The association of chest radiographic features with 12-week mortality in HIV-positive patients diagnosed with tuberculosis in hospital

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ABSTRACT:

The association of chest radiographic features with 12-week mortality in HIV-positive patients diagnosed with tuberculosis in hospital

Background: HIV-associated tuberculosis has a high mortality. Chest x-rays are an adjunct diagnostic tool for tuberculosis but has high inter-reader variability, which may be reduced with chest x-ray scoring systems. We analysed and scored chest x-rays of hospitalised patients with HIV-associated tuberculosis and assessed the relationship of these chest x-ray scores with 12-week mortality and biomarkers of tuberculosis dissemination.

Methods: In this cohort study, the chest x-rays of adult patients, admitted with a new diagnosis of microbiologically confirmed HIV-associated tuberculosis were scored using the Timika scoring system. We excluded patients without a valid test result for the 3 biomarkers of tuberculosis dissemination (urine lipoarabinomannan, TB blood culture and urine Xpert); valid chest x-ray; or who were lost to follow up.

Results: Amongst 364 included participants, 73 (20%) died and 291 (80%) survived. Median age was 36 years and median CD4 count 57cells/mm^3. 25% of participants had normal chest x-rays. No association was found between chest x-ray score and dissemination score. Higher chest x-ray score was associated with higher hazards of death using a multivariate analysis: every 10-point increase in chest x-ray score resulted in 9% increased hazards of death.

Conclusion: In this cohort, a higher Timika chest x-ray score was associated with higher hazards of death at 12-weeks.

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PART A: PROTOCOL

Research Topic: The association of chest radiographic features with 12-week mortality in HIV-positive patients diagnosed with tuberculosis in hospital.

Main hypothesis: Patients hospitalised with HIV-associated tuberculosis without features of pulmonary tuberculosis on chest radiograph have higher mortality at 12 weeks.

Background and Rationale

Epidemiology of Tuberculosis and HIV-associated tuberculosis

The World Health Organisation (WHO) estimated that 1.7 billion people are infected with *Mycobacterium tuberculosis* (MTB) and an estimated 10 million people (range, 8.9 -11.1 million) developed tuberculosis (TB) disease in 2019, globally. An estimated 1.4 million people died of tuberculosis in 2019.[(1)]

Even though 85% of people who develop tuberculosis can be successfully treated, tuberculosis remains one of the leading causes of death. [(1)] Risk factors for tuberculosis include HIV infection, diabetes, poor nutrition, poverty, and smoking.[(1)] In 2019, an estimated 8.2% (range 7.0 – 9.5%) of new tuberculosis cases were in HIV positive people with 18 times (range 15 - 21) greater risk of developing tuberculosis compared to the global population.[(1)] HIV positive individuals not on antiretroviral therapy (ART) with tuberculosis infection have a 3 -16 % annual risk of developing tuberculosis disease, compared to HIV-uninfected individuals with tuberculosis infection who have a lifetime risk between 5 and 10%.[(2)] A decline in CD4 count results in an increased risk of developing tuberculosis disease and patients with advanced HIV are at high risk for developing extra-pulmonary and disseminated TB.[(3)] Gupta-Wright and colleagues (2020) reported a 31% 8-week

mortality rate in hospitalized patients diagnosed with HIV-associated TB, with even higher mortality in patients with disseminated TB.[4]

Pathophysiology of disseminated tuberculosis

Disseminated tuberculosis is defined as the spread of *Mycobacterium tuberculosis* via the blood stream or lymphatic system to two or more non-adjacent sites/organs in the body.[5] In studies done by our research group we have applied a pragmatic definition that any patients with a diagnostic test positive for tuberculosis in blood (TB blood culture or Xpert Ultra) or urine (lipoarabinomannan, Xpert or Xpert Ultra) has disseminated TB.

Disseminated TB is characterized by blood stream spread of *Mycobacterium tuberculosis* to multiple sites/organs in the body.[6] The organs/sites generally infected includes the spleen, liver, kidneys and/or lymph nodes.[7] A meta-analysis found an increased risk of mortality within the first 30 days, associated with blood stream tuberculosis infection compared to the absence of blood stream infection.[8] Schutz (2019) and colleagues found a 12-week mortality of 21.5% amongst 576 patients hospitalized with a new diagnosis of HIV-associated TB in a South-African setting.[9] Furthermore 37.1 % of these deaths occurred within 1 week of enrolment. Thus, early diagnosis and initiation of anti-tuberculous drugs is essential to reduce mortality in patients hospitalised with HIV-associated TB. The majority of patients who die in hospital with HIV-associated TB have evidence of dissemination at post-mortem.[10]

Patients with disseminated TB may present with non-specific clinical presentation[6]; often have negative sputum smears[7] and confirmatory diagnostic tests such as

MTB blood cultures have long turn-around times.[7] This results in underdiagnosis and underestimation of the prevalence of HIV-associated disseminated TB.[8]

MTB blood cultures are frequently positive in patients with disseminated TB.[7] A prospective cohort study done in Uganda found 23% of the 367 HIV positive patients hospitalised with severe sepsis, had a positive MTB blood culture.[11] Similarly, a South-African study found 31% of 132 hospitalised patients with newly diagnosed HIV-associated TB had a positive MTB blood culture.[7] The drawbacks of this test are lack of availability in resource limited settings and the long-turnaround time.[7]

Kerkhoff and colleagues (2017) found that urinary lipoarabinomannan (LAM) lateral flow assay test had a 65.9 % (95% CI 49.4 – 79.9) sensitivity in MTB blood culture positive patients, and sensitivity was increased to 87.8% (95% CI 73.8 -95.9) when used in combination with a Urine Xpert test. Urine specimens have also been found to be easier to obtain than sputum in many hospitalised patients. Alere LAM provides a rapid result and can lead to early initiation of tuberculosis treatment. LAM testing is a cost-effective diagnostic tool.[1]

The use of Xpert testing on non-concentrated and concentrated urine yielded sensitivities of 53.7 % (95 CI 37.4 – 69.3) and 78% (95% CI 62.4 – 89.4) respectively in patients with a positive MTB blood culture. However, Urine Xpert is not routinely used as a diagnostic test for disseminated TB as it is not endorsed by WHO. Kerkhoff (2017) and colleagues found that Urine LAM, Urine Xpert and MTB Blood cultures were predictive measures of tuberculosis disease severity and death; and may be correlated with burden of mycobacterial load in the body.[7]

Limitations in chest radiography in the diagnosis of disseminated TB

Chest radiography is used as an adjunct in screening algorithms in the diagnosis of pulmonary TB (PTB). A systematic review on the use of scoring systems on chest radiography in the diagnosis of pulmonary TB, majority of which included in-patient studies, reported high sensitivity but very low specificity.[12] Additionally, there can be significant variability in interpretation of a chest x-ray, between readers. The use of radiographic scoring systems can potentially reduce variability between readers.[12] However, there are few studies that have systematically evaluated chest radiographic scoring systems for the diagnosis of tuberculosis in HIV-positive inpatients, the majority of whom have disseminated TB.

HIV positive patients with low CD4 counts, have a poor immune response and are less likely to have typical features of tuberculosis like cavitation, on chest radiographs.[3] Miliary patterns on chest x-ray are a typical feature of disseminated TB but are absent in many patients with disseminated TB. One small cohort study with 52 participants found only 29% of patients with disseminated TB had a miliary pattern on chest radiographs[13], indicating low sensitivity. In addition, a miliary pattern may be found in other disease processes such as cryptococcosis, which reduces its specificity. [13] Normal chest-rays can be found in up to 15% of HIV-positive patients with pulmonary TB.[13] Crump and Reller also found that a miliary pattern on chest radiograph was more common in non-mycobacteraemic TB patients.[13]

Timika scoring system

The Timika scoring system is a validated, standardised tool created to score the radiological severity and predict response to treatment in adult patients with pulmonary TB. This scoring system was first developed in a TB clinic in Papua,

Indonesia where 115 adult patients (>15 years) with smear positive pulmonary TB had serial chest x-rays at diagnosis, month 2 and 6 of tuberculosis treatment. Four years later, the scoring system was validated by reviewing a further 139 comparable adult patients with tuberculosis.[14]

Although the initial scoring system was developed in a setting with relatively low HIV prevalence, the subsequent validation dataset had a 13% increase in the number of participants with HIV-TB co-infection.[14]

An inpatient study conducted in Bangalore, Karnataka, India, recruited 60 HIV negative adults with pulmonary TB and calculated the Timika chest radiograph score for each chest radiograph. 58.3 % of these patients had cavitary disease and 41.7 % non-cavitary disease. 38.3 % of these chest radiographs had a Timika score of ≥ 71 and 61.7% a Timika score of < 71. This study found a statistically significant difference in chest radiograph score at month 2, with patients with a month 2 positive smear having a higher chest radiograph score compared patients with a month 2 smear negative result. The mean Timika chest radiograph score was also statistically significant in terms of outcome; patient with a favourable outcome at month 30 had a lower baseline Timika score compared to patients with an unfavourable outcome at month 30. The study also found that a Timika score of >71 was associated with increased symptom duration. This score of >71 was also associated with microbiological and clinical predictors of disease severity.[15]

Given the limited use of this scoring system in HIV-positive population, this study will aim to determine whether there is an association between chest radiograph score and 12-week mortality in patients hospitalised with HIV-TB.

Aims of this study

The aim of this study is to analyse chest radiographic features in patients with HIV-associated TB, admitted to hospital at the time of their tuberculosis diagnosis, and to assess the relationship of these features to 12-week mortality and markers of TB dissemination. We will determine the proportion of patients with a normal chest x-ray in this study population and assess the association of a normal chest x-ray with 12-week mortality. We also aim to establish whether chest radiographic features and scores are associated with 12-week mortality and with biomarkers of tuberculosis dissemination.

Our hypothesis is that patients hospitalised with HIV-associated tuberculosis without features of pulmonary tuberculosis on chest radiograph will have higher mortality at 12 weeks. The rationale is that patients who die within 12 weeks are more acutely ill with more severe immunosuppression and will be less likely to have typical features of pulmonary infection on chest radiograph due to a more impaired ability to mount an immune response to pulmonary infection (and will thus have a lower Timika score). We further hypothesize that patients who test positive for biomarkers of tuberculosis dissemination will have fewer changes typical of tuberculosis on chest radiograph than those who test negative for dissemination biomarkers and that there would be a downward trend in chest x-ray abnormalities from patients who test negative for all three biomarkers, compared to those who test positive for one, two or all three biomarkers.

Objectives

1. To ascertain what proportion of inpatients diagnosed with HIV-associated TB have no features of pulmonary TB on chest radiograph and whether this is associated with mortality.

2. To determine whether there is an association between TB dissemination marker score and chest radiograph score.

3.To determine the association between chest radiograph score and 12-week mortality in patients hospitalised with HIV-associated TB.

Study Design

Patients were enrolled in a prospective cohort study, KDH TB study, investigating the causes of mortality in patients hospitalized with HIV-associated tuberculosis. The observational study enrolled a total of 682 patients of which 567 were diagnosed with tuberculosis. Microbiological evidence of tuberculosis was found in 487 (84.5%) patients.[9] Patients had chest x-rays performed on admission to hospital and biomarkers of disseminated tuberculosis were evaluated on samples collected at enrolment. The three biomarkers were: urine LAM on neat urine, Xpert on concentrated urine samples and mycobacterial blood culture. This study will analyse the chest radiograph findings of patients with tuberculosis, microbiologically confirmed (MTB confirmed on Xpert test or culture from any clinical sample including sputum), from this cohort.

Study Population

Hospitalised, HIV positive adults (≥18years) newly diagnosed with tuberculosis recruited during KDH TB study between 2014 and 2016. The aim of the parent study was to improve the understanding of the pathophysiology contributing to the high

mortality in this patient population despite the initiation of anti-tuberculous drugs and to inform the development of new evidence-based treatment strategies.

