3

[c]Number of sub-optimal views included as negative feature = 3

[d]Any one of intra-abdominal lymph nodes (any location, any size), ascites, any splenic lesion, pericardial effusion (any size), any pleural effusion, any hepatic lesion

Supplemental Digital Content 7

Table. Diagnostic accuracy of independent point-of-care ultrasound predictors for the diagnosis of HIV-associated tuberculosis

Number of independent ultrasound predictors[a]	DOR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR (+) (95% CI)	LR (-) (95% CI)
One or more	**2.9** (1.9 - 4.4)	**67%** (60% - 74%)	**58%** (52% – 64%)	**53%** (49% – 58%)	**71%** (66% - 76%)	**1.6** (1.4 - 1.9)	**0.6** (0.4 – 0.7)
Two or more	**5.1** (3.0 – 8.7)	**33%** (27% – 41%)	**91%** (86% – 94%)	**72%** (63% – 80%)	**66%** (63% – 68%)	**3.7** (2.4 – 5.8)	**0.7** (0.7 – 0.8)
Three or more	**54.6** (3.3 – 914)	**10%** (6% - 15%)	**100%** (98% – 100%)	---	**61%** (60% – 62%)	---	**0.9** (0.9 – 1.0)

Abbreviations: DOR, Diagnostic Odds Ratio (ultrasound feature present versus absent); CI, Confidence interval; PPV, Positive predictive value; NPV, Negative predictive value; LR(+), Likelihood ratio for positive test; LR(-), Likelihood ratio for negative test;
aAscites, Intra-abdominal lymph-nodes (any size); Pericardial effusion (any)

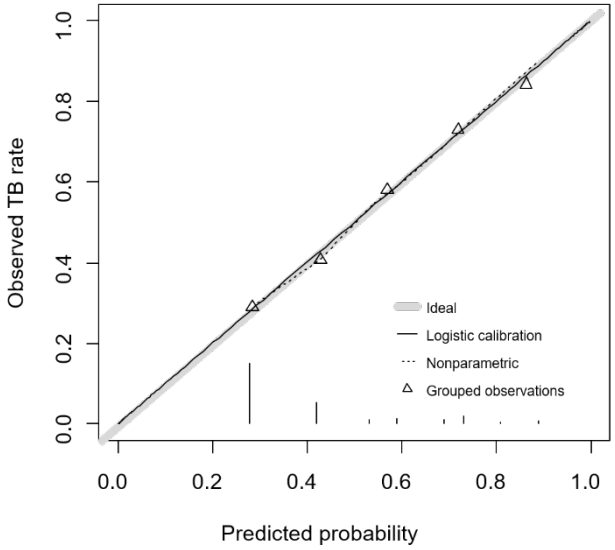
Supplemental Digital Content 8

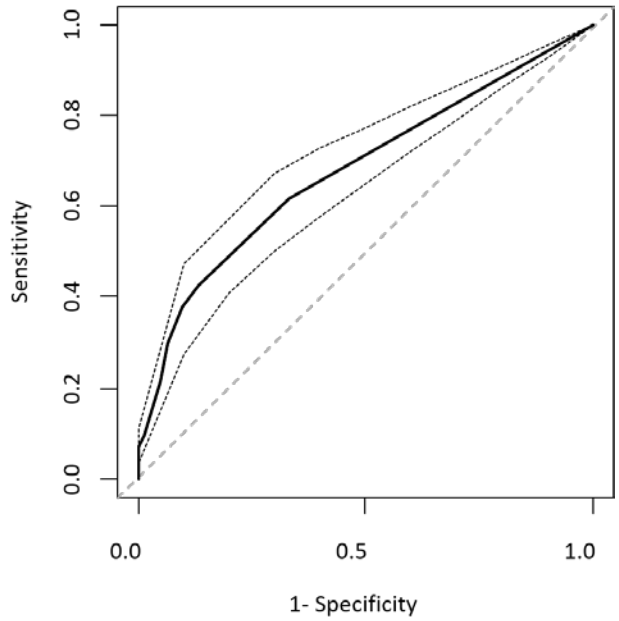
Figure. Validation plots for the assessment of variables included in a multivariable logistic regression model for the diagnosis of HIV-associated tuberculosis.

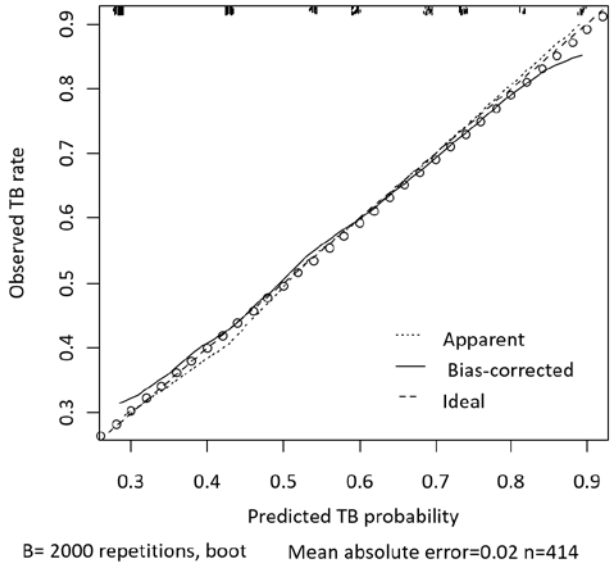
Upper, Calibration curve between observed and predicted probabilities. Parametric (solid black line) and non-parametric (dotted black line) lines were created by regression analysis. Grouped observations are indicated by black triangles and the ideal line (solid grey line) indicate agreement between observed and predicted probabilities.

Middle, Discrimination curve (solid black line) with 95% confidence bounds (black dotted lines). The diagonal line (grey dashed) represents the line of no discrimination (c-statistics = 0.50). The area under the receiver operating characteristic curve is 0.680 (95% Confidence Interval 0.631 to 0.729)

Lower, Bootstrap calibration curve using a smooth nonparametric calibration estimator (LOESS), with superimposed logistic calibration curve estimated by bootstrapping (2000 repetitions) an intercept and slope correction. The intercept of the calibration curve is - 0.0174, when the slope is fixed at 1. The ideal line (dashed line) indicate agreement between observed and predicted probabilities.







Abbreviations: TB, tuberculosis

APPENDIX TO CHAPTER 5

The use of point-of-care ultrasound in the clinical setting

Point-of-care ultrasound (PoCUS) can be used in various ways to diagnose HIV-associated tuberculosis. Theoretically, PoCUS has the potential to be used as a screening test in asymptomatic patients to identify those whom would benefit from a confirmatory test. This would imply that PoCUS has a sensitivity of at least 90% as suggested by the WHO.[1] There are currently no studies describing PoCUS as a screening test for tuberculosis in people living with HIV.

PoCUS can also be used as a secondary screening test. It is typically used after clinical examination and thus in symptomatic patients with a clinical suspicion of tuberculosis. In such a scenario, the threshold for a positive PoCUS finding needs to be maximised towards the highest specificity (i.e. low false-positive rate). In our study, two or more independent PoCUS predictors had a specificity of 91% (95% CI 86% to 94%) and thus has the potential to be used as a rule-in test for HIV-associated tuberculosis.

PoCUS also has the additional advantage that it can be used as a tool to identify additional sampling sites. The procedural use of PoCUS include lympnode biopsy, pericardiocentesis, thoracentesis and peritoneocentesis. This can increase the detection of tuberculosis cases.

Limitations

The reference standard was the detection of *Mycobacterium tuberculosis* from Xpert MTB/RIF or culture. Research specific samples included sputum (Xpert MTB/RIF and culture), concentrated urine (Xpert MTB/RIF), and blood (TB culture) samples. Any result from other specimens from any anatomical site obtained as part of the routine standard of care by hospital or clinic clinicians (nonstudy) were included if they were taken during admission or within 6 weeks after hospital discharge. This resulted in a discrepancy between ultrasound visualised pathology (ascites, pleural effusion, pericardial effusion) and relevant samples being collected as part of the clinical work-up for tuberculosis. Only two patients had ascitic fluid collected (of 54 patients with ultrasound visualised ascites) and only 24 patients had pleural fluid collected (of 72 patients with ultrasound visualised pleural effusions). The number of tuberculosis cases could thus have been underestimated leading to misclassification bias. Furthermore, about 30% of patients were unable to provide a sputum sample, increasing the underestimation of tuberculosis cases even more. These classification errors could lead to bias in the diagnostic accuracy estimates as any disagreement between the reference standard and the PoCUS test will be labelled as either a false-positive or a false-negative PoCUS result. It is not possible to predict whether the sensitivity and specificity will be biased upwards or downwards as the

direction depends on whether the errors are correlated.[2] If errors between the index test and the reference standard are positively correlated, the diagnostic accuracy estimates will be inflated.

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CHAPTER 6

A multi-parameter diagnostic clinical decision tree for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre

RESEARCH ARTICLE

A multi-parameter diagnostic clinical decision tree for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre [version 1; peer review: awaiting peer review]

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v1 **First published:** 17 Apr 2020, **5**:72 (https://doi.org/10.12688/wellcomeopenres.15824.1)
Latest published: 17 Apr 2020, **5**:72 (https://doi.org/10.12688/wellcomeopenres.15824.1)

Abstract

Background: Early diagnosis is essential to reduce the morbidity and mortality of HIV-associated tuberculosis. We developed a multi-parameter clinical decision tree to facilitate rapid diagnosis of tuberculosis using point-of-care diagnostic tests in HIV-positive patients presenting to an emergency centre.
Methods: A cross-sectional study was performed in a district hospital emergency centre in a high-HIV-prevalence community in South Africa. Consecutive HIV-positive adults with ≥1 WHO tuberculosis symptoms were enrolled over a 16-month period. Point-of-care ultrasound (PoCUS) and urine lateral flow lipoarabinomannan (LF-LAM) assay were done according to standardized protocols. Participants also received a chest X-ray. Reference standard was the detection of *Mycobacterium tuberculosis* using Xpert MTB/RIF or culture. Logistic regressions models were used to investigate the independent association between prevalent microbiologically confirmed tuberculosis and clinical and biological variables of interest. A decision tree model to predict tuberculosis was developed using the classification and regression tree algorithm.
Results: There were 414 participants enrolled: 171 male, median age 36 years, median CD4 cell count 86 cells/mm^3. Tuberculosis prevalence was 42% (n=172). Significant variables used to build the classification tree included ≥2 WHO symptoms, antiretroviral therapy use, LF-LAM, PoCUS independent features (pericardial effusion, ascites, intra-abdominal lymphadenopathy) and chest X-ray. LF-LAM was positioned after WHO

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

symptoms (75% true positive rate, representing 17% of study population). Chest X-ray should be performed next if LF-LAM is negative. The presence of ≤1 PoCUS independent feature in those with 'possible or unlikely tuberculosis' on chest x-ray represented 47% of non-tuberculosis participants (true negative rate 83%). In a prediction tree which only included true point-of-care tests, a negative LF-LAM and the presence of ≤2 independent PoCUS features had a 71% true negative rate (representing 53% of sample).
Conclusions: LF-LAM should be performed in all adults with suspected HIV-associated tuberculosis (regardless of CD4 cell count) presenting to the emergency centre.

Keywords
HIV, tuberculosis, algorithm, emergency, lipoarabinomannan, point-of-care, ultrasound, X-ray

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Author roles: van Hoving DJ: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Visualization, Writing – Original Draft Preparation; **Meintjes G**: Conceptualization, Funding Acquisition, Methodology, Resources, Writing – Review & Editing; **Maartens G**: Conceptualization, Data Curation, Methodology, Supervision, Writing – Review & Editing; **Kengne AP**: Conceptualization, Data Curation, Formal Analysis, Methodology, Supervision, Writing – Review & Editing

Competing interests: DJvH was donated a Mindray ultrasound machine from the RCA division of Ascendis Medical to use in a part of the study.

