

The Effect of Diabetes and HIV on radiographic manifestations of Pulmonary
Tuberculosis

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0.2 Declaration

I, ...*Natacha Berkowitz*..., hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: ...

Signed by candidate

Date: ...1 March 2017.....

0.3 Acknowledgments

This dissertation is entirely my own work and I was responsible for analysis of the data and writing of the manuscript. This study forms part of a larger study designed by Prof Oni, where I was responsible for recruitment and retention of participants, phenotypic characterization of all participants, and data management.

This study would not have succeeded if it were not for the clinical staff involved, especially Nonceba Gobe, Rene Goliath and Nashreen Omar-Davies. Thank you to doctors Oompie, Sood and Vorster for assisting in radiograph reading. Thank you to Prof Robert Wilkinson for supporting my public health pursuits within his research group. It has been a privilege to work with Prof Oni and I greatly appreciate her giving me the opportunity to work on the parent study, as well as for her guidance and mentorship, over the past 3 years.

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0.4 Abstract

Due to the epidemiological transition, diabetes prevalence in South Africa is increasing, while HIV prevalence remains high. Diabetes, along with HIV, has been found to be a significant risk factor for the development of tuberculosis. Early detection and treatment of tuberculosis is essential to prevent unwarranted morbidity and mortality. This hinges on efficient diagnostic methods and tools. The chest radiograph remains a cornerstone in pulmonary tuberculosis diagnosis, especially in those where microbiological evidence of disease is lacking.

A study was conducted to investigate the chest radiographic presentation of pulmonary tuberculosis in patients with diabetes, as well as to analyse the effect of HIV comorbidity on this association. The study was conducted in Khayelitsha, Cape Town, an area with a high tuberculosis, HIV and diabetes burden. A literature review was conducted to identify the key features of pulmonary tuberculosis on chest radiograph for patients with diabetes and HIV. We found that patients with diabetes were more likely to have lower lung field infiltrates and increased cavitation, with glycaemic control affecting the presence of these findings. Patients with HIV presented more often with features of primary tuberculosis on chest radiograph, namely hilar and/or mediastinal adenopathy, diffuse reticulonodular infiltrate, and lower lung field (LLF) infiltrates and cavities. These features were influenced by degree of immunosuppression. This review also found that there was no literature describing the influence of HIV on the chest radiographic features of tuberculosis in patients with diabetes.

This study was conducted between June 2013 - October 2015, where 377 patients with pulmonary tuberculosis, from Ubuntu and Site B primary care clinics in Khayelitsha, underwent posterior-anterior chest radiography. Chest radiographs were read using a CRRS tool.

Participants with diabetes and tuberculosis (TBDM) had a higher proportion of lower lung field opacification (76,2%: 95% CI: 56,3 – 96,1) and were 3,92 times more likely to have LLF cavitations than patients with TB only. TBDM participants with HbA1c levels over 10% had more frequent LLF involvement overall (90,9% vs 61,9% p=0,052) and isolated LLF involvement (27,3% vs 3,6%; p= 0,019) than TB only participants. Both TBDM and TBDM

participants with HIV (TBDMHIV) had higher proportions of isolated LLF lesions as compared to TB only participants (14,3% vs 3,6%; $p=0,093$ and 15,2% vs 3,6%; $p = 0,039$, respectively). As CD4 counts increased, there was an upward trend towards an increase in the proportion of cavitations for TBDMHIV participants, but this was not evident in participants with TB and HIV (TBHIV).

This study confirms the atypical nature of chest radiograph in persons with TBDM, TBHIV and TBDMHIV, with diabetes driving the presence of lower lung field involvement. These findings can be used in bi-directional screening algorithms for patients with diabetes, with or without HIV and highlights the important role of radiographic examination in pulmonary tuberculosis.

0.5 List of Abbreviations

2hBG	Two Hour Fasting Oral Glucose Tolerance Test
AIC	Akaike's Information Criterion
AIDS	Acquired Immune Deficiency Syndrome
ARV	Antiretroviral
CRF	Case Report Form
CRRS	Chest Radiographic Reading and Reporting System
CRW	Clinical Research Worker
FBG	Fasting Blood Glucose
HbA1c	Glycosylated haemoglobin
HIV	Human Immunodeficiency virus
LLF	Lower Lung Field
NCD	Non-Communicable Disease
OR	Odds Ratio
PTB	Pulmonary Tuberculosis
SDG	Sustainable Development Goals
T2DM	Type 2 Diabetes Mellitus
TB	Tuberculosis
TBDM	Patients With Tuberculosis And Diabetes
TBDMHIV	Patients With Tuberculosis, Diabetes And HIV
TBHIV	Patients With Tuberculosis And HIV
UCT	University of Cape Town
UL	Upper Lobe
WHO	World Health Organisation

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Part A: Protocol

A.1 Introduction

A.1.1 Background

Despite advances in scientific research, including improved diagnostic technologies, tuberculosis (TB) remains a leading cause of death globally, with 9.6 million incident cases and 1.5 million dying of TB in 2014(1). While efforts have been made to curb the epidemic through the roll-out of GeneXpert diagnostic tests, TB case notification and mortality remains high, and South Africa remains the 3rd highest burden TB country around the world(1). Human immunodeficiency virus (HIV) is the strongest known risk factor for TB(2) and considerable attention has been paid to integration of HIV/TB services, and roll-out of antiretroviral therapy in order to decrease TB risk and improve TB outcomes. Although advances have been made, TB incidence remains high and treatment success sub-optimal. There is therefore a need for research on other risk factors, largely neglected to date in TB national programs, that contribute to an increased TB risk and poorer clinical outcomes.

Rapid urbanization and epidemiological transition in South Africa has been accompanied by decreased physical activity, obesity, increased consumption of processed foods, and has contributed to an emerging epidemic of non-communicable diseases, such as Type 2 Diabetes Mellitus (T2DM)(3). In Khayelitsha, the setting for this study, the prevalence of T2DM is 13% among the population(4). The association between TB and diabetes (DM), and the need for integration of services, has been recently highlighted by the World Health Organisation (WHO) Collaborative Framework for Care and Control of Tuberculosis and Diabetes(5). From a systematic review of 13 observational studies, it has been suggested that persons with diabetes have a 3-fold increased risk of TB, especially in regions with high TB incidence(6).

A.1.2 Problem statement

The chest radiograph is still a valuable TB diagnostic tool. It is utilized when first line sputum testing is negative, unavailable or indeterminate. However, chest radiograph diagnosis can be difficult, due to inter-patient variability of chest radiograph manifestations, and patient characteristics such as age, sex, immune status and time to diagnosis(7, 8). The resulting atypical presentations may confound and delay diagnosis.

Compounding the increased risk of TB in DM is the atypical clinical presentation of persons with DM and TB (TBDM). Chest radiographs of TBDM patients have been noted to be dissimilar to normoglycaemic patients with TB. Persons with diabetes are more likely to have lower lobe opacification, multiple cavities and larger cavities(9-12). Glycaemic control appears to correlate with worsened manifestations on chest radiographs(9). This atypical presentation may delay diagnosis and treatment of TB in persons with DM, and potentially leading to worsened morbidity and mortality.

Numerous studies have compared the chest radiographs of persons with DM and normoglycaemic patients with TB, however, in our setting HIV is also prevalent. The characteristics of chest radiographs among patients with HIV and TB are well documented(13). In this study, we wish not only to describe the characteristics of chest radiographs of patients with diabetes and TB, but also to compare this to patients co-infected with HIV.

A.1.3 Research question

In patients who were recruited as part of a larger TB and DM study in Khayelitsha, is an atypical chest radiographic presentation of pulmonary TB more common in adult TB patients with DM when compared to those without DM?

Secondary Research question:

How does HIV co-infection influence this presentation of pulmonary TB in TB and TBDM patients?

A.1.4 Research justification

Patients with diabetes and tuberculosis may have features on chest radiograph that are unusual and may be overlooked at presentation, leading to misdiagnosis. These radiograph features may also be more severe in TBDM patients, than in normoglycaemic tuberculosis patients. With HIV infection also affecting radiographic presentation of TB, the HIV status of the TBDM patient may further augment the atypical findings on the chest radiograph.

A.1.5 Objectives

- Describe unique radiographic features of chest radiographs of TBDM patients;
- To compare the radiographic features of chest radiographs of TBDM patients and normoglycaemic TB patients;
- To compare radiographic features of TBDM patients with and without HIV and by doing so establish the effect of HIV on these features.

A.2 Methodology

A.2.1 Study design

This is a retrospective cross-sectional study where chest radiographs of HIV infected and uninfected patients with confirmed PTB will be collated from a larger TBDM prevalence study (HREC REF NO: 403/2011) investigating the prevalence of diabetes amongst TB patients and TB amongst patients with diabetes in Khayelitsha, Cape Town. As part of this parent study participants underwent chest radiography at time of TB screening. These chest radiographs will be read and reported by two clinicians, a research medical officer and a radiologist - not involved in care of patients and truly blinded to HIV or DM status.

A.2.2 Population and sampling

A.2.2.1 Characteristics of the study population

Participants were recruited from Ubuntu TB and ARV clinic, as well as Site B Community Health Centre in Khayelitsha as part of the larger study. All participants who were diagnosed with active pulmonary TB as per national protocol (see TB case definition below)(14) will be included in this study sample. Khayelitsha is a peri-urban

township with a population of approximately 500,000 people. The TB case notification rate is >1600/100 000 with an antenatal HIV prevalence of 28%(15). The age standardized DM prevalence in Khayelitsha is 13.1% (95% CI: 11.0–15.1%) (4). Participants were recruited between July 2013 and July 2015.

A.2.2.2 Parent study procedures

This parent study consisted of two arms. Arm A was a case control study screening patients who presented to Ubuntu TB and ARV clinic with respiratory symptoms for TB, investigating the prevalence of diabetes in TB patients. Arm B was a cross sectional component screening known diabetes patients at Site B Community Health Centre for TB, to investigate TB prevalence in patients with diabetes. Flow diagrams for each study arm are shown below. All participants in both arms were offered HIV screening using a rapid whole blood finger prick antibody diagnostic tests.

Figure A-1: Study Flow Arm A

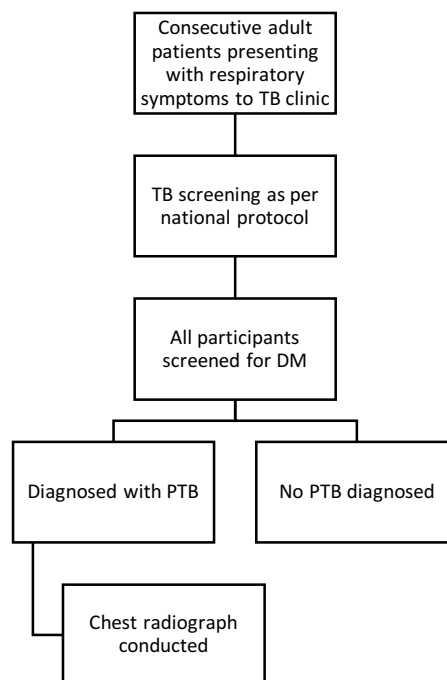
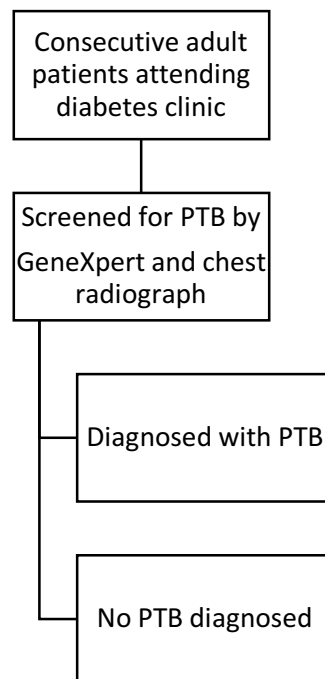


Figure A-2: Study Flow Arm B



A.2.2.3 Case definitions

The National TB protocol TB case definition was used(14):

- 1) smear microscopy positive for acid-fast bacilli;
- 2) isolation or identification of Mycobacterium tuberculosis by culture or Genexpert;
- 3) patients with compatible clinical signs and symptoms (including typical chest radiograph) and resolution of symptoms after TB treatment.

Participants who screened microbiologically negative for PTB, as well as having resolution of respiratory symptoms were regarded as PTB negative.

The diabetes case definition:

- 1) Participant is known to have DM prior to enrolment;
- 2) Participant is on oral antiglycaemic agents and/or insulin;

3) Participant meets the biochemical criteria for DM diagnosis as defined by the American Diabetes Association(16) namely a FBG of >7.0mmol/l, 2hBG of >=11.1mmol/l or an HbA1c of >6.5%.

A.2.2.4 Inclusion and exclusion Criteria

All male and female patients older than 18 years who were diagnosed with pulmonary TB (PTB) and underwent chest radiography, as part of the larger study, will be included in this study.

Participants who were diagnosed with PTB but did not have a chest radiograph, as well as those who refused HIV screening, will be excluded from the analysis.

A.2.3 Measurement

A.2.3.1 Research procedures

Written informed consent (Appendix D5) will be obtained from all participants by clinical research workers (CRW) in the language of the patient's choice. All consented participants underwent screening for DM as part of the parent study. This included phlebotomy for glycosylated haemoglobin (HbA1c), fasting blood glucose (FBG) and a two hour fasting oral glucose tolerance test (2hBG). The 2hBG was omitted in those participants with known DM.

A.2.3.2 Data collection

Basic demographic information and a standardized STEPs chronic disease risk factor tool (Appendix D3) will be completed by a clinical research worker for all participants. HIV status will also be recorded.

Chest radiographs will be analyzed using a standardized tool (Appendix D4). This tool will be used to record specific radiographic changes related to pulmonary TB, and where they are located: parenchymal abnormalities, lung field opacifications, hilar and mediastinal lymphadenopathy, cardiothoracic ratio, pulmonary effusion and cavitation. The chest radiograph will be read by two clinicians, a research medical

officer and a radiologist. They will each complete the reporting tool independently and be blinded to the DM and HIV status of the participant. If there is discordance between the reports the chest radiograph will be read a third time by a physician and consensus reached.

Variables for this analysis will be extracted from these tools. All data recorded will be anonymized and captured on an Access database.

A.2.3.3 Sample size and power calculations

The most common atypical feature of TBDM on chest radiograph is lower lobe involvement which occurs in between 50% – 86% for TBDM patients and 20% - 46% for patients with TB only (17-19). Using the upper limits of this prevalence to detect a difference at $\alpha = 0.05$ and 80% power, 37 TBDM and 37 TB only cases are needed. In this sample, we have 21 TBDM with completed chest radiographs and 84 non-DM TB. Due to this smaller sample the power decreases to 78% at $\alpha = 0.05$. This reduction in power will increase the probability of committing a Type II error, meaning that we may find no difference in radiographic manifestations of pulmonary TB between TB and TBDM, when indeed they are present. The analysis of the secondary objective may also be underpowered due to this small sample. We will take this into consideration when undertaking the interpretation of results.

A.2.3.4 Study variables

Study variables are listed in table 1. The primary outcomes will be atypical features of tuberculosis on chest radiograph (lower lobe opacification and cavities). Participant characteristics include demographic data as well as clinical information on DM (glycaemic control measured by HbA1c), TB sputum results and HIV status. Smoking status will be recorded as this may alter the features on chest radiograph.

Table A-1: Study Variables

Variable	Type	Definition	Categories
Age	Continuous	Independent	>18
Sex	Categorical	Independent	Male Female
Smoking	Categorical	Independent	Non Smoker (never smoked/ former smoker) Smoker (current smoker)
HIV status	Categorical	Independent	Negative Positive
CD4	Continuous	Independent	>20+
Previous TB	Categorical	Independent	No Yes
HbA1c	Continuous	Independent	>2+
Smear status	Categorical	Independent	Negative Scanty One plus Two plus Three plus Not done
Genexpert	Categorical	Independent	Negative Positive Indeterminate Not done
Sputum culture	Categorical	Independent	Negative Positive Not done
Chest radiograph features:		Dependent	
Parenchymal abnormalities	Categorical		No Yes
Location			Left Right
Airspace opacities			
Cavities	Categorical		No Yes
Location			Left Right
Miliary nodules	Categorical		No Yes
Pleural effusion	Categorical		No Yes
Location	Categorical		Left Right
Lymphadenopathy	Categorical		No Yes

Hilar	No Yes
Location	Left Right
Mediastinal	No Yes
Location	Left Right

A.2.4 Statistical analysis plan

A.2.4.1 Descriptive analysis

Participant characteristics will be described using means and standard deviations for normally distributed continuous variables, and medians and inter-quartile ranges for non-parametric continuous variables. Frequency tables with proportions will be used to describe categorical variables. These characteristics will be tabulated and stratified by DM status. Chest radiograph features also be described with simple proportions, tabulated and stratified by DM status. A third exploration will include stratification of chest radiograph features by HIV status. For the DM group, features on chest radiograph will also be stratified by glycaemic control which will be a function of HbA1c level according to the American Diabetes Association(16). We chose HbA1c as a measure of glycaemic control as it has been shown to accurately reflect average glycaemic control over the previous 8 – 12 weeks, as well as being a sensitive measure of diabetes severity(20). Differences between the groups will be determined using Chi squared tests and Fisher’s exact test for categorical data. Significance testing will be conducted using two sided p-values and 95% confidence intervals.