Inclusion Criteria

1. HIV positive

2. Adults ≥ 18 years

3. Hospitalized patients (inpatients) recruited as part of KDH TB study

4. Microbiologically proven tuberculosis (*Mycobacterium tuberculosis* confirmed on any clinical sample during index admission

5. Participants with valid results available for all the following three tests:

- Urine Xpert – on concentrated urine
- Mycobacterial blood culture
- Urinary lipoarabinomannan on neat urine

Exclusion Criteria

No chest radiograph performed or retrievable from time of admission (with +/- 5 days of admission date)

Definitions

Disseminated tuberculosis is defined as the spread of *Mycobacterium tuberculosis* via the blood stream or lymphatic system to two or more non-adjacent sites/organs in the body.[6]

TB dissemination score: A three-point dissemination score is to calculate the degree of tuberculosis dissemination. For each of the 3 tests (urine LAM, urine Xpert and TB

blood culture) that were positive, one point was awarded. The dissemination score ranges between 0 and 3.[9]

Chest radiographic features of pulmonary TB will be defined as any lung parenchymal changes in keeping with pulmonary TB including nodularity, consolidation and cavitation.

No chest radiograph features of pulmonary TB will be defined as the absence of any features suggestive of pulmonary TB (including areas of consolidation, nodularity, cavitations etc). Thoracic adenopathy and pleural effusion on their own will not be considered as features of pulmonary TB.

Outcomes: 12-week all-cause mortality. This outcome was selected, as it was the primary endpoint of the parent study.

Microbiologically confirmed tuberculosis: *Mycobacterium tuberculosis* confirmed on Xpert test or culture from any clinical sample including sputum.

Methods

Data obtained from the KDH TB study will be used for secondary data analysis. The KDH TB study recruited HIV positive adults with newly diagnosed HIV-associated tuberculosis in a secondary hospital in Cape Town, between 2014 and 2016.

Digital images of chest radiographs of each eligible participant will be reviewed independently by two medical doctors blinded to the outcome and clinical details of patients. The Timika scoring system will be used to score each chest radiograph. The chest radiograph score will be calculated as follows: two narrow, horizontal lines will be projected onto a digital CXR to divide the lung field into 6 zones. Each zone will be assessed for opacifications, distinguishing between homogenous- or nodular opacification. If homogenous opacification is present, the percentage diseased area

for the zone will be multiplied by 1.0. However, if mainly nodular opacification is present in a particular zone, the percentage of diseased area for that zone will be multiplied by 0.5. The product of the six zones is divided by 600 to calculate the total proportion of the lung affected (in percentage). The next step will be to assess for the presence of any cavitation and a value of 40 is added for the presence of at least 1 cavity. A cavity is defined as a hypodense area ≥ 1 centimetre in diameter within a nodule or area of consolidation. CXR score will then be calculated by adding the total proportion of lung affected (%) and 40 for the presence of cavitation, to give a maximum total out of 140. [15] An optimal cut off ≥71 indicates higher radiological severity of PTB**.**

Each reader will record their score on a CRF. If the first 2 reviewers Timika score differs by more than 10 points, a third reader will be asked to do an independent scoring of the same CXR. The 2 scores with the least difference in scoring will be used for data analysis. A modified Timika scoring system will be used. All data will be captured in a RedCAP database.

All statistical analyses will be done using R statistical software.

Retrospective power calculation:

We have a fixed sample size of n~370 patients who meet inclusion criteria and there was an overall 12-week mortality rate in the parent cohort of 21.5%.

There is no data on mortality in hospitalised HIV-TB patients with or without normal chest X-rays to guide our calculations. If we estimate finding ~30% mortality in patients with normal chest X-rays and ~ 15% mortality in patients with X-ray findings compatible with tuberculosis, we will have 90% power with two-sided alpha of 0.05 to detect this difference with this sample size.

A smaller difference of ~30% mortality vs 20% mortality will result in 49% power with two-sided alpha of 0.05 and with a more moderate difference of ~30% vs 17% mortality this sample size will have 75% power to detect the difference with a two-sided alpha of 0.05.

General Analytic plan

Descriptive statistics of the cohort will be analysed and presented with categorical variables presented as counts/percentages and continuous variables presented as median with interquartile ranges (IQR). Baseline characteristics, including chest radiograph score, will be stratified by survival or death at 12 weeks. Fisher's exact or Chi squared test will be used to compare categorical variables and the Wilcoxon rank sum test for continuous variables. A p-value of 0.05 will be considered significant.

Inter-reader agreement will be calculated with Pearson correlation coefficient and Kappa statistic.

To address aim 1: To ascertain what proportion of inpatients diagnosed with HIV-associated TB have no features of pulmonary TB on chest radiograph and whether this is associated with mortality:

Proportions of patients with features of pulmonary TB will be reported and proportions who died within 12 weeks will be compared using Chi squared or Fisher's exact tests. Kaplan Meier and Cox proportional regression analysis will be performed to assess 12-week survival. Chest radiograph score will be included as a categorical variable comparing chest radiograph features of pulmonary TB versus patients with no features of pulmonary TB. Hazard ratios with 95% confidence intervals will be estimated with Cox proportional regression analysis to assess

survival at 12-weeks and the model will be adjusted for variables including sex, age and HIV viral load.

To address aim 2: To determine whether there is an association between TB dissemination marker score and chest radiograph score.

The chest radiograph score values will be compared between patients who tested positive (for one or more) versus patients who tested negative for all of the three markers of dissemination (Urine LAM, Urine Xpert and TB blood culture), respectively, using the Wilcoxon rank sum test. A Jonckheere-Terpstra test will be performed to test for trend in chest radiograph scores across dissemination groups used as an ordered ordinal variable from 0 to 3.

To address aim 3: To determine the association between chest radiograph score and 12-week mortality in patients hospitalised with HIV-associated TB.

Kaplan Meier plots and Cox proportional regression analysis will be performed to assess 12-week survival. Chest radiograph score will be analysed as a categorical variable comparing score of <71 versus a chest radiograph score ≥ 71 and will also be assessed as a numerical continuous variable. Hazard ratios with 95% confidence intervals will be estimated with Cox proportional regression analysis to assess the survival at 12-weeks and the model will be adjusted for variables including sex, age and HIV viral load.

Ethical Approval

Ethical approval will be obtained from the University of Cape Town Human Research Ethics Committee prior to the start of the study.

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2. LITERATURE REVIEW

Introduction

Tuberculosis is a leading cause of death from an infectious agent globally[1] and the leading cause of hospital admissions in people living with HIV (PLHIV).[2] The probability of developing active disease is higher in PLHIV.[3] Patients hospitalised with HIV-associated tuberculosis have a high mortality risk. One post-mortem study found 94% of 240 adult patients who died during their admission in a medical ward, were HIV positive. Of whom 79 (37%) were smear positive and 110 (50%) culture positive for *Mycobacterium tuberculosis.* Of note, only 50% of tuberculosis cases were on antituberculosis treatment at the time of death.[4] Another post-mortem study reported 69% of inpatients with HIV died as a result of mycobacterial infections. Nine of the 39 inpatients had disseminated mycobacterial infections, which was not suspected as the cause of death ante mortem.[5] Additionally, HIV is a major risk factor for concurrent pulmonary tuberculosis(PTB) and extra-pulmonary tuberculosis(EPTB),[6] and concurrent PTB and EPTB results in a four-fold increase in mortality compared to PTB.[7] A meta-analysis of post-mortem studies conducted in resource limited settings found that 87.9% (95% CI = 82.2 – 93.7%) of tuberculosis in adults was disseminated TB and tuberculosis were undiagnosed in 45.8% (95% CI = 32.6 – 59.1%) of cases.[8] Among PLHIV, both TB- and antiretroviral therapy (ART) are necessary to lower mortality.[3, 8]

The World Health Organisation (WHO) currently recommends that all PLHIV be screened for tuberculosis at any contact with healthcare and that hospitalised PLHIV, have a sputum Xpert and urine lipoarabinomannan (LAM) test performed during their admission regardless of symptoms[3] because tuberculosis is a major contributor to

mortality in this population[8]. This recommendation is informed in part by the STAMP trial, which found that the addition of urine LAM and urine Xpert in the diagnostic algorithm led to an increase in tuberculosis diagnosis and treatment initiation and reduction in 56 day mortality in high-risk groups.[9] Early diagnosis of tuberculosis and prompt initiation of TB treatment is vital to reduce morbidity and mortality.[6, 10]

Pathophysiology

Tuberculosis is an infectious disease caused by the *Mycobacterium tuberculosis bacillus*[3]. Tuberculosis is spread when individuals with active tuberculosis disease cough or sneeze and infectious droplets are expelled into the air[3].When the bacteria are inhaled by other individuals, it may either stay dormant within the lungs (also known as latent TB) and reactivate at a later stage or it may directly cause active disease.

The lungs are the primary entry point of tuberculosis; however, the disease may spread via the blood to the lymph nodes, pleural space and other organs, which is known as EPTB. Disseminated TB refers to spread via the blood stream to multiple organs [11]. Disseminated TB is most commonly found in PLHIV with lower CD4 cell counts. One study showed more disseminated tuberculosis in participants with CD4 count of < 200 cell/mm3[10] and another study in patients with a CD4 count of < 50 cells/mm^3.[6] The natural progression of active tuberculosis disease is that without treatment up to 70% of individuals will die within 10 years.[3]

Clinical presentation of tuberculosis

Typical tuberculosis symptoms are cough, pleuritic chest pain, night sweats, prolonged fever reduced appetite and loss of weight.[6, 12] PLHIV are more likely to

have an atypical presentation.[8] Patients with disseminated TB may present with non-specific symptoms which may delay making a definitive diagnosis.[6]

TB screening and diagnostics

Sputum Microscopy

The combination of clinical symptoms and signs, sputum testing and chest radiographs are the most common methods of diagnosing tuberculosis. However, patients who are acutely ill or has disseminated disease are less likely to be able to provide a sputum sample for testing.[13] Sputum based microscopy is less sensitive in people with HIV-associated TB.[8]

Smear microscopy is still widely used as a diagnostic test for pulmonary TB.[1] Typically, 2 sputum samples are collected in patients with suspected TB. Sensitivity of smear microscopy is low in patients with HIV, with a sensitivity ranging between 22 and 43%.[14] Smear sensitivity may increase with induced sputum or bronchoscopy. However, these methods may not be available or feasible in resource limited settings. PLHIV frequently present with paucibacillary TB or are too sick to produce an adequate sputum sample.[15] This limits the use of sputum microscopy in hospitalised patients with suspected HIV-associated TB, who are acutely ill.

Chest radiographs

X-rays are electromagnetic beams that are passed through a patient and captured behind a patient on plain film or a digital detector.[16] In out-patient settings, chest x-rays can be used to triage patients with high risk of PTB. Chest x-rays can also be used as a diagnostic tool for PTB due to its high sensitivity.[16] A good knowledge of anatomy is needed to interpret an x-ray as it is a two-dimensional image of three-dimensional structures.

Chest x-rays as a diagnostic strategy for pulmonary TB, has a higher sensitivity when used in combination with clinical symptoms and signs. An additional benefit of chest x-ray is identifying other conditions beyond tuberculosis.[16] Chest x-ray findings may be affected by various factors such as immunosuppression and previous tuberculosis disease.[17] Radiological findings in keeping with active pulmonary disease are upper zone consolidation, cavities characterised with thick walls and bronchial dissemination nodules or miliary shadows.[11, 18] Patients with advanced HIV disease (AHD) (CD4 < 200 cells/mm) are less likely to have cavitation and more likely to have hilar or mediastinal lymphadenopathy[19] and extra-pulmonary features[11, 20, 21] as well as mid- and lower zone consolidation and a miliary pattern.[18] A study conducted in India found that radiological features in PLHIV are more likely to be atypical and involve the mid lower lung zones compared to HIV negative patients where the upper zones are more likely to be involved.[20]

A South-African prospective cohort study which included adult HIV positive patients admitted with ≥1 WHO danger signs found that the patients who had a chest x-ray categorised as “likely TB” was a good predictor of a confirmed TB diagnosis.[22] The chest radiographic features most commonly found associated with culture positive TB were diffuse micronodular infiltrates (odds ratio (OR) 6.45), enlarged hilar and mediastinal nodes (OR 2.34) and nodularity ≥ 3mm (OR 2.21).[22] In this study cavities in the lung were not a good predictor of culture positive TB. This contrasts with a Ugandan study, which included a majority HIV-negative people (72%), that found the commonest chest x-ray finding in drug-sensitive pulmonary TB was consolidation (74.8%), broncho-pneumonic opacifications (56.1%) and cavitation

(38.1 %). No difference in the proportion of patients with cavities was found between HIV negative- versus PLHIV, with median CD4 of 218 cells/mm^3 (range 2 – 896).[17] In PLHIV, cavities were found to be larger in patients with rifampicin resistant tuberculosis compared to drug-sensitive pulmonary TB (p = 0.004).[17] Yoo et al (2011) found that in a cohort of 334 patients hospitalised with HIV-associated TB, 16 % of patients had a normal chest x-ray.[23] Patients with normal chest-x-rays were more likely to be younger, have a lower CD4 count and be sputum smear negative, compared to patients with abnormal chest x-rays.[23] Chest radiographic features of tuberculosis also change depending on CD4 counts. Among 873 patients with HIV-associated PTB, patients with CD4 count < 50 copies/mL were more likely to have a normal chest x-ray compared to those with CD4 > 500copies/mL (21% vs 2%; p = 0.001).In patients with lower CD4 counts normal chest x-rays may lead to delayed diagnosis and delay in treatment initiation which affects patient outcomes.[23]

A survey conducted across 9 countries in sub-Saharan Africa reported the average availability of chest x-ray services were 26% (ranging between 0 and 94%), with radiological services more commonly available in urban secondary (74%) or tertiary (94%) health care facilities.[24] The lack of radiological services in resource limited areas impedes its utility as a screening or diagnostic tool for tuberculosis.[25] Computed Tomography (CT) of the chest is more sensitive than chest x-rays[11], but is costly and not widely accessible.

