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Post-Tuberculosis Metabolic Disease and Mortality Among Patients Treated for Tuberculosis

Argita Salindri

ABSTRACT

POST-TUBERCULOSIS METABOLIC DISEASE AND MORTALITY AMONG PATIENTS TREATED FOR TUBERCULOSIS

By

Argita Dyah Salindri

June 23, 2021

The global strategy to end tuberculosis (TB) includes interim goals to reduce TB incidence by 80% and TB mortality by 90% by the end of 2030. However, global TB control primarily focuses on achieving favorable TB treatment outcomes (i.e., microbial cure or treatment completion). Yet clinical and public health understanding of the long-term impacts of TB on post-TB health is severely limited. Emerging evidence suggests that TB patients may have increased post-TB risk of non-communicable diseases such as chronic obstructive pulmonary disease, type-2 diabetes mellitus (T2DM), cardiovascular disease, or stroke. Compared to general population mortality rates, patients previously treated for TB have approximately double the rate of all-cause mortality. However, guidance on how to improve patients' post-TB health is unavailable. Furthermore, the impact of comorbidity factors such as pre-existing T2DM, human immunodeficiency virus (HIV), and hepatitis C co-infections on post-TB health has not been assessed. To evaluate the relationship between comorbidities and post-TB treatment health, we conducted three observational studies (two retrospective and one prospective) in the country of Georgia.

In **Study 1**, we estimated the association between common comorbidities (T2DM, HIV, and hepatitis C co-infections) and post-TB mortality among patients with drug-resistant TB (DRTB) between 2009-2017 (n=1,032). Competing risks models were used to estimate the hazard rate of all-cause mortality post-TB treatment comparing DRTB patients with and without comorbidities. We reported a strong association between HIV co-infection and post-TB treatment mortality (adjusted hazard ratio [aHR] 4.40, 95% confidence interval [CI] 2.17 – 8.93). The hazard rate of post-TB mortality was non-significantly higher among patients with hyperglycemia (aHR 1.19, 95%CI 0.73 – 1.96) or hepatitis C co-infection (aHR 1.25, 95%CI 0.71 – 1.96) compared to those without hyperglycemia or hepatitis C.

In **Study 2**, we estimated the association between the use blood glucose-lowering agents with TB treatment outcomes among DRTB patients with hyperglycemia (n=128). Hyperglycemia was determined according to fasting blood glucose level at TB treatment initiation or a record of self-reported previous T2DM diagnosis from a health care provider. We evaluated TB treatment outcomes using three metrics, including 1) time to sputum culture conversion, 2) final TB treatment outcome, and c) mortality post-TB treatment. We used log-binomial and Cox proportional hazard regression models to determine the association between T2DM characteristics and the study outcomes. Among DRTB patients with T2DM with a record of blood glucose-lowering agents use (n=60), metformin use was associated with a reduced risk of poor TB treatment outcomes (adjusted risk ratio [aRR] 0.23, 95%CI 0.05 – 0.97).

In **Study 3**, we prospectively followed patients who were successfully treated for TB to evaluate cardio-metabolic risks post-TB treatment. Newly diagnosed adult pulmonary TB patients with a favorable outcome at the completion of TB treatment were eligible (n=105). For this study, interim data from study baseline and 6-month follow-up were used. We used log-binomial regression to estimate the risk ratio of metabolic syndrome at the end of TB treatment, comparing patients treated for drug-susceptible (DSTB) vs. DRTB. We also used general linear models to estimate the association between drug-resistant TB and visceral adipose index (VAI) at the end of TB treatment. Among a subset of individuals with 6-month follow-up information available (n=62), mixed models with random intercepts were used to estimate the association between drug-resistance and change in VAI. Compared to patients treated for DSTB, patients treated for DRTB had a higher prevalence of metabolic syndrome (aRR 2.29, 95%CI 0.50 – 10.37) and elevated VAI levels (adjusted mean difference 0.37, 95%CI -0.13 – 0.86) at the end of TB treatment. Although non-significant, the result from the mixed model suggested that the mean VAI among patients formerly treated for DRTB was higher by 0.60 (95%CI -0.20 – 1.40) points compared to those treated for DSTB.

Our preliminary findings suggest a clinical need for post-TB continued care even when patients have a successful TB treatment outcome. Importantly, patients with pre-existing comorbidities and DRTB, who may have increased risks of metabolic diseases or mortality post-TB treatment, should receive targeted follow-up care after TB treatment completion.

POST-TUBERCULOSIS METABOLIC DISEASE AND MORTALITY AMONG PATIENTS TREATED FOR TUBERCULOSIS

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PREFACE

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Author's Statement Page

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List of Abbreviations

ACS	acute coronary symptoms
aHR	adjusted hazard rate ratio
AIDS	acquired immunodeficiency syndrome
AMPK	AMP-activated protein kinase
aPR	adjusted prevalence ratio
ART	antiretroviral therapy
BGLA	blood glucose-lowering agents
BMI	body mass index
CFR	case fatality rate
CI	confidence interval
COPD	chronic obstructive pulmonary disease
cRR	crude risk ratio
DOT	directly observed therapy
DRTB	drug-resistant tuberculosis
DST	drug-susceptibility test
DSTB	drug-susceptible tuberculosis
FBG	fasting blood glucose
HCV	hepatitis C virus
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HR	hazard rate ratio
IQR	interquartile range

IR	incidence rate
IRR	incidence rate ratio
LMIC	low-middle income country
LTBI	latent tuberculosis infection
MDR TB	multidrug-resistant tuberculosis
Mtb	*Mycobacterium tuberculosis*
mROS	mitochondrial reactive oxygen species
mTOR	mammalian target of rapamycin
NCTLD	National Center for Tuberculosis and Lung Diseases
PET-CT	Positron Emission Tomography – Computed Tomography
PLHIV	people living with HIV
PR	prevalence ratio
PY	person-years
PZA	pyrazinamide
RIF	rifampicin
SCC	sputum culture conversion
SDG	sustainable development goals
SLD	second-line drugs
T2DM	type-2 diabetes mellitus
TB	tuberculosis
TBDM	tuberculosis-diabetes
TG	triglyceride
UK	United Kingdom

USA	United States of America
VAI	visceral adipose index
VECD	Vanderbilt-Emory-Cornell-Duke
WC	waist circumference
WHO	World Health Organization
XDR TB	extensively drug-resistant tuberculosis

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CHAPTER 1

Literature Review and Statement of Purposes

INTRODUCTION

Tuberculosis (TB) is a major public health problem, with nearly 1.5 million deaths reported annually [1]. Furthermore, TB surpassed human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) as a leading cause of death due to a single infectious disease [2]. The United Nations Sustainable Development Goals (SDGs) and the End TB strategy set ambitious goals to eliminate TB by 2035 [3]. However, despite advancement and progress made in the global TB elimination efforts, there is no indication that we will meet the End TB end goals. Importantly, the convergence of TB and chronic communicable or non-communicable disease epidemics poses critical public health challenges, especially among developing countries where the burdens of the two diseases are high [4]. In these settings, TB and chronic diseases are not only co-existing but also affecting the risk of each other.

While there is a large body of work addressing the potential impact of pre-existing comorbidities on TB treatment outcomes, studies assessing the long-term effects of pre-existing comorbidities are still limited. Moreover, there is an overall lack of research on the risk of chronic non-communicable disease progression after TB treatment. Thus, the overall dissertation goal is to address the current gaps of knowledge regarding metabolic diseases and health post-TB treatment. Specifically, we aimed to explore the importance of pre-existing comorbidities and drug-resistant TB (DRTB) on post-TB mortality and metabolic disease risks by conducting three observational studies among patients treated for TB disease in the country of Georgia. The three studies included: 1) a retrospective cohort study to estimate the association between pre-existing

comorbidities and post-TB mortality, 2) a retrospective cohort study of TB treatment outcomes among DRTB patients with hyperglycemia, and 3) a prospective cohort study to estimate the prevalence of metabolic syndrome and visceral adipose index (VAI) after TB treatment completion.

Study 1 Overview

Study 1 was conducted as part of the "*Hyperglycemia and risk of post-tuberculosis adverse health outcomes among patients treated for drug-resistant tuberculosis"* study in collaboration with the National Center for Tuberculosis and Lung Diseases (NCTLD), Tbilisi, Georgia. The study was funded by the Vanderbilt-Emory-Cornell-Duke (VECD) Fogarty Global Health Research program. The specific aims of study 1 were to:

1. Estimate the rates of post-TB mortality among patients treated with second-line TB drugs (SLDs)
2. Estimate the association between pre-existing comorbidities including hyperglycemia, hepatitis C virus (HCV), or HIV co-infection and the risk of post-TB mortality

Study 1 utilized data from NCTLD's TB online surveillance database and Georgia's death registry. Newly diagnosed adult pulmonary TB patients treated with SLDs reported to the surveillance system from 2009 – 2017 were included in the final analyses. Hyperglycemia was determined by fasting blood glucose level (i.e., ≥5.6 mmol/L) or a self-reported prior diabetes diagnosis at the beginning of TB treatment. HIV and HCV co-infection status were determined by laboratory test results recorded in the surveillance system. Unique national identifiers, patients' names, and date of birth were used to obtain patients' vital status from the National Statistics Office of Georgia after TB treatment ended.