A.2.4.2 Logistic regression

Univariate and multivariate logistic regression will be used to describe the association between individual radiographic characteristics and disease status, adjusting for *a priori* factors which may influence the outcome. These include age and sex, as well as smoking status and history of previous PTB which may independently change the chest radiograph appearance. Model fit will be assessed using likelihood ratio test for nested models and Akaike's Information Criterion (AIC) for non-nested models.

All data will be analyzed using STATA 13.1 (StataCorp, College Station, TX, USA).

A.3 Ethical considerations

A.3.1 Harm

There will be minimal risks to the participants. Phlebotomy will be conducted by a trained phlebotomist, ensuring that there is minimal pain incurred and low risk of soft tissue injury or hematoma. Chest radiographs are safe with minimal radiation exposure as according to the *United Nations Scientific Committee on the Effects of Atomic Radiation* a single chest radiograph exposes the patient to about 0.05 mSv, which is about the radiation dose people are exposed to naturally over the course of 10 days.

For the study personnel there is a risk of needle stick injury. This risk will be minimized by proper training on phlebotomy. If such an injury does occur then post-exposure prophylaxis, in the form of anti-retrovirals, and appropriate counselling and follow up will be provided for the staff member.

A.3.2 Autonomy

During the informed consent procedure, it will be emphasized to the participant that the choice to partake in the study is entirely at their own discretion and that their clinical care will not be negatively affected if they choose not to participate. They will also be provided with the choice to withdraw at any point.

A.3.3 Benefit

Participants will be screened for DM which is not part of routine TB care. They will be referred to the appropriate facility for further care if they are found to be diabetic.

Participants will be compensated with R30 for travel expenses.

A.3.4 Informed consent process

Informed consent will be taken by a trained community research worker. It will be conducted in the language most familiar to the participant. The nature of the study, procedures, risks, benefits and option to withdraw at any point will be explained to the participant. Informed consent documents will be signed by both the CRW and the participant and dated. For those participants who cannot sign, a thumb print will be taken. A copy of the informed consent document will be given to the participant to keep.

A.3.5 Privacy and confidentiality

Data collected is of a sensitive nature therefore privacy and confidentiality is of utmost importance. We will ensure that all data collected will be kept confidential by undertaking the following measures:

- 1) The participant's name will not be linked to the case report form (CRF) and each participant will be issued with a unique participant identification number and chest radiographs and samples will be labelled with this number;
- 2) all interviews and procedures will be conducted in a private space;
- 3) CRFs will be kept onsite in a locked filing cabinet with access only to the necessary study personnel.

A.4 Dissemination of results

Currently there are no clear screening guidelines to screen persons with DM for TB. This study will contribute to the diagnosis of TB in persons with diabetes and interpretation of ambiguous chest radiographs in persons with diabetes. The results will be written up and disseminated to the clinicians at Ubuntu TB clinic. We will also disseminate nationally and internationally through academic meetings and publication in a peer-reviewed journal.

A.5 Logistics

A.5.1 Timeline

Participants were recruited between July 2013 and October 2015, as part of the parent TB and DM study. Data collection was completed in September 2015.

A.5.2 Budget

The parent study was funded by a Wellcome Trust Strategic Funding Clinical Infectious Disease Research Initiative Fellowship to Dr Oni (Fund number: 412300). No additional funding is needed for this study.

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Part B: Literature Review

B.1 Introduction

Tuberculosis (TB) is an ancient disease, which has afflicted humanity for centuries. With the advent of contemporary public health measures and the discovery of effective chemotherapy in the mid 20th Century, TB became a treatable disease and incidence declined(1). However, TB rates in the 1990's began to climb again fueled by the Acquired Immune Deficiency Syndrome (AIDS) epidemic, especially in Sub-Saharan Africa(2). In 2015, 10.4 million people fell ill with TB globally(3), ranking TB as a leading cause of death worldwide, alongside Human Immunodeficiency Virus (HIV)(3).

TB has been highlighted by the United Nations under their Sustainable Development Goals (SDG) – under SDG 3: “Ensure healthy lives and promote well-being for all ages”(4). Included in this goal is the ending of the TB epidemic by 2030. Within the End TB strategy, the World Health Organization (WHO) aims to reduce TB deaths by 95% in 2035 (compared with 2015) and reduce the incidence rate to <10/100 000(5). These are ambitious targets, which can only be achieved through multi-sector collaboration.

Major strides have been made in TB control over the past two decades with a drastic decline in the mortality and incidence of TB, especially in the developed world(3). Unfortunately, approximately 4.3 million TB cases are missed by health care systems annually(3). Additional interventions, as well as improved implementation of existing programs, targeting populations who are vulnerable and at high risk of developing TB are needed. These populations include those who are living with HIV, prisoners, miners, the homeless, children and other clinical risk groups, such as those living diabetes (DM)(6).

Due to the epidemiological transition there is a changing burden of disease in the developing world. Non-communicable diseases (NCDs) now contribute to 50% of the morbidity in Sub-Saharan Africa(7), where HIV and TB is most prevalent. This increase in NCDs is due to rapid urbanization, accompanied by decrease in physical activity and the increase in consumption

of processed food and related obesity(8). South Africa has not been excluded from this NCD epidemic. NCDs make up four of the top 10 causes of death with diabetes moving from tenth to sixth position between 1997 and 2012(9). This changing burden of disease emphasizes the importance in understanding the interactions between NCDs and prevalent communicable diseases, in order to prevent disease spread and control disease burden.

B.1.1 Populations at risk

Both HIV and DM are well-recognized risk factors for developing TB. South Africa has the largest HIV epidemic worldwide with approximately 7 million people living with HIV and a population prevalence of 19.2%(10). People living with HIV are 26 times more likely to develop TB than those who are uninfected(3). TB incidence declines with the administration of ART but TB risk remains high(11). The adult DM prevalence in South Africa has been estimated at 7%(12) and due to the epidemiological transition this prevalence is set to increase. In South Africa, glycaemic levels in persons with diabetes are often poorly controlled(13), potentially increasing their risk of developing TB. A recent systematic review found that those living with DM have as much as three times greater risk of developing TB(14). This review had limited data on TB risk in Africa, where TB burden is highest with studies conducted in Africa since this review was published confirming this risk(15). The estimated proportion of TB due to DM in 2030 will be 12.6% (95% CI 9.2- 17.3%)(16) and a recent study conducted in Khayelitsha estimated that the population attributable risk of TB due to DM in this population was 14% (Oni et al, in press). Worryingly, a large percentage of DM in TB patients is undiagnosed(17, 18). While both HIV and DM are individually prevalent in South Africa, there is the potential for increasing co-prevalence as the HIV-infected population ages.

B.1.2 The utility of the chest radiograph in the diagnosis of TB

Early detection and treatment of TB is one of the pillars of the WHO End-TB strategy(5). Delayed diagnosis results in increased morbidity, mortality and transmission of TB(19-21). To aid rapid diagnosis it is essential to understand the clinical and radiographic presentation of TB, and how common comorbidities influence this presentation, as atypical presentations

may result in delayed diagnosis(21, 22). Chest radiographs have been a part of screening and diagnosis of TB since the early 20th century(23). The chest radiograph has been found to be highly sensitive in detecting TB but it's specificity is low(24). Modern technologies such as GeneXpert molecular diagnosis have revolutionized the diagnosis of TB but chest radiography still plays an important role. Chest radiographs remain essential in targeted high risk population screening(6) and in the diagnostic algorithm for smear negative TB(25, 26). Chest radiography is still a cost effective modality in low resource regions where GeneXpert may not be freely available(24).

The reliability of chest radiography as a TB diagnostic tool is reduced by inter-reader variability and radiographic presentations depends on underlying immune status and other co-morbidities. To address this, in 2005, the chest radiographic reading and reporting system (CRRS) was developed in Cape Town, with the aim of decreasing inter-observer discrepancies in chest radiograph reading and reporting for epidemiological studies(27). The reliability of this tool in diagnosing culture positive TB with typical radiographic manifestations has been validated in a the clinical setting(28). The limitation of this tool is that sensitivity and specificity declines when radiographic presentation of TB is atypical, such as in HIV-infected patients(28).

B.1.3 Typical radiographic features of TB

TB in adults classically presents in one of two ways on chest radiograph. Most common is the post primary pattern, which includes apical infiltrates and/or cavities in the upper lobes or infiltrates in the apical segments of the middle/lower lobes. Less common is the primary (atypical) pattern, presenting with atypical features on chest radiograph such as hilar and/or mediastinal adenopathy, diffuse reticulonodular infiltrate, as well as infiltrates and cavities of the lower/middle lobes or normal radiographs. Other patterns of presentation on chest radiograph include miliary pattern and pleural effusions(29).

B.2 Objectives

The primary objective of this literature review was to identify literature describing the chest radiographic manifestations of pulmonary tuberculosis in adult patients with drug sensitive or resistant tuberculosis and comorbid i) DM, ii) HIV and iii) DM and HIV.

The secondary objectives were to a) identify other parameters which may influence radiographic presentation in this population, and b) identify the most common atypical radiographic features within each group.

B.3 Methods

B.3.1 Search strategy

The Pubmed online database was used to search for relevant original studies and reviews. We searched using the following terms: “tuberculosis”, “diabetes mellitus or diabetes”, “radiograph*”, “chest x-ray”, “HIV or human immunodeficiency virus”. Combinations of the terms were used to find literature specific for:

- I. Features of TBDM chest radiography
- II. Features of tuberculosis and HIV (TBHIV) chest radiography

The bibliographies of relevant literature were also reviewed for possible studies to include in the literature review.

B.3.2 Inclusion criteria

All studies included were published in English. Studies were conducted on adult participants with a confirmed microbiological diagnosis of pulmonary tuberculosis. Definitions of tuberculosis, HIV and diabetes diagnosis needed to be provided. Outcomes needed to include analysis or description of chest radiograph features of pulmonary tuberculosis. Both descriptive and analytical studies were included. Literature that was accessible through the University of Cape Town (UCT) Library was included.

B.3.3 Exclusion criteria

Studies conducted on minors and that were not published in English were excluded.

B.3.4 Study Assessment

Studies were critically reviewed and assessed according to the study design presented and methodology. Limitations and potential areas of bias and confounding were assessed for each study and considered when discussing the literature.

B.4 Results

For the TBDM related search our search strategy identified 303 articles. Of these 21 met our inclusion criteria. An additional 2 studies were identified from the bibliographies of articles included in the review.

For the TBHIV related search, our search strategy identified 596 articles. Of these 40 met our inclusion criteria. 21 studies were not accessible through UCT libraries.

B.4.1 Radiographic features of TB in HIV-infected patients (TBHIV)

The literature divides the chest radiograph presentation of TB by typical (post primary) and atypical (primary) patterns, as well as individual features. Table B-1 lists the atypical features that each study found to be associated with HIV-infection. Cavitation was the most frequent presentation of pulmonary TB, irrespective of HIV infection (28-30, 32, 34, 37) with a prevalence ranging from 13%(30) – 70.8%(31) in HIV infected patients, and 38,8%(32) – 91,5%(31) in uninfected patients. All of the literature found that HIV-infected patients were more likely to have an atypical chest radiograph presentation(30-42). Table B-1 summarizes the literature found, which describes the radiographic differences between HIV-infected and uninfected patients with pulmonary TB. The literature found HIV-infected patients were more likely to have the following atypical features: adenopathy(30, 31, 33-35, 37, 38, 40, 42), middle/lower lobe infiltrates(35, 36, 39, 41), lobar consolidation(31, 37, 38) and lack of cavities(30-38, 40, 41). HIV-infected and non-infected patients had similar a predisposition

for pleural effusions and miliary pattern (30-34, 36, 38-40). The prevalence of these features amongst HIV-infected patients varied in the literature as follows: adenopathy 20,4%(31)– 31,1%(34), middle/lower lobe infiltrates 19%(35) – 48,8%(41), lobar consolidation 10%(37) – 42%(30), pleural effusions 11%(33)– 43%(34) and military pattern 6%(38) – 34,2%(32).

Three studies consolidated the atypical features into a count of overall atypical chest radiograph(31, 33, 39). The prevalence of having an overall atypical chest radiograph ranged from 14,2%(31) – 37,3%(33) in HIV-infected patients, and 0%(31) -23%(39) in HIV-uninfected patients. The only other risk factors which were also associated with HIV-infection in these studies were a lower level of sputum smear positivity(39) and lower tuberculin skin test positivity(33); a higher prevalence of drug use(31).

Table B-1: Summary of Studies Comparing Chest Radiograph Features of TB in HIV-infected and HIV uninfected Patients

AUTHOR	YEAR OF PUBLICATION	STUDY DESIGN	CITY, COUNTRY	STUDY SETTING	SAMPLE SIZE (HIV+/HIV-)	HIV+ MORE LIKELY TO HAVE ATYPICAL/ PRIMARY FEATURES ON CXR (YES/NO)*	HIV+ PREDOMINANT RADIOGRAPH FEATURES	FEATURES ASSOCIATED WITH IMMUNOSUPPRESSION
LONG ET AL(33)	1991	Cross-sectional	Haiti	Hospital	67/158	Yes	Adenopathy, Normal Radiograph	AIDS presents more frequently with primary/ atypical disease pattern***
BATUNGWANAYO ET AL(34)	1992	Cross-sectional	Kigali, Rwanda	Hospital	48/11	Yes	Adenopathy, Pleural Effusion	-
POZNIAK ET AL(35)	1995	Cross-sectional	Harare, Zimbabwe	TB Unit, City Health Department	202/220	Yes	Adenopathy, Pleural Effusion, Fewer Cavities	-
MLIKA-CABANNE ET AL(36)	1995	Cross-sectional	Tanzania and Burundi	Hospital	185/119	Yes	Adenopathy, Consolidation, Nodular Pattern, Fewer Cavities	-
TSHIBWABWA ET AL(37)	1997	Case control	Zaire and Zambia	Hospital	963/1000	Yes	Adenopathy, Pleural Effusion, Interstitial Pattern, Miliary Pattern, Lobar Consolidation	-
AWIL ET AL(38)	1997	Cross-sectional	Gulu District, Uganda	Hospital, In- and Out-Patient	92/61	Yes	Adenopathy, Lobar Consolidation	-
JOHNSON ET AL(39)	1997	Nested cross-sectional	Kampala, Uganda	Hospital	212/111	Yes**	Lower Lung Field Infiltrate	-
DE ALBUQUERQUE ET AL(40)	2001	Cross-sectional	Pernambuco, Brazil	Hospital Outpatient	39/236	Yes	Miliary Pattern, Adenopathy, Pleural Effusion*	-
ADERAYE ET AL(41)	2004	Cross-sectional	Addis Ababa, Ethiopia	Hospital Outpatient	94/69	Yes	Miliary Pattern, Interstitial Infiltrate, Pleural Effusion	-

PICON ET AL(31)	2007	Cross-sectional	Porto Alegre, Brazil	Hospital	113/118	Yes	Multifocal Disease, Adenopathy	-
CHAMIE ET AL(42)	2010	Cross-sectional	Kampala, Uganda	TB Referral Centre	873/1141	Yes	Normal Radiograph, Miliary Pattern, Adenopathy, Pleural Effusion	CD4 <150 more likely to have normal radiograph, miliary pattern, adenopathy and pleural effusion
ANGTHONG, ANGTHONG(32)	2011	Cohort	Nakon Nayok, Thailand	Hospital	38/139 (CD4 >200: 5; CD4 <200: 33)	Yes	Diffuse Nodular Opacities, Miliary Pattern	CD4 >200 more likely to have cavitation, mass lesion. CD4 < 200 more likely to have diffuse nodular opacities, miliary pattern
KISEMBO ET AL(30)	2012	Cross-sectional	Kampala, Uganda	Hospital	186/27 (CD4 >50: 80; CD4 <50: 102)	Yes	Miliary Pattern, Adenopathy	CD4 > 50: more consolidation, more cavities CD4 <50: more adenopathy

* Proportion of HIV infected participants with an atypical feature of tuberculosis on chest radiograph was greater than in HIV uninfected participants.

**Not statistically significant

***This study did not use the CD4 cell count as a measure of immunosuppression and stratified participants according to clinical AIDS status.

B.4.2 Radiographic features of TB in patients with Diabetes (TBDM)

Table B-2 summarizes the literature found describing the radiographic features of PTB in patients with diabetes. The most commonly reported findings on chest radiograph were the presence and location of lobar opacifications/lesions and cavities. Overall there was some variation in the findings in the literature with regards to the chest radiograph presentation of TB in patients with DM, however the majority of the literature found that TBDM patients had a predominance of lower lung field involvement and more frequent cavitation. Seventeen studies reported on location of opacifications/lesions related to TB. Of these, twelve found a predominance of lower lung field involvement in TBDM patients(43-54), with a prevalence ranging between 15,2%(50)– 29%(47). Of the eighteen studies that reported on the presence of cavitation, twelve studies found that TBDM patients have more frequent cavitation than non-TBDM patients(45, 47, 48, 50, 51, 53-59), with a prevalence of 19,2%(55) – 50,8%(60).