Urinary lipoarabinomannan (LAM)

Lipoarabinomannan (LAM) is a component of the outer cell wall of *Mycobacterium tuberculosis* (MTB) and may be excreted in urine.[26] The LAM assay is a point of care lateral flow strip that detects MTB in the urine of patients with active tuberculosis disease.[13] The Determine™ TB LAM Ag test (Alere LAM)is currently

the only LAM test approved by the WHO, although there are other test kits in production for example the FujiLAM.[27] The Alere LAM test is performed by applying 60microliters of urine onto the test strip. The test can be read between 25 mins and 35 mins after applying the urine and requires a reference card to interpret the test line result.[13]

Current recommendations by the WHO for the use of urinary LAM tests are:

> All HIV positive patients admitted to hospital with tuberculosis signs and symptoms or any patients who are seriously ill with advanced HIV, or a CD4 count < 200 cells/mm3 regardless of tuberculosis symptoms or signs[26]
>
> HIV positive patients presenting at outpatient facilities who have TB symptoms and signs, seriously ill or who have CD4 counts <100 cells/mm3 regardless of tuberculosis symptoms and signs[26]

Sensitivity of the urinary LAM is dependent on the level of immunosuppression. In patients with a CD4 <100 cells/mm LAM has a higher sensitivity.[13] A meta-analysis which included 5 studies of PLHIV with CD4 < 200 cells/mm^3, determined that the pooled sensitivity of LAM is 45% and pooled specificity 92%.[28] The use of urinary LAM led to increased diagnostic yield of tuberculosis amongst patients with AHD (CD4 <200) with symptoms and signs of tuberculosis.[13] Thus, in a subset of patients this is a valuable diagnostic tool. Performing LAM requires limited expertise, is quick and cost-effective. Unlike sputum, urine does not generate infectious particles.[29] Urine is mostly easily obtained and eliminates the need for sputum collection which is difficult in patients who are acutely ill[28, 29]. The use of LAM testing reduces the delay in tuberculosis treatment initiation[28].One study found that the use of LAM led to an increase in diagnostic yield of 19.9% if used in combination with clinical signs and sputum smears from 62.2% (95%CI: 54.1–69.8) to 82.1% (95%CI:

75.1–87.7). In the same study, combining LAM with sputum microscopy showed a higher diagnostic yield than each individual test, and this combination yielded 76.3% (95%CI: 68.8–82.7) of diagnoses in confirmed tuberculosis patients.[28]

Studies have found an increase in mortality in PLHIV with a positive urinary LAM, especially within the first 2 months (adjusted OR 2.7 (95% CI 1.5 to 4.9).[28] One randomised controlled trial (RCT) showed that the addition of urine lipoarabinomannan and urine Xpert in the diagnostic work-up for tuberculosis, reduced 56-day mortality (adjusted Risk Difference of –7.1%, 95% CI –13.7 to –0.4; p=0.036) in HIV positive inpatients with CD4 counts <100 cells/mm3.[9] One shortcoming of the urinary LAM test is that it cannot distinguish between Mycobacterium tuberculosis and nontuberculous mycobacteria.[30] The current urinary LAM test also requires a reader to decide whether a line is present or absent on the test strip, which may introduce inter-reader variability. However, this is mitigated by the use of a reference card with every use. [31]

Xpert and Xpert Ultra

MTB/Rif rapid molecular assay (Xpert) is a molecular test that uses 5 beacons to detects and amplifies *Mycobacterium tuberculosis* DNA at a specific sequence on the rpoB gene.[32] Xpert testing is able to detect MTB antigen as well as Rifampicin sensitivity. The Gene-Xpert diagnostic system was originally developed for anthrax detection by Cepheid (Sunnyvale, CA, USA).[32] Xpert Ultra, the newest version of this molecular test features additional probes that targets two multi-copy genes, *IS6110* and *IS1081* respectively. Xpert Ultra has bigger amplification targets and DNA reaction chamber compared to Xpert. Xpert Ultra has higher sensitivity and improved diagnostic accuracy for Rifampicin resistance detection compared to

Xpert.[33, 34] The WHO currently recommends Xpert or Xpert Ultra testing as the gold standard diagnostic test in sputum samples of symptomatic patients[35] .The benefits of Xpert testing system is that it is automated and a rapid diagnostic test that provides results within 2 hours.[29, 32] Certain machines have the capacity to run multiple cartridges concurrently.[32] The assay includes a reagent that reduces the infectious risk for the technician by reducing the viability of MTB in sputum.[32] Xpert testing requires technical expertise and laboratory equipment in order to utilise this diagnostic tool.[28] Xpert MTB/Rif assay in sputum samples have a sensitivity of 90% and specificity of 99%.[36] Xpert MTB/Rif assay can also be used in non-respiratory clinical specimens such as urine[8], blood[37] , pleural fluid, cerebrospinal fluid.[38] However, the WHO does not endorse the use of Xpert in certain non-sputum specimens currently. Boloko and colleagues reported that blood Xpert Ultra resulted in 37% diagnostic yield in 447 patients with microbiological confirmed HIV-associated TB. An increase in diagnostic yield was shown with a lower CD4 cell count and low haemoglobin.[37]

One diagnostic study in ambulatory HIV-infected patients, which collected a small urine volume found that the sensitivity of urine Xpert MTB/Rif was higher in patients with lower CD4 counts, with sensitivity = 44.4 % in patients with CD4 counts < 50 cells/mm^3 , compared to 25.0 % in patients with CD4 counts between 50 and 100 cells/mm^3 and 2.7% in CD4 counts $\geq$ 100 cell/mm^3.[29] Furthermore, this study found that urine Xpert positive patients had a higher mortality compared to Xpert negative patients, 25% and 1.5 % respectively.[29] Urine Xpert assays detects MTB DNA which imply renal tract involvement and indicates dissemination.[29] Urine Xpert tests were more likely to be positive in patients with advanced HIV. Even though this study found urine Xpert sensitivity was only approximately two thirds of urinary LAM

sensitivity, it is thought that sensitivity may be higher if larger urine volumes are used and if tested in hospitalised patients who are more likely to have disseminated TB.[29] A South-African study found that in patients with paucity of sputum, the urine MTB/Rif assay sensitivity in unconcentrated urine was 40% (95% CI 22 – 61%). Combined with urinary LAM, sensitivity improved to 70% (95 CI 48 -85%). Furthermore, urine MTB/Rif specificity was 98% (95% CI 95 -100).[15] Kerkhoff and colleagues found that combining urine MTB/Rif Xpert and urinary LAM as diagnostic tests increased the diagnostic yield to 88% in patients who were mycobacterial blood culture positive.[39]

A meta-analysis on the diagnostic yield of Xpert in lymph node samples, pleural fluid and CSF, compared to culture and composite reference standard (CRS) found the following: lymph node tissue or aspirates pooled sensitivity of 83.1% (95% CI 71.4 – 90.7) compared to 81.2% (72.4 – 87.7%) with cultures as reference standard compared to CRS, respectively. In CSF, pooled sensitivity was 80.5% (95% CI 59.0–92.2%) compared to culture and 62.8% (95% CI 47.7–75.8%) against CRS. Pooled sensitivity in pleural fluid was 46.4% (95% CI 26.3–67.8%) compared to culture and 21.4% (95% CI 8.8–33.9%) compared to CRS.[38] Xpert pooled specificity was more than 98.7% across all sample sites compared to CRS.[38]

TB blood culture

Disseminated TB is often referred to as MTB bloodstream infection, which is diagnosed with a positive mycobacterial blood culture.[39] Kerkhoff and colleagues found that patients who had a positive mycobacterial blood culture, were more likely to have advanced HIV with lower CD4 counts (median CD4 42 cells/mm^3; IQR 29 - 127) and had poorer outcomes compared to patients with a negative mycobacterial blood culture.[39, 40] The same study found delayed time to positivity with a median

time of 26 days (IQR 23 -31 days); which could lead to a delay in both diagnosis and time to treatment initiation [39] and in turn reduces its diagnostic value.[40] HIV-positive patients with a positive TB blood culture had 2.2 times (95%CI 1.0 – 5.2) higher risk of death within 90 days compared to patients with a negative TB blood culture.[39] A meta-analysis of MTB bloodstream infection found the risk of death in patients with HIV-associated TB was 2.5 times higher in patients with MTB bloodstream infection compared to those without (adjusted hazard ratio 2·48, 95% CI 2·05–3·08).[40] Additionally, a delay in the initiation of TB treatment of more than 4 days resulted in an increased mortality (odds ratio 3·15, 95% CI 1·16–8·84) compared to those with shorter treatment delay.[40] The benefits of mycobacterial blood cultures are that it is less invasive and safe[41], and requires only 3-5 ml of blood. The use of mycobacterial blood cultures also allows for drug sensitivities to be tested.[41]

Chest X-ray scoring systems

Chest x-ray scoring systems aim to improve the diagnostic accuracy of chest x-rays for pulmonary diseases, like pulmonary TB (PTB). A systematic review of chest x-ray scoring systems for the diagnosis of PTB determined that for hospitalised patients with suspected tuberculosis, sensitivity was high, but specificity was low. For this study population, chest x-ray scoring systems had a high negative predictive value (range 93 -100%) but low positive predictive value (range 8 -61%). Thus, the ability to rule out tuberculosis was high but too rule in was low. All chest x-ray scoring systems identified in this systematic review incorporated both clinical and radiological criteria.[42]

Timika scoring system

The Timika X-ray scoring system was developed in Timika, Papua province, Indonesia. The Timika scoring system was developed as a grading tool, using a

numerical score, to predict severity and evaluate treatment response in patients with smear positive pulmonary TB.[43]

The initial derivation study was conducted between 2003 and 2004 and a validation study was conducted between 2008 and 2009. The two studies enrolled 115 participants and 139 participants, respectively. The studies enrolled participants, 15 years and older with pulmonary TB, confirmed by smear positive sputum. All participants had postero-anterior chest x-rays performed at time of diagnosis, 2 months- and 6 months after TB treatment initiation.[43]

The derivation study showed that baseline cavitation (OR 3.26 95% CI 1.11 – 9.56) and total percentage of lung field affected (OR 1.9 95% CI 1.3 – 2.7) significantly predicted 2-month smear status.[43] Baseline cavitation was also significantly associated with higher smear microscopy grade ($p = 0.007$).[43] The derivation study determined that a scoring model including cavitation and percentage lung affected were significantly better in 2-month smear status prediction compared to using the variables alone. A chest x-ray score was generated using regression coefficients and weighted scoring using the following calculation: “Timika X-ray score” = total percentage affected lung + 40 in the presence of a cavity.[43] The derivation study determined that inter-reader agreement was relatively low, which has been documented in other studies when scoring is done by both radiologists and clinicians[43]. However, the validation study found that the scoring is still valid when read by a TB clinician which supports its use in a practical real-world setting. The validation study confirmed that the presence of cavitation in the lung fields are predictors of severity of disease characterised by worse lung function. This dataset includes a subset of HIV positive patients (13% of participants) and concluded that the scoring method was still valid for this population group[43].