Study 2 Overview

Study 2 was also conducted as part of the *"Hyperglycemia and risk of post-tuberculosis adverse health outcomes among patients treated for drug-resistant tuberculosis"* study. Study 2 specific aims were to:

1. Describe the use of blood glucose-lowering agents (BGLA) among DRTB patients with hyperglycemia
2. Estimate the association between BGLA and TB treatment outcomes, including time to achieve sputum culture conversion (SCC), final TB treatment outcomes, and post-TB mortality

We pooled a list of DRTB patients with hyperglycemia from the online TB surveillance database. We only included DRTB patients with hyperglycemia who started TB treatment in Tbilisi, Georgia, due to access to patients' medical charts. Similar to Study 1, hyperglycemia was defined by fasting blood glucose level at the beginning of TB treatment or a self-reported prior diabetes diagnosis from a healthcare provider. Hyperglycemia status was then classified as "pre-diabetes" and "diabetes." Diabetes characteristics, including diagnosis classification (newly diagnosed vs. known diabetes), years of living with diabetes, and records of BGLA use during TB treatment, were abstracted from patients' medical charts. Outcomes of interest for study 2 included a) time to achieve SCC, b) final TB treatment outcomes, and c) post-TB mortality. We defined time to achieve SCC as time (measured in days) from TB treatment initiation to the first of two consecutive negative culture results. Final TB treatment outcomes were grouped either as "favorable" or "poor" according to the World Health Organization (WHO) guideline for TB reporting. And post-TB mortality was determined as described in Study 1.

Study 3 Overview

Study 3 was conducted in conjunction with a currently funded R21 study entitled *"Pulmonary Impairment after Tuberculosis Treatment"* or PITT study. PITT study is a one-year prospective cohort study conducted among patients who were successfully treated for TB disease per WHO guidelines. The parent study aimed to assess lung function and impairment after successful TB treatment. However, study 3 specific aims were to:

1. Estimate the prevalence of metabolic syndrome and distribution of visceral adipose index (VAI) at the end of TB treatment
2. Estimate the association between DRTB, metabolic syndrome, and VAI at the end of TB treatment
3. Evaluate the impact of DRTB on changes in the VAI levels from the end of TB treatment vs. 6-months post-TB treatment completion.

Briefly, we enrolled patients to participate in PITT study at the end of TB treatment, and patients were followed up at 6- and 12-month post-TB treatment completion. Study 3 used PITT interim data collected during the study baseline and 6-month follow-up visits from December 2020 – March 2021. The primary study exposure for study 3 is the type of drug resistance (DRTB vs. drug-susceptible TB [DSTB]) determined by phenotypic drug-susceptibility test (DST) results. Study 3 outcomes included metabolic syndrome and VAI. Metabolic syndrome was determined by the presence of at least three of the following conditions: a) elevated plasma glucose level (i.e., glycated hemoglobin [HbA1c] ≥5.7%), b) elevated triglycerides (i.e., ≥1.69 mmol/L), c) lower high-density lipoprotein (HDL) (<1.03 mmol/L for male and <1.29 mmol/L for female), d) elevated blood pressure (i.e., ≥130/85mmHg), and e) central obesity (i.e., waist circumference

≥102 cm for men and ≥88 cm for women). Visceral adipose index was calculated as a secondary outcome.

BACKGROUND

Mounting evidence suggests that TB survivors frequently experience post-TB residual complications [5-11]. Therefore, public health efforts to understand TB burdens are now slowly shifting to include post-TB health as a new research focus. In recent years, international symposiums and workshops were held to set priorities, identify gaps and barriers in addressing post-TB health issues [12, 13]. However, to date, epidemiologic evidence on post-TB health is limited to retrospective cohort study designs. Moreover, risk factors of poor post-TB health (e.g., pre-existing comorbidities) are likely heterogeneous across different settings. Thus, expanding the current TB research scope to include post-TB health will better characterize the total public health burden due to the global TB epidemic. This expanded research agenda will be critical to identifying comprehensive efforts to accelerate the reduction of TB burdens.

Epidemiology of Tuberculosis and Comorbidities

Tuberculosis epidemiology

There were approximately 10.0 million (range 8.9 – 11.0 million) incident TB cases in 2019 [1]. In the same year, nearly half a million new rifampicin (RIF)-resistant TB cases were reported globally, 78% of which were multidrug-resistant TB (MDR TB) cases [1]. WHO estimated that MDR TB occurred among 3.3% of new TB cases (i.e., primary MDR TB) and 17.7% of individuals previously treated for TB disease (i.e., acquired MDR TB) [1]. It is well established

that TB disproportionately affects developing countries such as India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh, and South Africa, which contributes approximately two-thirds of the global TB burden [1]. Interestingly, the highest burden of acquired MDR TB was among former Soviet Union nations, contributing to more than half of the global MDR TB cases [14]. In the country of Georgia, TB was emerged as one of the major public health issues, especially among internally displaced persons, following the Soviet Union breakup [15]. In 2020, the estimated incidence of TB in Georgia was 74/100,000 populations, and TB mortality was around 4.1/100,000 populations. The national MDR TB burden was around 14/100,000 populations [1]. MDR TB was reported among 12% of newly diagnosed TB cases and 33% among previously treated TB cases [1].

Tuberculosis and diabetes

The convergence of TB and diabetes epidemics is more prominent in low- and middle-income countries (LMICs), where the two diseases are highly co-prevalent [16]. The latest figures indicated that currently, there are 463 million adults that are living with diabetes globally, and it is projected to increase up to 578 million in 2030 and nearly 700 million in 2045 [17]. Geographically, diabetes highly affects adults (i.e., 20 – 79-years-old) living in China, India, the USA, Pakistan, Brazil, Mexico, and Indonesia. Notably, 66.8% of people living with diabetes in these countries are undiagnosed, which could partially explain the high rates of premature deaths observed in these nations [17]. Although the prevalence of diabetes in the European region is relatively lower, a report showed that the prevalence of obesity and other metabolic disorders are slowly elevating among former Russian federation nations [18, 19], and thus likely increase the diabetes prevalence in the next couple of years. In 2016, diabetes affected 15% of Georgian adults

and contributed to 1% of adult mortality [20]. According to a previous study, the prevalence of diabetes was 11.6% among newly diagnosed TB patients [21].

Pre-existing diabetes (i.e., diabetes diagnosed before or at the time of TB treatment initiation) is associated with increased risk of TB infection, TB activation (i.e., from latent to active TB), and poor TB treatment outcomes. Previous studies showed that the odds of LTBI among adults with diabetes are twice the odds among those without diabetes [22, 23]. With a quarter of the global population is being infected by *Mycobacterium tuberculosis* (*Mtb*) [24, 25], the TB-diabetes burden is likely to persist if we do not incorporate aggressive efforts to suppress TB incidence in the current TB elimination efforts. We also know that an estimated 15-20% of all TB cases are attributed to diabetes [26]. More importantly, a recently published systematic review and meta-analysis reported that the estimated global prevalence of diabetes among patients with TB is twice the prevalence among the general population [27]. Previous studies also consistently reported that diabetes might double (and in some settings, triple) the risk of TB activation or primary progression [26, 28] and is associated with poor TB treatment outcomes (i.e., TB mortality, delayed sputum culture conversion, and TB relapse) [29-32].

Metformin as a candidate of host-directed adjuvant tuberculosis therapy

Numerous studies have documented the various benefits of metformin in terms of TB infection/disease control. A retrospective cohort study from Taiwan reported a lower TB incidence among metformin users compared to non-metformin users (hazard rate ratio [HR] 0.84, 95%CI 0.74 – 0.96) [33]. In the same study, individuals with high-dose metformin prescriptions also had lower TB incidence when compared to those with low-dose metformin prescriptions. Similarly, Pan et al. also reported a reduced incidence rate of TB among metformin users vs. sulfonylurea

users [34]. Metformin has been reported to have preventive effects on poor TB treatment outcomes. A hospital-based study from Taiwan highlighted the role of metformin in reversing the increased risk of mortality during treatment among TB patients complicated with diabetes (HR 0.56, 95%CI 0.39 – 0.82) [35]. Metformin usage among TB patients was also associated with faster culture conversion time [36, 37], higher TB treatment success rates, and reduced risks of TB relapse [37].