A few studies commented on other chest radiograph features of TB. Here, findings varied substantially. Jabbar(49) found that 32% of the diabetics in his sample had pleural effusions but he had no control group, whereas Tatar(56) found that TBDM patients were more likely to have pleural effusions. Both Wang(50) and Alavi(57) contradict this and found no association between TBDM and the presence of pleural effusion on chest radiograph. Wang(50) did find that TBDM was associated with more consolidation, with Alavi(57) finding more frequent miliary patterning in TBDM patients.

Table B-2: Summary of Studies Comparing Chest Radiograph Features of TB in Patients with and without Diabetes

AUTHOR	YEAR OF PUBLICATION	STUDY DESIGN	CITY, COUNTRY	STUDY SETTING	PREVALENCE OF DIABETES IN TB CASES	PREDOMINANCE OF LOWER LUNG FIELD INVOLVEMENT	MORE LIKELY TO HAVE CAVITATION
WEAVER(43)	1974	Cross sectional	Atlanta, Usa	TB Hospital	11% (23/205)	Yes	-
MARAIS(44)	1980	Cross sectional	Pelonomi, South Africa	Pelonomi Hospital	2.1% (9/436)	Yes	-
JAIN ET AL(61)	1985	Cross sectional	Jaipur, India	Hospital	6.9% (16/230)	No	No
MORRIS ET AL(46)	1992	Case Control	San Antonio, Usa	Chest Hospital		Yes	No
UMUT ET AL(45)	1994	Case Control	Istanbul, Turkey	Unclear – Possibly Hospital		Yes	Yes
AL-WABEL ET AL(62)	1997	Case Control	Abha, Saudi Arabia	Hospital		No	No
PEREZ-GUZMAN ET AL(47)	2001	Case Control	Mexico City, Mexico	Chest Hospital		Yes	Yes
SHAIKH ET AL(48)	2003	Cross sectional	Riyadh, Saudi Arabia	Chest Hospital	27% (187/692)	Yes	Yes
WANG ET AL(55)	2005	Cross sectional	Taiwan	Tertiary Care Referral Centre	21.5% (99/461)	No	Yes
JABBAR ET AL(49)	2006	Case Control	Karachi, Pakistan	Tertiary Hospital		Yes	-
TATAR ET AL(56)	2009	Case Control	Izmir, Turkey	TB Hospital	7.3% (78/1063)	-	Yes
WANG ET AL(50)	2009	Cross sectional	Kaohsiung, Taiwan	Tertiary Hospital With Primary Care Component	34% (74/217)	Yes	Yes
OZSAHIN ET AL(63)	2011	Cross sectional	Kastamonu, Turkey	TB Hospital – Males Only	10.1% (81/800)	-	No
CARREIRA ET AL(51)	2012	Case Control	Lisbon, Portugal	Respiratory Hospital		Yes	Yes
ALAVI ET AL(57)	2014	Cross sectional	Iran	TB Referral Hospital	24% (36/149)	-	Yes

SINGH ET AL(52)	2015	Cross sectional	India	Tertiary Care		Yes	-
MORENA- MARTINEZ ET AL(58)	2015	Cross sectional	Barcelona, Spain	All Patients On Program For Prevention And Control Of TB In Barcelona	5.9% (349/5849)	-	Yes
GIL-SANTANA ET AL(64)	2016	Case Control	Brazil	Primary Care		No	No

B.4.3 Radiographic features of TB in patients with diabetes and HIV (TBDMHIV)

There was a paucity of literature describing radiological features of TB in patients with both DM and HIV (TBDMHIV). Only one of the studies describing features of TB in HIV patients included recording DM status within the methods, and this was retrospectively retrieved from case records, however the results did not report DM prevalence in this cohort(31). Of the TBDM literature, nine studies mentioned HIV status. Of these, eight were retrospective record reviews. Four of these studies reported zero HIV-infected DM patients(46, 47, 50, 53) and three excluded HIV-infected patients from their sample(51, 57, 59). One study, conducted in Brazil, reported 98% of TBDM patients were tested for HIV with zero testing positive(64).

B.4.4 Additional variables influencing radiographic features of TB on chest radiograph

The literature search identified four additional variables that may alter the appearance of TB on chest radiograph. These were age, glycemic control, degree of immunosuppression and smear status.

The effect of age and glycemic status is summarized in Table B-3. We found two studies that investigated the association between glycaemic control in TBDM patients and radiographic manifestations of pulmonary TB. Both these studies showed that TBDM patients with good glycemic control (defined as an HbA1c <7.0%) had more normal radiographic presentations(54, 59).

Age has also been shown to modify the chest radiographic presentation of TB. Al-Tawfiq(65) found that older patients (>60 years) with TB presented with fewer cavities and more frequent mid- and lower zone lesions. This is similar to Chiang's(54) finding in 2014. Contrary to this finding, when stratifying by DM status, Perez-Guzman(53) found that older (>50 years) TBDM patients had a higher frequency of cavitation than their non-DM counterparts. They also found that lower lobe lesions increased with age for both TB and TBDM but more so with TBDM(53).

Table B-3: Summary of Studies Describing Effect of Age and Glycemic Control on Radiographic Presentation

AUTHOR	YEAR OF PUBLICATION	STUDY DESIGN	CITY, COUNTRY	STUDY SETTING	EFFECT OF AGE ON RADIOGRAPHIC PRESENTATION	EFFECT OF GLYCEMIC CONTROL ON RADIOGRAPHIC PRESENTATION
PEREZ-GUZMAN ET AL(53)	2000	Case Control	Mexico City, Mexico	Respiratory Hospital	Increase prevalence of lower lobe lesions for both TBDM and TB. TBDM have a higher prevalence of cavitation at an older age.	-
AL-TAWFIQ ET AL(65)	2009	Cross-Sectional	Dhahran, Saudi Arabia	Saudi Aramco Medical Services Organization	>60 years old had fewer cavities	-
PARK ET AL(59)	2012	Cross Sectional	Seoul, South Korea	Hospital Patients	-	Uncontrolled DM had more frequent cavities than non-DM
CHIANG ET AL(54)	2014	Case Control	Multiple Sites, Taiwan	Tertiary Hospital	>65 had higher prevalence of lower lung field opacity & fewer cavities (DM And Non- DM)	Where controlled TBDM had no increased risk, uncontrolled DM had 2.32 (95% CI 1.36 - 3.98) times greater risk of lower lung field opacities and 1.84 (95% CI 1.20–2.84) times greater risk of cavitation as compared to non-DM TB patients.

The degree of immunosuppression also appears to have an effect on chest radiograph features of TB. Presence of AIDS and CD4 count were used as a marker of immunosuppression in much of the TB/HIV literature. The effect of CD4 count is summarized in Table B-1 and Table B-4.

Long(33) found that there were no differences between HIV-infected patients with and without AIDS. This finding could be due to the small sample size of AIDS patients (10/225) entered into the study. By contrast, Batungwanayo(34) found that AIDS patients had more adenopathy and less cavitation. This study had a higher proportion of AIDS participants (73% of the HIV-infected cohort, 59% of the total sample). In these studies, there is a risk of misclassification of AIDS status as participants were classified using a clinical syndrome rather than a measurable biological marker, such as CD4 count. This may have contributed to the varied findings.

Later literature used the CD4 count as measure of immune status. These studies found that chest radiographs of those patients with a CD4 count of <200 cells/mm³ had more atypical features (or features of primary TB infection) than those participants with a better preserved immune system(30, 32, 33, 42, 66-70). Keiper(67) found that while a lower CD4 count was significantly associated with a more atypical pattern, the CD4 count for the typical chest radiographic TB pattern had a wider range (1- 990 vs 4 – 50). A study conducted in South Africa found that adenopathy, normal radiograph, reticulo-nodular pattern and lower- or mid-zone infiltrate had high specificities (76 – 100%) for CD4 <200 cells/mm³ (66).

Table B-4: Summary of Studies Describing Immune Status on Radiographic Presentation

AUTHOR	YEAR OF PUBLICATION	STUDY DESIGN	CITY, COUNTRY	STUDY SETTING	SAMPLE SIZE (CD4 >200 CELLS/MM ³ /CD4 <200 CELLS/MM ³)	EFFECT OF CD4 COUNT
POST ET AL(66)	1995	Case Control	Cape Town, South Africa	Hospital	150*	CD4 < 200: adenopathy, diffuse reticulonodular pattern, miliary pattern CD4>200: Upper zone infiltrates
KEIPER ET AL(67)	1995	Cross-Sectional	Philedelphia, United States	Hospital	11 / 26	CD4 <200: atypical pattern CD4 >200: typical pattern
ASIMOS ET AL(68)	1995	Cross-Sectional	Charlotte, United States	Hospital	7 / 16	CD4 < 200: atypical pattern CD4 >200: typical pattern
PERLMAN ET AL(71)	1997	Nested Cross Sectional	US Multicentre		30 / 98	CD4 < 200 had more adenopathy
ENG SAN, MUHAMAD(69)	2000	Cross-Sectional	Kota Bharu, Malaysia	Hospital	62 / 18	CD4 <200 more atypical pattern
BUSI RIZZI ET AL(72)	2003	Retrospective Cross Sectional	Italy	Hospital	50 / 159	Higher CD4 (on HAART) had post primary/typical pattern
DA SILVA ET AL(73)	2006	Cross-Sectional	Florianopolis, Brazil	Hospital	87*	CD4 <200 had more adenopathy
GARCIA ET AL(70)	2007	Cross-Sectional	Brazil	Hospital	11 / 27	CD4 <200: primary pattern CD4 >200: post primary pattern

*No further details given

Two studies assessed the association between sputum status and chest radiograph appearance. Both Al Tawfiq(65) and Ozsahin(63) found that positive sputum smears and cultures were associated with cavitation on chest radiograph. In addition to this Al Tawfiq(65) also noted that positive smears were associated with upper lobe infiltrates.

B.5. Discussion

B.5.1 TBHIV and TBDM alter the radiographic manifestations of PTB

This review reveals that both TBHIV and TBDM patients have unique TB presentations on chest radiograph. TBHIV patients typically have an atypical or primary infection presentation (which includes lower lung field involvement), with TBDM patients more likely to have lower lung field lesions and increased cavitation. While pleural effusions and miliary patterning were similarly distributed in HIV-infected and uninfected patients, the literature varied in this regard when comparing TBDM patients to non-DM TB patients.

Much of the TBDM literature did not account for HIV status. Apart from one study (which found zero HIV infection in the TBDM cohort) HIV status was either not mentioned, excluded, or not actively screened for (mostly due to the retrospective nature of the studies). As discussed earlier, with rising DM prevalence in high HIV and TB burden settings, we would expect a fair proportion of TB patients to have both HIV and DM. We would therefore expect that patients with TB, DM and HIV would also have an atypical radiographic presentation, the pattern of which may be unique to this population. This omission may account for some of the conflicting findings in the TBDM literature biasing the results.

B.5.2 Relevance of individual patient characteristics for the clinical interpretation of TB chest radiography

Studies by Batungwanayo(34), Mlika-Cabanne(36) and Aderaye(41) found that even though HIV-infected patients with TB often present atypically, the most common features of TB on chest radiograph in HIV-infected participants were similar to those in HIV-uninfected participants – those being cavitation, upper lobe infiltrates and parenchymal opacifications. This suggests that other factors may modify the features found on chest radiography of HIV-infected TB patients.

Chest radiography is used for both screening and diagnosis of TB. The literature suggests that in addition to HIV and DM, age, glycemic control, level of immunosuppression and smear status make the TB chest radiograph present more atypically. We have shown that TBDM and

TBHIV can present atypically on chest radiograph, however there is still a lot of typical radiographic presentation in these groups. Atypical presentations may be exaggerated in studies which erroneously select for one of the potential confounders or effect modifiers mentioned above.

For sensitive screening and accurate diagnosis clinicians need to understand the patterns of atypical presentation, and the variables associated with it. Ignorance of this may lead to a delayed or missed TB diagnosis, worsening morbidity and mortality. In circumstances where chest radiographs are being used for screening, algorithms may need to be adjusted for specific population groups.

B.5.3 Comparability to the South African setting and Limitations

While there is a definite pattern of atypical chest radiograph presentations for both TBDM and TBHIV patients the studies varied by location, population, case definitions and radiograph reporting methods.

Our study was conducted at a Primary Care clinic in Khayelitsha, a peri-urban South African township with a high TB and HIV burden. Amongst TB patients, TBDM co-prevalence has been reported to be 12.6% (95% CI 9.7–16.1%), with more than half of the TBDM patients also having HIV infection (Oni et al, in press). The literature varied substantially to our setting.

All the literature describing radiographic manifestations of TB in HIV-infected patients came from high TB incidence regions. This is in contrast to the TBDM literature where only 5(44, 49, 52, 61, 64) out of the 24 studies reviewed were conducted in regions with a high TB incidence. The DM prevalence in TB in these studies varied considerably (ranging from 2.1% (50) to 34%(55)). This may contribute to the variability of the findings amongst the TBDM literature.

The majority of the studies reviewed were conducted in a hospital setting. Participants in a hospital setting may be more morbid than those enrolled in a Primary Care setting. This may

impact on chest radiograph features, as their disease may be more advanced than what is found at the Primary Care level biasing the results.

While most of the literature used reliable and valid methods to diagnose TB and HIV, of concern is the variability in the literature of DM definition. Most of the studies were retrospective record reviews. In these studies, participants were classified as diabetic if records described a prior diagnosis of diabetes, use of anti-glycemic medication and/or a fasting blood glucose or OGTT result compatible with diabetes. Only 2 studies were prospective(52, 61) and performed DM screening on TB patients. The retrospective nature of the majority of the studies could lead to unreliable estimate of DM prevalence in the cohorts examined, as DM may be under diagnosed in regions where screening for DM in TB patients is not routine. Misclassification of TBDM patients could weaken the association between atypical TB chest radiograph features and TBDM.

The method and tools used to report chest radiograph findings varied substantially in the literature. There was no standardized or validated tool used in the majority of the studies, and while experts were most often asked to report on the radiographs, terminology and features reported often differed. This makes the comparison of some features difficult as they may have been under-reported as only a small number of studies explored these features. A standardized reporting method would make the literature increasingly comparable and valid. In regions with a low TB prevalence clinicians may be unfamiliar with the typical TB chest radiograph, increasing the risk of misclassification of radiographic features.

Our literature search was restricted to papers available on the UCT libraries database. There was a large body of earlier research that was not available to review. These omitted papers were mostly from the TBHIV literature. However, there was a strong consensus on the features of chest radiography of TB in HIV amongst the literature reviewed, hence we do not expect the omission of the unavailable resources to bias our results.

B.6 Conclusion

In this review, we have described the common radiographic findings of TB in both patients living with HIV or DM. We have found that patients with TBHIV have more atypical chest radiographs and that this may vary by immune status. Additionally, TBDM patients have more cavities and lower lobe infiltrates than the general TB population and this presentation may change according to glycemic control. Age and level of immunosuppression may be additional variables affecting chest radiograph features of TB.

We have identified that both DM and HIV modify the presentation of TB on chest radiograph and that an association with lower lobe lesions may be common to both TBDM and HIV-TB patients. Although there is global recognition of the rising threat of co-morbid HIV and NCDs, there is a distinct lack of literature surrounding TBDMHIV patients. Out of 63 studies reviewed only 9 alluded to TBDMHIV co-morbidity, and amongst these none revealed a high co-prevalence. Lack of HIV testing in DM patients or DM screening in TB patients may have resulted in this finding, as well as the low HIV prevalence in regions where the TBDM studies were conducted. With increasing co-morbidity of HIV and NCDs in high HIV prevalence settings clinicians need to understand how these two diseases interact, especially with TB and the features thereof.

Our study is a unique opportunity to examine the relationship between DM, HIV and TB in a high TB, HIV and DM prevalence setting. We recognize that there is a lack of literature describing TBDM and TBDMHIV chest radiography in this setting, where the TB burden is one of the highest in the world. Results from this study will provide clinicians with essential information to aid in the diagnosis of TB, which may otherwise be missed. This study aims to identify radiographic manifestations of TB in persons with DM, and how HIV co-infection influences this presentation.

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Effect of Diabetes and HIV on the radiographic manifestations of Pulmonary Tuberculosis

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Key Points:

- Diabetes predisposes to lower lung field lesions on the tuberculosis chest radiograph.
- Diabetes and HIV comorbidity modify this presentation and is associated with isolated lower lung field lesions.
- These findings can be used in bidirectional tuberculosis screening algorithms

Keywords: Diabetes (DM), Pulmonary Tuberculosis (pulmonary TB), Chest Radiography, HIV

Running title: Effect of DM and HIV on PTB radiographs

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C.1 Abstract

Background

The chest radiograph remains an important tool for tuberculosis (TB) screening and diagnosis. HIV and diabetes (DM) are well-recognized risk factors for TB, and while many studies have described the independent effects of DM and HIV on the chest radiographic presentation of TB, none have described the effect of DM and HIV comorbidity.

Methods

Posterior-anterior chest radiographic examinations were conducted on 337 pulmonary TB patients from primary care clinics in Khayelitsha, Cape Town. Features of chest radiographs of patients with pulmonary TB only (n=84) were compared to patients with comorbid HIV (TBHIV, n=199), DM (TBDM, n=21), and HIV and DM (TBDMHIV, n=33).