Kriel and colleagues conducted a study to validate the Timika scoring system in patients with pulmonary TB, aiming to refine the methods of applying the scoring system and additionally comparing cavity detection on chest x-ray with chest tomography[44]. The authors included 449 participants older than 18 years with confirmed smear- or culture positive pulmonary TB, without HIV infection and with available outcome data.

They found that the inter-reader reliability score was high (r = 0.86), however with only modest agreement on cavitations. This study concluded that overall, the score's predictive ability to predict outcomes is poor, but could contribute to assessing radiological severity of disease.[44]

The limitation of the Timika scoring system is that it was developed in a very specific subset of tuberculosis patients.[43] It has only been applied in a small subset of PLHIV which affects generalisability.[44] It requires further validation studies in this study population, who may have very subtle chest x-ray changes or features like pleural effusions or hilar lymphadenopathy.[43]

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Part B: Journal Manuscript

Title

Association between chest radiographic features and 12-week mortality in HIV-positive patients diagnosed with tuberculosis in hospital

Target Journal: The International Journal of Tuberculosis and Lung Disease

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ABSTRACT [202 of 200 words]

The association of chest radiographic features with 12-week mortality in HIV-positive patients diagnosed with tuberculosis in hospital

Background: HIV-associated tuberculosis has a high mortality. Chest x-rays are an adjunct diagnostic tool for tuberculosis but has high inter-reader variability, which may be reduced with chest x-ray scoring systems. We analysed and scored chest x-rays of hospitalised patients with HIV-associated tuberculosis and assessed the relationship of these chest x-ray scores with 12-week mortality and biomarkers of tuberculosis dissemination.

Methods: In this cohort study, the chest x-rays of adult patients, admitted with a new diagnosis of microbiologically confirmed HIV-associated tuberculosis were scored using the Timika scoring system. We excluded patients without a valid test result for the 3 biomarkers of tuberculosis dissemination (urine lipoarabinomannan, TB blood culture and urine Xpert); valid chest x-ray; or who were lost to follow up.

Results: Amongst 364 included participants, 73 (20%) died and 291 (80%) survived. Median age was 36 years and median CD4 count 57cells/mm^3. 25% of participants had normal chest x-rays. No association was found between chest x-ray score and dissemination score. Higher chest x-ray score was associated with higher hazards of death using a multivariate analysis: every 10-point increase in chest x-ray score resulted in 9% increase in hazards of death.

Conclusion: In this cohort, a higher Timika chest x-ray score was associated with higher hazards of death at 12-weeks.

BACKGROUND [304 words]

Tuberculosis is the leading cause of hospital admissions in people living with HIV (PLHIV) and has a high inpatient mortality of 20%.[1] Chest radiography is used as an adjunct to bacteriological testing in the diagnosis of pulmonary tuberculosis[2] It is readily available and widely utilised in hospital settings.[2] The strongest predictor of tuberculosis in patients hospitalised with one or more WHO danger sign, was having a chest x-ray categorised as “Likely TB”.[3] However, there can be significant variability in interpretation of a chest x-ray between readers. The use of radiographic scoring systems can potentially reduce this variability.[4] A systematic review of the use of scoring systems for chest radiography interpretation in the diagnosis of pulmonary tuberculosis, in which the majority studies were in-patient studies, reported chest x-ray scoring systems have a high sensitivity but very low specificity.[4] The Timika chest x-ray score was developed for patients with smear positive pulmonary tuberculosis, as a predictor of 8-week smear status and treatment outcome.[5] However, only a small subset of their study population was PLHIV. Additionally, very few studies have systematically evaluated chest radiographic scoring systems for the diagnosis of tuberculosis in PLHIV.[4] We analysed and scored chest x-rays of patients admitted to hospital with a new diagnosis of HIV-associated tuberculosis and assessed the relationship of the chest x-ray scores to 12-week mortality and markers of tuberculosis dissemination. We hypothesised that patients hospitalised with HIV-associated tuberculosis without typical features of pulmonary tuberculosis on chest radiography would have higher mortality at 12 weeks. Our rationale for this hypothesis was that patients who die within 12 weeks would be more acutely ill with more severe immunosuppression and

would therefore be less likely to have typical features of pulmonary infection on chest radiograph due to their impaired ability to mount an inflammatory response (and thus have a lower Timika score).

STUDY POPULATION AND METHODS [909 words]

Study design and population

This study is a secondary analysis of clinical-, biomarker- and radiographic data from an observational cohort study conducted at Khayelitsha District Hospital between January 2014 and October 2016.[6] The parent study was previously reported and recruited HIV positive adults (≥18years), with CD4 count <350µL/mL, hospitalised with a suspected new diagnosis of tuberculosis.[6] Patients had chest x-rays performed on hospital admission and clinical samples were collected to measure biomarkers of disseminated tuberculosis upon enrolment. Three biomarkers of disseminated tuberculosis were measured: urine LAM on neat urine, Xpert MTB/RIF (Cepheid) on concentrated urine and mycobacterial blood culture using Myco/F Lytic bottles (Becton Dickinson Biosciences). For this study, we selected all patients from the cohort who had microbiological confirmation of tuberculosis diagnosis, valid test results for all three of the biomarkers of dissemination and a chest x-ray performed during the admission. This study was approved by the University of Cape Town Human Research Ethics Committee (HREC 077/2023).

Study procedures

Digital images of chest x-rays of each eligible participant were independently reviewed by two medical doctors blinded to the outcome and clinical details of the participants. The Timika scoring system[5] was used to score each chest x-ray using a standardised electronic case report form on a REDCap database. The chest x-ray

zones were determined by superimposing two horizontal lines onto the digital chest x-ray to divide the lung field into 6 zones of similar sizes. Each zone was assessed, distinguishing between homogenous- or nodular opacifications. If a homogenous opacification was present, the percentage diseased area for the zone was multiplied by 1.0. For this study miliary patterns were included and scored as homogenous infiltrates. Nodular opacifications in a particular zone were multiplied by 0.5. If a zone contained both types of infiltrates, the predominant type of infiltrate was used to calculate the percentage lung field affected. The product of all six zones was divided by 600 to calculate the total proportion of the lung affected (as a percentage). However, if a particular zone's lung field was obscured in any way, for example by a pleural effusion, artefact or structural lung changes, that zone was not assessed. The denominator was then changed to exclude that zone. Furthermore, chest x-rays were assessed for the presence of any cavitation and a value of 40 added for the presence of at least 1 cavity. The chest x-ray score was then calculated by adding the total proportion of lung affected (%) and 40 for the presence of cavitation, to give a maximum total out of 140.The derivation study determined that a weighted chest x-ray score of 71 predicted smear outcome significantly better than simply using the affected area of lung fields.[5]

If the two readers' Timika score differed by more than 10 points, a third reader performed independent scoring of the same chest x-ray. The two scores with the least difference were used to calculate an average and this was used for data analysis. A third reader 's interpretation was used to reach consensus on interpretation of chest x-ray patterns in some cases where the first 2 readers disagreed.

Radiologic diagnostic definitions

Predefined definitions were used when analysing the chest x-rays. Chest radiographic features of pulmonary tuberculosis were defined as any lung parenchymal changes in keeping with pulmonary tuberculosis including nodularity, consolidation and cavitation. Thoracic adenopathy and pleural effusion on their own were not considered as features of pulmonary tuberculosis as these were regarded as extrapulmonary features of tuberculosis. A miliary pattern was defined as tiny, diffusely distributed pulmonary opacifications that were uniform in size and less than 3mm in diameter. A nodular pattern was defined as discrete pulmonary opacifications more than 3 mm in diameter. Homogenous infiltrates were defined as an increase in pulmonary parenchymal attenuation, obscuring airway walls and margins of vessels. Cavities were defined as gas-filled spaces, seen as lucency or areas with low attenuation within pulmonary consolidation, with a diameter of 1 centimetre or more. Pleural effusions were defined as pleural opacification with a possible meniscus.

Statistical analysis

Patient characteristics are presented as counts with percentages for categorical variables and median with interquartile ranges (IQR) for continuous variables. Patient characteristics, including chest radiograph score, were categorised by 12-week survival outcome. Data were compared using Fisher's exact or Chi squared test for categorical variables and the Wilcoxon rank sum test for continuous variables. A p-value of less than 0.05 was considered significant. Cohen's Kappa statistic was used to determine inter-reader agreement. The three markers of dissemination (urine LAM, urine Xpert and TB blood culture) were allocated 1 point for each positive test so that a patient could have a score ranging from 0 to 3 and

this was used in analyses as an ordinal variable. A Kruskal-Wallis test was performed to determine whether there was a difference between chest x-ray scores across dissemination score groups. A Jonckheere-Terpstra test was performed to test for trend in chest radiograph scores across dissemination groups.

Kaplan Meier and Cox proportional regression analyses were performed to assess association of chest radiograph score with 12-week survival. The hazard ratio with 95% confidence intervals was estimated with Cox proportional regression analysis and the model was adjusted for variables including sex, age and HIV viral load. The Cox analysis was performed with chest radiograph score as a categorical variable, comparing score of <71 versus a score ≥ 71, and repeated using the score as a numerical continuous variable. Data were analysed using R Statistical Software R (version 4.3.2).

RESULTS [666 words]

A total of 659 participants were enrolled in the parent study[6], 495 (65%) participants had microbiologically confirmed MTB. We excluded 104 participants without valid results for all three-dissemination marker tests: 12 (2.4%) without TB blood culture results, 15 (3%) without urine Xpert results and 77 (15.6%) without urine LAM results. An additional 20 (4%) participants without chest x-rays were excluded. Seven participants (1.4%) were lost to follow up and were excluded.

Figure 1: Study Flowchart

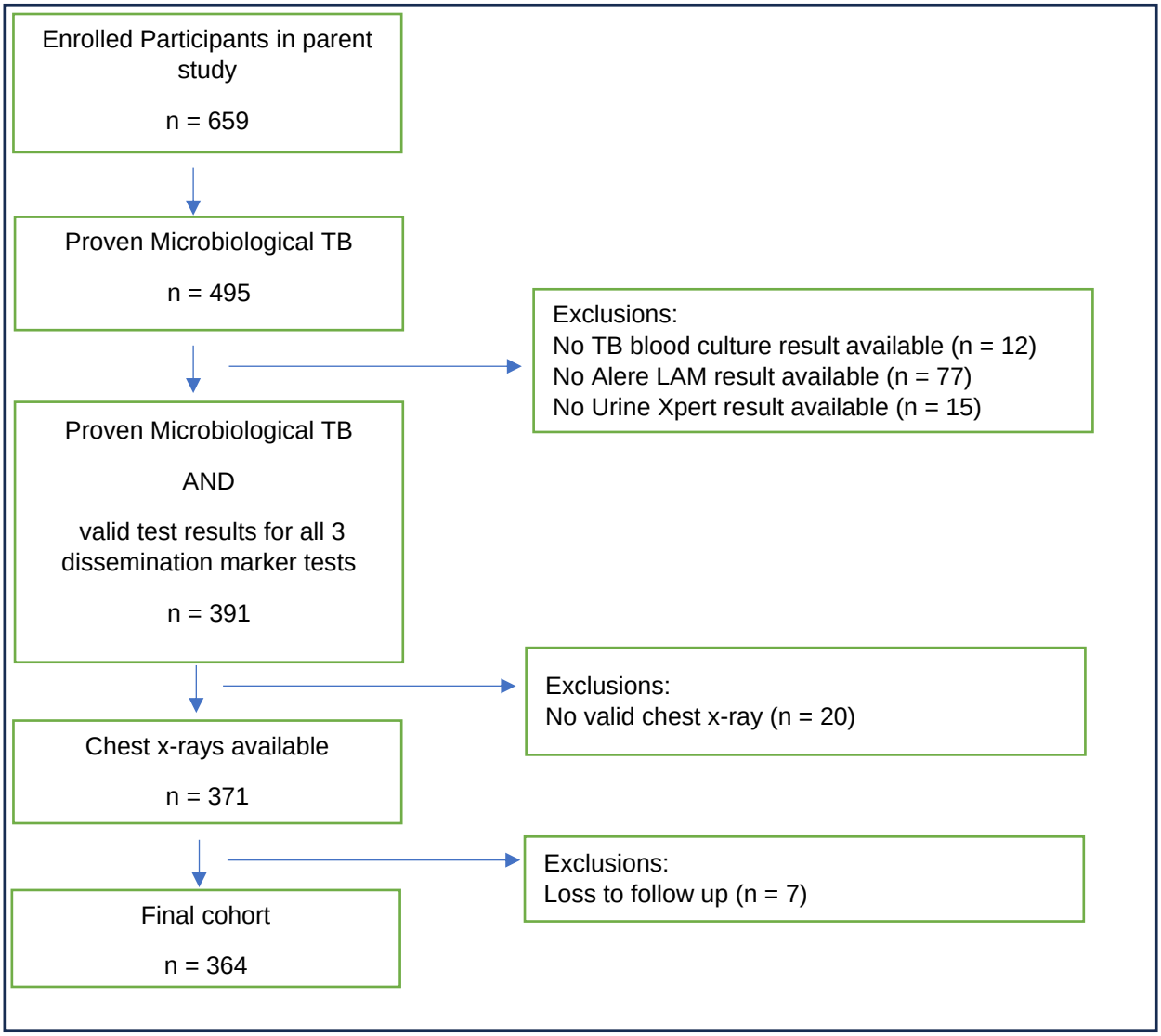


Table 1: Demographic and clinical characteristics, categorised by 12-week outcome