Despite these evident benefits of metformin on TB infection/disease control, the biological mechanism underlying metformin properties remains unclear. Metformin works in several different pathways, including inducing anti-inflammatory effects [38], inhibiting the signaling of mammalian target of rapamycin (mTOR) [39], or increasing the mitochondrial reactive oxygen species (mROS), which is critical in killing *Mtb* bacteria [38]. Furthermore, murine studies showed that metformin is effective in reducing the inflammatory response caused by TB by activating the autophagy macrophages as the result of the stimulated expression of AMP-activated protein kinase (AMPK) [40, 41] [36]. This property will inhibit the growth rate of MTB and thus preventing further damages to the lungs. Interestingly, results from several preclinical studies found that the drug-drug interaction between metformin and rifampicin can also suppress the intracellular growth of MTB [42, 43]. With the current evidence, many TB researchers and clinicians proposed metformin as a candidate for host-directed adjuvant therapy among individuals with latent tuberculosis (LTBI) or TB disease. However, whether other blood glucose-lowering agents also have similar properties in controlling TB infection or TB disease remains unexplored.

Tuberculosis and HIV co-infection

The TB and HIV syndemic is one of the significant barriers of the TB elimination program. It continues to place an immense public health burden, especially in the health care system. In 2020, there were approximately 37 million people living with HIV globally, 1.5 million of which were newly diagnosed [44]. The majority of people living with HIV (PLHIV) are concentrated in the African (67%) or Southeast Asian (15%) nations [45, 46], regions that also have high TB burdens. Despite the low prevalence, the epidemic of HIV/AIDS in the country of Georgia is expected to rise with the increasing number of people who inject illicit drugs over the past few decades (the latest estimates suggested that there were approximately 52,500 people who inject drugs in 2016) [47]. In 2020, there were approximately 8400 (range 7300 – 9300) PLHIV in the country of Georgia, 76% (range 66 – 85) of which know their status, and 65% (57 – 72) were on antiretroviral therapy (ART) [48]. The prevalence of HIV/AIDS among TB patients in the country of Georgia ranged from 1.7 – 2.2% [49].

Immunodeficiency among PLHIV has a critical impact on host susceptibility toward *Mtb*, making it the key driver of the TB-HIV co-epidemic [50-52]. To date, HIV is the strongest risk factor for TB progression, as shown by previous studies suggesting that the risk of TB progression among PLHIV can be up to 20-fold compared to those without HIV co-infections [53]. Moreover, among PLHIV, TB is the leading cause of death [54]. In 2019, WHO reported 208,000 TB deaths associated with HIV/AIDS [1]. Among PLHIV, the use of ART was shown to have a protective effect on TB reactivation (67% risk reduction) [55] and TB/HIV associated deaths (64 – 95% risk reduction [50, 56].

Tuberculosis and HCV co-infection

The epidemiology of and clinical assessment on TB and HCV comorbidities has not been well described. However, the risk of TB may be high in settings where HCV co-infection is prevalent due to the common risk factors such as injection drug use or poor infection control measures [57]. Globally, around 3% of the world's population is infected with HCV, the majority of which reside in regions like Eastern Europe, Africa, as well as the southern and eastern part of Asia [58]. There are approximately half a million HCV-related deaths reported annually [58]. Chronic infection of hepatitis C virus was affecting approximately 5.4% of Georgian adults (around 150,000 persons) in 2015 [59]. In the country of Georgia, the prevalence of HCV co-infection among patients with TB was 21% [60]. Studies assessing the clinical impact of HCV on TB manifestation are limited [61-63]. However, it is consistently reported that HCV co-infection may increase the risk of hepatitis during treatment with anti-TB drugs, which could, in turn, increase the risk of poor TB treatment outcomes [60, 63, 64].

Post-Tuberculosis Health

Post-tuberculosis mortality

The case fatality rate (CFR) of TB reported in the WHO annual report only included deaths reported at the beginning or during TB treatment. Consequently, the programmatic report on TB mortality might be severely underestimated because deaths after lost to follow-up or failed treatment were not included [65]. More importantly, recent findings suggested that patients previously treated for TB disease may have increased risks of post-TB mortality [6]. With at least 9 million TB survivors re-entering the global population each year, it is critical to understand the

risk of and factors associated with post-TB mortality to estimate the total public health burden caused by TB disease.

The increased risk of post-TB mortality among patients treated for TB has been well documented. For example, a recent meta-analysis reported a pooled standardize mortality ratio of 2.91 (95%CI 2.21 – 3.84) comparing former TB patients to the general population. Moreover, two retrospective cohort studies conducted in Ethiopia and the United States of America (USA) reported that the post-TB mortality rate was highest during the first 5-years after TB treatment completion [66, 67]. Thus, it is likely that patients treated with TB may have increased mortality risk due to residual complications associated with TB disease.

Socio-demographic characteristics (i.e., lower socioeconomic status, lower education attainment), as well as behavioral risk factors including smoking, alcohol abuse, and drug use, are common risk factors for post-TB mortality [66, 68, 69]. Importantly, post-TB mortality rates were significantly higher among patients living with HIV or those who were diagnosed with MDR TB [68]. Moreover, several studies included in the systematic review paper by Romanowski et al. reported that chronic non-communicable diseases such as lung cancer, chronic obstructive pulmonary disease (COPD), congestive heart failure, pneumonia, diabetes, and cardiovascular diseases are common causes of death after TB treatment [70-73].

Metabolic disease risks post-tuberculosis treatment

Patients treated for TB disease may also be at a greater risk of developing post-TB chronic non-communicable diseases such as type-2 diabetes mellitus (T2DM), cardiovascular diseases, or stroke [5, 8-10, 74]. The bi-directional relationship between TB and diabetes has been long postulated; however, understanding of the extent and biological pathways TB may increase the

risk of metabolic diseases remains limited [75-77]. The lack of studies assessing diabetes risk post-TB may be due to the multiple causative components of diabetes and the complex nature of its pathology [8, 78-80].

To date, there are only a few studies that are looking at TB as the risk factor for diabetes progression. For example, a recently published study using data from Taiwan suggested that the incident rate of diabetes among patients previously treated for TB was higher when compared to the national estimates [8]. Similarly, a study using primary care data from the United Kingdom (UK) reported that the incidence rate of diabetes was substantially higher among individuals with a history of TB disease compared to those without (incidence rate ratio [IRR] 5.65, 95%CI 5.19 – 6.16) [79]. Additionally, a nationwide retrospective cohort study from Denmark showed that the rate of diabetes incidence was slightly higher among individuals with a history of TB disease (incidence rate [IR] 4.56/100,000 person-years [PY]) compared to those without TB history (IR 4.01/100,000 PY) [78]. Despite these accumulating evidence to support TB as a risk factor for diabetes progression, the biological pathway to explain how TB increases the risk of diabetes progression post-TB treatment remains a critical gap.

Inflammation during TB disease may also increase the risk of cardiovascular diseases, including acute coronary syndrome (ACS) or stroke. However, studies examining the risk of post-TB cardiovascular diseases are limited. A retrospective cohort study conducted among newly diagnosed TB patients from Taiwan documented a higher ACS incidence among patients treated for TB patients vs. the general population (2.10 vs. 1.51 per 1000 person-years) [11]. After adjusting for potential confounders, the incidence of ACS among TB patients was 1.4-fold the incidence among the non-TB group. Another study from Taiwan estimated the risk of ischemic stroke among patients treated for TB during a 3-year follow-up [74]. The study reported a higher

risk of stroke among TB patients (6.0%) vs. the non-TB comparison cohort (3.7%). The study also reported a 50% increased risk of stroke among patients treated for TB disease vs. the non-TB group after adjusting for potential confounders (aHR 1.52, 95%CI 1.21 – 1.91) [74].

While few studies may identify diabetes and cardiovascular diseases as common causes of post-TB mortality, it is still unknown to what extent TB increases the risk of these chronic conditions. With the complex pathophysiology of these chronic diseases, it is hard to measure the onset of chronic disease or its progression without routine screenings. Due to the long progression of cardio-metabolic diseases, studies examining the risk of chronic diseases post-TB can be resource exhaustive and may not be feasible in resource-limited settings. Measuring markers risk factors of cardio-metabolic disease such as metabolic syndrome and visceral adipose index could provide a snapshot of patients' cardio-metabolic risk profile after TB treatment. Tailoring these simple and inexpensive measures to post-TB treatment may help clinicians to routinely monitor cardio-metabolic disease risks over time to prevent premature deaths, especially among patients treated for TB disease.