Results

Compared to TB only, participants with TBDM had a higher proportion of lower lung field (LLF) cavitation (23,8%; 95% CI: 3,9–43,7%; p=0,041) and were 3,92 times more likely to have LLF cavitations. TBDMHIV had higher proportion of isolated LLF lesions as compared to TB only participants (15,2% vs 3,6%; p=0,039). As CD4 counts increased, there was an upward trend towards an increase in the proportion of cavitations for TBDMHIV participants, but this was not evident in TBHIV participants.

Conclusions

This study reveals the atypical nature of chest radiographs in persons with TBDM, TBHIV and TBDMHIV. The presence of DM drives the LLF cavitation in TB chest radiographs. HIV and DM comorbidity alters this radiographic pattern, although isolated LLF lesions are still more prominent in TBDMHIV. These findings should inform bidirectional TB screening algorithms in patients with DM with or without HIV-infection, and highlights the important role of radiographic examination in TB screening in populations undergoing epidemiological transition.

C.2 Background

Although tuberculosis (TB) infection rates are declining globally, TB still contributes to significant morbidity and mortality worldwide, with 10.4 million individuals falling ill from TB in 2015(1). While HIV is a well-recognised risk factor for the development of TB, diabetes (DM) has recently been highlighted as an additional significant risk factor(2). This is increasingly pertinent as the HIV-infected population is becoming ever more at risk of developing non-communicable diseases such as DM. This is due to prolonged lifespans secondary to improved access to effective antiretrovirals (ARVs), biomedical interactions and societal and behavioural changes (such as increasing urbanisation and more sedentary lifestyles)(3, 4).

Early detection and treatment of TB is essential, and is one of the pillars of the World Health Organisation (WHO) End-TB strategy(5). Studies have shown that decreasing the delay between presentation, diagnosis and treatment reduces TB transmission and improves patient outcomes(6, 7). New diagnostic technologies, such as GeneXpert, may decrease the delay to treatment through rapid diagnosis(8), however chest radiography still has a role in TB diagnosis. The WHO recently released recommendations on the use of chest radiography in TB diagnosis and screening(9). Where the chest radiograph was previously placed at the end of diagnostic algorithms, its importance higher up in the algorithm is now recognised, especially when bacteriological confirmation of disease is not available(10). Clinicians need to be adept at reading and interpreting pulmonary TB (PTB) chest radiographs so as not to delay diagnosis and treatment. Undiagnosed TB may result in increased morbidity and mortality, especially in HIV-infected patients(11). The rapid epidemiological transition occurring in many countries with a high TB burden is accompanied by a change in patterns of TB co-morbidity with an increasing prevalence of TB/DM/HIV comorbidity. This changing patterns of disease, impact on the clinical presentation of PTB including the chest radiograph presentation.

The chest radiographic presentation of PTB in the HIV-infected population has been well described with most patients presenting with features of primary infection, such as hilar adenopathy, pleural effusions, lobar consolidation and miliary disease on chest radiograph(12), with increasingly atypical features as CD4 counts decline(13, 14). Similar

studies have been conducted in patients with DM and TB (TBDM)(15, 16). However, the majority of these studies have been conducted in areas of low TB incidence with only one early, small study being from South Africa(17). TBDM patients have been reported to present with more lower lung field opacifications and increased cavitation(15, 16, 18). It has been suggested that the abnormalities found in both HIV-infected patients with TB (TBHIV) and TBDM patients are due to immune dysregulation and suppression of cell mediated immunity(19). The combined effect of DM and HIV comorbidity on the chest radiographic features of PTB has not yet been described, as the TBDM literature have either excluded HIV-infected participants or have been retrospective in nature, with no active screening for HIV.

The primary objective of this study is to describe the atypical chest radiographic features of PTB in patients with comorbid DM in a population with a high TB, DM and HIV burden. The secondary objectives are to investigate the effect of concurrent HIV infection on these characteristics and how disease severity (HIV or DM) effects this presentation.

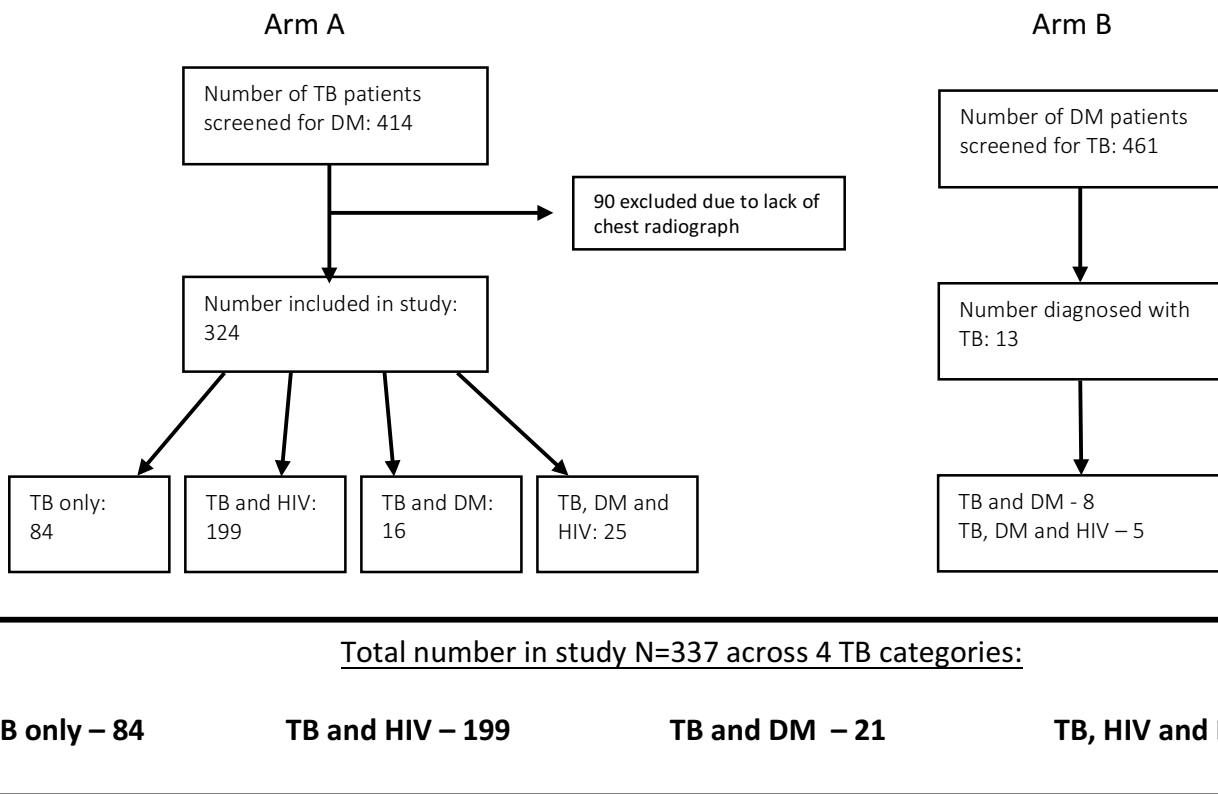
C.3 Methods

C.3.1 Study setting and recruitment

As part of a larger study investigating the association between DM and TB, participants with TB were recruited between June 2013 and October 2015. Participants were recruited from two arms of the parent study: the first arm being a cross-sectional study screening TB patients for DM (Arm A), the second arm, a cross-sectional study screening DM patients for TB (Arm B). All participants were recruited from Khayelitsha, a predominantly Black-African, peri-urban township in Cape Town, South Africa, with a population of approximately 390,000 people(20). In Khayelitsha the TB case notification rate is >1600/100 000(21) with an antenatal HIV prevalence of 37,1%(22), and the DM prevalence is approximately 13%(23). Participants who were recruited from arm A were recruited from Ubuntu clinic, a primary care TB and ARV clinic, whereas the arm B participants were recruited from the DM clinic at Site B primary health care facility. Both clinics are situated on the same property and service the same population.

To investigate the chest radiographic features of PTB in DM patients, we included all participants from both arms of the parent study who had a proven diagnosis of TB (as per national guidelines) and a chest radiograph taken at recruitment. Breakdown of the recruitment process is shown in Figure 1.

Figure C-1: Participant recruitment diagram



C.3.2 Data Collection

Clinical characteristics:

Participants in arm A were screened for DM using a fasting blood glucose (FBG) test, two hour oral glucose tolerance test (2hBG) and glycosylated haemoglobin (HbA1c) test.

Participants were considered diabetic if they had a prior diagnosis of DM, were on oral anti-glycaemic agents or insulin, or met the biochemical criteria of DM by any of the three tests above, as per the American Diabetes Association guidelines (FBG >7.0mmol/l, 2hBG \geq 11.1mmol/l or an HbA1c >6.5%)(24). Participants from arm B were recruited from a DM clinic and all had a prior diagnosis of DM. All participants underwent spontaneous, or if required, induced sputum. Sputum samples underwent GeneXpert processing and drug resistance testing, and were sent for smear microscopy if the GeneXpert result was positive or if participants were HIV-infected. Participants were classified as having TB if they were found to be smear microscopy positive for acid-fast bacilli; have isolation or identification of *Mycobacterium tuberculosis* by culture or Genexpert or have TB compatible clinical signs and symptoms (including typical chest radiograph) and resolution of symptoms after TB treatment. All participants in both arms were offered HIV testing and counselling. A standardized STEPs chronic disease risk factor tool(25) was completed by a clinical research worker for all participants.

Chest radiography:

Posterior-anterior chest radiographic examinations were conducted by an experienced radiographer on all participants. All radiographs were read by two clinicians, a radiologist and an infectious disease research medical officer. Both readers were blinded to the DM and HIV status of the participant. If there was discordance between the two reports, a third reader was used and consensus was reached. A standardized chest radiographic reading and reporting system (CRRS) tool was used to report on all chest radiographs(26). The CRRS has been previously validated for epidemiological research(26) and was chosen to decrease inter-reader variability. It consists of a tick sheet to capture the absence or presence and lobar location of opacifications, cavitations, miliary disease, pleural effusions and lymphadenopathy.

C.3.3 Statistical Analysis

Participant characteristics were described using descriptive statistics. The clinical and radiographic characteristics of participants in the four TB categories were compared using Pearson's chi-squared test and Fischer's exact test, as well as Wilcoxon rank-sum test for continuous data. The most common atypical radiographic features were identified for each TB category. Variation in chest radiographic features by CD4 cell count and glycaemic control were also described. Participants with HIV were categorised into CD4 cell counts of above and below 500cells/mm³ based on national ARV initiation criteria at the time of the study(27) and an HbA1c of 10% or more was considered severe hyperglycaemia. Multivariate logistic regression was used to determine the association between the identified common atypical TB chest radiographic features (lower lung field opacification and consolidation and isolated lower lung field lesions) and DM status, and to examine the potential effect modification of HIV infection on this association through the addition of an interaction term. Smoking status and previous history of TB were found to have significant associations with the atypical presentations and were added to the final models. Odds ratios and 95% confidence intervals were calculated. Significance testing was performed using two sided P-values of <0.05. The presence of upper lobe cavitation is traditionally the most pathognomonic feature of PTB. In our literature review we found that lower lung field cavitation was the most common atypical finding in TBDM. As the prevalence of lower lung field cavitation varied between 50% – 86% for TBDM patients and 20% - 46% for patients with TB only, a sample of 37 TBDM and 37 TB only participants was needed to detect a difference in lower lobe cavitation at a power of 80% and alpha at 0,05(15, 18). All statistical analysis was conducted on Stata 13.1 (StataCorp, College Station, TX, USA).

C.4 Ethics

All participants underwent informed consent in their first language and the study was approved by the University of Cape Town Human Research Ethics Committee (HREC 373/2016).

C.5 Results

A total of 337 participants with TB were included in the study. Participants were stratified by TB category: participants with only TB (24,9%), participants with TB and HIV (TBHIV: 59,1%), participants with TB and DM (TBDM: 6,2%) and participants with TB, DM and HIV (TBDMHIV: 9,8%).

C.5.1 Participant characteristics

Diabetes: There were 54 patients with DM in the study. The proportion of DM patients did not differ between males and females, with 58,7% being female ($p=0,153$). DM participants were overall older than non-DM (median age: 45,4 IQR: 37,0–57,2 vs 35,6 IQR: 29,6–42,1 years; $p < 0,001$), with the TBDM category having the oldest participants amongst the TB categories (Table C-1). DM diagnosis was largely driven by HbA1c (table XX). The median HbA1c for all participants with DM was 7,4% (IQR: 6,6 – 10,9%), with 52,4% of the TBDM and 28,6% of the TBDMHIV participants having had an HbA1c of 10% or greater.

HIV: The overall HIV prevalence in this sample was 68,8% ($n = 232$; 95% CI: 63,9 – 73,8%). A recent CD4 cell count was recorded for 209 of these participants. The overall median CD4 cell count was low (203 cells/mm³; range 2-1172 cells/mm³). No significant differences in CD4 cell count were found by gender, age or DM status. As shown in Table C-1, median CD4 cell counts did not differ between TBHIV and TBDMHIV categories ($p=0,263$).

Given the potential impact of smoking and a previous TB history on chest radiograph presentation, these variables were further explored below:

Smoking: Less than a quarter of the overall sample were smokers (Table C-1). TBDMHIV participants had a significantly lower proportion of smokers (6,1% 95% CI: 2,5 – 14,7%) as compared to any other TB category (Table C-1). Overall and across all four TB categories, males were more likely to smoke than females [35,2% (95% CI: 28,5 – 41,9%) vs 4,2% (95% CI: 0,87 – 7,5%), $p < 0,001$]. Median ages for smokers and non-smokers were similar for all TB

categories, apart from the TB only category where non-smokers were significantly younger than smokers (median age: 28,7 vs 42,0 years; $p=0,045$).

Previous TB: The majority of participants were previously TB naïve (70%; 95% CI 64,7 – 74,9%), with no differences in this proportion in HIV-infected and uninfected participants (31,9% vs 23,8%; $p= 0,131$), or participants with or without DM (22,2% vs 30,7%; $p=0,208$). Participants in the TBHIV category had a higher reported prevalence of previous TB compared to TB only or TBDMHIV participants (Table C-1: $p= 0,027$ and $p=0,026$ respectively). Participants with previous TB were slightly older than TB naïve participants (median age: 37,9 IQR: 32,4–46,4 vs 35,7 IQR: 28,4–43,1 years, $p=0,011$).

Table C-1: Participant characteristics by TB category

	OVERALL (n=337)	TB (n=84)	TBHIV (n=199)	P-value*	TBDM (n=21)	P-value*	TBDMHIV (n= 33)	P-value*
Sex								
Female (%)	145 (43,0)	18 (21,4)	99 (49,8)	0,000	10 (47,6)	0,015	18 (54,6)	0,000
Male (%)	192 (57,0)	66 (78,6)	100 (50,3)		11 (52,4)		15 (45,4)	
Age (median years)	36,6 (30,0- 43,9)	31,0 (25,3 - 42,5)	36,5 (31,5 - 41,6)	0,002	54,8 (45,3 - 60,5)	0,000	40,8 (32,8 - 51,4)	0,005
Age category (%)				0,000		0,000		0,000
<25 years	23 (6,8)	13 (15,5)	5 (2,5)		1 (4,8)		4 (12,1)	
25 – 35 years	109 (32,3)	35 (41,7)	68 (34,2)		1 (4,8)		5 (15,2)	
35 – 45 years	121 (35,9)	19 (22,6)	88 (44,2)		3 (14,3)		11 (33,3)	
>45 years	84 (24,9)	17 (20,2)	38 (19,1)		16 (76,2)		13 (39,4)	
HbA1c - median (IQR) (n = 372)^a	5,8% (5,5 - 6,1)	5,7% (5,5 – 6,0)	5,7% (5,4 - 5,7) (n = 195)	0,772	10% (6,75 - 12,2) (n = 20)	0,000	6,9% (6,5 - 9,5)	0,000
CD4 cell count – median (cells/mm³) (n = 209)^a	203 (85 -332)	NA	203,5 (85 -337) (n=182)	-	NA	-	202 (74-309) (n=27)	
CD4 <500 cells/mm³ (%) (n =209)^a	194 (92,8)	NA	169 (92,9) (n=182)	-	NA	-	25 (92,6) (n=27)	
Smoker (%) (n = 330)^a	73 (22)	22 (26,5) (n=83)	45 (23,2) (n=194)	0,556	4 (20) (n=20)	0,548	2 (6,1) (n=33)	0,014
Previous TB history (%)	99 (29,4)	18 (21,4)	69 (35,2)	0,027	7 (33,3)	0,252	5 (15,2)	0,442
DM diagnosis		NA	NA					
Known DM								
New DM diagnosis	28 (8,3) 26 (7,7)				15 (71,4) 6 (28,6)		13 (39,4) 20 (60,6)	
Microbiological ly confirmed TB	261 (77,4)	66 (78,6)	146 (73,4)	0,356	18 (85,7)	0,347	27 (81,8)	0,483

IQR: inter-quartile range; HbA1c: Glycosylated haemoglobin

*as compared to TB only participants

^awhere participant data are missing, the denominator is provided in parenthesis

Table C-2: Diabetes diagnosis by diagnostic test

		OVERALL (N=54)	NEW DM (N=26)	OLD DM (N=28)
Diagnostic test (%)	FBG	42,6 (29,2 – 56,8)	30,8 (14,2 – 51,8)	53,6 (33,9 – 72,5)
	2hBG	18,5 (9,3 – 31,4)	34,6 (17,2 – 55,7)	3,6 (0,09 – 18,3)
	Hba1c	87,0 (75,1 - 94,6)	76,9 (56,3 – 91,0)	96,4 (81,7 – 99,9)

C.5.2 Radiographic findings

Overall, the most common radiographic finding was opacification (81%; 95% CI: 76,8 – 85,2%), with the distribution of opacification being predominantly upper lobe (UL) (71,2%; 95% CI: 66,4 – 76,1%). Almost half of the sample had cavitation (47,8%; 95% CI: 42,4 – 53,1%), the majority of which were UL. However 10,4% (n=35; 95% CI: 7,1 – 13,7%) of the sample had lower lung field (LLF) cavitation. Although pleural effusions (24%; 95% CI: 19,5 – 28,6%) and lymphadenopathy (37,9%; 95% CI: 32,7 – 43,1%) were found fairly frequently in the overall sample, the proportion of these findings did not vary significantly across the TB categories (Table C-3). The most infrequent radiographic finding was a miliary pattern (2,7%; 95% CI: 0,9 – 4,4%) and this did not vary by TB category (Table C-3).