Characteristics	**All**[1] n = 364	**Died**[1] n = 73	**Survived**[1] n = 291	**p-value**[2]
Sex • Female • Male	 180 (49) 184 (51)	 39 (53) 34 (47)	 141 (48) 150 (52)	 0.4
Age, years	36 [31 – 43]	38 [32 – 47]	35 [30 – 42]	0.016
CD4, cells/mm^3	57 [21 -116]	37 [13 – 99]	62 [23 – 125]	0.009
HIV Viral load (Log 10)	5.25 [3.97 - 5.76]	5.38 [3.90 - 6.02]	5.21 [3.98 - 5.74]	0.3

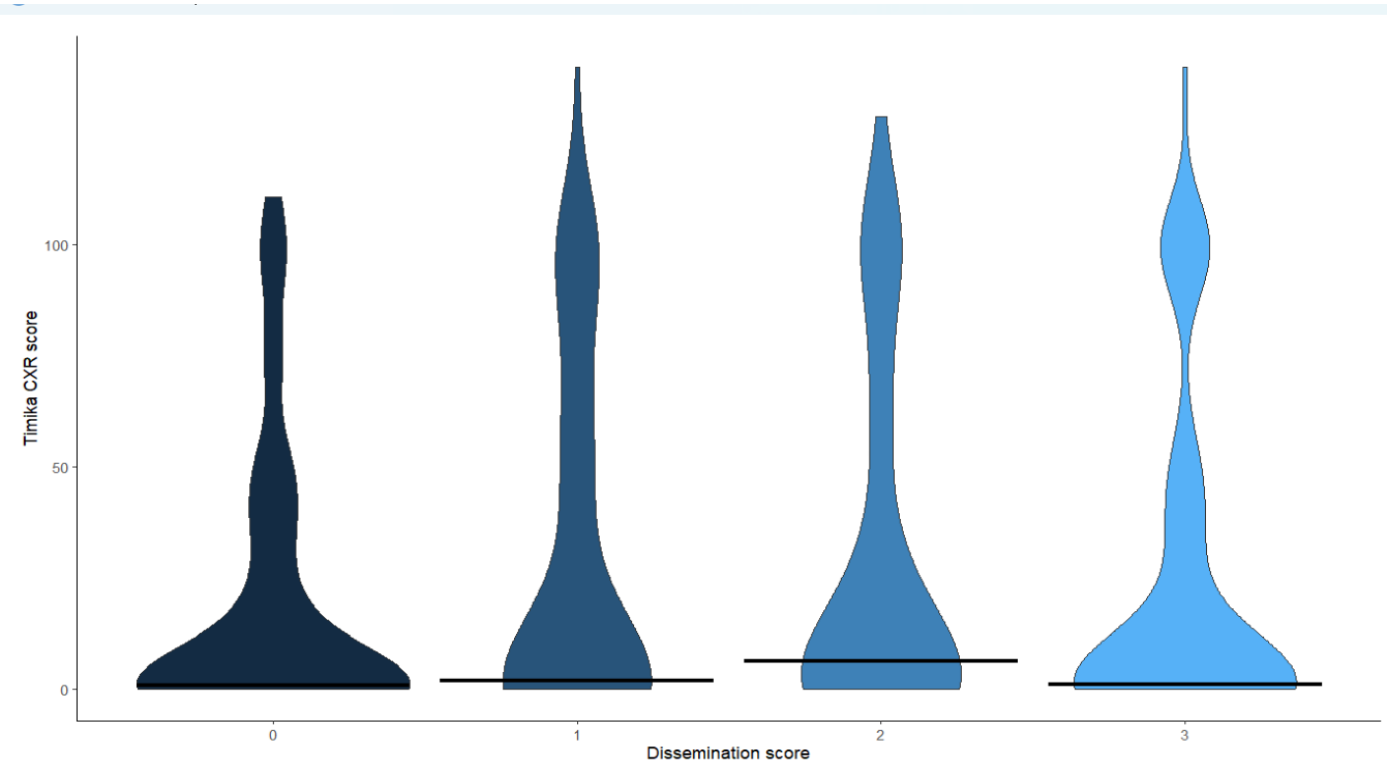
ART status • Interrupted • Naïve • On ART	 78 (22) 159 (44) 124 (34)	 18 (25) 28 (39) 25 (35)	 60 (21) 131 (45) 99 (34)	 0.6
Diabetes • Yes • No	 9 (2.5) 346 (97)	 6 (8.5) 65 (92)	 3 (1.1) 281 (99)	 0.003
Hypertension • Yes • No	 17 (4.8) 339 (95)	 10 (14) 62 (86)	 7 (2.5) 277 (98)	 <0.001
Epilepsy • Yes • No	 9 (2.5) 347 (97)	 1 (1.4) 71 (99)	 8 (2.8) 276 (97)	 0.7
Cough • Yes • No	 238 (68) 114 (32)	 46 (65) 25 (35)	 192 (68) 89 (32)	 0.6
Heart rate, beats per minute	107[98, 120]	112 [98 – 124]	106 [98 – 120]	0.2
Respiratory rate, breaths per minute	20 [20 - 25]	20 [20 – 26]	20 [20 – 24]	0.8
Peripheral lymphadenopathy • Yes • No	 63 (17) 300 (83)	 12 (16) 61 (84)	 51 (18) 239 (82)	 0.8
Splenomegaly • Yes • No	 20 (5.6) 338 (94)	 4 (5.7) 66 (94)	 16 (5.6) 272 (94)	 0.9
Hepatomegaly • Yes • No	 131 (37) 224 (63)	 28 (41) 41 (59)	 103 (36) 183 (64)	 0.5
Cardiac failure • Yes • No	 20 (5.5) 341 (94)	 10 (14) 62 (86)	 10 (3.5) 279 (97)	 0.002
TB blood culture • MTB • Negative	 164 (45) 200 (55)	 46 (63) 27 (37)	 118 (41) 173 (59)	 <0.001
Urine Xpert • MTB • Negative	 202(55) 164 (45)	 50 (69) 23 (32)	 152 (52) 139 (48)	 0.012
Alere LAM • Positive • Negative	 153 (42) 211 (58)	 35 (48) 38 (52)	 118 (41) 173 (59)	 0.3
Sputum smear • Positive • Negative	 128 (47) 142 (53)	 25 (58) 18 (42)	 103(45) 124 (55)	 0.12
Sputum Xpert • MTB • Neg • Inconclusive	 228 (78) 62 (21) 1 (0.3)	 41 (89) 5 (11) 0 (0.0)	 187 (76) 57 (23) 1 (0.4)	 0.2
Sputum TB culture • AFB • MTB • Negative • Contaminated	 1 (0.4) 231 (86) 25 (9.3) 11 (4.1)	 0 (0.0) 41 (100) 0 (0.0) 0 (0.0)	 1 (0.4) 190 (84) 25 (11) 11 (4.8)	 0.023
[3]Timika score[4]	2 (0 - 42)	16 (0 - 71)	1.3 (0 - 34)	0.016

[1]Median [IQR]; n (%)
[2]Wilcoxon rank sum test; Pearson's Chi squared test; Fisher's exact test
Abbreviations: ART - antiretroviral treatment; CD4 - cluster of differentiation 4; LAM - lipoarabinomannan; TB – tuberculosis; MTB – *Mycobacterium tuberculosis;* AFB – Acid fast bacilli.
[3] Timika chest x-ray score – Not normally distributed – shown as median and IQR in table. See below means (IQR):
All participants: 25.3 (0 – 42)
Participants who died: 36.6 (0 – 71)
Participants who survived: 22.5 (1 – 34)
[4]Timika chest x-ray score displayed as a boxplot (Figure 1 - Supplementary appendix)

The median age of all participants was 36 years, with 51% male. Seventy-three (20%) of participants died and 291(80%) survived. Median CD4 count was lower in the group who died compared to those who survived (37cells/mm^3 versus 62 cells/mm^3 respectively). Most participants (44%) were ART naïve. A significantly higher number of participants who died had a positive TB blood culture (63% vs 41%) and a positive urine Xpert (68% vs 52%). Low numbers of participants had comorbid diabetes or hypertension, but these co-morbidities were significantly more frequent in those patients who died.

Based on the predefined definition of pulmonary tuberculosis on chest x-ray, 116 (31.9%) chest x-rays were assessed as normal and 248 (68.1%) were assessed as abnormal. Of those with normal chest x-rays, none had pleural effusions and 25 (6.9%) had lymphadenopathy present. Thus, 25% of all participants had normal chest x-rays taking into account these additional features. There was no statistically significant difference in outcome between participants with a normal baseline chest x-ray compared to participants with abnormal chest x-rays (Table 2 available in Supplementary Appendix). A miliary pattern was identified in 9% of all chest x-rays and was not significantly different between those who died versus those who survived (Chi-square test – p-value = 0.105)

Figure 2: The association between TB dissemination biomarker score and chest x-ray score.



Violin plot of the distribution of Timika chest x-ray score stratified by dissemination scores between 0 and 3. Kruskal-Wallis test applied and there was no significant difference in x-ray score between groups (p-value = 0.42)

A three-point dissemination biomarker score was used to allocate a score to each participant. Distribution of dissemination score was as follows: a score of 0 in 104 (29%); a score of 1 in 83 (23%); a score of 2 in 95 (26%) and a score of 3 in 82 (23%). A third of participants who died had a dissemination score of 3 and a third of participants who survived had a dissemination score of 0 (Table 3 available in the Supplementary Appendix). There was no significant difference between the chest radiograph scores across dissemination score groups and no significant upward or

downward trend across groups (Kruskal Wallis test – p = 0.419 and Jonckheere-Tepstra test – p = 0.282).