Grant information: GrM was supported by the Wellcome Trust (098316 and 203135), the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant No 64787), NRF incentive funding (UID: 85858), and the South African Medical Research Council through its TB and HIV Collaborating Centres Programme with funds received from the National Department of Health (RFA# SAMRC-RFA-CC: TB/HIV/AIDS-01-2014). GaM was supported by NRF incentive funding (UID: 85810). The opinions, findings and conclusions expressed in this manuscript reflect those of the authors alone.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: van Hoving DJ, Meintjes G, Maartens G and Kengne AP. **A multi-parameter diagnostic clinical decision tree for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre [version 1; peer review: awaiting peer review]** Wellcome Open Research 2020, **5**:72 (https://doi.org/10.12688/wellcomeopenres.15824.1)

First published: 17 Apr 2020, **5**:72 (https://doi.org/10.12688/wellcomeopenres.15824.1)

Introduction

Tuberculosis remains an important cause of morbidity and mortality globally, despite ongoing control efforts[1]. The early diagnosis and successful treatment of people with tuberculosis should reduce the risk of mortality and morbidity, and decrease the transmission of tuberculosis[2]. Factors associated with delays in the diagnosis of tuberculosis include the limitations of tuberculosis diagnostic tests, limited availability of these tests in high burden settings, and the reduced diagnostic performance of tuberculosis tests in people living with HIV (PLWH)[3–5]. In PLWH with advanced immunosuppression, the diagnosis of active tuberculosis is challenging due to more atypical clinical presentations; other opportunistic infections with similar presentations; high proportion with inability to produce sputum or negative sputum smears; and high rates of extra-pulmonary and disseminated tuberculosis[6–11]. Autopsy studies in HIV-positive adults report a very high proportion with tuberculosis (32% to 47%), almost half (46%) of which was undiagnosed pre-mortem[12].

The WHO recommends that HIV-positive patients should be systematically screened for active tuberculosis when visiting a healthcare facility[2]. Many patients access the healthcare system through hospital emergency centres. The prevalence of HIV-related admissions to emergency centres varies, with up to 43% documented in Uganda[13]. These patients are often severely ill and would benefit from prompt diagnosis and treatment of tuberculosis to decrease mortality[14].

The use of point-of-care diagnostic tests would facilitate rapid diagnosis of tuberculosis. Lateral flow lipoarabinomannan (LF-LAM) is currently the only true point-of-care test, with other tests (e.g. smear microscopy, Xpert MTB/RIF, Xpert MTB/RIF Ultra, GeneXpert OMNI, and portable digital chest X-ray) being near point-of-care tests[15]. Point-of-care ultrasound (PoCUS) is also a potentially useful test for extra-pulmonary or disseminated tuberculosis[16]. No evidence-based algorithm incorporating clinical information, individual PoCUS features, and urine LF-LAM for diagnosing tuberculosis in HIV-positive patients currently exists. We performed a cross-sectional diagnostic study and developed a multi-parameter clinical decision tree to facilitate rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre.

Methods

Study setting and participants

Khayelitsha is a township with a mix of formal and informal housing in Cape Town, South Africa. The Khayelitsha Health sub-district has an antenatal HIV prevalence of 34%[17], and an annual tuberculosis notification rate of 917 per 100,000 persons[18]. The emergency centre of Khayelitsha Hospital (a district-level hospital) manages ± 35,000 patients per annum with an admission rate around 30%. The HIV prevalence of patients managed in the resuscitation unit is 23%[19].

Inclusion criteria were adults (≥18 years); HIV-positive (HIV-status was determined by laboratory confirmation or from the clinical records), and presence of at least one symptom of the WHO's recommended four-symptom screening rule for tuberculosis in PLWH (cough of any duration, fever, drenching night sweats, or weight loss)[20]. Exclusion criteria were: presenting to the emergency centre more than 24 hours before screening; received anti-tuberculosis treatment within 3 months of screening; pregnant; main clinical presentation of meningitis syndrome or new focal neurology; trauma, gynaecological or psychiatric presentation. Data from this cohort relating to LF-LAM and PoCUS were previously published[21,22]. These manuscripts described the use of LF-LAM in an acute care setting and identified PoCUS features independently associated with HIV-associated tuberculosis[21,22].

All participants provided written informed consent using a two-phase consent process. Severely ill participants were provided with a short one-page consent form indicating what extra tests would be done and that these would be used to facilitate diagnosis of tuberculosis and for research purposes. Full consent was obtained once patients had recovered and agreed to participate. The study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 697/2015).

Procedures and samples

Consecutive patients evaluated at the emergency centre were screened for eligibility from June 2016 through October 2017. A standardized data collection form was used to record demographic and clinical information. Urine, sputum and blood samples were obtained from all patients whenever possible (see *Extended data*)[23]. Fresh urine samples were tested using the Xpert MTB/RIF assay (GX4) (Cepheid Inc., Sunnyvale, CA, USA) and for the presence of LAM (Alere Determine™ TB LAM Ag test, Alere Inc., Waltham, MA, USA); LF-LAM was performed in the emergency centre[21]. Sputum specimens were tested using the Xpert MTB/RIF assay (GX4) and cultured in mycobacterial growth indicator tubes (MGIT; Becton Dickson, Sparks, MD, USA). Mycobacterial blood cultures were performed using the BACTEC MYCO/F Lytic blood culture bottle (Becton Dickson, Sparks, MD, USA). The MTBDR*plus* assay (Hain Lifescience, Nehren, Germany) were used to identify culture isolates as *M. tuberculosis* complex. Complete blood count and CD4 cell count were done as part of routine clinical care. CD4 cell count results were accepted if performed within 3 months of enrolment. The National Health Laboratory Service performed all the tests.

Ultrasound examination was performed in the emergency centre and the findings documented on a standardized assessment form. A single, emergency physician (with adequate training and credentials as specified by the International Federation of Emergency Medicine's Emergency Ultrasound Special Interest Group[24]) performed the ultrasound examination using either a Mindray M5™ ultrasound system with a 3C5s (2.5–6.5 MHz) convex probe and a 7L4s (5.0–10 MHz) linear probe (Mindray DS USA, Inc., Mahwah, NJ, USA) or a NanoMaxx™ ultrasound system with a L38n (10-5 MHz) linear array probe and a C60n (5–2 MHz) curved array probe (SonoSite Inc., Bothell, WA, USA). Ultrasound examinations

were performed before any specimens were collected. At the time of the ultrasound, the point-of-care sonographer had access to the clinical information but not to results from the reference standard (detection of *M. tuberculosis* from Xpert MTB/RIF and/or culture on any specimen obtained from any anatomical site).

Chest x-rays were reviewed by a single radiologist using a standardized assessment form (see *Extended data*[23]. Chest x-rays were classified as unlikely tuberculosis, probable tuberculosis, and likely tuberculosis. The radiologist had no access to clinical information or the reference standard.

Statistical analyses

The sample size was determined with the aim of including more than the recommended 10 candidate predictors (including interaction terms) from multivariable logistic regression analyses[25]. The tuberculosis prevalence in HIV-positive patients in the emergency centre is around 25%,[19] and a sample size of 400 HIV-positive participants was deemed adequate to include 100 tuberculosis cases. Data were analysed with the use of SAS/STAT® software (Version 9.4 of the SAS System for Windows [Copyright © 2019 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA]), R statistical software version 3.4.3 (2017-11-30) [The R® Foundation for Statistical Computing Platform], and SPSS Statistics for Windows, Version 25.0 (IBM Corp. Released 2017. Armonk, NY: IBM Corp.). Group comparisons used $\chi 2$ test and variants for qualitative variables and Student's t-test or non-parametric equivalents for continuous variables. Results are presented as count (percentages), mean and standard deviation (SD) or median and 25th-75th percentiles as appropriate.

Logistic regressions models were used to investigate the independent association between prevalent microbiologically confirmed tuberculosis and clinical and biological variables of interest. Candidate variables included WHO symptom screen (presence of cough, ≥1 present, ≥2 present, ≥3 present), antiretroviral therapy status (currently on antiretroviral medicine), presence and number of WHO danger signs (≥1 present, ≥2 present, ≥3 present)[26], number of individual PoCUS features (≥1 present, ≥2 present, ≥3 present), number of PoCUS features independently associated with tuberculosis (≥1 present, ≥2 present, ≥3 present), urinary LF-LAM, haemoglobin, chest X-ray (possible tuberculosis, likely tuberculosis, possible and likely tuberculosis) and CD4 cell count (<100 cells/mm^3, 100–200 cells/mm^3, >200 cells/mm^3). Individual PoCUS features included any sized pericardial effusion, pleural effusion, ascites, any focal splenic lesion, and any sized intra-abdominal lymphadenopathy. PoCUS features independently associated with tuberculosis (pericardial effusion of any size, ascites, intra-abdominal lymphadenopathy of any size) were determined by multivariable logistical regression[22].

For correlated variables, when more than one index was significant in a univariate model, the one with more significant effect on the $-2\log\hat{L}$ statistic was first entered into the multivariable model. However, in the final model, the effect of substituting variables was also assessed. When more than one correlated variable was significant in multivariable models, the final model selected was the one associated with the smallest Akaike's information criterion (AIC), a statistic derived from the $-2\log\hat{L}$ statistic. Multivariable model building was based on the combination of significant variables in univariable models (based on a threshold p<0.10). A model comprising WHO screening symptoms and history of current antiretroviral therapy use was used as starting model[20]. The ability of logistic regression models to discriminate between participants who had and those who did not have microbiologically confirmed tuberculosis was assessed using area under the receiver operating characteristic curves (AUC) and the relative integrated discrimination improvement (RIDI) which measures the percentage increase in discrimination when an extra variable is added to a prediction model[27,28]. AUC comparisons used nonparametric methods[29]. Bootstrap techniques were used to derive the 95% confidence interval (CI) for the RIDI estimates, which were based on 1000 replications.

We developed a decision tree model to predict microbiologically confirmed tuberculosis, including variables from the best performing multivariable logistic regression model, using the classification and regression tree (CART) algorithm and *rpart* package (version 4.1-11) of the R statistical software. The CART algorithm builds a tree model through recursive partitioning, through which process the data is successfully split into increasingly homogenous subgroups. At each stage (also known as node), the algorithm selects a predictor and a cut-point associated with the best ability of the predictor to discriminate participants with tuberculosis from those without. This was less an issue in the current analyses with no continuous predictor. However, for class variables with more than two levels, the algorithm could collapse levels in order to achieve the best discrimination. The CART starts with one predictor, then adds other predictors (and nodes) until reaching homogenous groups or having subgroups with few participants (<5), or exhaustion of predictors which can contribute further to subgroups refinement. Due to the small size of the achieved tree, no pre- or post-pruning was applied. CART uses a generalization of the binomial variance (Gini index) for its impurity function, and employs a 10-fold cross-validation to estimate error rates. The algorithm code is available as *Extended data*[30].

Results

Study population

We screened 556 patients; 414 (74.5%) of whom were enrolled (Figure 1). The prevalence of microbiologically confirmed tuberculosis was 41.5% (n=172): both Xpert MTB/RIF and culture positive n=93, 54.1%; only Xpert MTB/RIF positive n=32, 18.6%; only culture positive n=47, 27.3%. A median of 3 samples (25th–75th percentile, 2–4) were obtained from participants for culture and/or Xpert MTB/RIF (Table 1). At least two samples were obtained from two or more different anatomic sites in 350 (84.5%) participants.