C.5.2.1 Atypical radiographic presentations

TBDM: All of the TBDM participants had some parenchymal abnormality, with 76,2% (95% CI: 56,3 – 96,1%) and 23,8% (95% CI: 3,9 – 43,7%) having LLF opacification and cavitation respectively. TBDM participants were found to have more frequent LLF cavities (Table C-3; p=0,041) than TB only participants. Isolated LLF opacification was present in 14,3% (n=3; 95% CI: 2,0–30,6%) of TBDM participants, compared to 3,6% of TB only patients (p=0,093). If LLF opacification and cavitation were pooled, TBDM participants had a higher proportion of pooled LLF involvement compared to participants with TB only (76,2% vs 61,9%; p = 0,220) but this difference was not significant. However, TBDM participants with an HbA1c of more than 10% (n=11) had higher proportion of pooled LLF involvement (90,9% vs 61,9% p=0,052) and isolated LLF involvement (27,3% vs 3,6%; p= 0,019) compared to TB only participants.

TBHIV: TBHIV participants had a high proportion of normal chest radiographs (15,1%; 95% CI 10,1 – 20,1%) and hence, fewer parenchymal abnormalities when compared to participants

with TB only (84,9% vs 96,4%; $p=0,006$). Strikingly, the proportion of UL cavitation amongst TBHIV participants was almost 50% lower than TB only participants (35,2 vs 64,3; $p < 0,001$). No significant difference was found between TBHIV and TB only participants with regard to LLF opacification or cavitation (Table C-3). The degree of immunosuppression affected radiographic presentation. As the CD4 cell count increased, chest radiographs became less atypical, with more frequent UL opacification (73,3%; 95% CI: 56,0 – 90,1%). However, cavitation did not follow this trend with proportion of UL and LLF cavitation remaining similar for TBHIV participants with CD4 cell counts above and below 500 cells/mm³ (Figure 3), with UL cavitation remaining significantly lower than TB only participants (Tables 3 and 4).

TBDMHIV: TBDMHIV participants presented similarly to the TBHIV category, namely, a high proportion of normal chest radiographs (15,2%; 95% CI 5,1-31,9%); fewer parenchymal abnormalities (84,9% vs 96,4%, $p=0,006$) and less frequent UL involvement (opacifications or cavitations, Table C-3) than TB only participants. The TBDMHIV category had the lowest proportion of radiographs with LLF opacification (33,3%; 95% CI: 16,4 - 50,3%) and cavitation (6,1%; 95% CI: 2,5 – 14,7%) of all the TB categories (Table C-3). Additionally, the TBDMHIV category had a higher proportion of participants with isolated LLF lesions ($n=5$) as compared to the TB only ($n=3$) category (15,2% vs 3,6%; $p=0,039$), and this proportion was similar to that of the TBDM category ($n=3$, 14,3%; $p = 0,626$). Only 8 TBDMHIV participants had a CD4 cell count greater than 500cells/mm³. When stratified by CD4 count a similar pattern to TBHIV is observed, specifically, increasing UL opacification as CD4 count increased (Figure 4). There was a decline in frequency of LLF opacifications for TBDMHIV participants with a CD4 cell count of >500cells/mm³ ($n=1$) (Table C-5). In contrast to TBHIV participants, the proportion of UL and LLF cavitation increased for TBDMHIV participants as CD4 cell count increased (Figure 5) with no significant difference in frequency of UL and LLF cavitation between TBDM and TBDMHIV participants with a CD4 cell count >500mm³ (Table C-5).

Table C-3: Radiographic features of Pulmonary TB across TB categories

FEATURE (%)	OVERALL (N=337)	TB (N=84)	TBDM (N=21)	P-value ^a	TBHIV (N=199)	P-value ^a	TBDMHIV (N= 33)	P-value ^a
Normal radiograph	38 (11,3)	3 (3,6)	1 (4,8)	0,508	30 (15,1)	0,006	5 (15,2)	0,039
Any opacification	273 (81,0)	76 (90,5)	21 (100)	0,156	150 (75,4)	0,004	26 (78,8)	0,123
Upper lobe opacification	240 (71,2)	73 (86,9)	17 (81,0)	0,348	129 (64,8)	0,000	21 (63,6)	0,004
Lower lung field opacification	181 (53,7)	51 (60,7)	16 (76,2)	0,187	103 (51,8)	0,167	11 (33,3)	0,008
Any cavitation	161 (47,8)	57 (67,9)	11 (52,4)	0,184	83 (41,7)	0,000	10 (30,3)	0,000
Upper lobe cavitation	143 (42,4)	54 (64,3)	11 (52,4)	0,315	70 (35,2)	0,000	8 (24,2)	0,000
Lower lung field cavitation	35 (10,4)	6 (7,1)	5 (23,8)	0,041	22 (11,1)	0,314	2 (6,1)	0,598
Miliary pattern	9 (2,7)	3 (3,6)	0	0,505	5 (2,5)	0,439	1 (3,0)	0,680
Pleural effusion	81 (24)	16 (19,1)	6 (28,6)	0,249	53 (26,6)	0,175	6 (18,2)	0,914
Lymphadenopathy	127 (37,9)	31 (36,9)	7 (33,3)	0,761	75 (38,1)	0,853	14 (42,4)	0,581

^acompared to TB category

Figure C-2: Proportion of opacification for TBHIV participants by increasing CD4 cell count compared to TB participants

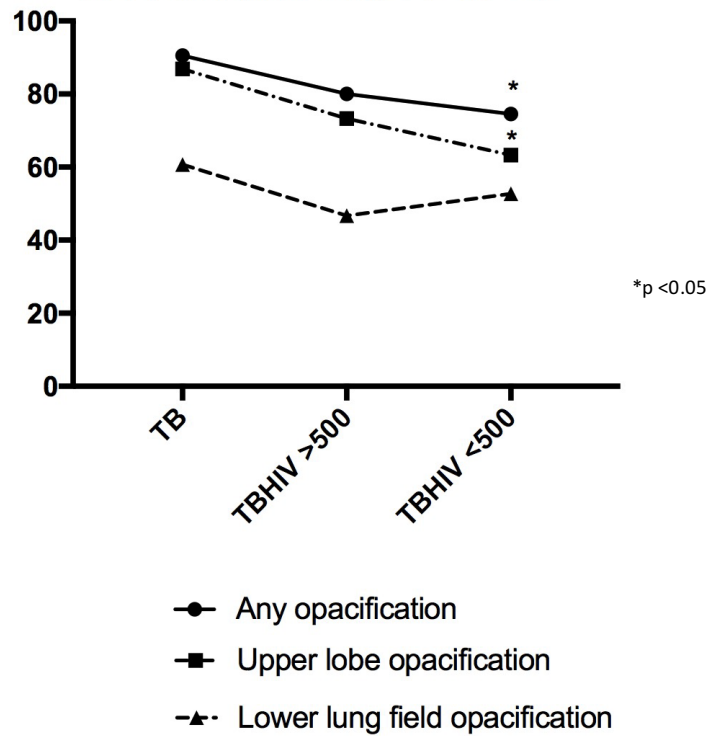


Figure C-3: Proportion of cavitations for TBHIV participants by increasing CD4 cell count compared to TB participants

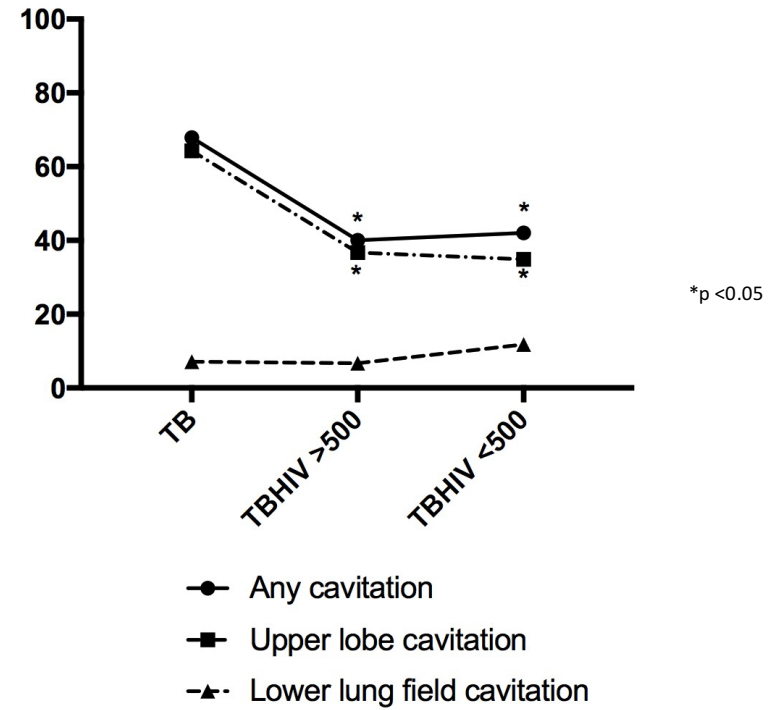


Figure C-4: Proportion of opacification for TBDMHIV participants by increasing CD4 cell count compared to TBDM participants

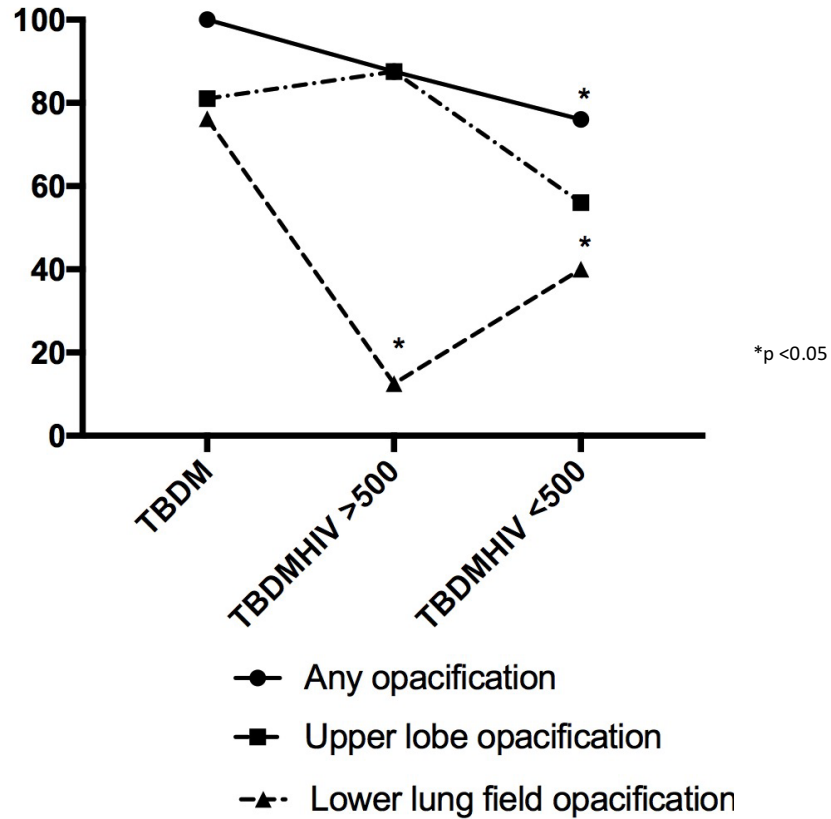


Figure C-5: Proportion of cavitations for TBDMHIV participants by increasing CD4 cell count as compared to TBDM participants

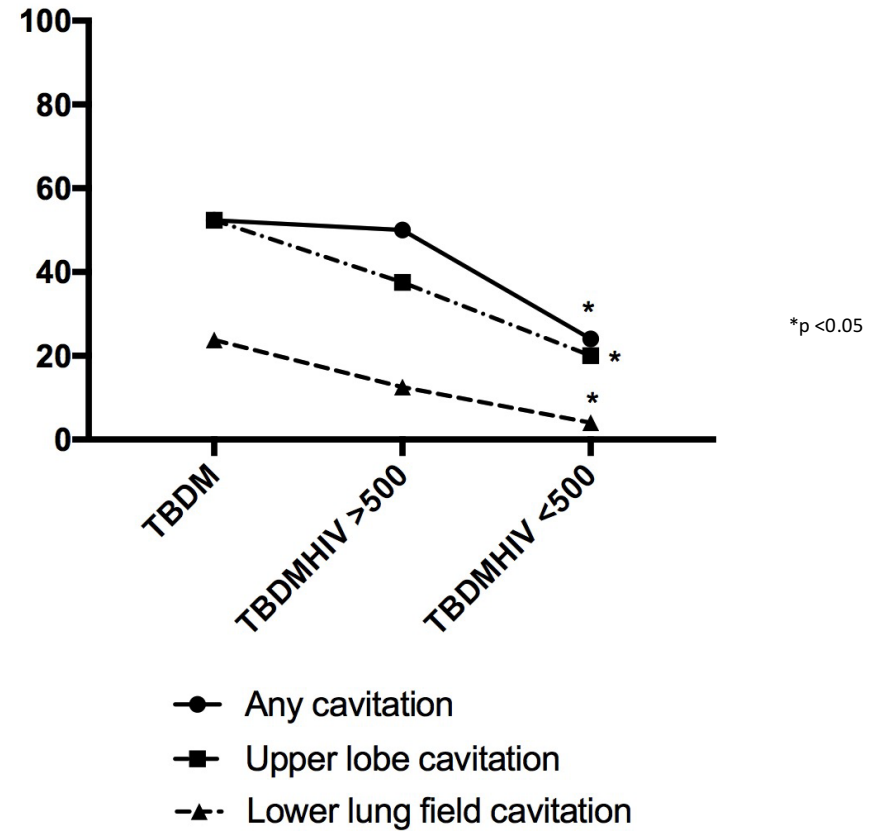


Table C-4: Radiographic features across TB, TBHIV and TBDMHIV categories when CD4 cell count <500 cells/mm³

FEATURE (%)	TB (N=84)	TBHIV (N=169)	P-value	TBDM (N=21)	TBDMHIV (N=25)	P-value
Parenchymal abnormality	81 (96,4)	142 (84)	0,004	21 (100)	21 (84)	0,078
Any opacification	76 (90,5)	126 (74,5)	0,003	21 (100)	19 (76)	0,019
Upper lobe opacification	73 (86,9)	107 (63,3)	<0,001	17 (81,0)	14 (56)	0,072
Lower lung field opacification	51 (60,7)	89 (52,7)	0,225	16 (76,2)	10 (40)	0,014
Any cavitation	57 (67,9)	71 (42)	<0,001	11 (52,4)	6 (24)	0,047
Upper lobe cavitation	54 (64,3)	59 (34,9)	<0,001	11 (52,4)	5 (20)	0,022
Lower lung field cavitation	6 (7,1)	20 (11,8)	0,247	5 (23,8)	1 (4)	0,060

Table C-5: Radiographic features across TB, TBHIV and TBDMHIV categories when CD4 cell count >500 cells/mm³

FEATURE (%)	TB (N=84)	TBHIV (N=30)	P-value	TBDM (N=21)	TBDMHIV (N=8)	P-value
Parenchymal abnormality	81 (96,4)	27 (90)	0,176	21 (100)	7 (87,5)	0,309
Any opacification	76 (90,5)	24 (80)	0,133	21 (100)	7 (87,5)	0,576
Upper lobe opacification	73 (86,9)	22 (73,3)	0,087	17 (81,0)	7 (87,5)	0,721
Lower lung field opacification	51 (60,7)	14 (46,7)	0,182	16 (76,2)	1 (12,5)	0,011
Any cavitation	57 (67,9)	12 (40)	0,007	11 (52,4)	4 (50)	0,258
Upper lobe cavitation	54 (64,3)	11 (36,7)	0,009	11 (52,4)	3 (37,5)	0,134
Lower lung field cavitation	6 (7,1)	2 (6,7)	0,647	5 (23,8)	1 (12,5)	0,483

C.5.3 Effect of HIV on TBDM presentation

The results from Table C-6 highlight LLF involvement as a characteristic feature of the TBDM chest radiograph. Multivariate logistic regression was used to explore the associations between LLF involvement (opacifications, cavitations and isolated lesions) and TB category, as compared to TB only. After adjustment for HIV status, TBDM had a 3,92 fold increased odds of LLF cavitation as compared to TB only participants (95% CI 1,04–14,73; p=0,043). While not significant there appears to be an increasing trend of isolated LLF lesion in TBDM participants (aOR 4,90; 95% CI 0,90-26,5; p=0,065). A previous history of TB was the only other variable with a significant positive association with LLF lesions (both opacification and cavitation, aOR 2,18, 95% CI 1,30-3,64; p=0,003 and aOR 2,14 95% CI 1,02-4,46; p=0,043 respectively). There was no significant association between TBHIV or TBDMHIV and LLF involvement.