Metabolic syndrome

Metabolic syndrome is an established risk factor for diabetes, cardiovascular diseases, and stroke [81]. Metabolic syndrome is a composite of five disorders: 1) elevated blood glucose level (i.e., fasting plasma glucose ≥100mg/dL), 2) elevated triglycerides (i.e., ≥150mg/dL), 3) lower high-density lipoprotein (HDL) (female <40 mg/dL, male <50 mg/dL), 4) elevated blood pressure (≥130/85 mmHg), and 5) central obesity (waist circumference ≥88cm for female and ≥102cm for male) (**Table 1.1**). A cross-sectional study from Mexico reported that hypertension, diabetes, obesity, and dyslipidemia were common among TB patients. However, the study did not estimate

the relative measure of these diseases occurrences comparing the TB group to the non-TB group [82]. To our knowledge, currently, no study is looking at the prevalence of metabolic syndrome at the end of- or post-TB treatment. Such studies will help establish the potential biological pathways to explain how TB can increase cardio-metabolic diseases such as diabetes, acute coronary symptoms, and stroke.

Visceral Adipose Index (VAI)

Visceral adipose index (VAI) is a novel gender-specific marker of adipose distribution and function estimated using anthropometric (waist circumference [WC] and body mass index [BMI]) and metabolic (triglyceride [TG], and high-density lipoprotein cholesterol [HDL-C]) variables developed by Amato and colleagues [83]. VAI can be calculated using these formulas:

$$VAI_{MALE} = \left(\frac{WC}{39.68 + (1.88 \times BMI)}\right) \times \left(\frac{TG}{1.03}\right) \times \left(\frac{1.31}{HDL}\right)$$

$$VAI_{FEMALE} = \left(\frac{WC}{36.58 + (1.89 \times BMI)}\right) \times \left(\frac{TG}{0.81}\right) \times \left(\frac{1.52}{HDL}\right)$$

VAI is a reliable and routinely applicable indicator of visceral fat function associated with the risk of cardio-metabolic diseases [84]. Although there is no clinical cut-off point to quantify the cardio-metabolic risks, VAI has a good predictive power of T2DM compared to BMI [85]. In addition, among a cohort of TB patients in India, higher VAI levels (VAI >5.0) were associated with poor TB treatment outcomes [86].

BIOLOGIC PLAUSIBILITY

Tuberculosis and cardio-metabolic diseases post-tuberculosis treatment

The complex immune response during a TB episode may affect the host metabolisms and subsequent risk of cardio-metabolic diseases [5, 16]. During the early phase of active TB episode where bacterial replications are high and not well-controlled by anti-TB drugs, pro-inflammatory cytokines such as IFN-γ, IL-6 and IL-12 are being upregulated continuously, and this will lead to persistent systematic inflammation, a potential mediator for various chronic diseases including diabetes and CVD [5, 87-89].

Previous studies also suggested that MTB infection can elevate blood glucose levels at least temporarily in animal and human studies [90-92]. However, once TB bacteria infection is well-controlled with the use of anti-TB drugs, blood glucose levels are likely to reverse to normal level (i.e., stress hyperglycemia) [5]. During this stress hyperglycemia period, vascular cell tissues could pick up the high blood glucose concentration as an epigenetic imprint which would alter the proatherogenic gene expression during the subsequent normoglycemic periods [16]. A few studies also suggested that substantial proportions of patients with pre-diabetes or diabetes diagnosed at TB treatment initiation (i.e., newly diagnosed) had persistent hyperglycemia at the end of TB treatment (range 26-43%) [16]. This persistent hyperglycemia is likely to push individuals with normoglycemia to subclinical diabetes and pre-diabetes to overt diabetes (**Figure 1.1**).

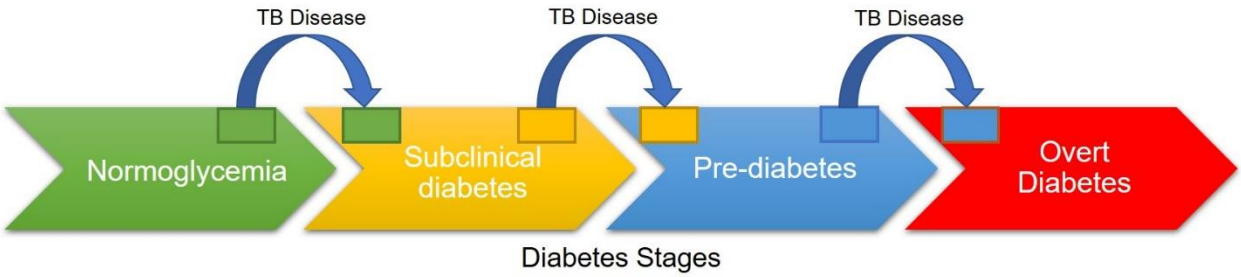

Figure 1.1 Plausible carry-over effects of TB disease on diabetes manifestation. During a TB episode, stress hyperglycemia may tip patients with normal blood glucose to the pre-diabetes category, and those with pre-diabetes to overt diabetes category. In this case, subclinical diabetes is marked by a degree of insulin resistance and increased secretion of insulin and amylin by β-cells in attempt to offset the glucose metabolism, thus blood glucose level may still within the normal range among these individuals.

Infectious agents like *Mtb* may also promote the formation of atherosclerotic lesions as a response to the persistent systemic inflammation during the chronic infection phase [10, 93-97]. Inflammation response such as dysregulation of lipid synthesis may alter vessel vasomotion in the endothelium and promote atherosclerotic formation [98, 99]. These atherosclerotic lesions will then narrow the surface of arteries (i.e., atherosclerosis) which could eventually increase the risk of subsequent cardiac events [10]. Although rare, *Mtb* could also reside in the atherosclerosis plaque [100] and cause direct damages to not only the coronary vessels but also the myocardium (i.e., tuberculous myocarditis) [10, 101-103]. Furthermore, although findings are inconsistent, TB drugs may also increase the blood total cholesterol level in some settings [104].

Post-tuberculosis mortality

As TB commonly attacks the lungs, it is not surprising that a proportion of successfully treated TB patients may suffer from low or medium grades of lung damages (e.g., fibrosis, bronchiectasis, airflow obstruction) despite favorable TB treatment outcomes [105, 106]. A prospective study from South Africa among patients with pulmonary TB showed that only 32% of thoracic lesions were resolved entirely at 1-year follow-up after TB treatment completion [106].

Positron Emission Tomography-Computed Tomography (PET-CT) readings from this study showed that cavitation, consolidation, and nodules were still prominent among a few patients at follow-up visits post-TB treatment. These residual lung damages will likely affect pulmonary functions and prevent individuals from returning to their normal lives. If left untreated, these thoracic impairments may lead to chronic pulmonary diseases, contributing to at least 3.5 million deaths worldwide annually [5].

TB may also increase the severity of other pre-existing comorbidities. For example, among patients with pre-existing diabetes, the prolonged pro-inflammatory responses during a TB episode may alter patients' blood glucose control. Poor blood glucose control was associated with increased risks of diabetes complications such as neuropathy, nephropathy, retinopathy, heart complications, or diabetes mortality. A retrospective study conducted in Israel found that the post-TB mortality risk among people living with diabetes was lower compared to the general population [72].

TB recurrence (i.e., relapse or re-infection) could also explain the observed increased risk of post-TB mortality among TB survivors [107]. A study from Ethiopia, for example, reported a higher CFR five-year post-TB treatment among TB survivors with a history of recurrent TB (14.9%) compared to those with no record of recurrent TB (4.9%) [108]. The role of diabetes-TB recurrence on long-term mortality may be significantly attenuated in regions with a low TB burden [108]. The weakened immune among patients with pre-existing comorbidities may lead to a higher bacterial load during a TB episode [41, 109]. This may explain why TB patients with pre-existing comorbidities such as diabetes and HIV required a longer time to achieve bacterial clearance [110-113]. The high bacterial load among patients with pre-existing comorbidities could lead to Th1

cell dysfunction [114, 115] and altered chemotaxis of neutrophils [116] that would delay response to MTB infection [41].

GAPS OF KNOWLEDGE

Mounting evidence suggests that TB burden is currently fueled by the converging epidemics of TB and chronic non-communicable diseases. This looming co-epidemic, especially among low- and middle-income countries (LMICs), prevents us from achieving the End-TB and the United Nations Millennium Development Goals. However, to date, the focus of the global TB elimination program is only to reduce TB incidence and TB mortality. Currently, there is no clinical guideline to follow-up patients after TB treatment completion. Thus, knowledge regarding post-TB health is limited, especially in resource-limited settings.