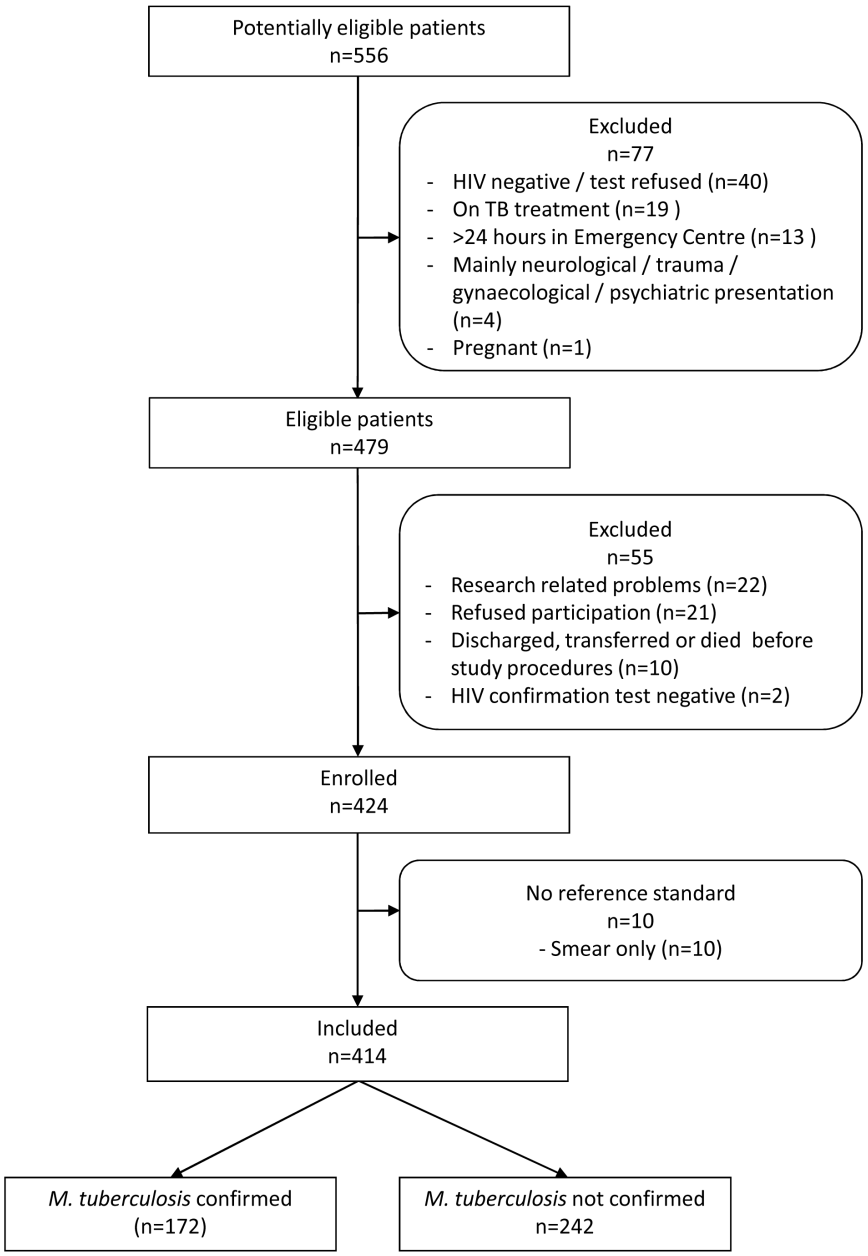

Figure 1. Flow diagram of study participants.

Demographic and clinical characteristics of participants with and without confirmed tuberculosis are presented in Table 2. The median CD4 cell count was 86 cells/mm^3 (25th–75th percentile, 30–218). The alternative diagnoses and the reasons for a clinical tuberculosis diagnosis in participants without microbiologically confirmed tuberculosis are presented in Table 3 and Table 4. The all-cause in-hospital mortality was 7.2% (n=30), 15 of whom had confirmed tuberculosis (representing 8.7% in hospital). These individual-level data are available at Zenodo[31].

Univariable associations

Univariable associations between microbiologically confirmed tuberculosis and clinical variables are presented as odds ratio (OR) with 95% CI and summarized in Table 5. The presence of two or more WHO screening symptoms (1.83 (1.04–3.22)), one or more PoCUS individual features (2.89 (1.87-4.47)), one or more PoCUS independent features (2.89 (1.92-4.35)), urinary LF-LAM (6.70 (3.99-11.25)), current antiretroviral therapy use (0.46 (0.31-0.68)), CD4 cell count less than 100 cells/mm^3 (1.98 (1.32-2.95)), and chest x-ray reported as 'likely tuber-

Table 1. Total clinical samples sent for mycobacterial testing (includes 'research' and 'routine' samples).

Sample	Sample Samples obtained within 24 hours						Total samples obtained during admission					
	No. (%) patients producing ≥ 1 sample	Total no. samples	Total no. Xpert tests done	Total no. cultures done	No. positive culture and Xpert tests (%)	No. (%) TB patients with ≥1 positive culture or Xpert test	No. (%) patients producing ≥ 1 sample	Total no. samples	Total no. Xpert tests done	Total no. cultures done	No. positive culture and Xpert tests (%)	No. (%) TB patients with ≥1 positive culture or Xpert test
Urine	266 (69.5)	278	277	1	57 (20.5)	55 (37.2)	312 (75.4)	328	322	6	64 (19.5)	62 (36.0)
Sputum*	227 (59.3)	349	289	232	202 (38.8)	88 (59.5)	291 (70.3)	581	466	441	291 (32.2)	112 (65.1)
Blood	312 (81.5)	343	0	343	66 (19.2)	61 (41.2)	345 (83.3)	404	0	404	72 (17.8)	64 (37.2)
Fine needle aspirate (FNA)	4 (1.0)	4	2	4	5 (83.3)	3 (2.0)	10 (2.4)	12	5	6	9 (81.8)	5 (2.9)
Cerebrospinal fluid (CSF)	13 (3.4)	13	12	4	0 (0.0)	0 (0.0)	31 (7.5)	32	27	13	0 (0.0)	0 (0.0)
Pleural fluid	15 (3.9)	16	3	14	8 (47.1)	8 (5.4)	24 (5.8)	27	4	26	13 (44.8)	12 7.0)
Pericardial fluid	0 (0)	0	0	0	0 (0.0)	0 (0.0)	2 (0.5)	2	0	2	2 (100)	2 (1.2)
Ascitic fluid	1 (0.3)	1	0	1	1 (100)	1 (0.7)	2 (0.5)	2	0	2	1 (50.0)	1 (0.6)
Other (swab, tracheal aspirate)	1 (0.3)	1	1	1	0 (0.0)	0 (0.0)	2 (0.5)	2	1	2	0 (0.0)	0 (0.0)
	383 (92.5)	1005	584	600	339 (28.6)	148 (86.0)	414 (100)	1390	825	902	452 (26.2)	172 (100)

TB = Tuberculosis

* Culture and Xpert MTB/RIF done on sputum taken on the same day were counted as two samples

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Table 2. Demographic and clinical characteristics of study population.

Characteristics at enrolment (n (%) unless otherwise specified)	All (N=414)	*M. tuberculosis* confirmed (N = 172)	*M. tuberculosis* not confirmed (N = 242)	p-value□
Age (years) (Median (Q_1-Q_3))	36 (30 – 43)	35 (30 – 42)	36 (31 – 44)	0.12
Gender: Male	171 (41.3)	71 (41.3)	100 (41.3)	0.99
Current cough of any duration	352 (85.0)	148 (86.0)	204 (84.3)	0.62
WHO symptom screen ≥ 1 present	414 (100)	172 (100)	242 (100)	---
WHO symptom screen ≥ 2 present	347 (83.8)	152 (88.4)	195 (80.6)	0.03
WHO symptom screen ≥ 3 present	239 (57.7)	104 (60.5)	135 (55.8)	0.34
Currently on antiretroviral therapy	195 (47.1)	62 (36.0)	133 (55.0)	<0.01
WHO danger signs ≥ 1 present	320 (77.3)	138 (80.2)	182 (75.2)	0.22
WHO danger signs ≥ 2 present	170 (41.1)	74 (43.0)	96 (39.7)	0.49
WHO danger signs ≥ 3 present	61 (14.7)	24 (14.0)	37 (15.3)	0.71
PoCUS individual features ≥ 1 present	264 (63.8)	133 (77.3)	131 (54.1)	<0.01
PoCUS individual features ≥ 2 present	140 (33.8)	86 (50.0)	54 (22.3)	<0.01
PoCUS individual features ≥ 3 present	64 (15.5)	47 (27.3)	17 (7.0)	<0.01
PoCUS independent features ≥ 1 present	217 (52.4)	116 (67.4)	101 (41.7)	<0.01
PoCUS independent features ≥ 2 present	80 (19.3)	58 (33.7)	22 (9.1)	<0.01
PoCUS independent features ≥ 3 present	17 (4.1)	17 (9.9)	0 (0.0)	<0.01
Urine lateral flow lipoarabinomannan (LF-LAM) positive*	94 (22.9)	71 (41.8)	23 (9.5)	<0.01
Hemoglobin (g/dl) (Mean ± SD)#	9.7 ± 2.7	9.0 ± 2.4	10.2 ± 2.7	<0.01
CD4 cell count < 100 cells/mm^3†	219 (53.7)	108 (63.9)	111 (46.4)	<0.01
CD4 cell count 100 to 200 cells/mm^3†	77 (18.6)	32 (18.6)	45 (18.6)	1.0
CD4 cell count > 200 cells/mm^3†	117 (28.3)	32 (18.6)	85 (35.1)	<0.01
Chest x-ray: Possible tuberculosis	109 (26.3)	39 (22.7)	70 (28.9)	0.16
Chest x-ray: Likely tuberculosis	150 (36.2)	98 (57.0)	52 (21.5)	<0.01

SD = Standard Deviation; Q1-Q3 = 25th – 75th percentile; WHO symptom screen = Cough of any duration, fever, drenching night sweats, weight loss; WHO danger signs = Respiratory rate > 30/min, Heart rate > 120/min, Temperature > 39°C, being unable to walk unaided; PoCUS = Point-of-Care Ultrasound; PoCUS individual features = Pericardial effusion (any size), pleural effusion, ascites, any splenic lesion, intra-abdominal lymphadenopathy (any size); PoCUS independent features = Pericardial effusion (any size), ascites, intra-abdominal lymphadenopathy (any size); * N=411; # N=410; † N=408; □ Comparison between *M. tuberculosis* confirmed and *M. tuberculosis* not confirmed

culosis' (4.81 (3.13-7.40)) were significantly associated with confirmed tuberculosis.

Multivariable model

Measures of model performance are summarized in Table 6. The initial model (WHO screening symptoms ≥2, antiretroviral therapy use) had poor discriminatory power in predicting confirmed tuberculosis with an AUC of 0.615. The addition of either PoCUS independent features or PoCUS individual features to the initial model both improved model goodness of fit and its discriminatory power, however the model with PoCUS independent features had a greater AUC and a smaller AIC. The further addition of urinary LF-LAM and chest x-ray improved the model. Adding CD4 cell count did not improve the performance of the model (Table 6).

Based on RIDI% estimates, adding urinary LF-LAM, PoCUS independent features, and chest x-ray to the initial and subsequent models conferred similar levels of improvement for tuberculosis prediction (Table 7). Change in RIDI% was meaningless when CD4 cell count was added to the model comprising WHO symptoms screen, antiretroviral therapy use, PoCUS independent features, urinary LF-LAM and chest X-ray (RIDI% 2.6 (2.4-2.7)).

Prediction tree

Significant variables (Model F in Table 7) were included in the splitting process to build the classification tree for micro-biologically confirmed tuberculosis. The CART created for confirmed tuberculosis is shown in Figure 2, and the CART as applied to a theoretical cohort of 1000 patients is presented in

Table 3. Distribution of alternative diagnoses in participants without microbiologically confirmed tuberculosis.