Table C-6: Multivariate Logistic Regression

	LOWER LUNG FIELD OPACIFICATION						LOWER LUNG FIELD CAVITATION				ISOLATED LOWER LUNG FIELD LESIONS			
	OR (95% CI)	P-value	aOR	P-value			OR (95% CI)	P-value	aOR	P-value	OR (95% CI)	P-value	aOR	P-value
TBDM	0,79 (0,44 - 1,43)	0,437	1,71 (0,56 - 5,21)	0,346			1,41 (0,58 - 3,43)	0,450	3,92 (1,04 - 14,73)	0,043	2,28 (0,95 - 5,50)	0,065	4,90 (0,90 - 26,5)	0,065
TBHIV	0,58 (0,34 - 0,93)	0,025	0,63 (0,37 - 1,09)	0,099			0,94 (0,44 - 2,02)	0,880	1,39 (0,53 - 3,63)	0,499	1,73 (0,68 - 4,42)	0,248	2,67 (0,76 - 9,42)	0,127
TBDMHIV	-		0,46 (0,20 - 1,03)	0,061	-				0,63 (0,13 - 2,80)	0,076	-		1,75 (0,59 - 5,21)	0,319
Previous TB history	2,00 (1,22 - 3,25)	0,006	2,18 (1,30 - 3,64)	0,003			2,28 (1,11 - 4,68)	0,025	2,14 (1,02 - 4,46)	0,043	0,76 (0,31 - 1,85)	0,547	0,74 (0,30 - 1,83)	0,509
Smoker	0,64 (0,38 - 1,07)	0,091	0,51 (0,29 - 0,88)	0,016			0,90 (0,38 - 2,17)	0,820	-		0,56 (0,19 - 1,68)	0,302	-	
Age	1,00 (0,98 - 1,02)	0,747	-				1,01 (0,99 - 1,04)	0,512	-		1,02 (0,98 - 1,05)	0,304	-	
Sex	1,10 (0,72 - 1,72)	0,646	-				1,07 (0,52 - 2,21)	0,847	-		0,62 (0,29 - 1,35)	0,229	-	

OR = Odds Ratio; aOR = adjusted Odds Ratio; CI = Confidence Interval

C.6 Discussion

C.6.1 Lower lung field involvement in TBDM and TBDMHIV

Lower lung field involvement is considered an atypical presentation of pulmonary TB. We found that a high proportion (76,2%) of TBDM participants in our study had LLF opacification, with 23,8% having LLF cavitation. LLF cavitation was almost four times more likely in TBDM participants as compared to TB only. In TBDMHIV participants, diabetes, as compared to HIV-related immunosuppression, appears to be the driver of LLF cavitation, as on adjustment for the degree of the immunosuppression, TBDMHIV participants had an increased frequency of LLF cavitation, similar to TBDM. This finding is supported by Perez(15) and Chiang(28), who both conducted studies in high TB incidence settings. DM is now a well-recognized risk factor for TB and a recently completed study from Khayelitsha revealed that 61,5% of DM found amongst TB participants was undiagnosed (Oni et al, in press), emphasizing the need to screen TB patients for DM. This finding of increased LLF cavitation on the TB chest radiograph in TBDM patients may be utilized in targeted DM in TB screening algorithms by suggesting potential DM comorbidity. Furthermore, this has the potential to improve DM outcomes by earlier case detection and treatment.

As reported in previous studies (18, 28), this study found a high proportion of isolated LLF opacification in TBDM and TBDMHIV patients (14,3% and 15,2% respectively). A recent study that screened DM patients for TB in South Africa found a significant prevalence of both asymptomatic and smear microscopy negative TB in TBDM patients (Berkowitz et al, under review). Given the risk that atypical TB presentations may lead to a delayed diagnosis of PTB, contributing to continued transmission and increased mortality (6, 7), these findings highlight the need for clinicians to be aware of, and investigate, atypical presentations of TB in DM comorbid patients in order to avoid delays in TB diagnosis.

C.6.2 The presence of HIV-infection modifies the atypical TBDM chest radiographic features

TBHIV participants in this study presented similarly to what has been described in previous literature, specifically, they had more normal chest radiographs(29), fewer upper lobe infiltrates(30-32) and fewer cavities(29, 30, 33, 34) compared to HIV-uninfected TB patients. To our knowledge, our study is the first which describes the interaction between DM and HIV with regards to the TB chest radiograph presentation. We found that TBDM was significantly associated with LLF cavitations, however no statistically significant associations were found between TBDMHIV and LLF involvement. This suggests that the presence of HIV infection suppresses the typical TBDM chest radiograph appearance. While the body of evidence supporting LLF involvement in TBDM participants is strong, there are a number of studies that disagree with this finding(35-38). Of these, the only study which actively screened for HIV was by Gil-Santana et al(36) and found that none of the sample were infected. Of the remaining studies one did not mention HIV testing(37) and the others were retrospective record reviews(35, 38). This could have resulted in misclassification of HIV status if results were not recorded or patients not tested. Lack of ascertainment of HIV status amongst TBDM participants may have led to these studies not finding the typical LLF lesion predominance in TBDM patients.

C.6.3 Degree of diabetes glycemic control and HIV immunosuppression influences chest radiograph presentation

We found that TBDM participants with poorer glycemic control had a higher proportion of both combined and isolated LLF involvement. This is similar to a study conducted in Taipei, where TBDM participants with an HbA1c of greater than 9% were 1,62 times more likely to have LLF opacities as compared to TB participants without DM(39). There is also a suggestion that DM patients with poorer glycemic control may be at increased risk of developing TB disease(40). This is of particular importance in our setting given the poor degree of glycemic control achieved by many DM patients in South Africa (41).

The chest radiographic presentation of PTB in HIV-infected patients at varying degrees of immunosuppression, primarily using the CD4 cell count, has been well described(13, 34, 42, 43). Most often the CD4 cell count is used as a marker of degree of immunosuppression. Studies have found that HIV-infected patients with low CD4 counts were more likely to have atypical radiographic presentations with fewer cavities, increased hilar adenopathy and more diffuse interstitial infiltrates, rather than the typical post-primary pattern of apical cavitation and infiltrates. Very few describe the distribution of lesions by CD4 cell count and when they do the description is often limited to describing lesions in either upper or lower zones, but not both. Our study is unique as we have described the changing distribution of both cavitations and opacifications within the lung with increasing CD4 cell count. Whilst our study found that the frequency of cavitation decreased as CD4 count declined in TBDMHIV participant, this was not observed in TBHIV participants. This is contrary to previous findings and could be attributed to omission of DM status from HIV chest radiograph.

C.6.4 Strengths and limitations

This is one of the few studies to explore the effect of the interaction between TB, DM, and HIV on chest radiography, on a relatively large sample of TB patients with both HIV and DM, in a setting with a significant burden of all 3 diseases. As the risk of TB is increased in both of these conditions, it is likely that there will be an increasing proportion of patients, especially in the study setting, that have both these diseases and TB. It is important to have a better understanding of the chest radiographic presentation of TB in these patients to prevent misdiagnosis, delayed treatment, and unwarranted morbidity and mortality.

Our study had a number of limitations. Of the potential participants from the parent study, ninety were excluded from the analysis due to lack of chest radiography, potentially biasing our findings. However, the omitted participants were similar to the study sample with regards to age, sex, and TB category distributions, as well as median HbA1c and CD4 cell count values (data not shown). Therefore, we would expect minimal variation in chest radiograph findings between these two groups. Diabetes screening was conducted at TB diagnosis which may have resulted in over diagnosis of DM due to stress induced hyperglycemia(44), potentially resulting in the difference in radiological presentation

between DM and non-DM participants being due to TB disease severity rather than DM. We were limited on the number of TBDM participants resulting in the study being marginally underpowered, with our sample size resulting in a 78% power. This could have resulted in some of our results not being statistically significant, especially when investigating rarer radiological findings. Additionally, as we extracted the participants' CD4 count retrospectively some of the participant data was missing, reducing our ability to detect statistically significant differences between study categories, particularly the TBDMHIV category, when stratifying by CD4 count. In addition to CD4 count, other variables that may influence chest radiographic presentation that were not collected include a prior history of trauma, lung or heart disease, exposure to chemicals or hazardous working environments and duration and extent of smoking. Finally, there was a risk of misclassification of radiographic findings due to inter-reader variability. We attempted to reduce this by using a third reader, however this may not have eliminated misclassification bias completely.

C.7 Conclusion

This study confirms that LLF involvement, particularly cavitation and isolated lesions, on the chest radiograph is associated with DM in patients with PTB. Co-infection with HIV has a significant effect on this relationship and results in the modification of typical TBDM chest radiograph patterns, although isolated LLF lesions are still more prominent in DM with or without HIV-infection. This may explain some of the varied findings in previous studies. Factors which may alter the presentation of TBDM radiography include HIV status (and the degree of immunosuppression) and glycemic control. Therefore, atypical lesions on the chest radiograph of patients with DM and/or HIV should alert attending clinicians to possible active TB disease and promote microbiological testing. Additionally, in patients with proven TB, atypical lesions could prompt the screening for both DM and HIV, especially in high DM and HIV burden settings. As our study had a relatively small sample of TBDMHIV patients, further research is needed to fully understand how DM and HIV interact to influence the presentation of PTB. These findings should inform the development of bidirectional TB screening algorithms in patients with DM, with or without HIV-infection, and highlights the important role of radiographic examination in TB screening in this population.

C.8 Acknowledgements¹

NB was responsible for the data collection, analysis and writing of the manuscript. RG was responsible for study coordination and staff management. VS and FO assisted in chest radiograph reading and reporting. TO, NL and RJW were responsible for design of parent study, as well as reviewing and editing of the final manuscript.

C.9 Funding

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C.10 Conflict of Interest²

The authors report no potential conflict of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

¹Authors to be included in final submission to Clinical Infectious Disease: Natacha Berkowitz (NB), Rene Goliath (RG), Vishesh Sood (VS), Ferdie Oompie (FO), Tolullah Oni (TO), Naomi Levitt (NL) and Robert J Wilkinson (RJW).

²For the purposes of this dissertation forms have not been submitted to Clinical Infectious Disease

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Part D Appendices

D.1 Protocol for parent study

OVERALL RESEARCH OBJECTIVE

The main aim of this study is to investigate the incremental yield and best screening algorithms for diabetes mellitus (DM) and tuberculosis (TB) among a) newly diagnosed TB patients and b) patients attending diabetic clinics, respectively.

Specific aims:

- To assess the prevalence of DM among Cases
- To evaluate the best performing algorithms to diagnose DM in Cases
- To measure the prevalence of TB among diabetes patients

BACKGROUND

TB remains a leading cause of death globally, with an estimated 8.8 million new cases reported every year, threatening the goal of global TB elimination by year 2050 (1). Tackling this challenge will require not only improvements in diagnostic and treatment services, but identification and reduction of risk factors that increase susceptibility for TB. Medical conditions that impair immune function, such as malnutrition, alcoholism or HIV co-susceptibility for TB. Medical conditions that impair immune function, such as malnutrition, alcoholism or HIV co-infection, can increase the likelihood of infection or reactivation of latent TB. Increasing evidence suggests that DM is also a significant risk factor for TB. In a recent systematic review, the relative risk for TB in diabetic patients was 3.1 (2). The strength of this link was influenced by geographic/ethnic differences, and

young people were at particularly high risk; in India, DM is thought to be associated with nearly 15% of pulmonary Cases (3). However, these studies had a number of limitations. In particular, very few were carried out in low-income countries, with none in Africa, raising uncertainty about the strength of DM-TB association and benefit of bi-directional screening for DM and TB in these settings with high TB/HIV prevalence and AN increasing burden of DM. Practical guidance on when to suspect, and how best to diagnose, diabetes in TB patients, and how to confirm or exclude it are lacking. The World Health Organisation recommends HbA1c as a diagnostic test for diabetes with a cut-off value of 6.5% (4). However, less clear is the diagnostic value of results below the WHO cut-off (there is an argument for population-specific cut-off values). A study conducted on a population of mixed-ancestry in Cape Town showed that this cut-off value was sub-optimal (erasmus). Furthermore, it is not known how the diagnostic performance of the cut-off is affected by acute illness, such as TB. A point of care HbA1c test could make diabetes screening more effective and potentially more affordable. A study comparing POC devices found that only Afinion and DCA Vantage met the diagnostic performance criteria (6). There are also insufficient data on which to base TB screening guidelines for diabetic patients.

The growing epidemic of diabetes as a threat to TB control

While 95% of patients with TB live in the low- and middle-income countries, 70% of patients with DM also live in these same countries. It is estimated that, worldwide in 2010, there were 285 million people with DM, and the number predicted to rise to 438 million by 2030, making DM one of the most common NCDs globally (7). The majority of this increase will occur in developing countries where TB remains endemic. Diabetes on this scale is likely to pose another threat for global TB control. The potential public health and clinical importance of this seems to have been largely ignored until recently. Recent joint consultations by International Union Against Tuberculosis and Lung Disease, the World Diabetes Foundation and the World Health Organization emphasised the urgent need for research in this area (10). There is also a paucity of data on whether DM is associated with a high prevalence of subclinical TB among patients attending diabetic clinics, as observed in HIV-1 co-

infected persons (13). This has major importance when considering TB screening strategies, with potential for amplification by transmission within diabetic clinics and also potentially serious consequences of false-negative TB screening. Research is therefore required to develop appropriate and evidence-based guidelines for TB screening among persons with DM.

Diabetes, HIV and TB in South Africa and the Western Cape

South Africa is among the 22 high TB burden countries globally, and also has the highest urban: rural ratio in sub-Saharan Africa, with 62% of the population being urban dwellers (17). Urbanisation, in addition to rapid epidemiological and demographic transition has resulted in a rising burden of NCDs. The global burden of disease study demonstrated that in Southern Africa, while HIV and TB rank first and 4th in the top ten causes of morbidity, 50% of the causes of morbidity are non-communicable; diabetes is ranked 7th. A community survey conducted in 2008/9 from Cape Town townships showed a DM prevalence of 13.1% (95% CI: 11.0- 15.1) (26).

Mortality rates from NCDs are also increasing; diabetes was the 6th leading causes of mortality in South Africa in 2010. In the Western Cape, the province within which Cape Town is located, TB and DM are the top 2 causes of mortality. Against this background, the prevalence of HIV, the strongest known risk factor for TB, remains high, with 70% of TB cases being HIV-infected in Khayelitsha

PLAN OF INVESTIGATION

Setting and study population

The study will be conducted in Khayelitsha, reputed to be the fastest growing township in South Africa. Khayelitsha is located 30km from Cape Town with a population of over 500,000 predominantly black Africans, HIV antenatal prevalence of 28% and a TB case-notification rate of >1600/100,000 (27). The largest TB clinic in Khayelitsha is Ubuntu clinic. A survey by the Department of Health reported 1187 newly registered TB cases were diagnosed at Ubuntu clinic in 2011.

Study design

DM Screen in TB patients

We will recruit consecutive TB cases at Ubuntu clinic, prior to initiation of TB treatment. We will record demographic, medical and drug history, HIV status and (if positive) date of initiation of ART, regimen, and CD4 count, as well as clinical, microscopic and radiological characteristics of TB at diagnosis. In addition we will document other risk factors for diabetes, such as family history, body mass index (BMI), and waist circumference. Before starting TB chemotherapy blood samples will be collected to measure fasting blood glucose and oral glucose tolerance test, and HbA1c performed using Afinion, a validated HbA1c Point of care test. Patients will be considered to be diabetic if 1) the diagnosis is known at presentation, 2) they are taking oral hypoglycaemic therapy and/or insulin or 3) as per case definitions. Previous studies have suggested that it may be more reliable to screen for diabetes later in the course of TB treatment rather than at the start (11), because inflammation/cytokine stimulation associated with active TB may elevate blood glucose levels resulting in false positive diabetes diagnoses if tests are performed too early. We will, therefore, repeat fasting blood glucose and HbA1c tests after 2 months of TB treatment. Patients with a new diagnosis will be referred to the DM clinic for standard care. HIV testing is offered routinely to all patients with TB.