Importantly, while studies investigating the impact of pre-existing comorbidities on final TB treatment outcomes are abundant, it is unknown whether pre-existing comorbidities can also increase the risk of all-cause mortality post-TB. Among the ten studies included in the recent meta-analyses [6], three of which were from high TB-burden countries (i.e., China and India), pre-existing comorbidities diagnosed prior or at the beginning of TB treatment were not well characterized despite the consistent mentions that diabetes and cardiovascular diseases are two most likely causes of post-TB mortality. Only one study reported an increased risk of death due to diabetes after TB treatment [117]. Additionally, one study also reported an increased risk of post-TB mortality among patients treated for MDR TB [118]. However, the effect of pre-existing comorbidities on post-TB mortality among patients with DRTB is unknown.

The majority of studies assessing the potential use of metformin as a host-directed adjuvant therapy for TB were conducted among patients with DSTB. The use of glucose-lowering agents

among patients with DRTB and T2DM is not well described. Metformin was able to reverse the increased risks of TB reactivation, TB relapse, or TB mortality [35, 37]. However, whether metformin can accelerate microbial clearance remains inconclusive. Only one study suggested that the use of metformin was associated with a higher rate of sputum culture conversion among patients with M/XDR TB [36]. Furthermore, whether metformin or other glucose-lowering agents are preventive of post-TB mortality is unknown. Among patients with diabetes, hypoglycemic agents may prevent patients from developing diabetes complications. Thus, among TBDM patients, it is plausible that hypoglycemic agents would also prevent premature post-TB deaths associated with diabetes complications. Additionally, if TB recurrence is indeed a mediator in the causal pathway from diabetes to post-TB mortality, the use of metformin during TB treatment is expected to prevent the TB recurrence, ultimately suppressing the risk of post-TB mortality.

While emerging evidence suggests that patients treated for TB may be at risk of chronic non-communicable diseases, studies reporting the incidence of cardio-metabolic diseases are scarce. The majority of post-TB health studies relied on retrospective data due to the long progression of metabolic diseases such as diabetes. Moreover, whether patients treated for DRTB poses a greater risk of metabolic diseases post-TB remains unknown. Understanding to what and by which extent TB can increase the risk of post-TB metabolic diseases is critical to determine the potential preventive actions to avoid premature deaths among patients treated for TB disease. Furthermore, the lack of guidelines on how to follow-up TB patients after treatment is one of the key reasons why it is difficult to measure metabolic disease incidence after TB treatment. Screening for simple and inexpensive markers/indicators of metabolic diseases such as metabolic syndrome and visceral adipose index after TB treatment completion may provide a snapshot of cardio-metabolic risk profile after TB treatment completion.

STATEMENT OF PURPOSES

The overall goal of this dissertation is to explore the potential complications after TB treatment, an area that is often neglected in TB research. To achieve our goals, we conducted three observational studies (two retrospective studies and one prospective cohort study) among patients treated for TB disease in the country of Georgia **(Figure 1.2)**. In Georgia, National Center for Tuberculosis and Lung Diseases (NCTLD) acts as the Georgian National TB program executor and treats approximately n=4,000 TB patients annually, including ±300 DRTB patients. The NCTLD manages two hospitals (one for DSTB and one for DRTB), one ambulatory clinic, and a national reference laboratory for TB. The three dissertation studies included adult pulmonary TB patients (≥16 years old) with bacteriological confirmation before or at TB treatment initiation.

Study 1 aimed to determine whether pre-existing comorbidities such as hyperglycemia, HCV, or HIV co-infection are associated with higher post-TB mortality rates among patients treated for DRTB. This study will help identify groups of patients that are at increased risk of post-TB mortality. Identifying the high-risk group will help clinicians and public health experts design and tailor a clinical or public health intervention to reduce post-TB mortality rates.

Study 2 aimed to evaluate T2DM characteristics associated with poor TB treatment outcomes, measured in three different metrics: 1) time to achieve sputum culture conversion, 2) final TB treatment outcomes, and 3) post-TB mortality. More specifically, we described the use of blood glucose-lowering agents among DRTB patients with T2DM and its effects on TB treatment outcomes. Although emerging studies reported potential roles of metformin as host-directed therapy for TB treatment, our study provides evidence from a cohort of DRTB patients with T2DM.

Study 3 aimed to estimate the prevalence of metabolic syndrome and VAI levels at the end of TB treatment among a cohort of patients successfully treated for TB disease. This study will help establish one of the plausible biological pathways explaining how TB increases the risk of chronic non-communicable diseases, specifically cardio-metabolic diseases. Results from Study 3 will highlight the importance of DRTB on cardio-metabolic risks post-TB treatment. Study 3 will also be one of the first few studies to provide epidemiologic evidence on the risk of cardio-metabolic diseases post-TB treatment with a prospective cohort study design.

Altogether, the three dissertation studies will increase awareness on post-TB complications and contribute new knowledge to better understand the possible clinical long-term impacts of TB disease. Results from this dissertation also underscore the importance of a life-course approach to fully understand the consequences of TB disease. Lastly, this dissertation will also provide epidemiologic evidence to advocate clinicians and public health experts to formulate recommendations and guidelines to continue care after TB treatment to prevent post-TB metabolic diseases and deaths.

Figure 1.2 Framework depicting each post-TB study area and potential study questions addressed

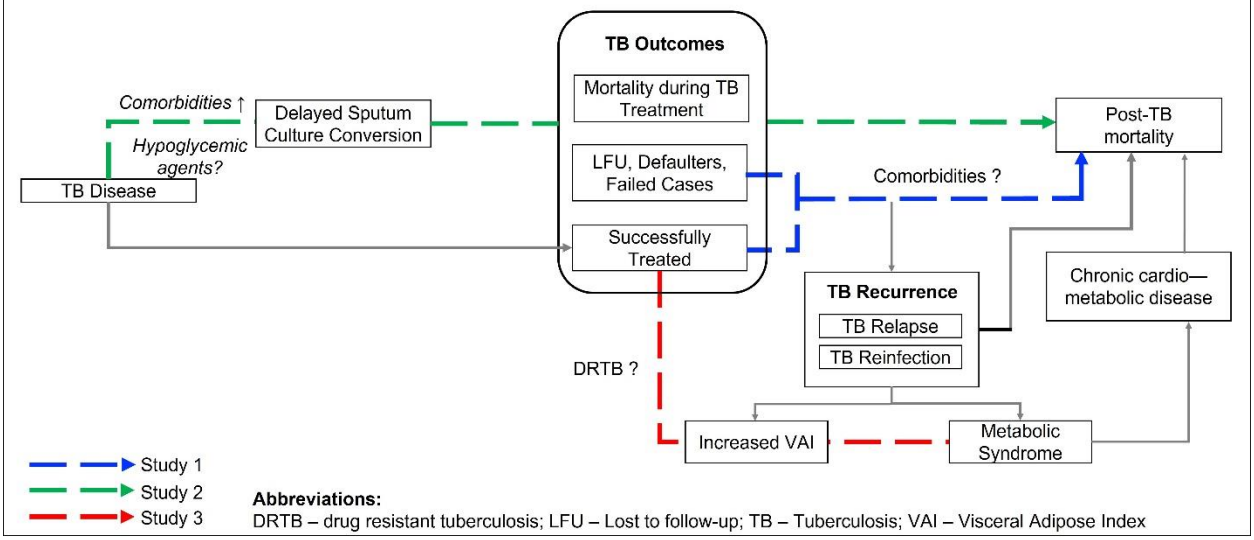

Table 1.1 Definitions of metabolic syndrome according to multiple guidelines

Metabolic Syndrome Measures*	Categorical Cut Points		
	Definition 1 AHA	**Definition 2 IDF†**	**Definition 3 AACE**
Elevated waist circumference	≥102cm (male) ≥88cm (female)	White: ≥102cm (male), ≥88cm (female) Other races: ≥90cm (male), ≥80 (female) or BMI ≥30kg/m^2	US Standard >102cm for male >88cm for female
Elevated triglycerides	≥150mg/dL or on treatment to reduce blood lipid levels	≥150mg/dL or on treatment to reduce blood lipid levels	≥150mg/dL
Reduced HDL-C	<40mg/dL for male <50 mg/dL for female or on treatment to reduce cholesterol levels	<40mg/dL for male <50mg/dL for female or on treatment to reduce cholesterol levels	<40mg/dL form male <50mg/dL for female
Hypertension	Systolic ≥130mmHg and/or diastolic ≥85mmHg or on treatment for hypertension	Systolic ≥130mmHg and/or diastolic ≥85mmHg or on treatment	Systolic ≥130mmHg and/or diastolic ≥85mmHg
Elevated blood glucose	FBG ≥100mg/dL or on treatment to lower blood glucose level	FBG ≥100mg/dL or previous diagnosis of DM	FBG ≥110 mg/dL

Abbreviations:
AHA – American Heart Association; AACE – American Association of Clinical Endocrinology; DM – diabetes mellitus; IDF – International Diabetes Federation; FBG – fasting blood glucose; HDL-C – high density lipoprotein plasma concentration
*Metabolic syndrome was defined as having at least 3 of the listed measures
†IDF definition was a little different as it requires the person to have an elevated waist circumference or BMI ≥30kg/m^2 in addition to 2 other measures

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CHAPTER 2

Paper 1: Comorbidities and mortality post-tuberculosis treatment: A retrospective cohort study among patients previously treated with second-line tuberculosis drugs in Georgia

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ABTSRACT

Background: We aimed to determine the relationship between pre-existing comorbidities (including hyperglycemia, hepatitis C virus [HCV], and human immunodeficiency virus [HIV]) with rates of all-cause post-TB mortality.