Alternative diagnoses	n (%)
Lower Respiratory Tract Infection / Pneumonia	96 (39.7)
Clinical diagnoses of tuberculosis	63 (26.0)
Gastro-enteritis (acute & chronic)	13 (5.4)
Pneumocystis pneumonia	11 (4.5)
Renal failure (acute & chronic)	8 (3.3)
Bronchiectasis	6 (2.5)
Dysentery	3 (1.2)
HIV wasting syndrome	3 (1.2)
Undifferentiated abdominal pain	3 (1.2)
Appendicitis	2 (0.8)
Congestive cardiac failure	2 (0.8)
Delirium	2 (0.8)
Empyema	2 (0.8)
Gallstones	2 (0.8)
Kaposi sarcoma	2 (0.8)
Urosepsis	2 (0.8)
Bronchitis	1 (0.4)
Chronic Lymphocytic Leukaemia	1 (0.4)
Colon carcinoma	1 (0.4)
Constipation	1 (0.4)
Chronic Obstructive Pulmonary Disease (COPD) exacerbation	1 (0.4)
Cor Pulmonale	1 (0.4)
Duodenitis	1 (0.4)
E. coli bacteraemia	1 (0.4)
Interstitial lung disease	1 (0.4)
Liver carcinoma	1 (0.4)
Lung abscess	1 (0.4)
Meningitis	1 (0.4)
Non-tuberculous mycobacterial infection (disseminated)	1 (0.4)
Pelvic inflammatory disease	1 (0.4)
Progressive multifocal leukoencephalopathy	1 (0.4)
Scleroderma	1 (0.4)
Thrombotic thrombocytopenic purpura	1 (0.4)
Vitamin B12 deficiency	1 (0.4)
Unknown diagnosis	4 (1.7)
	242 (100)

HIV = Human Immunodeficiency Virus

Figure 3. The CART analysis suggest that once screened via WHO symptoms as eligible for further diagnostic investigations, the number of WHO symptoms present does not add further to the discrimination of people with tuberculosis from those without. Furthermore, CART positions urinary LF-LAM as the next screening test after WHO symptoms, with 75% of people with positive urinary LF-LAM test (17% of all those with positive WHO symptoms) having a definitive diagnosis of microbiologically confirmed tuberculosis (Figure 2 and Figure 3). For those with negative urinary LF-LAM, CART positions chest x-ray as the next screening test. Chest x-ray appears twice, but with complementary and not overlapping contributions. The first appearance of chest x-ray (after those with negative urinary LF-LAM) serves to separate participants with 'likely tuberculosis' on chest x-ray from those with 'possible or unlikely tuberculosis' on chest x-ray. The presence of one or no PoCUS independent features in those with 'possible or unlikely tuberculosis' on chest x-ray (47% of the starting sample) isolates 83% of this subgroup (representing 39% of the starting sample) where tuberculosis was not microbiologically confirmed (Figure 2 and Figure 3). The second appearance of chest x-ray occurs in participants with ≥2 PoCUS independent features and serves to separate those with 'possible tuberculosis' on chest x-ray from those with 'unlikely tuberculosis' on chest x-ray. The validation for the decision tree is presented in Figure 4.

We created a second decision tree to make it more clinically applicable by removing the history of antiretroviral therapy (ART) status, because ART interruption is often not disclosed and ART status may be unavailable in confused patients (Figure 5 and Figure 6). The branch on the original tree relating to antiretroviral therapy no longer expands, narrowing down what to decide for the 24% of the sample with negative urinary LF-LAM and 'likely tuberculosis' on chest x-ray. Just over half (56%) of these participants will have confirmed tuberculosis.

We created a third prediction tree by only excluding chest x-ray, which is not a true point-of-care test (Figure 7 and Figure 8). CART positions PoCUS as the next screening test for those with a negative urinary LF-LAM. The presence of two or less independent PoCUS features (75% of the starting sample) had a true negative rate of 71% (representing 53% of the starting sample) in the subgroup where tuberculosis was not microbiologically confirmed.

Discussion

We developed a prediction tree to diagnose HIV-associated tuberculosis in an emergency centre in a high burden setting. The variables selected on multivariable analysis for inclusion in the final model were the presence of >2 WHO screening symptoms, current antiretroviral therapy use, urinary LF-LAM, independent PoCUS features, and chest x-ray. The CART analysis positioned urinary LF-LAM as the first test to perform in participants with positive WHO screening symptoms, followed by chest x-ray. We also developed a simplified prediction tree by excluding chest x-ray, which is not a true point-of-care test: CART positioned PoCUS as the next screening test for those with a negative urinary LF-LAM.

Table 4. Reason for diagnosis of tuberculosis without microbiological confirmation.

Diagnostic test	n
Suggestive formal abdominal ultrasound done in radiology department	19
Suggestive chest X-ray	9
Positive urine lateral flow lipoarabinomannan (LF-LAM)	7
Suggestive formal abdominal ultrasound and suggestive chest X-ray	6
Not improving on empiric antibiotics	4
Raised adenosine deaminase (ADA) in effusion fluid (pleural or ascitic)	4
Cerebrospinal fluid suggestive of tuberculous meningitis (TBM)	4
Suggestive chest X-ray and positive urine LF-LAM	3
Suggestive formal abdominal ultrasound and positive urine LF-LAM	2
Psoas abscess on formal ultrasound	2
Caseous necrosis on biopsy (histology)	1
Suggestive computer tomography (CT) scan of abdomen	1
Suggestive chest X-ray and raised ADA in effusion fluid	1
Total	63

Table 5. Univariable associations between microbiologically confirmed tuberculosis and clinical variables.

Variables	Subgroups	Odds Ratio (95% CI)	p-value	AUC	AIC	-2 Log L	Likelihood ratio (χ^2 (p-value)	Calibration (χ^2 (p-value)
Intercept only			0.0006		564.03	562.03		
Presence of cough		1.15 (0.66-2.00)	0.623	0.509	565.79	561.79	0.243 (0.622)	NA
WHO symptoms screen	1 (reference)	1.00	0.128	0.540	562.65	556.65	4.312 (0.116)	0.0 (>0.999)
	2	1.84 (0.96-3.52)						
	3 or more	1.77 (0.99-3.18)						
	≥ 2 vs. < 2	1.83 (1.04-3.22)	0.036	0.539	561.39	557.39	4.645 (0.031)	NA
	≥ 3 vs. < 3	1.21 (0.81-1.80)	0.342	0.523	565.13	561.13	0.904 (0.342)	NA
WHO danger signs	Absent (reference)	1.00	0.511	0.542	566.63	558.63	2.327 (0.507)	0.0 (>0.999)
	1	1.31 (0.77-2.23)						
	2	1.52 (0.86-2.68)						
	3 or more	1.14 (0.59-2.22)						
	≥ 1 vs. < 1	1.34 (0.83-2.15)	0.230	0.525	564.57	560.57	1.462 (0.226)	NA
	≥ 2 vs. < 2	1.15 (0.77-1.71)	0.494	0.517	565.57	561.57	0.467 (0.474)	NA
	≥ 3 vs. < 3	0.90 (0.52-1.57)	0.706	0.507	565.89	561.89	0.143 (0.705)	NA
PoCUS individual features	Absent (reference)	1.00	<0.0001	0.679	522.43	514.43	46.52 (<0.0001)	0.0 (>0.999)
	1	1.74 (1.04-2.91)						
	2	3.08 (1.72-5.52)						
	3 or more	7.87 (4.05-15.28)						
	≥ 1 vs. < 1	2.89 (1.87-4.47)	<0.0001	0.616	541.88	537.88	24.149 (<0.0001)	NA
	≥ 2 vs. < 2	3.48 (2.27-5.33)	<0.0001	0.638	531.65	527.65	34.387 (<0.0001)	NA
	≥ 3 vs. < 3	4.98 (2.74-9.03)	<0.0001	0.602	534.32	530.32	31.71 (<0.0001)	NA

Variables	Subgroups	Odds Ratio (95% CI)	p-value	AUC	AIC	-2 Log L	Likelihood ratio (χ^2 (p-value)	Calibration (χ^2 (p-value)
PoCUS independent features	Absent (reference)	1.00	<0.0001	0.673	510.29	502.29	58.66 (<0.0001)	0.0 (>0.999)
	1	1.87 (1.18-2.96)						
	2	4.69 (2.57-8.56)						
	3 or more	>999.99 (<0.001-999.99)						
	≥ 1 vs. < 1	2.89 (1.92-4.35)	<0.0001	0.629	538.98	534.98	27.05 (<0.0001)	NA
	≥ 2 vs. < 2	5.09 (2.96-8.73)	<0.0001	0.623	526.90	522.90	39.13 (<0.0001)	NA
	≥ 3 vs. < 3	>999.99 (<0.001-999.99)	0.975	0.549	535.14	531.14	30.89 (<0.0001)	NA
Urinary LAM		6.70 (3.99-11.25)	<0.0001	0.663	506.21	502.21	59.82 (<0.0001)	NA
Hemoglobin (per unit lower)		0.999 (0.996-1.001)	0.247	0.376	564.51	560.51	1.518 (0.218)	24.78 (0.0017)
Antiretroviral therapy status		0.46 (0.31-0.68)	0.0001	0.596	550.07	546.07	14.89 (<0.0001)	NA
CD4 cell count	>200 cells/mm^3 (reference)	1.00	0.0006	0.599	551.39	545.39	15.57 (0.0004)	0.0 (>0.999)
	<100 cells/mm^3	2.58 (1.59-4.20)						
	100-200 cells/mm^3	1.89 (1.03-3.47)						
Chest X-ray	Unlikely TB [9] (reference)	1.00	<0.0001	0.704	506.473	500.47	60.48 (<0.0001)	0.0 (>0.999)
	Possible TB	1.94 (1.12-3.34)						
	Likely TB	6.46 (3.90-10.70)						
	Possible TB vs Unlikely & Likely TB	0.73 (0.46-1.15)	0.175	0.530	563.09	559.09	1.86 (0.172)	
	Likely TB vs. Unlikely and Likely TB	4.81 (3.13-7.40)	<0.0001	0.677	510.18	506.18	54.78 (<0.0001)	NA
	Possible & Likely TB vs. Unlikely TB	3.86 (2.44-6.12)	<0.0001	0.641	528.04	524.04	33.16 (<0.0001)	NA

CI = Confidence Interval; AUC = Area under the receiver operating characteristics curves; AIC = Akaike information criterion; WHO symptom screen = Cough of any duration, fever, drenching night sweats, weight loss; WHO danger signs = Respiratory rate > 30/min, Heart rate > 120/min, Temperature > 39°C, being unable to walk unaided; PoCUS = Point-of-Care Ultrasound; PoCUS individual features = Pericardial effusion (any size), pleural effusion, ascites, any splenic lesion, intra-abdominal lymphadenopathy (any size); PoCUS independent features = Pericardial effusion (any size), ascites, intra-abdominal lymphadenopathy (any size); LAM = Lateral flow lipoarabinomannan; TB = tuberculosis

Table 6. The performance of multivariable models predicting microbiologically confirmed tuberculosis.

Model	Variables in the model	AUC (95% CI)	AIC	-2 Log L	Likelihood ratio χ^2	Calibration χ^2 (p-value)
A	WHO symptom screen ≥2, ART use	0.615 (0.564-0.665)	547.80	541.80	18.12 (<0.0001) DF2	1.386 (0.500)
B	A + PoCUS independent features	0.703 (0.653-0.753)	503.66	491.66	69.30 (<0.0001) DF5	4.330 (0.741)
C	A + PoCUS individual features	0.701 (0.650-0.751)	514.26	502.26	58.70 (<0.0001) DF5	5.391 (0.612)
D	A + urinary LAM	0.736 (0.688-0.784)	489.64	481.64	79.32 (<0.0001) DF3	3.981 (0.408)
E	D + PoCUS independent features	0.773 (0.727-0.819)	463.38	449.38	111.57 (<0.0001) DF6	5.259 (0.628)
F	E + Chest x-ray	0.820 (0.779-0.862)	433.99	415.99	144.966 (<0.0001) DF8	7.429 (0.386)
G	F + CD4 cell count	0.821 (0.780-0.863)	435.99	413.99	146.970 (<0.0001) DF10	8.753 (0.363)

p-values for AUC comparisons: 0.0009 (B-A), 0.0001 (C-A), <0.0001 (D-A), <0.0001 (E-A), <0.0001 (F-A), <0.0001 (G-A); 0.868 (C-B), 0.237 (D-B), 0.0003 (E-B), <0.0001 (F-B), <0.0001 (G-B), 0.181 (D-C), 0.0002 (E-C), <0.0001 (F-C), <0.0001 (G-C), 0.0052 (E-D), <0.0001 (F-D), <0.0001 (G-D), 0.002 (F-E), 0.0022 (G-E), 0.8223 (G-F); Overall p<0.0001 for difference across all AUC.

AUC = Area under the receiver operating characteristics curves; CI = Confidence interval; AIC = Akaike information criterion; WHO symptom screen = Cough of any duration, fever, drenching night sweats, weight loss; ART = Antiretroviral therapy; PoCUS = Point-of-Care Ultrasound; PoCUS individual features = Pericardial effusion (any size), pleural effusion, ascites, any splenic lesion, intra-abdominal lymphadenopathy (any size); PoCUS independent features = Pericardial effusion (any size), ascites, intra-abdominal lymphadenopathy (any size); LAM = Lateral flow lipoarabinomannan

Table 7. Relative integrated discrimination improvement (RIDI, %) statistic comparing different models.