Survival analyses

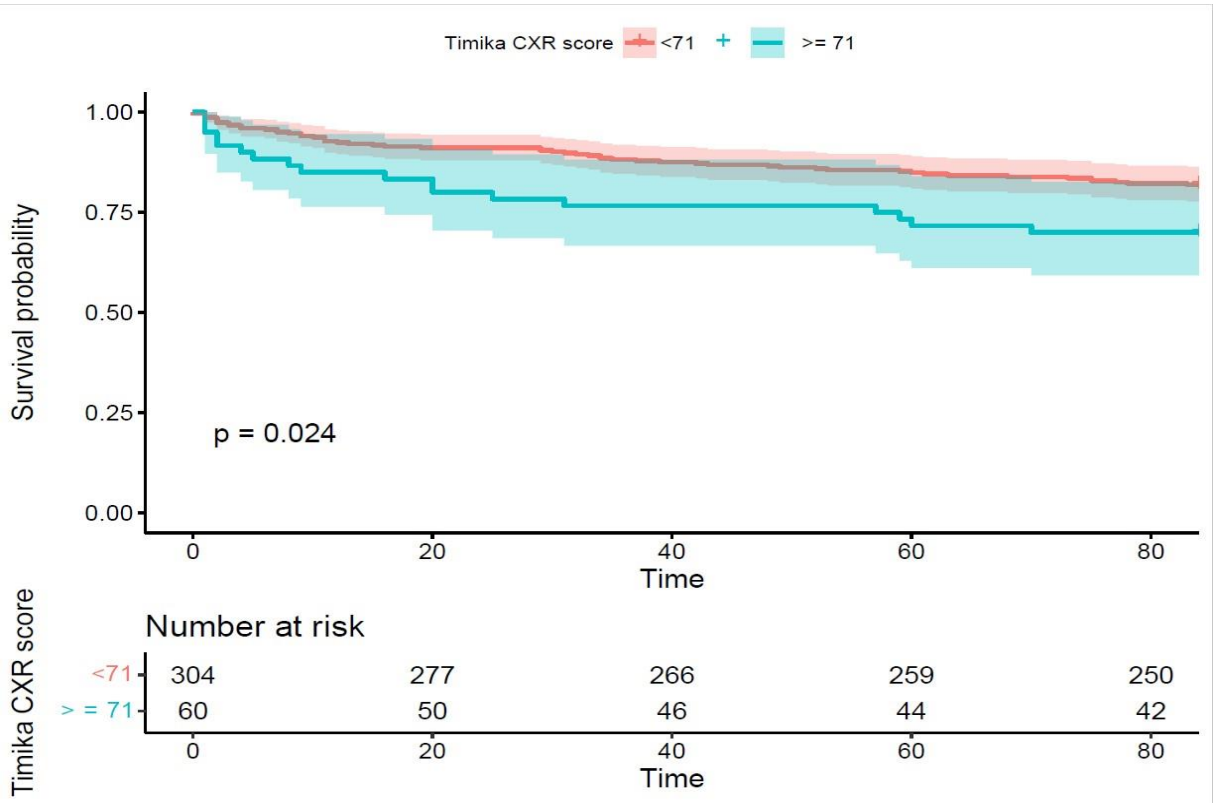


Figure 3: Kaplan Meier (KM) curve showing survival over 84 days for participants with a Timika chest x-ray score <71 versus ≥ 71.

Table 2: Cox proportional hazards regression analyses

Variable	Univariate Analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
CXR score – categorical[1]	1.84	1.08 -3.13	0.025	1.84	1.08 – 3.14	0.026
Sex (male)				0.77	0.48 – 1.23	0.269
Age[2]				1.33	1.07 – 1.64	0.009
HIV viral load[3]				1.00	1.00 – 1.00	0.187
CXR score – Continuous[4]	1.09	1.03 – 1.51	0.002	1.09	1.03 -1.15	0.003

Sex (male)				0.77	0.48 – 1.23	0.281
Age[2]				1.30	1.05 -1.61	0.014
HIV viral load[3]				1.00	1.00 -1.00	0.164

1. CXR score as a binary categorical variable with CXR score ≥ 71 vs CXR < 71
2. Age: per 10-year increase
3. HIV viral load: per 1000 copies/mL increase
4. CXR score as a continuous variable: per 10-point increase

Abbreviations: CXR – chest x-ray; HR – Hazard ratio; CI – confidence interval

Participants with Timika score ≥ 71 (KM cumulative survival estimate = 0.70; 95% CI 0.59 – 0.83) had a lower survival at Week 12 compared to participants with Timika score < 71 (KM cumulative survival estimate = 0.82; 95% CI 0.78 – 0.86; p = 0.024) (Kaplan Meier curve - figure 3).

In Cox proportional hazards regression analysis, the chest x-ray score was first used as a binary categorical variable (<71 versus ≥71) and then as a continuous numerical variable. Both univariate models were run as well as multivariate models adjusting for sex, age and HIV viral load. The hazard ratios (HR) for chest x-ray score as binary variable in both the unadjusted and adjusted model were similar at a HR of 1.84 (95%CI 1.08 – 3.13; p = 0.025) and 1.84 (95%CI 1.08 – 3.14; p = 0.026), respectively. Thus, patients with a chest x-ray score ≥ 71 had 84% higher hazards for mortality compared to patients with a chest x-ray score <71. The proportional hazard assumption was not violated (Global Schoenfeld test; overall model; p = 0.079)

Using chest x-ray score as a continuous variable, both the unadjusted and adjusted models yielded a HR of 1.09 (95%1.03 – 1.51; p = 0.002 and 1.03 -1.15; p = 0.003, respectively). Thus, for every 10-point increase in chest x-ray score, the hazards for deathincreased by 9%. The proportional hazards assumption was not violated (Overall model; p = 0.054).

Inter-reader agreement varied depending on the different chest x-ray features. Inter-reader agreement (Cohen's Kappa) was 0.75 for area of the lung affected; 0.81 for cavities and 0.96 for total score (see Table 4 in Supplementary Appendix).

DISCUSSION [735 words]

We assessed chest x-rays using the Timika chest radiograph score in a large cohort of adults with advanced HIV disease who were hospitalised with a new diagnosis of tuberculosis. A quarter of patients had a normal chest x-ray. We found no association between chest x-ray score and biomarkers of tuberculosis dissemination. Patients with a higher chest x-ray score had a significantly higher hazards of death. Hazards for mortality increased by 9% with every 10-point increase in chest x-ray score. Inter-reader agreement varied between the three aspects of chest x-rays which were assessed.

Nearly a third (31.9%) of chest x-rays had normal lung fields and were classified as normal using the pre-defined definition. In our study, the presence of lymphadenopathy and pleural effusions in isolation, was not regarded as a feature of pulmonary tuberculosis. This initially resulted in an overestimation of the proportion of patients with a normal chest x-ray. When taking into account those with lymphadenopathy and pleural effusions, 25% of chest x-rays were normal. Other studies found a lower proportion of normal chest x-rays between 15% and 21%. [7, 8] Patients with severe immunosuppression with lower CD4 counts, like this cohort, are more likely to have normal chest x-rays.[9]

No significant difference was found in chest x-ray scores across the dissemination score groups. Although we hypothesised that participants with a higher

dissemination score would have a lower chest x-ray score related to poorer immune response, the patient population were all very ill and the majority had a very low CD4 count and so the range of CD4 counts may not have been wide enough to capture a significant difference between dissemination score groups.

In survival analyses, a higher chest x-ray score was associated with higher hazards of death. This disproves our initial hypothesis that a lower chest x-ray score would be associated with higher mortality. This may be due to our categorisation of chest x-rays with a miliary pattern as homogenous infiltrates that then received a high score. Miliary infiltrates have not previously been included in the scoring system[5, 10]. However, we decided to include chest x-rays with a miliary pattern in our scoring as miliary tuberculosis is an important feature of disseminated tuberculosis and is associated with higher mortality.[11]

Our study has some limitations. Firstly, there was varying inter-reader agreement, ranging from substantial to almost perfect inter-reader agreement. A third reader was needed to score 5% of the chest x-rays and gave input in an additional 10% of chest x-rays to reach consensus on interpretation thereof. This may explain the high inter-reader agreement in the total chest x-ray scores. This finding is in keeping with other studies.[5, 10] Secondly, the derivation and validation studies of the Timika scoring system excluded or only included a small subset of PLHIV, thus it has not been well validated for study population like ours. Thirdly, two independent medical officers scored the chest x-rays instead of radiologists which may influence the accuracy of the interpretation of some chest x-ray findings due to lack of expertise. However, in a resource limited setting most chest x-rays are not reported by a radiologist and thus our procedures are more representative of a real-world scenario. In addition,

dissemination score is used pragmatically and has not been shown to have a direct correlation of clinically confirmed TB at multiple anatomical sites. Lastly, the Timika score does not take into account thoracic lymphadenopathy which is an important radiographic feature of pulmonary TB.[3] We found that patients with higher chest x-ray score had higher hazards of death. However, a 10-point difference in Timika score may carry a different clinical implication for patients with low-moderate scores versus higher scores. This variability needs to be considered when interpreting the scores. This chest x-ray scoring system may potentially be useful in identifying those at higher risk for mortality, in combination with other clinical variables. Further modifications are needed to adjust this chest x-ray scoring system to take into account the specific radiographic features of tuberculosis that are frequent in PLHIV, such as miliary infiltrates and thoracic lymphadenopathy. This is important if the scoring system is to be used as a diagnostic or predictive tool in this patient population. Alternatively, derivation and validation studies are required to the develop new scoring system for this patient population. Computer-aided software for tuberculosis (TB) detection in chest radiographs shows promise, but its sensitivity is notably lower PLHIV and those with smear-negative TB.[12]

Acknowledgements

Marcia Vermeulen, Charlotte Schutz and Graeme Meintjes were involved with study conception and planning of the study. Amanda Jackson assisted with the design of the data capture sheets and set up of the REDCap database. Marcia Vermeulen and Alexis Maseko conducted the chest x-ray analysis and scoring. Linda Boloko assisted as the third reader of chest x-rays. Marcia Vermeulen wrote the first drafts

of the manuscript. Charlotte Schutz helped with reviews of the first drafts of the paper and Graeme Meintjes reviewed the final draft.

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Part C:

Supplementary Appendix

Title

Association between chest radiographic features and 12-week mortality in HIV-positive patients diagnosed with tuberculosis in hospital

Table of Contents

Figure 1: Boxplot of the distribution of chest x-ray scores categorised by 12-week outcome

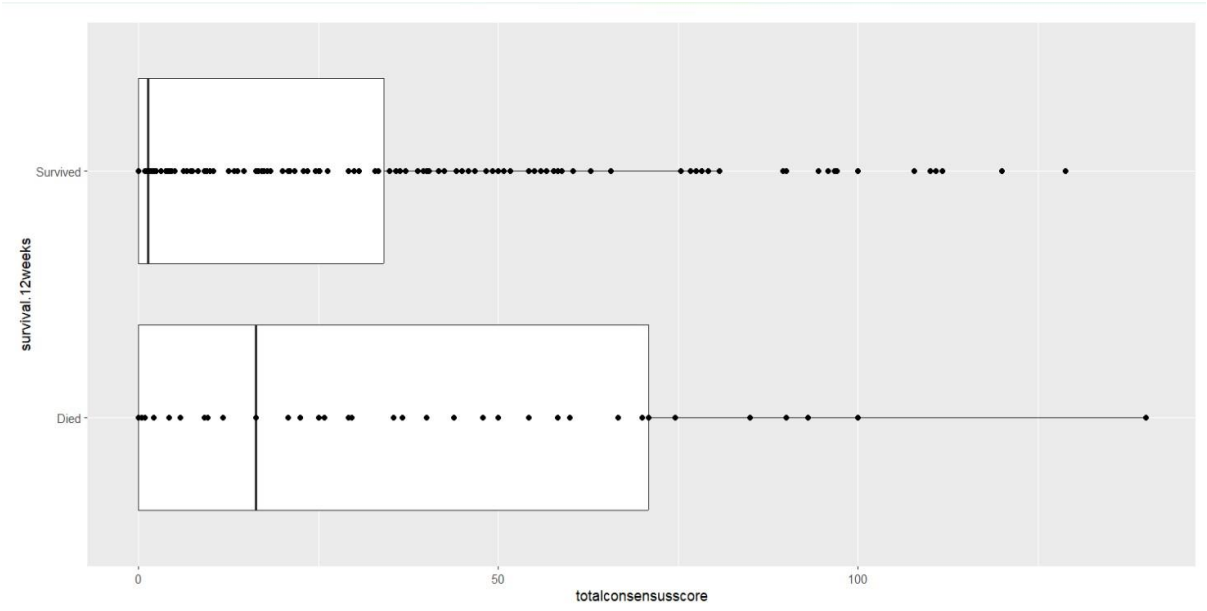

Table 2: Proportion of participants with normal versus abnormal chest x-rays, categorised by survival outcome at 12 weeks.

	Died	Survived	Total	p-value*

Normal CXR	24 (20.7)	92 (79.3)	116	
Abnormal CXR	49 (19.8)	199 (80.2)	248	
	73	291	364	0.9471

*Chi-squared test

Table 3: Distribution of dissemination score, stratified by 12-week outcome.