Methods: We conducted a retrospective cohort study among patients treated for TB in the country of Georgia during 2009-2017. Eligible participants were >15years with newly diagnosed laboratory-confirmed pulmonary TB who received second-line treatment. Exposures included hyperglycemia, HCV, and HIV serologic status. The outcome was mortality post-TB treatment determined (through November 2019) by cross-validating vital status with the Georgia's national death registry. We estimated hazard rate ratios (HR) and 95% confidence intervals (CI) of post-TB mortality among participants with and without pre-existing comorbidities using cause-specific hazard regression.

Results: Among 1032 with vital status determined and were included in analyses, 34 (3.3%) participants died during treatment and 87 (8.7%) died after TB treatment. Among those who died post-TB treatment, median time to death was 21 months (IQR 7–39) after TB treatment. Adjusting for age and gender, the hazard rates of mortality post-TB treatment were higher among participants with hyperglycemia (adjusted hazard ratio [aHR] 1.19, 95%CI 0.73–1.96), HCV (aHR 1.25, 95%CI 0.71–2.19), or HIV co-infection (aHR 4.40, 95%CI 2.17–8.93) compared to those without.

Conclusions: In our cohort, post-TB mortality occurred most commonly in the first three years after TB treatment ended. Additional post-TB care/follow-up may reduce rates of death post-TB treatment.

Keywords: post-TB mortality; comorbidities; hyperglycemia, HIV, hepatitis C

INTRODUCTION

Tuberculosis (TB) remains a leading cause of infectious disease death with an estimated death toll of nearly 1.5 million annually [1]. The END TB strategy includes a goal to reduce TB mortality by 95% in 2035 (i.e., compared to 2015) [2]. Unfortunately, the reported number of TB deaths is likely to be underestimated because deaths after lost to follow-up or treatment failure may not be captured nor included in the programmatic TB report [3]. To meet the END TB goals, it is important to better understand TB-related mortality including deaths occurring after the completion of TB treatment.

It is well documented that patients treated for TB disease may have higher mortality rates compared to the general population [3, 4]. Mounting evidence suggests that despite microbial cure and a favorable final clinical outcome, patients formerly treated for TB disease may still be exposed to various residual effects or complications post-TB treatment such as lung impairments and other chronic non-communicable diseases risks related to the host inflammatory response during and after an active TB episode [5-8]. However, little is known regarding the relationship between pre-existing comorbidities (i.e., comorbidities diagnosed prior to or at the beginning of TB treatment) and post-TB mortality. It is critical to identify host-related risk factors including pre-existing comorbidities associated with post-TB mortality to help reduce excess TB-related mortality.

Previous studies have consistently reported that older age, male gender, lower education level or socioeconomic status, alcohol abuse, tobacco smoking, and poor TB treatment outcomes were associated with increased risks of post-TB mortality [4, 9-13]. Highly drug-resistant forms of TB such as multidrug-resistant TB (MDR TB) and human immunodeficiency virus (HIV) co-infection were also associated with higher rates of mortality post-TB treatment [9, 14, 15].

However, whether other common comorbidities such as hyperglycemia (i.e., elevated blood glucose with or without diabetes/pre-diabetes) or positive anti hepatitis C antibody (e.g., hepatitis C co-infection [HCV]) diagnosed before or at the time of TB treatment initiation are also predictive of post-TB mortality is unknown. Understanding the extent to which these pre-existing comorbidities are associated with post-TB mortality is critical to help identify preventive strategies to reduce post-TB mortality rates.

Given the existing knowledge gaps, we aimed to a) estimate the rate of all-cause mortality post-TB treatment among patients treated with second-line TB drugs (SLDs) and b) determine the association between pre-existing comorbidities and mortality post-TB treatment using data of patients treated with SLDs from the country of Georgia.

METHODS

Study Design and Setting

We conducted a retrospective cohort study among adults (≥16 years old) newly diagnosed and laboratory-confirmed pulmonary drug-resistant TB (DRTB). Patients treated with SLDs and reported to the Georgia National Center for Tuberculosis and Lung Disease surveillance system from January 2009 – December 2017 were eligible to be included in the study. Per Georgian National TB guidelines, a complete blood count, chest X-ray, sputum smear and culture, and drug susceptibility testing (DST) were performed among all TB patients at treatment initiation. Fasting blood glucose (FBG) level at treatment initiation was only recorded in the surveillance system for patients treated with SLDs. Among patients with DRTB, routine care at TB treatment initiation also included diagnostic tests to screen for chronic comorbidities including HIV and viral hepatitis (hepatitis C and B) infections. In Georgia, all culture-confirmed patients are recommended to be

hospitalized until their sputum smears convert to negative and they demonstrate clinical improvements. After hospital discharge, a monthly visit to a directly observed therapy (DOT) ambulatory clinic is required for all patients with DRTB.

Definitions and Study Measures

The primary exposures of this study were the presence of comorbidities diagnosed before or at TB treatment initiation, including a) hyperglycemia, b) hepatitis C, and c) HIV co-infection. Hyperglycemia was determined by either a self-reported previous diagnosis of type-2 diabetes (T2DM), or a fasting blood glucose (FBG) level. Patients with FBG level ≥5.6 mmol/L or a self-reported prior T2DM diagnosis were categorized as patients with "hyperglycemia." Patients with FBG level <5.6mmol/L and no history of diabetes diagnosis were classified as "non-hyperglycemic." We also categorized T2DM status in three different levels: 1) no diabetes, 2) pre-diabetes (FBG ≥5.6 mmol/L), and 3) diabetes (previous diagnosis of T2DM from a healthcare provider or FBG ≥7.0 mmol/L). HIV and hepatitis C co-infections were determined by the antibody test results recorded in the surveillance system. We also defined "any comorbidity" if patients had a record of either elevated baseline fasting blood glucose at TB treatment initiation, previous diagnosis of diabetes, positive anti-hepatitis C or hepatitis B antibody, HIV positive, self-reported cardiovascular disease, kidney disease, ulcer, pancreatitis, chronic obstructive pulmonary disease, silicosis, or sarcoidosis. We then grouped the number of comorbidities as "none", "1 – 2", and "≥3".

Our primary study outcome, all-cause mortality post-TB treatment, was determined by cross-referencing patients' national unique identifier (or name and date of birth) with mortality status recorded in the death registry managed by the National Statistics Office of Georgia. The

study person time was measured from the beginning of TB treatment initiation until November 13th, 2019, when mortality queries were cross-referenced with the vital registry's office. Covariates included in our study (e.g., final TB treatment outcomes, self-reported smoking status) were defined according to surveillance records.

Statistical Analyses

Chi-square/Fisher's exact and Wilcoxon rank-sum tests were performed to assess the associations between patients' characteristics including comorbidities status and mortality post-TB. Poisson regression was used to estimate rates of all-cause mortality post-TB treatment (expressed by 1,000 person-years). Univariate and multivariable proportional hazard models with competing risks were used to estimate the hazard rate ratios (HR) of all-cause mortality post-TB comparing patients with and without pre-existing comorbidities [16]. In the cause-specific models estimating the HR of mortality post-TB treatment, patients were censored if they a) died during TB treatment (i.e., competing risk) or b) survived until the day mortality status was verified. Proportional hazard assumptions were assessed using 1) Kolmogorov-type supremum and 2) Schoenfeld's residuals tests [17]. Purposive covariates selection was used to determine variables included in the final multivariable models after considering the established risk factors identified in previous studies, the observed bivariate associations, and directed acyclic graph [18].

Subgroup analyses were performed to determine whether the effect of pre-existing comorbidities on all-cause mortality post-TB treatment varied among those who had favorable vs. poor TB treatment outcomes. Additionally, we assessed the interactions between pre-existing comorbidities and smoking to determine if the association between pre-existing comorbidities and all-cause mortality post-TB treatment varied by smoking status. We assessed the statistical

interaction by including the cross-product terms in the multivariable models. Statistical analyses were performed using SAS version 9.4 (Cary, North Carolina).