Model	Variables in the model	A	B	C	D	E	F	G
A	WHO symptom screen ≥2, ART use	NA	252.6 (242.3-262.8)	224.4 (214.8-233.9)	334.5 (321.0-348.0)	483.0 (464.6-501.5)	653.9 (629.5-678.3)	674.2 (649.0-699.4)
B	A + PoCUS independent features		NA	-7.77 (-8.37 to -7.17)	25.3 (23.5-27.1)	65.0 (63.4-66.5)	113.4 (111.1-115.8)	119.0 (116.6-121.4)
C	A + PoCUS individual features			NA	37.1 (35.0-39.3)	80.7 (78.6-82.8)	133.7 (130.8-136.6)	139.8 (136.8-142.8)
D	A + urinary LAM				NA	34.3 (33.4-35.1)	73.8 (72.3-75.3)	78.4 (76.8-80.0)
E	D + PoCUS independent features					NA	29.4 (28.7-30.1)	32.8 (32.0-33.5)
F	E + Chest x-ray						NA	2.6 (2.4-2.7)
G	F + CD4 cell count							NA

WHO symptom screen = Cough of any duration, fever, drenching night sweats, weight loss; ART = Antiretroviral therapy; PoCUS = Point-of-Care Ultrasound; PoCUS individual features = Pericardial effusion (any size), pleural effusion, ascites, any splenic lesion, intra-abdominal lymphadenopathy (any size); PoCUS independent features = Pericardial effusion (any size), ascites, intra-abdominal lymphadenopathy (any size); LAM = Lateral flow lipoarabinomannan.

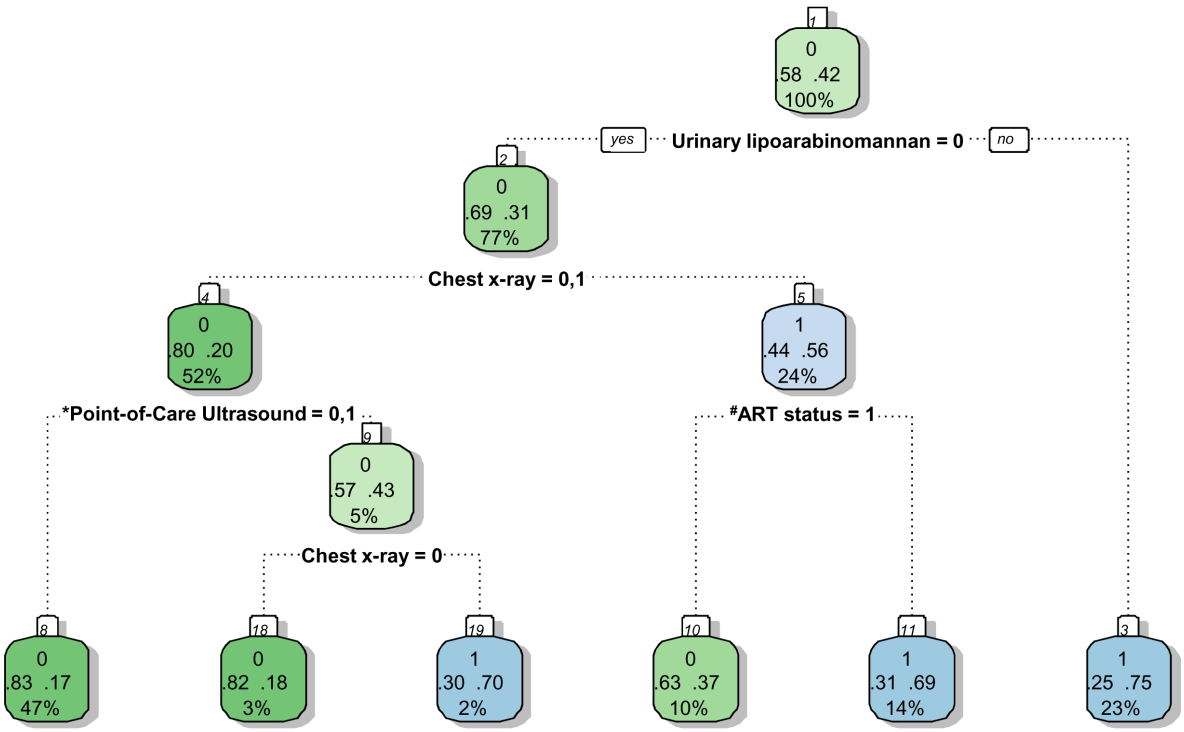

Figure 2. Prediction tree for microbiologically confirmed tuberculosis. Totals might not add up due to rounding. *Point-of-Care ultrasound = Independent point-of-care ultrasound features (ascites, any size pericardial effusion, any size intra-abdominal lymphadenopathy; #ART = Anti-retroviral therapy. Predictor coding: Urinary lipoarabinomannan: 0 = Negative, 1 = Positive; Chest x-ray: 0 = Unlikely tuberculosis, 1 = Possible tuberculosis, 2 = Likely tuberculosis; Point-of-Care ultrasound: 0 = None present; 1 = ≥1 feature present, 2 = ≥2 features present; ART status: 0 = Not on ART, 1 = Currently on ART. Explanation of node: Number in small white block represents the number of the node in the recursive partitioning; Bottom number in big coloured block represents the percentage of the entire dataset that passes through this particular node (e.g. 100% in block 1); Middle numbers in big coloured block represent the proportion with the outcome (right) and without the outcome (left) within the subgroup (e.g. in block, 58% without tuberculosis and 42% with tuberculosis in block1); Top number in big coloured block represent the presence (1) or absence (0) of the outcome in the majority of the observations in that block (e.g. majority in block 1 without tuberculosis (58% versus 42%)); The colour of the block has no particular meaning.

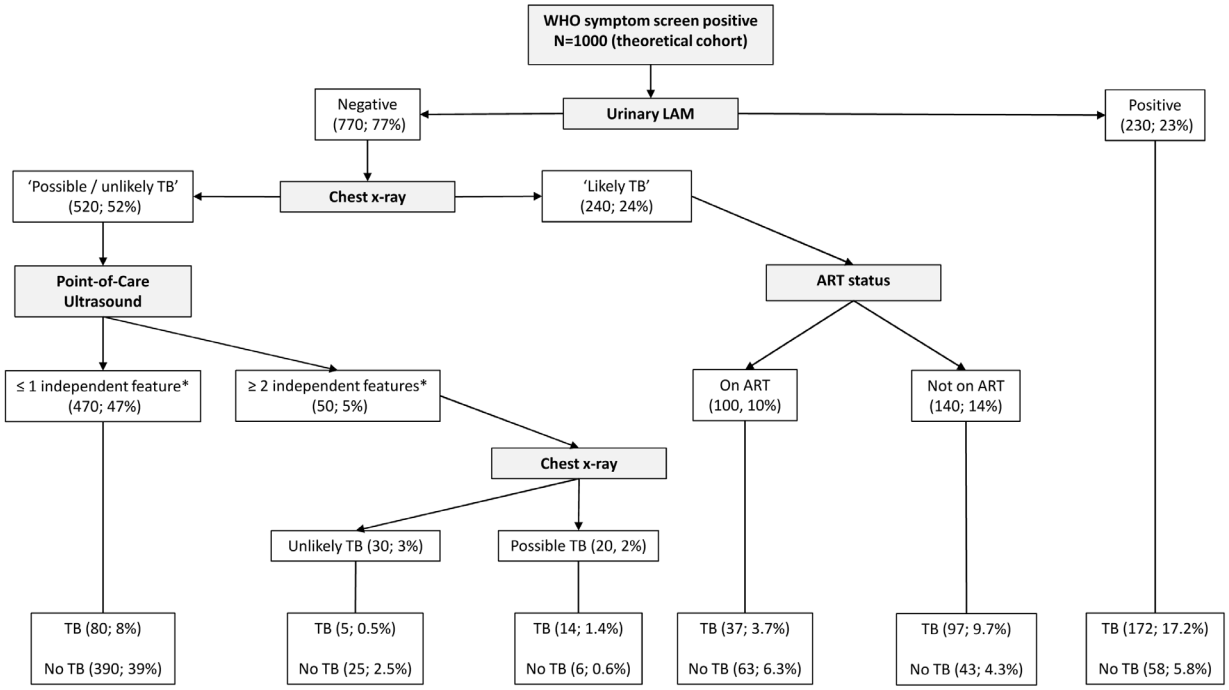

Figure 3. Prediction tree for microbiologically confirmed tuberculosis applied to a theoretical cohort of 1000 patients. Nodes show the number of patients and percentage of total sample size (n, %). Totals might not add up due to rounding. *Independent point-of-care ultrasound features (ascites, any size pericardial effusion, any size intra-abdominal lymphadenopathy; LAM = lateral flow lipoarabinomannan; TB = tuberculosis; ART = anti-retroviral therapy.

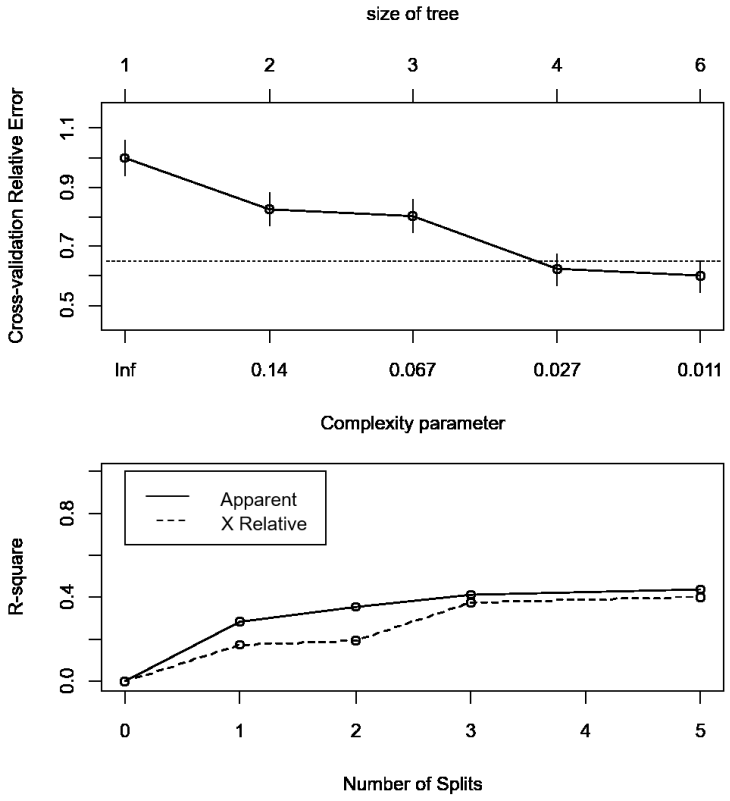

Figure 4. Cross-validation results of the decision tree. Upper panel: cross-validation relative error vs. numbers of split and complexity parameter; Lower panel: apparent R-square and R-square from cross-validation vs the number of splits.