Dissemination score	All (n =364)	Died (n = 73)	Survived (n = 291)	P- value
0	104 (29%)	9 (12%)	95 (33%)	0.003
1	83 (23%)	21 (29%)	62 (21%)	
2	95 (26%)	19 (26%)	76 (26%)	
3	82 (23%)	24 (33%)	58 (20%)	

Table 4: Inter-reader agreement

	Kappa	Lower CI	Upper CI	Interpretation
Total area lung affected	0.75	0.68	0.82	Substantial agreement

*Cavities present	0.81	0.71	0.91	Almost perfect agreement
Total score	0.96	0.93	0.98	Almost perfect agreement

Cohen's Kappa statistic
*Number of participants = 363

UCT HREC Approval Letters

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/home/human-research-ethics

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20 February 2023

HREC REF: 077 /2023

Dr C Schultz
CIDRI-IDM
Email: Charlotte.schultz@uct.ac.za Student: mcvermeulen8@gmail.com

Dear Dr Schultz

PROJECT TITLE: ASSOCIATION BETWEEN CHEST RADIOGRAPHIC FEATURES AND 12-WEEK MORTALITY IN HIV-POSITIVE PATIENTS DIAGNOSED WITH TUBERCULOSIS IN HOSPITAL- SUB-STUDY LINKED TO 057 /2013-
(MASTER'S DEGREE-DR MARCIA VERMEULEN)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2024.

Please submit a progress form, using the standardised Annual Report Form (FHS016) if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Marcia Vermeulen will also be involved in this study.

Please quote the HREC REF 077 /2023 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

HREC/ref 077.2023

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/ref 077.2023

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	28.02.2025
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	14/1/2024

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

HUMAN RESEARCH ETHICS COMMITTEE
11 JAN 2024
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Please clarify your plan for research-related activities during COVID-19 lockdown

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	10 Jan 2024		
HREC REF Number	077/2023	Current Ethics Approval was granted until	28 Feb 2024
Protocol title	Association between chest radiographic features and 12-week mortality in patients with HIV-positive patients diagnosed with Tuberculosis in hospital		
Principal Investigator	Dr. Charlotte Schutz		
Department / Office Internal Mail Address	Institute of Infectious Disease and Molecular Medicine Faculty of Health Sciences IDM building Room N2.09.2 University of Cape Town		
1.1 Does this protocol receive US Federal funding?		☐ Yes	✓ No

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
✓	Data collection is complete, data analysis only

Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository.

N/A

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval		364
Total number of records or specimens collected, reviewed or stored since last progress report		NA
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	☐ Yes	✓ No

4. Signature

Signature of PI	C Schutz	Date	10 Jan 2024

Data Capture Form

Timika 1

New Chest X-ray ID number	_____ (CXR_ _ _)
CXR ID NUMBER (initial KDH TB study ID)	_____
Date of CXR	_____ (dd-mm-yyyy)
Date of reading	_____ (dd-mm-yyyy)
Quality review	
Film Quality	◯ Optimal ◯ Sub-optimal ◯ Unreadable
1. Penetration	◯ Good penetration ◯ Under-penetrated ◯ Over-penetration
2. Rotation	◯ No rotation ◯ Rotated towards the right ◯ Rotated towards the left
3. Inspiration	◯ Good inspiratory effort ◯ Poor inspiratory attempt ◯ Hyper-inflated (Good inspiratory effort defined as diaphragm should be intersected by the 5th-7th anterior ribs in mid-clavicular line.)
Radiograph completely Normal	◯ Yes ◯ No
What percentage of the lung appears completely normal?	_____ (%)
Abnormalities consistent with TB	◯ Yes ◯ No

Total percentage of affected lung per zone

Please indicate the predominant infiltrate in the zone as N for nodular or H for homogenous; multiply percentage by 0.5 if nodular.
Please indicate if pleural effusion is obscuring greater than a costophrenic angle of a lung zone by entering PE and DO NOT SCORE THAT ZONE; the denominator will need to be adjusted accordingly.

perc_affected

Upper Zone Right (%)

_____

Upper Zone Right N/H

◯ N
◯ H

Upper Zone Right Corrected %

_____
(enter N/A if H)

Upper Zone Left (%)

_____

Upper Zone Left N/H

◯ N
◯ H

Upper Zone Left Corrected %

_____
(enter N/A if H)

Middle Zone Right (%)

_____

Middle Zone Right N/H

◯ N
◯ H

Middle Zone Right Corrected %

_____
(enter N/A if H)

Middle Zone Left (%)

_____

Middle Zone Left N/H

◯ N
◯ H

Middle Zone Left Corrected %

_____
(enter N/A if H)

Lower Zone Right (%)

_____

Lower Zone Right N/H

◯ N
◯ H

LowerZone Right Corrected %	_____ (enter N/A if H)
Lower Zone Left (%)	_____
Lower Zone Left N/H	◯ N ◯ H
LowerZone Left Corrected %	_____ (enter N/A if H)
Total	_____
Total: percentage	_____

Cavities

Are there any cavities >= 1cm in diameter visible?	◯ Yes ◯ No
If yes please add 40 to Total percentage of affected lung and indicate new total	_____

State number and size of cavities in each zone

cavities	
Right upper	_____
Left upper	_____
Right middle	_____
Left middle	_____
Right lower	_____
Left lower	_____

Size of cavities in cm

RU Size

_____

LU Size

_____

RM Size

_____

LM Size

_____

RL Size

_____

LL Size

_____

Lymph nodes

The presence of lymph nodes will only be scored if the diameter is > 10mm
The following scoring system will be used:

1+ = lymph nodes present in one location

2+ = lymph nodes present in two locations

3+ = lymph nodes present in more than two locations

Are there any lymph nodes visible?

- ◯ Yes
- ◯ No

If yes, please indicate grade

- ◯ 1+
- ◯ 2+
- ◯ 3+

If yes, please indicate location:

- ☐ right hilar
- ☐ left hilar
- ☐ right med
- ☐ left med
- ☐ other

Pleural effusion

Is there any pleural effusion visible?

- ◯ Yes
- ◯ No

If yes, please indicate grade of pleural effusion

- ◯ 1+
- ◯ 2+
- ◯ 3+

Any pleural effusion will be documented, using the following grading system:

1+ = costrophrenic angle
2+ = up until 1/3 of the lung
3+ = greater than 1/3 of the lung

Side (pleural effusion)	◯ right ◯ left ◯ both
CXR has a miliary pattern?	◯ Yes ◯ No ◯ Unsure

Reviewer details

Reviewer Initials	_____
Reviewer Date	_____
Total % lung affected	_____
Cavities	◯ Yes ◯ No (if 'yes', add 40)
Total score reviewer 1	_____
First reviewer score	_____
Second reviewer score	_____
Total consensus score	_____
Third reviewer score	_____ (Only needed if difference between first and second reviewer scores > 10)

Timika 2

New Chest X-ray ID number	_____ (CXR_ _ _)
CXR ID NUMBER (initial KDH TB study ID)	_____
Date of CXR	_____ (dd-mm-yyyy)
Date of reading 2	_____ (dd-mm-yyyy)
Quality review	
Film Quality 2	◯ Optimal ◯ Sub-optimal ◯ Unreadable
1. Penetration 2	◯ Good penetration ◯ Under-penetrated ◯ Over-penetration
2. Rotation 2	◯ No rotation ◯ Rotated towards the right ◯ Rotated towards the left
3. Inspiration 2	◯ Good inspiratory effort ◯ Poor inspiratory attempt ◯ Hyper-inflated (Good inspiratory effort defined as diaphragm should be intersected by the 5th-7th anterior ribs in mid-clavicular line.)
Radiograph completely Normal 2	◯ Yes ◯ No
What percentage of the lung appears completely normal (2)?	_____ (%)
Abnormalities consistent with TB	◯ Yes ◯ No

Total percentage of affected lung per zone

Please indicate the predominant infiltrate in the zone as N for nodular or H for homogenous; multiply percentage by 0.5 if nodular.
Please indicate if pleural effusion is obscuring greater than a costophrenic angle of a lung zone by entering PE and DO NOT SCORE THAT ZONE; the denominator will need to be adjusted accordingly.

Upper Zone Right (%)	_____
Upper Zone Right N/H	◯ N ◯ H
Upper Zone Right Corrected %	_____ (enter N/A if H)
Upper Zone Left (%)	_____
Upper Zone Left N/H	◯ N ◯ H
Upper Zone Left Corrected %	_____ (enter N/A if H)
Middle Zone Right (%)	_____
Middle Zone Right N/H	◯ N ◯ H
Middle Zone Right Corrected %	_____ (enter N/A if H)
Middle Zone Left (%)	_____
Middle Zone Left N/H	◯ N ◯ H
Middle Zone Left Corrected %	_____ (enter N/A if H)
2.3 Lower Zone Right (%)	_____
Lower Zone Right N/H	◯ N ◯ H
LowerZone Right Corrected %	_____ (enter N/A if H)

Lower Zone Left (%)	_____
Lower Zone Left N/H	◯ N ◯ H
LowerZone Left Corrected %	_____ (enter N/A if H)
Total	_____
Total: percentage	_____

Cavities

Are there any cavities >= 1cm in diameter visible?	◯ Yes ◯ No
If yes please add 40 to Total percentage of affected lung and indicate new total	_____

State number and size of cavities in each zone

cavities	
Right upper	_____
Left upper	_____
Right middle	_____
Left middle	_____
Right lower	_____
Left lower	_____

Size in cm

RU Size

_____

LU Size

_____

RM Size

_____

LM Size

_____

RL Size

_____

LL Size

_____

Lymph nodes

The presence of lymph nodes will only be scored if the diameter is > 10mm
The following scoring system will be used:

1+ = lymph nodes present in one location

2+ = lymph nodes present in two locations

3+ = lymph nodes present in more than two locations

Are there any lymph nodes visible?

- ◯ Yes
- ◯ No

If yes, please indicate grade

- ◯ 1+
- ◯ 2+
- ◯ 3+

If yes, please indicate location:

- ☐ right hilar
- ☐ left hilar
- ☐ right med
- ☐ left med
- ☐ other

Pleural effusion

Is there any pleural effusion visible?

- ◯ Yes
- ◯ No

If yes, please indicate grade of pleural effusion

- ◯ 1+
- ◯ 2+
- ◯ 3+

Any pleural effusion will be documented, using the following grading system:

1+ = costrophrenic angle
2+ = up until 1/3 of the lung
3+ = greater than 1/3 of the lung

Side (pleural effusion)	◯ right ◯ left ◯ both

Reviewer details

Reviewer 2 Initials	_____
Reviewer 2 Date	_____
Total % lung affected 2	_____
Cavities 2	◯ Yes ◯ No (if 'yes', add 40)
Total score second reviewer	_____

Author's Guidelines

The International Journal of Tuberculosis and Lung Disease

INSTRUCTIONS FOR AUTHORS

The *International Journal of Tuberculosis and Lung Disease* (*IJTLD*), publishes Editorials, Original Articles, Minireviews, Letters and Correspondence of significance on TB and the entire spectrum of lung diseases in adults and children. The IJTLD welcomes submissions on basic, translational, clinical, epidemiological and programmatic research relevant to the Union's mission to find health solutions for these conditions, including the development of vaccines, diagnostics and medicines for the prevention, management and control of TB and other respiratory diseases.

SUBMISSION OF ARTICLES

Articles should be submitted online via Manuscript Central: http://mc.manuscriptcentral.com/ijtld.

Before submitting your article, please read and carefully follow the Instructions for Authors outlined below.

All articles must be submitted in English. When necessary, authors are encouraged to seek professional editing service before submission. If the quality of the English is not considered to be adequate, the manuscript will be returned the authors without review. Authors may be offered the opportunity to re-submit a revised version that has been edited for English language.

Manuscripts may only be actively under consideration by one journal at any given time.

FAST TRACK REVIEW

For exceptional articles of major scientific or public health interest, the Editor-in-Chief may decide to proceed with fast-track review, aiming to reach a first decision within one week. If you believe your article requires fast-track review, please state this in the cover letter of your manuscript along with detailed justification(s).