TB screen in diabetic patients

We will recruit consecutive diabetes patients presenting to the primary care diabetes clinic at Khayelitsha day hospital. A TB screen will be performed as per case definition. We will also record demographic, medical and drug history, previous TB and TB contact history, as well as HbA1c to measure glycaemic control at the time of recruitment.

<i>Diabetes screen in TB patients (Baseline and 2 months after TB diagnosis)</i>	<ul style="list-style-type: none"> • Diabetes symptoms (nocturia, polyuria, polydipsia) • Random glucose measures on finger prick sample • Fasting glucose • Oral glucose tolerance test • HbA1c • Urine dipstick
<i>TB screen in diabetics</i>	<ul style="list-style-type: none"> • TB symptoms (cough, duration of cough, night sweats, fever, loss of weight or appetite, haemoptysis, pleuritic chest pain) • Spontaneous (or induced if necessary) sputum sent for smear microscopy and culture • Chest radiography • IGRA / TST
<i>Definition of TB disease</i>	<ul style="list-style-type: none"> • Smear microscopy positive for acid-fast bacilli • Isolation/identification of <i>Mycobacterium tuberculosis</i> by culture or Xpert MTB/RIF • In addition to these definitions, an exploratory endpoint will include patients without a definitive TB diagnosis but with compatible clinical symptoms with symptom resolution after TB therapy
<i>Subclinical TB</i>	<ul style="list-style-type: none"> • TB culture positivity in absence of the currently recommended TB symptom screen (cough of any duration, fever, weight loss, night sweats)
<i>Diabetes / Impaired glucose tolerance (IGT)</i>	<ul style="list-style-type: none"> • -Fasting plasma glucose 7.0mmol/l (IGT 5.6-6.9 mmol/l) • -Glucose tolerance test ≥ 11.1mmol/l (IGT 7.8-11.0 mmol/l) • -HbA1c $>6.5\%$ (IGT 5.7-6.4%)

Table 1: Case definitions

Subclinical TB disease Smear microscopy positive for acid fast bacilli Isolation

Outcome measures

Primary outcome

- The best performing screening algorithm to diagnose DM in TB patients

Secondary outcomes

- Accuracy of established intensified TB case-finding strategies among diabetics
- Prevalence of diabetes in TB patients
- Prevalence of TB in diabetics, including subclinical TB disease
- Effect of diabetes on TB clinical presentation (number and duration of pulmonary TB symptoms, TB

sputum smear and microscopy results, CXR findings, non-pulmonary manifestation of disease) and/or treatment outcomes of TB (2 month TB sputum clearance, completion and cure rates)

Sample size and power calculations

Assuming a DM prevalence of 24% in TB patients (based on a TB/DM risk ratio of 2 and DM prevalence in the general population of 12%), in order to have 80% statistical power and a screening

tool sensitivity of 60%, a sample size of 1537 TB cases is required in Malawi and South Africa respectively. This sample size will provide sufficient power to define and investigate different potential DM screening algorithms in TB patients.

For the TB prevalence in DM patients study, a sample size of 457 diabetic patients is required, assuming TB prevalence of 5% and at 2% precision.

Statistical analysis plan

The prevalence of diabetes and TB will be based on the respective gold standard diagnostic tests. We will calculate the sensitivity, specificity, positive and negative predictive values of the screening tools to diagnose

DM in TB cases, and TB in DM patients, using OGTT and TB culture gold standards, respectively. Among TB cases, risk factors associated with DM will be analysed using logistic regression. The model will be built manually with nested models compared using the likelihood ratio test. The Akaike's Information Criterion (AIC) will be used to compare non-nested models with a significantly lower AIC (>10%) indicating an improved model. In addition, outlying and influential observations will be identified and potential effect modification assessed using interaction variables.

To develop and evaluate a DM screening algorithm in TB cases, based on symptom screening, HIV status, ART, age, sex, BMI, waist circumference, and DM family history. Multivariable logistic regression analysis will then be performed to develop diagnostic models for DM using OGTT findings as the gold standard. A reduced clinical model without additional investigations will first be derived. These tests will then be added singly to the reduced clinical model and then simultaneously to explore the added predictive value of a single test and of combined tests, respectively. The ability of a multivariable model to discriminate persons with DM from those without will be assessed using Receiver Operator Characteristic curve and Area Under the Curve analysis. Significance testing will be done using a combination of two-sided p-values ($p < 0.05$) and 95% confidence intervals. All data will be analysed using STATA 12.0 (StataCorp, College Station, TX, USA)

Ethical considerations

This study has received ethics approval from the University of Cape Town Human Ethics committee (HREC Ref: 403/2011). A written consent will be obtained from all participants who will be given detailed explanation of the study. An information sheet will be available both in English and Xhosa.

Study timeline

Task	Dec 2013	2014				2015			
<u>Preparatory phase</u> <ul style="list-style-type: none"> Staff recruitment & training Questionnaire development 									
<u>Study recruitment and follow up</u> <ul style="list-style-type: none"> Identification and assessment of participants Sample collection Follow-up of participants 									
Sample and Data analysis									
<u>Report writing</u> <ul style="list-style-type: none"> Preparation for presentation and publication Submission of report 									

Anticipated overall outputs and impacts

This study performed at such an important time, with respect to the emerging diabetes epidemic in sub-Saharan Africa, will provide an invaluable set of data to document the strength of association between diabetes and TB and its clinical/public health impact. Data of this quality does not exist in the current literature on the link between TB and diabetes in sub-Saharan Africa; hence we expect this study to produce a series of high quality publications. Additionally, the study is designed to raise further research questions and provide some of the ground work needed, for example to determine 1) optimal screening models 2) whether diabetes influences recurrence of TB or anti-tuberculosis drug resistance 3) the need to develop randomised controlled trials of efficacy of interventions, such as TB chemoprophylaxis in people with diabetes, to improve outcomes of both diseases.

Contingency plans

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D.2 Ethical approval for dissertation study



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



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17 June 2016

HREC REF: 373/2016

Dr T Oni
Public Health & Family Medicine
Room 4.41, Entrance 5
Falmouth Building

Dear Dr Oni

PROJECT TITLE: EFFECT OF DIABETES AND HIV ON RADIOGRAPHIC MANIFESTATIONS OF PULMONARY TUBERCULOSIS- LINKED TO 403/2011 (Masters candidate- Dr N Berkowitz)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee 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 30th June 2017.

Please submit a progress form, using the standardised Annual Report Form 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)

We acknowledge that the student Dr N Berkowitz will be involved in this study.

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

Please quote the HREC REF in all your correspondence.

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

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 373/2016

D.3 Case Report Form (including STEPs Questionnaire) for parent study

Participant Study Number: DBSN- ____

Chronic Disease Risk Factor Surveillance

Survey Information

Date	Response	Code
1	Interviewer name	I3
2	Date of consent dd mmm yyyy	I4

Participant Study Number: DBSN - ____		
Consent, Interview Language and Name	Response	Code
3	Consent has been read and obtained Yes 1 No 2 If No, END	I5
4	Consent Language <i>(interview should be done in the same language as the consent)</i> Xhosa 1 English 2	I6
5	Time of consent (24 hour clock) hrs mins	I7
6	Participant Surname	I8
7	Participant First Name	I9
8	Sex Male 1 Female 2	C1
9	What is your date of birth? <i>Don't Know 77 777 7777</i> dd mmm yyyy	C2
10	Have the TB screening tool questions and vital signs been recorded Yes 1 No 2 If No, obtain and record on TB screening tool	I10
Additional Information that may be helpful		
11	Contact phone number(s) <i>(where possible, also give a second telephone number of friend/relative)</i>	I11
12	Home address	I12

Location and Date	Response	Code
Appointment date for completion of steps 1-3 and TB results (48 hours – 1 week later as per clinic protocol)	dd mmm yyyy	I13

Record and file identification information (I5 to I13) with consent form but separately from the rest of the completed questionnaire.

Step 2 Behavioural Measurements

CORE: Tobacco Use			
Now I am going to ask you some questions about various health behaviours. This includes things like smoking, drinking alcohol, eating fruits and vegetables and physical activity. Let's start with tobacco.			
Question	Response	Code	
21	Do you currently smoke any tobacco products , such as cigarettes, cigars or pipes?	Yes 1 No 2 <i>If No, go to q26</i>	T1
22	Do you currently smoke tobacco products daily ?	Yes 1 No 2 <i>If No, go to q26</i>	T2
23	How old were you when you first started smoking daily?	Age (years) Don't know 77 <input type="text"/> <i>If Known, go to q25</i>	T3
24	Do you remember how long ago it was? <i>(RECORD ONLY 1, NOT ALL 3)</i>	In Years <input type="text"/>	T4a
	<i>Don't know 77</i>	OR in Months <input type="text"/>	T4b
	<i>Don't know 77</i>	OR in Weeks <input type="text"/>	T4c
25	On average, how many of the following do you smoke each day? <i>(RECORD FOR EACH TYPE)</i> <i>Don't Know 77</i>	Manufactured cigarettes <input type="text"/>	T5a
		Hand-rolled cigarettes <input type="text"/>	T5b
		Pipes full of tobacco <input type="text"/>	T5c
		Cigars <input type="text"/>	T5d
		Other <input type="text"/>	T5e
		Other (please specify):	T5other
EXPANDED: Tobacco Use			
Question	Response	Code	
26	In the past, did you ever smoke daily ?	Yes 1 No 2 <i>If no, go to q29</i>	T6
27	How old were you when you stopped smoking daily?	Age (years) Don't Know 77 <input type="text"/> <i>If known, go to q29</i>	T7
28	How long ago did you stop smoking daily? <i>(RECORD ONLY 1, NOT ALL 3)</i> <i>Don't Know 77</i>	Years ago <input type="text"/>	T8a
		OR Months ago <input type="text"/>	T8b
		OR Weeks ago <input type="text"/>	T8c

CORE: Alcohol Consumption		
The next questions ask about the consumption of alcohol. See bottom of page for definitions.		
Question	Response	Code
29	Have you ever consumed an alcoholic drink such as beer, wine, spirits, cider? Yes 1 No 2 <i>If No, go to q37</i>	A1a
30	Have you consumed an alcoholic drink within the past 12 months? Yes 1 No 2 <i>If No, go to q37</i>	A1b
31	During the past 12 months, how frequently have you had at least one alcoholic drink? Daily 1 5-6 days per week 2 1-4 days per week 3 1-3 days per month 4 Less than once a month 5	A2
32	Have you consumed an alcoholic drink within the past 30 days? Yes 1 No 2 <i>If No, go to q37</i>	A3
33	During the past 30 days, on how many occasions did you have at least one alcoholic drink? Number Don't know ?? <input type="text"/>	A4
34	During the past 30 days, when you drank alcohol, on average, how many standard alcoholic drinks did you have during one drinking occasion? Number Don't know ?? <input type="text"/>	A5
35	During the past 30 days, what was the largest number of standard alcoholic drinks you had on a single occasion, counting all types of alcoholic drinks together? Largest number Don't Know ?? <input type="text"/>	A6
36	During the past 30 days, how many times did you have for men: five or more for women: four or more standard alcoholic drinks in a single drinking occasion? Number of times Don't Know ?? <input type="text"/>	A7

Alcoholic drink definitions
 1 drink = 1 glass of wine OR 1 small bottle of beer /cider OR 1 tot of spirit
 1 litre bottle of wine = 5 drinks
 1 litre bottle of spirits (e.g. vodka, whisky, brandy, gin) = 12 drinks
 1 large bottle of beer or cider = 2 drinks

CORE: Physical Activity		
<p>Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.</p> <p>Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.</p>		
Question	Response	Code
Work		
43	<p>Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like carrying or lifting heavy loads, digging or construction work for at least 10 minutes continuously?</p> <p>Yes 1</p> <p>No 2 If No, go to q46</p>	P1
44	<p>In a typical week, on how many days do you do vigorous-intensity activities as part of your work?</p> <p>Number of days <input type="text"/> <input type="text"/></p>	P2
45	<p>How much time do you spend doing vigorous-intensity activities at work on a typical day?</p> <p>Hours : minutes hrs mins</p>	P3 (a-b)
46	<p>Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking or carrying light loads for at least 10 minutes continuously?</p> <p>Yes 1</p> <p>No 2 If No, go to q49</p>	P4
47	<p>In a typical week, on how many days do you do moderate-intensity activities as part of your work?</p> <p>Number of days</p>	P5
48	<p>How much time do you spend doing moderate-intensity activities at work on a typical day?</p> <p>Hours : minutes hrs mins</p>	P6 (a-b)
Travel to and from places		
<p>The next questions exclude the physical activities at work that you have already mentioned.</p> <p>Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship.</p>		
49	<p>Do you walk or use a bicycle for at least 10 minutes continuously to get to and from places?</p> <p>Yes 1</p> <p>No 2 If No, go to q52</p>	P7
50	<p>In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?</p> <p>Number of days</p>	P8
51	<p>How much time do you spend walking or bicycling for travel on a typical day?</p> <p>Hours : minutes hrs mins</p>	P9 (a-b)

CORE: Physical Activity, Continued			
Question		Response	Code
Recreational activities			
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure).			
52	Do you do any vigorous-intensity sports, fitness or recreational (<i>leisure</i>) activities that cause large increases in breathing or heart rate like <i>running</i> or <i>football</i> for at least 10 minutes continuously?	Yes 1 No 2 <i>If No, go to q55</i>	P10
53	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (<i>leisure</i>) activities?	Number of days _____	P11
54	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes hrs mins	P12 (a-b)
55	Do you do any moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities that cause a small increase in breathing or heart rate such as <i>brisk walking, cycling, swimming, softball</i> for at least 10 minutes continuously?	Yes 1 No 2 <i>If No, go to q58</i>	P13
56	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities?	Number of days _____	P14
57	How much time do you spend doing moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities on a typical day?	Hours : minutes hrs mins	P15 (a-b)

Sedentary behaviour			
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television, but do not include time spent sleeping.			
58	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes hrs mins	P16 (a-b)

ANNEXURE: To be completed by staff member

DIABETES HISTORY			
Question		Response	Code
59	Any family history of diabetes?	Yes 1 No 2	R1
60	Have you ever had diabetes during pregnancy (gestational diabetes)?	Yes 1 No 2	R2

Step 3 Biochemical Measurements

CORE: Blood Glucose and HbA1c			
Question	Response		Code
61	Blood pressure	____ / ____	J1
62	During the past 8-12 hours have you had anything to eat or drink, other than water? <i>If yes, complete STEPS 1 and 2, and re-schedule appointment for blood tests:</i> Date: _____ dd mmm yyyy	Yes 1 No 2	B1
63	Fasting blood glucose and HbA1c	Hours : minutes	B5
		Fasting glucose mmol/l	
		HbA1c %	
64	Oral glucose tolerance test	Hours : minutes	B6
		2 hour glucose mmol/l	
CORE: Blood Lipids			
65	Total cholesterol	mmol/l	B8
EXPANDED: Triglycerides and HDL Cholesterol			
66	Triglycerides	mmol/l	B10
67	HDL Cholesterol	mmol/l	B11
Return Date	Response		Code
68	Appointment date for diabetes and cholesterol results (1 week)	dd mmm yyyy	B12
69	Appointment date for completion of step 4 (2 months)	dd mmm yyyy	B13

Step 4 Repeat Biochemical Measurements			
Date		Response	Code
70	Interviewer name		I17
71	Date	dd mmm yyyy	I18
RECENT DIABETES AND TUBERCULOSIS HISTORY			
Are you currently receiving any of the following treatments/advice for diabetes prescribed by a doctor or other health worker?			
72	Diabetes pills or insulin	Yes 1 No 2	H8a
Are you currently receiving treatment for tuberculosis?			
73	TB medication	Yes 1 No 2	H8b
Are TB culture results (from baseline TB screen) available?			
74	TB culture results	Positive 1 Negative 2 Pending 3 Not done 4	H8c
Have presenting TB symptoms resolved?			
75	TB symptoms resolved	Yes 1 No 2	H8d
FINAL TB GROUP ASSIGNMENT			
76	Final Study group assignment	TB case 1 TB control 2 Excluded 3	I19

CORE: HbA1c			
Question		Response	Code
77	Blood pressure	____/____	J2
78	During the past 8-12 hours have you had anything to eat or drink, other than water? <i>If yes, do not proceed. Re-schedule appointment.</i> Date: dd mmm yyyy	Yes 1 No 2	B14
79	HbA1c <i>Participant to be recalled for results only if new diabetes diagnosis made</i>	Hours : minutes [][] . [][] HbA1c % [][][][] . [][]	B15

D.4 Chest Radiograph Report Form

Date of CXR (dd/mmm/yyyy)

--	--	--	--	--	--	--	--	--	--

Date of Reading (dd/mmm/yyyy)

--	--	--	--	--	--	--	--	--	--

CXR Reader

Reader 1 Reader 2 Reader 3 Consensus

CXR Views

PA Lateral

1. Parenchymal abnormalities

Yes

No

Location of parenchymal abnormalities

Left

Right

Bilateral

Airspace Opacities present

Yes

No

Maybe

Not visible

Location of Airspace Opacities

RUL

LUL

RML

RLL

LL

Opacities calcified

Yes

No

Cavities present

Yes

No

Maybe

Not visible

Location of Cavities

RUL

LUL

RML

RLL

LL

Bilateral military nodules

Yes

No

Maybe

Not visible

2. Pleural effusion (abnormalities)

Yes

No

Maybe

Not visible

Location of pleural effusion

Left

Right

Bilateral

3. Lymphadenopathy visible

Yes No Maybe Not

Location of lymphadenopathy

Right hilar Left hilar Bilateral
 Right mediastinal Left mediastinal

4. Cardio thoracic ratio increased (>0.5)

Yes No

R_{MCD} cm

L_{MCD} cm

MTD cm

R_{MCD} = Right Max. Cardiac Diameter

L_{MCD} = Left Max. Cardiac Diameter

MTD = Max. Thoracic Diameter

5. Overall assessment: CXR normal?

Yes No

TB-related CXR findings?