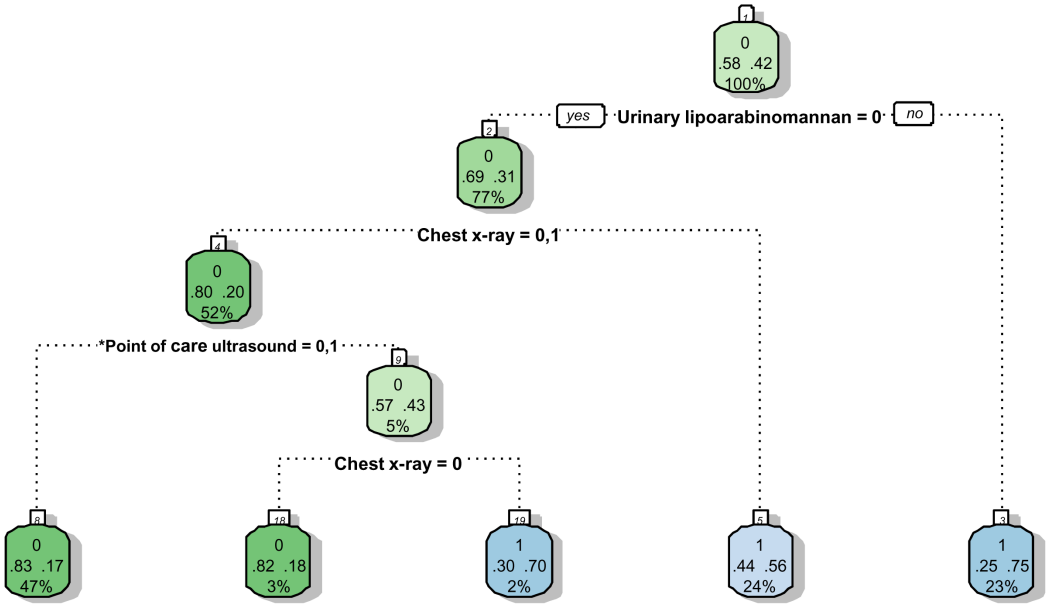

Figure 5. Prediction tree with antiretroviral status removed for microbiologically confirmed tuberculosis. Totals might not add up due to rounding. *Point-of-Care ultrasound = Independent point-of-care ultrasound features (ascites, any size pericardial effusion, any size intra-abdominal lymphadenopathy. Predictor coding: Urinary lipoarabinomannan: 0 = Negative, 1 = Positive; Chest x-ray: 0 = Unlikely tuberculosis, 1 = Possible tuberculosis, 2 = Likely tuberculosis; Point-of-Care ultrasound: 0 = None present; 1 = ≥1 feature present, 2 = ≥2 features present. Explanation of node: Number in small white block represents the number of the node in the recursive partitioning; Bottom number in big coloured block represents the percentage of the entire dataset that passes through this particular node(e.g. 100% in block 1); Middle numbers in big coloured block represent the proportion with the outcome (right) and without the outcome (left) within the subgroup (e.g. in block, 58% without tuberculosis and 42% with tuberculosis in block1); Top number in big coloured block represent the presence (1) or absence (0) of the outcome in the majority of the observations in that block (e.g. majority in block 1 without tuberculosis (58% versus 42%)); The colour of the block has no particular meaning.

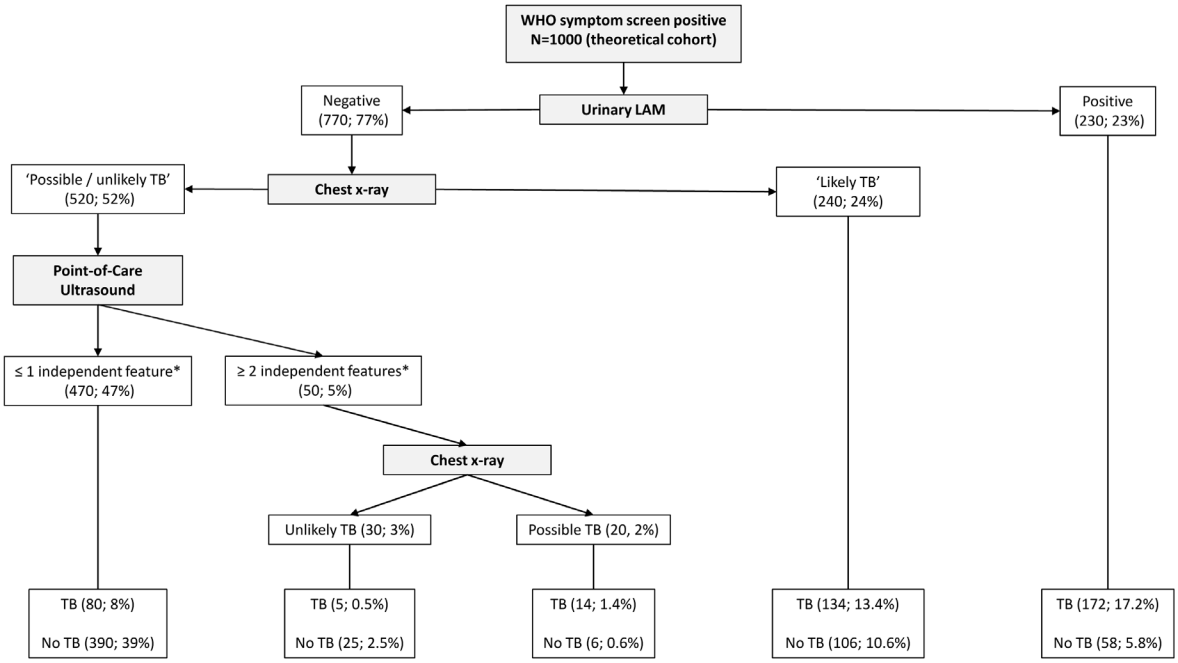

Figure 6. Prediction tree with antiretroviral status removed for microbiologically confirmed tuberculosis applied to a theoretical cohort. Nodes show the number of patients and percentage of total sample size (n, %). Totals might not add up due to rounding. *Independent point-of-care ultrasound features (ascites, any size pericardial effusion, any size intra-abdominal lymphadenopathy; LAM = Lateral flow lipoarabinomannan; TB = tuberculosis.

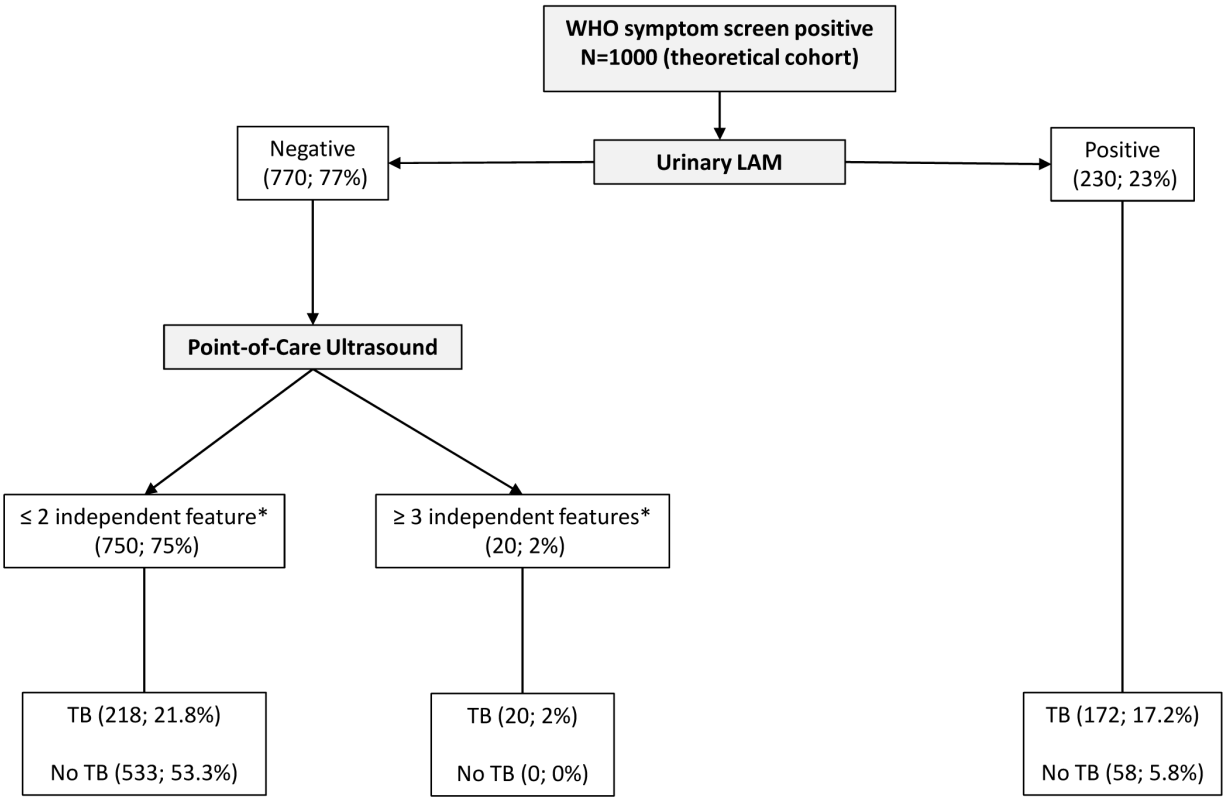

Figure 7. Prediction tree of point-of-care only tests for microbiologically confirmed tuberculosis applied to a theoretical cohort. Nodes show the number of patients and percentage of total sample size (n, %). Totals might not add up due to rounding. *Independent point-of-care ultrasound features (ascites, any size pericardial effusion, any size intra-abdominal lymphadenopathy); LAM = lateral flow lipoarabinomannan; TB = tuberculosis.

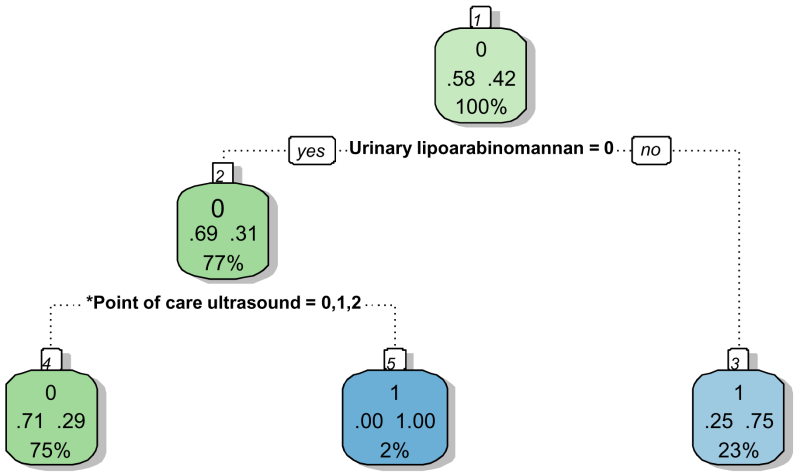

Figure 8. Prediction tree with chest x-ray removed for microbiologically confirmed tuberculosis. *Point-of-Care ultrasound = Independent point-of-care ultrasound features (ascites, any size pericardial effusion, any size intra-abdominal lymphadenopathy). Predictor coding: Urinary lipoarabinomannan: 0 = Negative, 1 = Positive; Point-of-Care ultrasound: 0 = Negative; 1 = ≥1 feature present, 2 = ≥2 features present, 3 = ≥3 features present. Explanation of node: Number in small white block represents the number of the node in the recursive partitioning; Bottom number in big coloured block represents the percentage of the entire dataset that passes through this particular node(e.g. 100% in block 1); Middle numbers in big coloured block represent the proportion with the outcome (right) and without the outcome (left) within the subgroup (e.g. in block, 58% without tuberculosis and 42% with tuberculosis in block1); Top number in big coloured block represent the presence (1) or absence (0) of the outcome in the majority of the observations in that block (e.g. majority in block 1 without tuberculosis (58% versus 42%)); The colour of the block has no particular meaning.

The use of urinary LF-LAM was the predictor with the best ability of creating pure groups (either with or without tuberculosis); classifying almost 25% of the study sample (75% of which were true positives) regardless of their CD4 cell count. The false positive rate of 25% is less than a recent Cochrane review, in which 33% of participants with tuberculosis symptoms had a false positive urinary LF-LAM result for microbiologically confirmed tuberculosis[32]. However, inappropriate exclusions (e.g. participants unable to produce sputum), different enrolment criteria and different CD4 cell counts could potentially explain the high false negative rate seen in the Cochrane review[32]. Another urine-based LAM assay, Fujifilm SILVAMP TB LAM (FujiLAM; Fujifilm, Tokyo, Japan), has higher sensitivity but somewhat lower specificity than the LF-LAM assay we used[33].

Urinary LAM is underutilized despite it being affordable, fast, non-invasive, and simple[34]. Only three high TB/HIV burden countries (Eswatini (formerly Swaziland), South Africa, and Uganda) had national roll-outs of LF-LAM testing by the end of 2018[34]. Urine LF-LAM is a simple point-of-care test achievable in acute care settings[21], which has been shown to reduce mortality in high-risk HIV-positive inpatients[35,36].