AUTHORSHIP

The journal recommends the International Committee of Medical Journal Editors' criteria for authorship (http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html). The ICMJE recommends that authorship be based on the following four criteria:

1.Substantial contributions to the conception or design of the work; or the acquisition,analysis, or interpretation of data for the work; AND

2.Drafting the work or revising it critically for important intellectual content; AND

3.Final approval of the version to be published; AND

4.Agreement to be accountable for all aspects of the work in ensuring that questionsrelated to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

When a multicentre group has conducted the study, all individuals who accept direct responsibility for the manuscript should be identified.When submitting a group author manuscript, the corresponding author should clearly identify all individual authors, as well as the group name.

FORMAT OF SUBMITTED ARTICLES

The formats for different types of articles are summarised as follows:	Word limit	Figure or Table*	References	Online Supplementary Data	Abstract
Article type					
Original Article (including also Systematic Reviews and Meta-Analysis)	2500	5	35 (70 for Systematic Reviews and meta-analyses)	Accepted	Yes (200 words)
Review (State of the Art, Guidelines/Consensus)	3500	5	70	Accepted	Yes (200 words)
Minireview	2000	3–4	40	Accepted	Yes (200 words)
Letter	1100	1	15	Accepted	No
Correspondence	600	1	10	Not accepted	No
Editorial	500-1400	1-2	25	Accepted	No

* Number stated refers to the maximum number of figures and/or tables combined.

Original Articles and Reviews

Original Articles (including also Systematic Reviews and Meta-Analyses) should not exceed 2,500 words (excluding abstract, references, Tables and Figure captions) and should have a structured summary of 200 words, up to 35 references and between 5 moderate-sized tables/figures. Please see examples of table sizes that will fit this layout within the section 'Figures and Tables'. Tables that are too large to be published on a print journal page should be included as Supplementary Data (see details below). Clinical trials must be registered in a WHO compliant clinical trial registry and reported according to CONSORT guidelines. Epidemiological studies should be conducted and reported according to STROBE guidelines. Systematic reviews and Meta-Analyses will only be considered if they provide insight beyond that available in the source studies. Reporting should follow PRISMA guidelines. Meta-Analysis of observational data should follow MOOSE guidelines. A completed PRISMA or MOOSE checklist should be included with the submission.

Abstract

An informative abstract of no more than 200 words that can be understood without reference to the text should be included. For optimal clarity, the author should use the headings Background, Methods, Results and Conclusion. Abstracts will be translated into French (authors are welcome to provide their own translation).

Main text headings: Three categories of heading are used.

Major headings (e.g., **METHODS**, **RESULTS**) are in Arial 12 bold caps. Minor heading 1 (e.g., *Study population and materials)* in Arial 12 italics. Minor heading 2 (e.g., *Human subjects)* in Times Roman 12 italics.

Sections should follow the usual conventions

Introduction (does not require a heading): This should include the aim, objectives and/or hypotheses for the manuscript, preceded by their rationale.
Methods: This should include a description of the study design, study population, intervention, exposures, outcomes and other relevant variables, where applicable. Details of the statistical analysis plan and sample size and study power should also be included. Methods should be described in a manner that is conducive to replication. Details of ethics approval (or a statement as to why it was not required) should be provided in the Methods section of all research studies. All studies involving human subjects should include details of informed consent.
Results*:* Present the results in logical sequence, referencing figures and tables (see information below on submitting figures and tables). For complex tables only highlight the most important results.
Discussion*:* Bring the reader back to your initial aims, objective or hypothesis, showing how this study has improved our understanding of the topic. **Conclusions***:* optional, but if used, please briefly highlight the single most significant aspect of this study.
Reviews (State of the Art, Viewpoints, Guidelines) are aimed to inform and educate readers and should stimulate debate around clinical and scientific topics. The IJTLD also welcomes suggestions for review articles on different aspects of TB and across the breadth of respiratory medicine. Authors proposing an unsolicited review should explain in a cover letter why the topic is timely and relevant and include a maximum of 5 examples of their own recent published work supporting their expertise in this field. Submitted reviews should not overlap with recently published ones on similar topics. Review articles should represent the state of the art in their specific field. The literature review should be up-to-date. Systematic review methods are encouraged but are not mandatory. The IJTLD strongly encourages the use of new imaginative figures and of a pivotal figure to summarise key concepts or conclusions of the review.
Review articles (see Table above) should not exceed 3,500 words (excluding abstract, references, tables and figure captions) and should have a structured summary of 200 words, up to 70 references and 5 moderate-sized tables/figures.
Minireviews are focused, expert reviews on cutting-edge issues. One-page proposals will be considered, and will be judged on 1) the scientific importance and novelty of the subject matter, 2) its relevance to the readership of the Journal, and 3) the expertise of the proposed authors. Minireviews are expected to draw conclusions and make recommendations that are based on the evidence presented.
Text up to 2,000 words, a structured summary of 200 words, 3–4 moderate-sized tables/figures and up to 40 references.

Forum: Letters and Correspondence
Letters include research letters, case studies and other forms of short communication to the Editor. Research letters are preliminary studies or short reports presented in the shorter format of a Letter to the Editor. Case studies are considered only if they contain original and innovative material, ideally discussing cases in the form of a mini-review of the available literature on the topic. Patient consent should be provided (or, in the case of death, the consent of a relative). Letters do not include an abstract or text headings and start 'Dear Editor,…'. They should not exceed 1,100 words (excluding references, tables and figure captions) and should have no more than 15 references. One figure or one table is mandatory.
Correspondence is designed to discuss relevant articles, guidelines, documents or other topical matters recently published in the IJTLD, or other journals or media. Correspondence in response to an article published in the IJTLD should be submitted within 3 months of the publication date of the original article. All Correspondence is sent

to the authors of the original article for a reply and these exchanges are prioritised for publication in the next available issue of the *Journal*. Correspondence does not include an abstract or text headings and starts 'Dear Editor, …'. They should not exceed 600 words (excluding references, tables and figure captions) and should have no more than 10 references. One figure or one table is highly recommended.

Editorials

Editorials are usually invited by the Editorial Board to allow experts to concisely discuss the findings of an Original Article (sharing their perspective on how the publication advances the field and highlighting the need for specific further research). Unsolicited Editorials are also of interest and can highlight a key initiative or paradigm shift. Editorials do not include an abstract and are between 500–1,400 words (excluding references, tables and figure captions) and should have no more than 25 references and 1-2 figures/tables.

Papers that do not conform to these guidelines will either be rejected, or returned to the authors for revision prior to peer review.

FORMATTING

Authors should submit a single Word document (.doc or .docx) – this document should include the title page, abstract text, references, tables and figures with legends. For ease of peer review, the article should have 1.5 or double spacing and continuous line numbering.

Title page: This should contain:

1) a concise, informative title of not more than 110 characters and spaces, without abbreviations

2) the names and affiliations of all contributing authors, clearly indicating who is linked to each institution

3) a running head of not more than 45 characters and spaces

4) a word count of the summary, a word count of the text, number of references, tables and figures

5) 3-5 keywords that do not appear in the title

6) the name, full address and contact details of the corresponding author.

ACKNOWLEDGEMENTS: Acknowledge only those people who have made substantial contributions to the study, with their consent. All sources of support in the form of grants, author contributions and all conflicts of interest should also be mentioned.

REFERENCES: The accuracy of references is the responsibility of the author. Please use superscript numbers in the text, and they must be numbered in the order in which they are cited. References that are cited more than once retain the same number for each citation. The references list at the end of an article should be arranged in numerical order.

References to an article: should include the names of the authors, followed by their initials. List all authors when three or fewer - see the example below:

Gordon JB, Bennett AM. Tuberculosis in reindeer. Scand Rev Respir Dis1978; 96 (Suppl): 217-219.

When there are more than three authors, list only the first author and add 'et al.'

References to a piece of work: (book/monograph) should include the names of the authors, the title of the piece of work, the ISSN number of the publication, the name of the Editor, the place and year of publication, the number of the volume and the first and last page numbers.

References to a chapter in a book: should include the names of the authors, the title of the chapter with the word "In" preceding the reference of the work e.g.

Girling DJ. The chemotherapy of tuberculosis. In: Ratledge C, Stanford J, Grange JM, eds. Biology of the mycobacteria. London, UK: Academic Press, 1989: pp 285-323.
Electronic references should be given only when an original citation is unavailable; please provide as much information as possible, including html address.
References to an article yet to be published: should give the name of the journal as '(In Press)' and include the article DOI.
Personal communications: should be given in the text with the name of the individual cited and with his/her consent.

FIGURES AND TABLES

Tables and figures should be self-explanatory and easily understood as a standalone element. Numbering of tables/figures corresponds to where they are first cited in the text. All abbreviations included in the title or in the Table/Figure, even if explained in the text of the article, should be expanded in a footnote to be understandable without referring to the text.
Tables: A short descriptive title should appear above the table. Each column should have a short or abbreviated heading. All abbreviations should be explained in a clear legend below the table. Tables should not have shading or bolding.
Explanations of data should be included in the legend and linked to the respective element by a number (1, 2, 3 etc). Tables should be treated as a standalone item, so references should be included in their entirety in the legend and not added to the Reference list at the end of the article. Please note that the number and size of the tables need to be accommodated within the pages allocated for each type of article.
Examples of table sizes:
Small table with 4-5 columns and 4-5 rows = **1/4 page** in a typeset article Moderate table with 4-6 columns and 10-12 rows = **1/2 page** in a typeset article Large table with 6-10 columns and 12-16 rows = **1 full page** in a typeset article

If there is the need to refer to very large datasets, the excess material can be included as Supplementary Data (please note charges below). The figures and tables in Supplementary Data should be numbered as Figure S1, Table S1 etc (to avoid confusion over labelling of the figures and tables in the main body of the article). Alternatively, the data can be hosted via a service such as Figshare (https://figshare.com) with a link embedded in the text.
Figures: These should be referred to consecutively in the text. They can be inserted into the Word document at the end of the References or uploaded separately as image files (.jpg, .ppt, .gif, .tif or .bmp). A brief explanatory legend should be provided for every figure to ensure it can be understood as a standalone item.
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Line drawings, flow charts and histograms: Must be supplied either as .doc or .xls files. For optimal clarity they should be in black and white, with solid black lines, and avoid shading.
Scans, photographs, or X-rays: Should be supplied at a resolution of a least 300 dpi (preferably 500 dpi) as TIFF or JPEG files suitable for reproduction. Photo-micrographs should have internal scale markers where appropriate. X-ray fim should bring out the detail with the area of importance clearly indicated. Techniques (staining, magnification, etc) should be defined.
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Avoid abbreviations in the title or summary. Abbreviations or unusual terms should be described the first time of use. Symbols and units of measure must conform to recognised scientific use, i.e. SI units. For more detailed recommendations, authors may consult the Royal Society of Medicine publication *Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors.* Designation of diseases must conform to the International Classification of Diseases. Designation of micro-organisms must conform to the norms of biology. Proprietary names of drugs, instruments, etc. should be indicated by the use of initial capital letters. Names of instruments should be accompanied by the manufacturer's name, city, state and country.

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Manuscripts should conform to the *Uniform Requirements for Manuscripts submitted to Biomedical Journals* (http://www.icmje.org/index.html). Authors should ensure they follow the relevant recommendations and guidelines for reporting their findings (CONSORT, STARD, MOOSE, STROBE, PRISMA, STREGA). Articles on clinical research should conform to the standards defined in the Helsinki Declaration, as revised in 2013 (www.wma.net/en/30publications/10policies/b3/index.html).

Stigmatising language: Authors are advised to avoid terms that may be perceived to be stigmatising, such as "TB suspect" or "defaulter". Authors can refer to the following publications: Zachariah R. et al., Language in tuberculosis services: can we change to patient-centred terminology and stop the paradigm of blaming the patients? Int J Tuberc Lung Dis 2012; 16: 714–717

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Although every effort is made to ensure that drug doses are presented accurately, readers are advised that dosage should be followed in conjunction with the drug manufacturer's published literature.

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Any specific issue related to the checklist should be addressed to the Editors-in-Chief in the accompanying covering letter. All other correspondence should be sent directly to:

The Editorial Office, The Union, 2 rue Jean Lantier, 75001 Paris, FRANCE. e-mail: journal@theunion.org

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