Yes No

Activity of TB findings

Active Inactive N/A

8. Technical aspects

PA Acceptable Poor but readable Not acceptable No readable

Lateral Acceptable Poor but readable Not acceptable No readable

9. Comments:

Initials and date of doctor completing the task above

Database Capture: Initials and date of person completing data entry

D.5 Informed Consent for parent study (English Version)

UNIVERSITY OF CAPE TOWN



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Epidemiology of Diabetes, TB and HIV co-infection in a high HIV/TB burden setting

INFORMED CONSENT and INFORMATION FORM

My name is _____

I wish to invite you to participate in a study that is trying to assess the interaction between tuberculosis (TB), HIV and Diabetes. This study is being run by the Institute of Infectious Diseases and Molecular Medicine at the University of Cape Town. Dr Tolullah Oni is the Principal Investigator.

Firstly, I wish to explain to you why this research is being done:

TB is a disease that is seen very commonly in Cape Town and, indeed, in most of South Africa. It is caused by bacteria that people breathe in and the infection results in cough, fevers and weight loss. The disease is more common, and can be more severe, in patients with HIV infection.

Diabetes is a disease caused by the body's inability to control sugar levels. This disease is becoming increasingly more common worldwide and in South Africa due to reduced exercise and increasing obesity. Previous studies have shown that like HIV, diabetes increases the risk of TB. But there are a few things we still do not know:

1. We do not know how much diabetes (and risk factors such as high cholesterol) there is in this setting.
2. We also do not know if the increasing levels of diabetes are contributing significantly to the TB epidemic, and
3. Although we know that HIV increases the risk of TB, we do not know if having HIV and diabetes further increases the TB risk. In other words, we do not know if a person with HIV and diabetes is at higher risk of TB than a person with HIV and no diabetes.

In addition, we know that low vitamin D levels increase the risk of TB. We also know that many people in Cape Town have very low levels of vitamin D. Therefore we also wish to measure your vitamin D level and see how this affects the risk of TB and recovery from TB in TB patients.

We request your participation in this study either because you are being investigated for TB, because you have been diagnosed with diabetes, or because you have recently undergone HIV counselling and testing (HCT).

As part of this study, we will check your blood for HIV and check your sputum (spit) for TB. We will also check you for Diabetes using the following tests:

We will ask you to come again before eating or drinking anything and check your sugar level that morning and 2 hours later after giving you a sugary drink. You must not eat or drink anything else during these 2 hours. We will also do an HbA1c test, a blood test for diabetes and check your blood cholesterol level. We will pay 30 Rand travel expenses for this purpose.

We will also take blood to measure your vitamin D level. If we diagnose HIV, we will give you a referral letter to take to any HIV clinic of your choice where baseline bloods will be done and your eligibility for antiretroviral therapy can be assessed. If TB is found, we will also give you a letter to take to your nearest TB clinic as it will be important to start TB treatment as soon as possible. If diabetes is diagnosed, we will write a referral letter for you and advise you of your nearest diabetes club where you can be started on appropriate treatment and receive regular advice on diet and lifestyle. If high cholesterol is diagnosed, we will write a referral letter to your ARV clinic or day hospital for further management.

It is entirely up to you to decide whether or not to take part in this study. If you do decide to take part, you will be asked to sign this consent form. We will then request you to provide us with an extra blood sample (15ml) that can be taken at the same time as your normal blood tests and may perform a Chest X Ray.

Although blood testing very rarely causes problems, if anything goes wrong the University provides insurance to cover this possibility. This study will also be monitored by the Research Ethics Committee of the University of Cape Town. Their job is to ensure your safety and protect you during the study.

The decision to participate is entirely your own. IF YOU DECIDE NOT TO PARTICIPATE, YOUR TREATMENT WILL NOT BE DIASDVANTAGED IN ANY WAY. In addition, at any point during the study you are free to withdraw without telling us why.

Throughout the study your privacy will be maintained and nobody other than the doctors and nurses looking after you will know that you are participating. Samples will be labelled with code numbers and hence the laboratory staff will not know your identity. When the results of the study become available, names of the participating patients will not be included.

Do you have any questions? During the study you may contact either the **Human Research Ethics Committee** (021 406 6492) or **Dr Tolullah Oni** (021 406 6079) if you have further questions. Please remember that Dr Oni will not be directly responsible for your medical care which will be conducted by your regular doctors and nurses.

Consent to participate in the study:

I have read the above / have had the above read to me. I have had the opportunity to discuss the study with _____ and have also had the opportunity to ask any questions. I consent to take part in this study:

Name _____

Signature _____

Date _____

Name of Staff member consenting _____

Signature _____

Date _____

Witness (if participant is illiterate)

Name _____

Signature _____ Date _____

D.6 Detailed statistical analysis

Descriptive statistics and univariate analysis:

Participants were stratified by TB category, that is, participants with only TB, TB and HIV (TBHIV), TB and diabetes (TBDM) or TB, diabetes and HIV (TBDMHIV). Participant characteristics and chest radiograph features (outcome measure) were described using simple proportions for the overall sample, as well as by stratifying by these TB categories. Proportion of radiograph features by TB category were compared using Chi squared test and Fishers exact test as appropriate.

Multivariate analysis and model building:

Multivariate analysis was used to calculate the association between participants with TBDM, TBHIV and TBDMHIV and atypical features of TB chest radiography: lower lung field opacification, lower lung field cavitation and isolated lower lung field lesions. Models were built using forward selection methods. The log likelihood ratio test was used to compare nested models, by testing the difference in the deviance between the two. The model with a log likelihood ratio test with a high Chi squared value and a P-value < 0.05 was considered the most appropriate model. The Akaike's Information Criterion (AIC) was used to compare non-nested models with the best model having the lowest AIC. HIV and the interaction between DM and HIV were included in the model so as to explain the association between atypical radiographic findings and participants with TBHIV and TBDMHIV. Other interactions between confounding and exposure variables were tested but none were found to be significant. To assess overall fit of the models the Pearson goodness-of-fit test was used with a P-value > 0.05 indicating good fit.

Model diagnostics:

The three aspects of the model which need to be checked are: the form of the linear predictor, the adequacy of the link function and outlying or influential observations. The form of the linear predictor was checked by creating scatterplots of the Pearson and deviance residuals against the index number, where a systematic pattern would denote an incorrect model. The logit link function is deemed appropriate for all binary responses and hence does not require checking. Observations where their standardised residuals lay outside $+2$ and -2

were considered outlying observations. These were visualized by creating a scatterplot of standardized residuals against the index number. Influential observations were identified using the h_i , a measure of how far the covariate pattern lies from the average covariate pattern and hence how influential it is on the model. Multicollinearity was assessed using the variance inflation factor (VIF) with a VIF $\ggg 10$ indicating the presence of multicollinearity.

Model Building Table D-7.1: Lower lung field opacities and TB category

Lower Lung Field opacities				Log likelihood ratio test		
MODEL	vs	Variable included	log likelihood	AIC	χ^2	P-value
A	-	Lower lung field (LLF) opacity + Diabetes	-227.4112	458.8223	-	-
B	A	Age Category	-226.1947	462.3894	2.43	0.4875
C	A	Smoker	-225.7972	457.5943	3.23	0.0724
D	A	Previous TB	-223.5753	453.1507	7.67	0.0056
E	A	sex	-227.3298	460.6595	0.16	0.6866
F	A	Age	-227.2344	460.4688	0.35	0.5521
G	A	HIV	-224.727	455.454	5.37	0.0205
Model D: LLF opacity + Diabetes + Previous TB +...						
H	D	Age Category	-222.457	456.9139	2.24	0.5247
I	D	Smoker	-221.1893	450.3787	4.77	0.0289
J	D	sex	-223.5726	455.1452	0.01	0.9411
K	D	Age	-223.5586	455.1173	0.03	0.8550
L	D	HIV	-220.2727	448.5453	6.61	0.0102
Model L: LLF opacity + Diabetes + Previous TB + HIV+...						
M	L	Age Category	-219.6594	453.3187	1.23	0.7466
N	L	Smoker	-217.4147	444.8294	5.72	0.0168
O	L	sex	-220.1067	450.2135	0.33	0.5646
P	L	Age	-220.2587	450.5175	0.03	0.8675
Model N: LLF opacity + Diabetes + Previous TB + HIV+ Smoker...						
Q	N	Age Category	-216.44	448.88	1.95	0.5830
R	N	Sex	-217.3756	446.7512	0.08	0.7798
S	N	Age	-217.3902	446.7805	0.05	0.8250

Final model:
 logit opLL DM hi DMhi prevTB smoker, or

Iteration 0: log likelihood = -227.71326
 Iteration 1: log likelihood = -215.58356
 Iteration 2: log likelihood = -215.54396
 Iteration 3: log likelihood = -215.54395

Logistic regression	Number of obs	=	330
	LR chi2(5)	=	24.34
	Prob > chi2	=	0.0002
Log likelihood = -215.54395	Pseudo R2	=	0.0534

opLL	Odds Ratio	Std. Err.	z	P> z	[95% Conf. Interval]	
DM	1.708838	.971891	0.94	0.346	.5605123	5.209745
hi	.6340933	.174845	-1.65	0.099	.3693539	1.088588
DMhi	.2692039	.1882827	-1.88	0.061	.0683512	1.06027
prevTB	2.176119	.5696798	2.97	0.003	1.302714	3.635098
smoker	.5062937	.1430596	-2.41	0.016	.2909947	.8808867
_cons	1.561431	.3812124	1.83	0.068	.9676266	2.519635

. estat gof

Logistic model for opLL, goodness-of-fit test

number of observations =	330
number of covariate patterns =	14
Pearson chi2(8) =	6.72
Prob > chi2 =	0.5666

Model Building Table D-7.2: Lower lung field cavities and TB category

Lower Lung field Cavities				Log likelihood ratio test		
MODEL	vs	Variable included	log likelihood	AIC	χ ²	P-value
A	-	Lower lung field cavity + Diabetes	-109.1866	222.3732		
B	A	Age Category	-109.1264	228.2527	0.12	0.9893
C	A	Smoker	-109.1762	224.3525	0.02	0.8854
D	A	Previous TB	-106.589	-106.589	5.20	0.0226
E	A	sex	-109.1555	224.3111	0.06	0.8031
F	A	Age	-109.0932	224.1865	0.19	0.6656
G	A	HIV	-109.1811	224.3623	0.01	0.9166
Model D: Diabetes + Previous TB +...						
H	D	Age Category	-106.4944	224.9888	0.19	0.9793
I	D	Smoker	-106.4848	220.9697	0.21	0.6481
J	D	sex	-106.5874	221.1749	0.00	0.9557
K	D	Age	-106.5866	221.1732	0.00	0.9447
L	D	HIV	-106.5552	221.1104	0.07	0.7949

As no additional variables improved the baseline model, HIV and DM*HIV were added to model D to create model M

Lower Lung field Cavities				Log likelihood ratio test		
MODEL	vs	Variable included	log likelihood	AIC	χ ²	P-value
M	-	Diabetes + Previous TB + HIV + DM*HIV	-104.8373	219.6747	-	-
N	M	Age Category	-104.6142	225.2284	0.45	0.9305
O	M	Smoker	-104.6589	221.3178	0.36	0.5503
P	M	sex	-104.8371	221.6743	0.00	0.9835
Q	M	Age	-104.7822	221.5643	0.11	0.7397

Model Building Table D-7.3: Isolated Lower lung field Involvement and TB category

Isolated Lower Lung field Involvement				Log likelihood ratio test		
MODEL	vs	Variable included	log likelihood	AIC	χ^2	P-value
A	-	Isolated lower lung field involvement + Diabetes	-94.31578	192.6316		
B	A	Age Category	-92.82771	195.6554	2.98	0.3953
C	A	Smoker	-93.91446	193.8289	0.80	0.3703
D	A	Previous TB	-94.19769	194.3954	0.24	0.6270
E	A	sex	-93.7355	193.471	1.16	0.2813
F	A	Age	-94.21573	194.4315	0.20	0.6546
G	A	HIV	-93.43261	192.8652	1.77	0.1838

As no additional variables improved the baseline model, HIV and DM*HIV were added to model A to create model H

Isolated Lower Lung field Involvement				Log likelihood ratio test		
MODEL	vs	Variable included	log likelihood	AIC	χ^2	P-value
H	-	Isolated lower lung field involvement + Diabetes + HIV + DM*HIV	-93.01717	194.0343		
I	H	Age Category	-91.07533	196.1507	3.88	0.2743
J	H	Smoker	-92.65318	195.3064	0.73	0.3935
K	H	Previous TB	-92.79132	195.5826	0.45	0.5015
L	H	sex	-92.73194	195.4639	0.57	0.4501
M	H	Age	-92.95765	195.9153	0.12	0.7301

No other variables significantly improved the model. However, when running model diagnostics model H had a poor fit (Pearson's Chi <0.001). We therefore included previous TB in the model as this had contributed to atypical lower lung field lesions in both the previous models.

Final Model:
 logit onlyLL DM hi DMhi prevTB, or

Iteration 0: log likelihood = -95.84989
 Iteration 1: log likelihood = -93.06948
 Iteration 2: log likelihood = -92.791721
 Iteration 3: log likelihood = -92.791318
 Iteration 4: log likelihood = -92.791318

Logistic regression	Number of obs	=	330
	LR chi2(4)	=	6.12
	Prob > chi2	=	0.1906
Log likelihood = -92.791318	Pseudo R2	=	0.0319

onlyLL	Odds Ratio	Std. Err.	z	P> z	[95% Conf. Interval]
DM	4.899458	4.222524	1.84	0.065	.9048006 26.53037
hi	2.667727	1.716598	1.52	0.127	.7558232 9.415916
DMhi	.3581676	.368689	-1.00	0.319	.04763 2.693347
prevTB	.7367688	.3411842	-0.66	0.509	.2972729 1.826027
_cons	.0397895	.0236191	-5.43	0.000	.0124306 .1273629

. estat gof

Logistic model for onlyLL, goodness-of-fit test

number of observations =	330
number of covariate patterns =	8
Pearson chi2(3) =	2.72
Prob > chi2 =	0.4365

D.7 Manuscript preparation instructions (Clinical Infectious Disease)

ARTICLE TYPES

Papers may be submitted in the following categories. The editors reserve the right to change the category for consistency with *CID* style.

Major Articles

Report clinically relevant investigations or observations within *CID*'s scope of interests.

Format guide:

- Word limit: 3000 words (excluding the abstract and references).
- Key points should be summarized on the title page in 40-words or less.
- References: 40 or less.
- Abstract: Up to 250 words, structured using the headings Background, Methods, Results and Conclusions.
- Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

MANUSCRIPT FORMAT AND STRUCTURE

Please refer to a recent issue of *Clinical Infectious Diseases* for guidance on style and layout of articles. Also refer to the Article type section for guidance on relevant information for each article type.

File Formats

The preferred format for submitting manuscripts online is Microsoft Word (.doc files). PDF files are not acceptable for submission.

File Contents

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Gorecki DC, Monaco AP, Derry JM, Walker AP, Barnard, EA, Barnard, PJ. Expression of four alternative dystrophin transcripts in brain regions regulated by different promoters. *Hum Mol Genet*, **1995** ; 155: 505-511.

Francis V, Bastin M. (2000) Gene targeting in rat embryo fibroblasts promoted by the polyomavirus large T antigen. *Nucleic Acids Res* [in press].

Maniatis T, Fritsch EF, Sambrook J. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, **1982** .

Huynh TV, Young RA, Davis RW. DNA Cloning. In: Glover DM. *DNA Cloning - A Practical Approach*. Vol 1. Oxford, UK: IRL Press; **1988** :49-78.

Public Health Service Task Force. Recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal

HIV-1 transmission in the United States. Available at: <http://www.aidsinfo.nih.org>. Accessed 24 April 2002.

Lyon DJ, Cheng AFB, Norrby SR. Mechanisms of cefotaxime resistance in blood culture isolates of *Enterobacter* high prevalence of extended-spectrum β -lactamases [abstract C43]. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, 1995:47.

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For human genes, use genetic notation and symbols approved by the Human Gene Mapping Workshop (see Wain HM, Bruford EA, Lovering RC, Lush MJ, Wright MW, Povey S. Guidelines for human gene nomenclature. *Genomics* 2002; 79:464–70). Human gene names and loci should be written in italicized capital letters and Arabic numerals. Human protein product names are not italicized. For human mutation nomenclature, see Antonarakis et al. (Recommendations for a nomenclature system for human gene mutations. *Hum Mutat* 1998; 11:1–3).

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