The performance of PoCUS when chest x-ray is available is limited (Figure 2 and Figure 3). One of every 11 PoCUS examinations will be 'positive' (i.e. two or more PoCUS independent features), but then an evaluation of the chest x-ray would still be needed to refine the classification of patients with and without tuberculosis. A 'negative' PoCUS examination (i.e. the presence of ≤1 PoCUS independent feature) will only rule out 39% of all patients with a clinical suspicion of tuberculosis. This supports other studies and the current WHO guidelines that ultrasound is an additional diagnostic tool and should not replace chest x-ray as the initial imaging step to diagnose tuberculosis in HIV-positive patients[20,37]. However, chest x-ray is not a true point-of-care test, unlike PoCUS. In acute care settings where chest x-ray is not readily available PoCUS has a 100% true positive rate when all 3 of the independent features were detected, indicating its potential value as a rule-in test; however, 39 PoCUS examinations will need to be performed to confidently diagnose one additional patient in those who had a negative LAM. The presence of ≤2 PoCUS independent features will rule out 53% of patients with a clinical suspicion of tuberculosis in situations where chest x-ray is not available; however, the high false negative rate (29%, 218/750) indicates that PoCUS cannot be used as a rule-out test and these patients will need to undergo further testing.

The use of urinary LF-LAM should be prioritised in all HIV-positive patients (regardless of CD4 cell count and clinical condition) who presents to the emergency centre with WHO tuberculosis symptoms. Although a result can be obtained after 25 minutes, a major time increasing factor would be to get a urine sample. The history of current use of ART should be obtained if the patient's condition allows, as it further refines the diagnostic ability of the algorithm by increasing both the true positive and the true negative rate. Chest x-ray should still be performed if available. In these settings, the value of PoCUS becomes doubtful due to the low positive yield (5%) and the further interpretation of a chest x-ray to better classify cases and non-cases. Although 47% of patients will have negative results for urinary LF-LAM, chest x-ray and PoCUS, the true negative rate is only 83%, too low to confidently rule tuberculosis out. In emergency centres without chest x-ray availability (e.g. limited resources, restricted radiology consulting times), physicians can confidently diagnose tuberculosis in patients where all three independent PoCUS features are present (true positive rate 100%). However, only 2% of the PoCUS examinations are expected to be positive and one can argue whether the time spend to perform the PoCUS is worthwhile. The 71% true negative rate again indicates the need for further diagnostic testing.

Our study has some limitations. Our findings may not be generalizable as the study was conducted in a single emergency centre in a high TB/HIV-prevalence setting; a single, experienced operator performed all the PoCUS examinations; and the chest x-rays were interpreted by a single experienced radiologist. The main strength of our study is the robust microbiologic reference standard composed of TB culture and Xpert MTB/RIF performed on multiple samples from different anatomic sites. However, it is still possible that some TB cases were missed by the reference standard. The study was also performed under routine conditions experienced in the emergency centre. Lastly, robust analytic strategies were used to develop and validate the diagnostic decision tree.

Conclusion

We developed a near-patient and point-of-care decision tree for the diagnosis of HIV-associated tuberculosis in acute care settings. Implementing this decision tree following screening via WHO symptoms can allow immediate initiation of TB treatment within the emergency centre in about a quarter of suspected patients among whom 75% would have microbiologically confirmed tuberculosis, or withhold such treatment in nearly half of suspected patients, among whom less than 18% will have microbiologically confirmed tuberculosis. Urinary LF-LAM had a 75% true positive rate, representing 17% of participants with positive WHO screening symptoms regardless of CD4 cell count and its use should be prioritised. The contribution of PoCUS in the context of urinary LF-LAM and chest X-ray availability was limited, due to the low positive yield, the need for further chest x-ray interpretation and the high false negative rate. In acute care settings without chest x-ray availability, PoCUS has a 100% true positive rate, but will only affect 2% of eligible patients. The role of PoCUS in diagnosing HIV-associated tuberculosis in the emergency centre needs to be further investigated.

Data availability

Underlying data

Zenodo: Rapid diagnosis of HIV-associated tuberculosis in the emergency centre. https://doi.org/10.5281/zenodo.3734101[31].

This project contains the following underlying data:

- HIV-TB_diagnostic_algorithm_data.csv. (Data used for diagnostic algorithm.)
- HIV-TB_diagnostic_samples.csv. (Data of diagnostic samples taken.)
- HIV-TB_diagnostic_algorithm_codebook.docx. (codebook for diagnostic algorithm data.)
- HIV-TB_diagnostic_samples_codebook.docx. (codebook for diagnostic samples.)

Extended data

Zenodo: Case report form: Rapid diagnosis of HIV-associated tuberculosis in the emergency centre. https://doi.org/10.5281/zenodo.3738912[23].

Zenodo: Code: Rapid diagnosis of HIV-associated tuberculosis in the emergency centre. https://doi.org/10.5281/zenodo.3739005[30].

Reporting guidelines

Zenodo: TRIPOD checklist for 'A multi-parameter diagnostic clinical decision tree for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre'. https://doi.org/10.5281/zenodo.3738999[38].

Acknowledgements

We acknowledge the valuable contribution of Rene Goliath (research clinical site coordinator, Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease & Molecular Medicine, University of Cape Town), the staff of the National Health Laboratory Service (NHLS), and the staff and administration of Khayelitsha Hospital.

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APPENDIX TO CHAPTER 6

The use of the prediction tree to facilitate empiric tuberculosis treatment decisions

Empiric tuberculosis treatment is defined as the initiation of anti-tuberculosis treatment in patients who do not have microbiologically confirmed tuberculosis, based on clinical and/or radiological features. International and national clinical algorithms exist which indicate the initiation of anti-tuberculosis treatment for HIV-positive persons where chest X-ray findings are suggestive of tuberculosis and where symptoms are not alleviated by broad spectrum antibiotics.[1,2] This is used in combination with two sputum smear microscopy tests (or a single sputum Xpert test) that are negative for *Mycobacterium tuberculosis*. In the South African primary care setting, 15% of patients initiated on anti-tuberculosis treatment were done so empirically.[3] Chest X-ray findings suggestive of tuberculosis informed the majority of the decisions and overall 13% of empirically-treated patients had subsequent microbiological confirmation of tuberculosis.[3] This low percentage was probably in part related to many patients not having tuberculosis culture sent for confirmation. The third prediction tree we developed used only rapid point-of-care tests and excluded confirmatory tests such as Xpert and tuberculosis culture. The point-of-care only decision tree could enable clinicians to facilitate decisions for the initiation of empiric anti-tuberculosis treatment.

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CHAPTER 7

Conclusion

South Africa has a high burden of HIV-associated tuberculosis with one of the highest mortality rates in the world.[1] Early diagnosis and initiation of anti-tuberculosis treatment is needed to decrease the related morbidity and mortality. Rapid diagnosis of HIV-associated tuberculosis is thus essential, but can only be accomplished with effective implementation of point-of-care testing. In South Africa, many HIV-positive patients, some severely ill, present to emergency centres with the suspicion of having active tuberculosis. The emergency centre is therefore strategically placed to achieve earlier tuberculosis diagnosis, if point-of-care testing can be accomplished within the often chaotic acute care environment. Rapid and early diagnosis of HIV-associated tuberculosis can expedite the initiation of anti-tuberculosis treatment. This has the potential to reduce transmission, limit morbidity and decrease mortality.[2,3] It is, however, important to note that the initiation of empiric anti-tuberculosis treatment (based on pre-specified criteria rather than clinician decision) has not been shown to decrease mortality at 6 months or at 12 months.[4–6]

The lateral flow lipoarabinomannan (LF-LAM) strip for urine is a potential point-of-care test that can be used in the acute care setting to decrease the diagnostic time of HIV-associated tuberculosis. However, data regarding its diagnostic accuracy in everyday acute care settings are lacking, as most studies are laboratory-based.[7] Ultrasound has emerged as a potential diagnostic tool for extrapulmonary tuberculosis in HIV-positive patients,[8] and individual features have been grouped into a point-of-care protocol, the Focused Abdominal Sonography for HIV/TB (FASH) protocol.[9] Although ultrasound was quickly incorporated into clinical practice, robust evidence for its use has been lacking. Furthermore, it is unclear which specific ultrasound features are adequate predictors of tuberculosis and whether single or grouped (≥1) individual features should be used.

The effective introduction of point-of-care ultrasound (PoCUS) into Cape Town emergency centres, together with the high prevalence of HIV-associated tuberculosis, provided an ideal setting for the conduct of a diagnostic study of HIV-associated tuberculosis that included PoCUS and a point-of-care urine LF-LAM assay. The overall aim of the study was to develop an evidence-based algorithm for the rapid diagnosis of tuberculosis in HIV-positive patients presenting to an emergency centre. In order to reach the aim, we first determined the diagnostic accuracy of urinary LF-LAM in HIV-positive patients presenting to the emergency centre and evaluated the interobserver agreement between emergency centre physicians and laboratory technologists. Secondly, we determined the diagnostic performance of PoCUS for HIV-associated tuberculosis using a robust (microbiological) reference standard. This included determining the diagnostic accuracy of individual PoCUS features, performing an external validation of the FASH protocol, and determining independent PoCUS predictors of HIV-associated tuberculosis that can be used by emergency centre practitioners. Lastly, we incorporated clinical

features, urine LF-LAM, individual POCUS features, and chest x-ray into near-patient and point-of-care clinical decision trees to rapidly diagnose HIV-associated tuberculosis in the emergency centre.

This thesis reports on the development of near-patient and point-of-care clinical decision trees to rapidly diagnose HIV-associated tuberculosis in the emergency centre. In Chapter 3, existing evidence for the use of ultrasound to diagnose extrapulmonary tuberculosis involving the abdomen was synthesised. Chapter 4 reports the findings on the diagnostic accuracy of urine LF-LAM done by emergency physicians in a real world setting. Chapter 5 reports the diagnostic performance of individual PoCUS features, the FASH protocols, and independent PoCUS predictors. Chapter 6 reports the development of a multi-parameter clinical decision tree (incorporating clinical information, individual PoCUS features, and point-of-care performed urinary LF-LAM) for the diagnosis of HIV-associated tuberculosis.

The systematic review reported in **Chapter 3** estimated the diagnostic accuracy of abdominal ultrasound across studies for detecting abdominal tuberculosis or disseminated tuberculosis with abdominal involvement in HIV-positive individuals. The five studies that compared abdominal ultrasound with the higher-quality reference standard (bacteriological confirmation of *Mycobacterium tuberculosis* from any clinical specimen) had a pooled sensitivity of 63% and a pooled specificity of 68%. Four studies used a lower-quality reference standard (clinical diagnosis of tuberculosis without microbiological confirmation), with a pooled sensitivity of 68% and pooled specificity of 73%. These estimates are based on data that is limited, varied, and of low-certainty. The low sensitivity of abdominal ultrasound means clinicians should not use a negative test result to rule out tuberculosis, but rather consider the result in combination with other diagnostic strategies (e.g. clinical features, chest X-ray, LF-LAM, and Xpert MTB/RIF).

The development of a low-cost, rapid, lateral flow assay has made urine LAM a useful point-of-care test.[10] However, studies to date have all been laboratory-based and none were performed in the emergency care setting. In **Chapter 4**, we described the performance of urine LF-LAM in HIV-positive adults presenting to the emergency centre and evaluated the interobserver agreement between emergency centre physicians and laboratory technologists. The study included 411 participants with similar sensitivity ($p = 1.0$) and specificity ($p = 0.23$) between point-of-care (sensitivity 42%, specificity 91%) and laboratory-performed (sensitivity 42%, specificity 88%) urine LF-LAM. The modest sensitivity is similar to that described previously,[7] and indicates that it should not be used as a rule-out test for HIV-associated tuberculosis. The similar diagnostic accuracy of urine LF-LAM between clinicians and laboratory technicians, together with the moderate agreement [$\kappa = 0.62$], indicates that urinary LF-LAM can be reliably performed at the point-of-care.