Sensitivity Analyses

Sensitivity analyses were performed to quantify systematic errors due to a) distribution assumptions used in the regression analyses, b) misclassification of hyperglycemia status and c) unmeasured or unknown confounders. We ran additional log-binomial models to estimate the cumulative risks of post-TB mortality comparing patients with comorbidities to those without comorbidities. To quantify systematic errors due to plausible hyperglycemia misclassifications, we performed probabilistic bias analyses using beta distribution [19]. To quantify systematic errors due to unmeasured/unknown confounders, we calculated E-value. E-value is an estimate of the minimum strength of association between unmeasured/unknown confounders with study exposures as well as study outcome to explain away the observed association between pre-existing comorbidities and all-cause mortality post-TB treatment [20].

RESULTS

Study Population and Characteristics

During the study period, there were 5,385 DRTB treatment episodes recorded in the surveillance system, 31.5% (1,697/5,385) of which were classified as new cases (i.e., no prior history of TB treatment) (Figure 2.1). Of these, 1,416 (83.4%) met our eligibility criteria and were submitted to Georgia's vital registry. We excluded 384 patients without unique national identifier (and no record match according to name and date of birth), leaving 1032 patients (72.9%) included in our analyses. Patients excluded due to missing vital status were similar to patients included in

the analyses in regard to age and gender distribution as well as prevalence of MDR or pre-XDR TB, cavitary disease, hyperglycemia, hepatitis C and HIV co-infections. Among patients included, the majority were male (73%), with a median age of 35 years old (interquartile range [IQR] 26 – 49) (Table 2.1). The majority of patients had multidrug-resistant TB (MDR TB) (44.7%, 461/1032) or pre-extensively drug-resistant TB (pre-XDR TB) (29.1%, 300/1032) (combined prevalence 73.7%). The prevalence of hyperglycemia, hepatitis C, and HIV co-infections were 22.7% (95% confidence interval [CI] 20.2 – 25.3), 14.7% (95%CI 12.8 – 17.1), and 3.8% (95%CI 2.7 – 5.1), respectively. Among patients included in the final analyses, 34 (3.3%) died during TB treatment with a median time to death of 4 months (IQR 1 – 7) after TB treatment initiation. Among 998 (96.7%) TB survivors, there were 87 (8.7%) post-TB deaths that occurred during 4,857 person-years of follow up (age-adjusted post-TB mortality rate 17.9, 95%CI 17.0 – 18.0) (Table 2). The majority (66.7%, 58/87) of post-TB mortality occurred in the first three years with a median time to death of 21 months (IQR 7 – 39) after TB treatment was completed and/or stopped (Figure 2.2).

Pre-Existing Comorbidities and All-Cause Mortality Post-Tuberculosis Treatment

Among 231 TB survivors with hyperglycemia, there were 27 deaths post-TB treatment during 1,129 person-years (post-TB mortality rate 23.91, 95%CI 16.40 – 34.87) (Table 2.2). The post-TB mortality rates among TB survivors with pre-diabetes and diabetes was 21.92 (95%CI 12.73 – 37.75) and 26.12 (95%CI 15.47 – 44.10), respectively. Among 147 TB survivors with hepatitis C co-infection, there were 18 deaths post-TB treatment during 701 person-years (post-TB mortality rate 25.68, 95%CI 16.18 – 40.76). Among 32 TB survivors with HIV co-infection, there were 9 deaths post-TB treatment during 130 person-years (post-TB mortality rate 69.23, 95%CI 36.02 – 133.10).

After adjusting for age and gender, the hazard rate of post-TB mortality among TB survivors with any hyperglycemia was 1.19 (95%CI 0.73 – 1.96) times the hazard rate among those without hyperglycemia. Similarly, the hazard rate of all-cause post-TB mortality among TB survivors with hepatitis C co-infection was 1.25 (95%CI 0.71 – 2.19) times the hazard rate among those without hepatitis C co-infection after adjusting for age and gender. The hazard rate of all-cause post-TB mortality among TB survivors with HIV co-infection was significantly higher (adjusted hazard rate ratio [aHR] 4.40, 95%CI 2.17 – 8.93) compared to those without HIV co-infection. Our estimates did not change substantially in the fully adjusted models (Supplemental Table 1).

Any pre-existing comorbidities diagnosed before or at the time of TB treatment initiation were common in our cohort of TB survivors (60.5%, 624/1032). Among TB survivors with pre-existing comorbidities, the majority (61.9%, 386/624) had one or two diseases at the beginning of TB treatment. The hazard rate of all-cause mortality post-TB among TB survivors with any comorbidities was 1.34 (95%CI 0.84 – 2.15) times the hazard rate among those without any comorbidities after adjusting for age and gender (Table 2). After adjusting for age and gender, the hazard rate of mortality post-TB was higher among TB survivors with one or two pre-existing comorbidities (aHR 1.28, 95%CI 0.77 – 2.13) compared to those without pre-existing comorbidities. Similarly, the hazard rate of mortality post-TB was higher among TB survivors with three or more pre-existing comorbidities (aHR 1.46, 95%CI 0.82 – 2.59) compared to TB survivors without pre-existing comorbidities.

Results From Interaction, Bias, and Sensitivity Analyses

The association between hyperglycemia and mortality post-TB treatment was similar among TB survivors with favorable (aHR 1.08, 95%CI 0.45 – 2.59) and poor TB treatment outcomes (aHR 1.04, 95%CI 0.55 – 1.94) (Table S2.2). Among TB survivors who had favorable TB treatment outcome, those with hepatitis C co-infection had higher hazard rate of mortality post-TB treatment compared to those without hepatitis C co-infection (aHR 1.78, 95%CI 0.70 – 4.54). However, among TB survivors who had poor TB treatment outcome, those with hepatitis C co-infection had similar hazard rate of mortality post-TB treatment compared to those without hepatitis C co-infection (aHR 0.94, 95%CI 0.46 – 1.92). Compared to TB survivors without HIV co-infection, those with HIV co-infection had a higher hazard rate of mortality post-TB treatment regardless of their final TB treatment outcome ($aHR_{favorable}$ 2.84, 95%CI 0.37 – 21.87; aHR_{poor} 3.49, 95%CI 1.61 – 7.57).

We found that the multiplicative effects of hyperglycemia and smoking, or HIV co-infection and smoking on mortality post-TB treatment were non-significant (statistical interaction $p>0.05$) (Table S2.3). However, the hazard rate ratio of all-cause mortality post-TB comparing TB survivors with hepatitis C co-infection to those without was 3.12 (95%CI 0.32 – 21.02) among non-smokers vs. 1.70 (95%CI 0.78 – 3.74) among smokers (statistical interaction $p=0.04$).

In the log-binomial models, the risk of post-TB mortality among TB survivors with hyperglycemia was 1.59 (95%CI 1.00 – 2.52) the risk of those without hyperglycemia. The risk of post-TB mortality was also higher among TB survivors with hepatitis C (crude risk ratio [cRR] 1.54, 95%CI 0.92 – 2.58) or HIV co-infection (cRR 3.63, 95%CI 1.99 – 6.61). The median of bias-adjusted RRs after accounting for hyperglycemia misclassification was 1.84 (2.5^{th} – 97.5^{th} percentile 1.10 – 2.92) in the non-differential model vs. 1.75 (2.5^{th} – 97.5^{th} percentile 1.01 – 3.00)

in the differential models. This indicates that misclassification of hyperglycemia in our cohort may result in bias towards the null. The E-values of ≥2.56, ≥2.43, and ≥6.72 could explain away the observed association for hyperglycemia, hepatitis C, HIV co-infection, and post-TB mortality risk.

DISCUSSION

In our large cohort of DRTB patients treated with SLDs, we reported nearly 10% mortality post-TB treatment among TB survivors vs. 3% mortality during TB treatment. Furthermore, we found that post-TB mortality occurred most commonly in the first three years after TB treatment ended. Additionally, we observed an overall trend toward increased post-TB mortality rates among patients with HIV co-infection compared to those without HIV co-infection.

We reported a higher rate of all-cause mortality rate in our cohort of patients treated for DRTB when compared to mortality rates of Georgia's general population in 2019 (17.3 vs. 12.5/1,000 person-years) [21]. This is consistent with previously published studies in different settings [3, 4, 10, 14, 22-24]. However, our cohort did not include non-TB individuals as a control group. Thus, we were not able to draw direct comparison on mortality rates between TB patients vs. non-TB individuals in Georgia.