The use of ultrasound to diagnose extrapulmonary tuberculosis has been included in the WHO diagnostic algorithm for seriously ill HIV-positive patients with a positive tuberculosis symptom screen.[11] Heller et al developed the FASH protocols that are easily learned and quick to perform PoCUS protocols.[9,12] The FASH-protocols were developed based on clinical intuition and their diagnostic accuracy was not reported.[9,12] As described in Chapter 3, the use of ultrasound is based on low quality of evidence, and no large prospective studies have been done to evaluate the diagnostic accuracy of PoCUS to diagnose HIV-associated tuberculosis. The study reported in **Chapter 5**, described the diagnostic accuracy of individual PoCUS features and the FASH protocols in relation to a reference standard of microbiologically-confirmed HIV-associated tuberculosis. We subsequently identified independent PoCUS predictors with logistic regression and compared the c-statistic between the derived logistic model and the FASH protocol. The prevalence of tuberculosis was 42% in the 414 participants. The performance of individual PoCUS features (at least one feature present) and a positive FASH-protocol were similar (sensitivity 73% versus 71%; specificity 54% versus 57%). The presence of two or more independent PoCUS predictors (intra-abdominal lymphadenopathy, ascites, pericardial effusion) had 91% specificity with a c-statistic of 0.680 (c-statistic of FASH-protocol 0.630). The moderate discrimination suggested that further refinement and possible inclusion of other clinical features should be investigated.

Urinary LF-LAM is currently the only true point-of-care test for HIV-associated tuberculosis, with other tests (e.g. smear microscopy, Xpert MTB/RIF, Xpert MTB/RIF Ultra, GeneXpert OMNI, and portable digital chest X-ray) being near point-of-care tests.[13] As eluded to earlier, the use of point-of-care diagnostic tests would benefit patients presenting to the emergency centre and the need for an evidence based clinical algorithm which incorporates clinical information, individual PoCUS features, and urine LF-LAM is needed for diagnosing tuberculosis in HIV-positive patients. **Chapter 6** describes the development of multi-parameter clinical decision trees (point-of-care and near-patient) to rapidly diagnose tuberculosis in HIV-positive patients presenting to the emergency centre. We used classification and regression tree (CART) analysis to develop the decision tree models from the best performing clinical and biological variables in the logistic regression models that were independently associated with prevalent microbiologically confirmed tuberculosis. The presence of $\geq$2 WHO screening symptoms, urinary LF-LAM, current antiretroviral therapy use, independent PoCUS features, and chest X-ray were the selected variables for inclusion in the final model. Urinary LF-LAM was positioned after WHO symptoms as the next screening test, playing a pivotal role in classifying almost 25% of the study sample (75% of which were true positives) regardless of their CD4 cell count. The performance of PoCUS when chest x-ray facilities are available is limited, and supports the current WHO guidelines that ultrasound is an additional diagnostic tool and should not replace chest x-ray as

the initial imaging step to diagnose tuberculosis in HIV-positive patients.[11] On the other hand, PoCUS had a 100% true positive rate for diagnosing tuberculosis in settings where chest X-ray is not readily available, suggesting that it can be used as a rule-in test for HIV-associated tuberculosis; however, 39 PoCUS examinations will need to be performed to diagnose one patient with HIV-associated tuberculosis in those with a negative urinary LF-LAM result. The contribution of PoCUS was therefore limited and its role in diagnosing HIV-associated tuberculosis needs be re-evaluated.

The diagnostic specificity was only moderate. Abdominal ultrasound had a pooled specificity of 68% and point-of-care urinary LAM 90.5%. The presence of any 2 independent PoCUS predictors had 91% specificity and the prediction tree, which only included true point-of-care tests, had a 71% true negative rate. The specificity of a diagnostic test should be high to avoid the need for further confirmatory tests. WHO recommends a specificity of 98% when compared against a microbiological reference standard, which is similar to Xpert MTB/RIF to diagnose tuberculosis.

Our study has limitations affecting the generalisability of the results. The study was conducted in a single emergency centre in a high TB/HIV-prevalence setting. Urine LF-LAM and PoCUS might not be applied to all participants in real-life scenarios as patients with a clear non-tuberculosis diagnosis after the clinical examination (e.g., fever from upper respiratory tract infection) will be managed accordingly. PoCUS examinations were performed by a single, experienced operator, and a second reviewer and or sonographer would have enhanced the generalizability of the features. Similarly, chest x-rays were interpreted by a single experienced radiologist.

The generalizability of the decision tree needs to be evaluated in a new population by external validation before implemention in a clinical setting.[14] The performance of the decision tree on a new data set should asses both calibration (comparing predicted and observed outcomes) and discrimination (the ability to differentiate between patients with or without microbiologically confirmed tuberculosis). The decision tree is also at risk of overfitting. A decision tree is overfitted if it gives highly accurate output on training data, but low accurate output on test data. A comparison between the accuracy of the decision tree on a data test set and the training data set is lacking. Lastly, the study did not evaluate the effect on clinical or patient-centred outcomes, and is therefore unable to asses if the point-of-care diagnostic tests used actually impact the initiation of anti-tuberculosis treatment or mortality.

Our study did not analyse the detection of drug-resistant tuberculosis. South Africa has a high incidence rate of rifampicin mono-resistant and multidrug-resistant tuberculosis (19 per 100 000 population), with 3.4% of new tuberculosis cases being classified as rifampicin-resistant.[1] The decision

tree and its individual components (urine LAM, point-of-care ultrasound, chest X-ray) lack the ability to diagnose drug resistance and molecular or culture-based tests are required for this.

The main strength of our study is the robust microbiologic reference standard composed of tuberculosis culture and Xpert MTB/RIF performed on multiple samples from different anatomic sites. However, it is still possible that some tuberculosis cases might have been missed by the reference standard. It was also a pragmatic study as the study was performed under routine conditions experienced in the emergency centre. Lastly, robust analytic strategies were used to identify PoCUS predictors and to develop and validate the diagnostic decision tree.

Impact of the studies on the field

Urinary LF-LAM has been extensively studied. Our study reported in Chapter 4 indicated that there is no diagnostic accuracy advantage in laboratory-performed versus point-of-care-performed urine LAM tests in emergency care centres in high burden settings. This real-world evaluation of the assay provides reassurance that it can be reliably used in the busy emergency centre. Anti-tuberculosis treatment can thus be appropriately initiated within a short period of presenting to the health care system in patients with a positive assay. The initiation of treatment while in the emergency centre has the potential to further reduce the mortality of HIV-associated tuberculosis already associated with the use of urinary LAM assays.[15,16] Additional benefits of an early diagnosis would include the decreased need for further (unnecessary) testing, while infectious patients can also be appropriately identified to decrease transmission. A negative urine LF-LAM should not be used to rule tuberculosis out. Further appropriate testing is thus needed in a filtered (LAM-negative) group ensuring that limited resources are not overstrained. It can also serve as a reminder for physicians to consider a broader differential diagnosis.

The study reported in Chapter 6, reiterate the pivotal role of urinary LF-LAM. It is useful in HIV-positive patients regardless of CD4-count, indicating that the expanded use of urine LAM (beyond the seriously ill or severely immunocompromised patient)[17] in hospital settings is justifiable. The results of our study together with the recent development of more sensitive urine LAM assay,[18] could lead to guidelines being amended, and might strengthen the case for national roll-outs of LF-LAM testing in high burden settings. This will positively impact patients in health care systems where CD4 counts are not routinely available.

Ultrasound has been widely used and advocated as a diagnostic option in HIV-associated tuberculosis, despite the lack of large prospective studies using a robust reference standard. The review described in Chapter 3 indicated that ultrasound should not be used as a standalone test, and that high quality

evidence is needed to define ultrasound's role in diagnosing HIV-associated tuberculosis. This paved the way for the study reported in Chapter 5, which was the largest study of ultrasound (either PoCUS or formal ultrasound examination done in radiology departments) for the diagnosis of tuberculosis in an HIV-positive population with a microbiological reference standard. Our study indicated that ultrasound for the diagnosis of HIV-associated tuberculosis can be used at the point of care. However, both individual PoCUS features and the FASH-protocol only had moderate sensitivity and care must be taken to not rule tuberculosis out after a negative PoCUS examination. The most significant independent predictors of tuberculosis were ascites, pericardial effusion and any size intra-abdominal lymphadenopathy, but only the presence of 2 or more of these independent PoCUS predictors had sufficient specificity to suggest that PoCUS can be used as a rule-in tuberculosis test in emergency centres in high-burden settings. The results reported in Chapter 5 thus concur with the review in Chapter 3, that PoCUS results should be interpreted in combination with other diagnostic strategies. However, the decision tree reported in Chapter 6 indicated the limited contribution of PoCUS when urinary LF-LAM and chest X-ray can be performed. The low positive yield and the high false negative rate suggest that PoCUS might be considered as an additional test in situations or settings where urinary LF-LAM and chest x-ray are not available.

Recommendations for further research

The development of LF-LAM strips for urine has enables the diagnosis of HIV-associated tuberculosis at the point of care. South Africa is one of the high TB/HIV burden countries that had implemented LF-LAM testing,[19] but the uptake of the test on ground level needs to be determined.[20] User perspectives on LF-LAM can identify barriers to implementation, for example, busy emergency centre physicians might still opt for laboratory-performed testing in order to avoid performing the 25 minute test. Not only will this decrease the amount of time spend per patient, but it will also lessen the cognitive load on physicians who now also need to remember that they have began running a test. This could affect how close to the bedside the test will be used.[21]

Pragmatic studies are also needed to further ascertain the uptake and evaluate the fidelity of use of urinary LF-LAM in real-life scenarios. The benefits of using a non-invasive specimen, like urine, to test for tuberculosis is well known, but obtaining a urine specimen can be challenging in the acute care setting. For example, bedridden patients would need to be catheterised, thus losing the non-invasive benefit and further increasing the workload of the acute care team. The fast turnaround time of LF-LAM is another benefit that might get lost in everyday practice. Once the decision is made to initiate anti-tuberculosis treatment, it can be commenced within a few hours if the drugs are available, yet, it often only occurs the next day despite the test being performed at the point of care.[21] Lastly, although

urinary LF-LAM is a straightforward and easy-to-use test, the visibility of the graded bands can be challenging, especially in reading faint results (e.g. grade 1). Anecdotally, many junior physicians are instructed to perform a LF-LAM test without adequate training on how to interpret the graded bands. Most worrisome is that many don't even know that a reference card is needed and interpret any faint line as a positive result. Operational research should address all these issues.

The crucial role that urine LF-LAM played in the study reported in Chapter 6, together with the recent development of more sensitive urine LAM assay,[18,22] suggests the potential expanded use of urine LAM. One can argue that every HIV-positive patient (regardless of CD4 cell count and clinical condition) who present in a hospital setting with WHO tuberculosis symptoms should get a urinary LF-LAM test.

The role of ultrasound (PoCUS and examinations done in radiology suit) in diagnosing HIV-associated tuberculosis are yet to be precisely defined. A randomized controlled trial is planned that aims to determine whether ultrasound, added to chest X-ray and microbiological tests, has an impact on the proportion of correctly managed patients with suspected extrapulmonary tuberculosis.[23] The study will also assess the impact on morbidity and mortality. More high quality evidence is needed and future studies should be large prospective multi-centre studies that use a robust reference standard to ensure that tuberculosis is correctly diagnosed. Studies should be well-designed to avoid incorporation bias and to ensure that a representative sample of participants are recruited. Studies performed in settings with different prevalences of tuberculosis and alternative diagnoses will also increase generalisability.

In our study reported in Chapter 5, the independent PoCUS predictors of tuberculosis after multivariable logistic regression were ascites, pericardial effusion and any size intra-abdominal lymphadenopathy. These are different to what other studies have identified.[24,25] Various thresholds are currently used to define a positive PoCUS feature, preventing meta-analyses being performed. For example, the threshold to determine the presence of intra-abdominal lymphadenopathy include any size (our study), greater than 10mm,[26] equal to or greater than 10mm,[24] equal to or greater than 12mm,[25] and greater than 15mm.[27,28] In our study, the presence of two or more of the independent PoCUS predictors suggests that PoCUS can be used as a rule-in tuberculosis test in emergency centres in high burden settings. Further research needs to be done to externally validate the findings, particularly pragmatic studies to assess the performance and uptake by different physicians.

The development of the point-of-care and near-patient decision tree needs further refinement and external validation. This would also allow the determination of the diagnostic accuracy of various alternative models. Its impact on patient-centred outcomes like mortality need to be determined, as well as a cost-effectiveness analyses undertaken. Only through ongoing innovation and intensified

research can we ensure a world free of tuberculosis and optimum mitigation of its consequences in the medium term.

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