In our cohort, we reported more than 4-fold increased hazard rates of all-cause mortality post-TB treatment among DRTB patients with HIV co-infection compared to those without. A prospective cohort study conducted in Vietnam reported that the hazard rate of post-TB deaths among TB patients with HIV was 5.1 (95%CI 4.2 – 6.3) times the hazard rate among TB patients without HIV [23]. However, this hazard estimation combined patients who died before and after TB treatment. Another study using data from the HIV Epidemiology in South American networks reported a higher hazard rate of death at five years after TB treatment completion among HIV

patients with TB diagnosis within 30 days of enrollment to the HIV clinic vs. those without TB (aHR 1.57, 95%CI 1.25 – 1.99) [25]. Moreover, in the subgroup analyses among TB-HIV patients, a lower CD4 count was associated with higher rates of deaths post-TB treatment (aHR 1.57, 95%CI 1.41 – 1.76). Unlike this South America's study, which only included drug-sensitive TB (DSTB) patients, our study only included patients with DRTB, and this may partially explain the higher effect of HIV on post-TB mortality reported in our study. Our study also had longer follow-up time (up to eight years of follow-up time) and we utilized competing risks model to distinctly modeled the hazard rates of deaths observed during and after TB treatment.

Hyperglycemia has been associated with increased risk of mortality during treatment [26-28]. However, the association between hyperglycemia and the risk of post-TB mortality is not well characterized. We reported a non-significantly higher hazard rate of mortality post-TB treatment among TB survivors with hyperglycemia compared to those without hyperglycemia. A retrospective cohort study using data from the vital statistics conducted in Israel found that there were less deaths due to diabetes among patients successfully treated for TB disease compared to the general population [4]. In contrast, a prospective study conducted in Mexico reported a higher proportion of non-TB related deaths post-TB treatment among patients with T2DM (4.9%) compared to those without T2DM (2.5%) [29]. The inconsistent findings of the association between pre-existing T2DM and the risk of post-TB deaths may be affected by the use of blood glucose-lowering agents and blood glucose management during and post-TB treatment. For instance, metformin use was associated with reduced mortality risk among patients in a study conducted in Taiwan [30]. Further study to assess the impact of T2DM clinical characteristics including the use of blood glucose-lowering agents during TB treatment on the risk of mortality post-TB deaths are still warranted.

In our cohort, TB survivors with hepatitis C co-infection had 25% higher rate of post-TB mortality compared to those without hepatitis C co-infection. To our knowledge, to date, there is no other study that is looking at the long-term outcomes of TB patients with hepatitis C co-infection. It is well known that anti-tuberculosis therapy, especially SLDs, may cause hepatotoxicity and could lead to adverse TB treatment outcomes [31, 32]. Hepatotoxicity could also lead to permanent damage to the liver and may increase the risk of deaths [33]. However, more epidemiologic evidence is needed to link hepatitis C to mortality post-TB treatment.

There are several plausible biological pathways to explain how pre-existing comorbidities could increase the risk of post-TB mortality. First, patients with pre-existing comorbidities may experience prolonged chronic and systematic inflammation during TB treatment, which could lead to severe disease manifestations/complications [34, 35] which, in turn, will increase the risk of mortality post-TB treatment. Second, patients successfully treated for TB with pre-existing comorbidities like T2DM may have carry over or residual complications such as proatherogenic and abnormal lipid plasma profile, an established risk factor for cardiovascular diseases [36]. Third, patients with pre-existing comorbidities may also have increased risks of TB relapse (i.e., re-infection or recurrent after treatment) due to their impaired immune system [37-39]. Further clinical studies are needed to establish which of these pathways is more common among TB survivors with pre-existing comorbidities.

Our study subjects to several limitations. First, we performed our analyses among patients treated from 2009 – 2017. Thus, cohort effects associated with changes in national TB treatment guidelines and/or care for TB patients with pre-existing comorbidities are likely affected our reported estimates. Second, we did not have data on the cause of death. Thus, we were not able to assess whether or not mortalities post-TB treatment observed in this study are directly associated

with pre-existing comorbidities. Third, we did not have clinical information on pre-existing comorbidities such as CD4 counts, blood glucose level during TB treatment, viral load, or whether or not patients were on medications to manage their comorbidities. Fourth, we identified several plausible sources of systematic errors, including bias due to misclassification of hyperglycemia status. However, after running the sensitivity analyses, we did not see any significant difference in our estimates that would change the study's overall findings.

In conclusion, we reported an overall higher rate of post-TB deaths vs. deaths during TB treatment among patients treated for DRTB. More importantly, the majority of post-TB deaths were observed three years after TB treatment was stopped. Aggressive approaches to identify patients at greatest risk of mortality after TB treatment are needed to help prevent premature deaths. Additionally, continuous care for pre-existing comorbidities during and after TB treatment may reduce the increased risks of deaths post-TB treatment.

Chapter 2 References

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TABLES AND FIGURES

Figure 2.1 Study flow

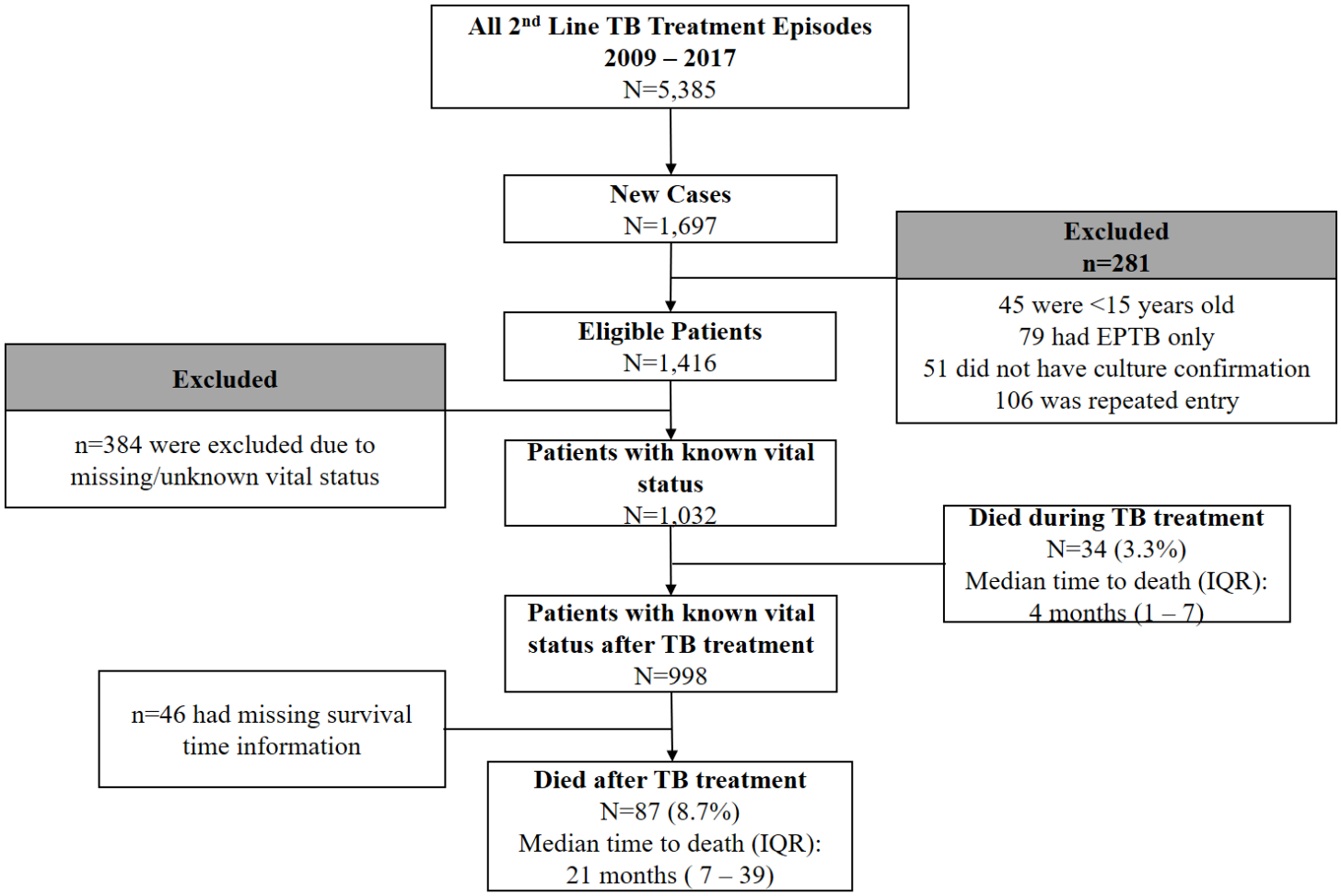


Table 2.1 Demographic and clinical characteristics of patients treated with second-line tuberculosis drugs according to comorbidity status, Georgia, 2009 – 2017 (N=1,032)

Characteristics	Total N=1032 N (%)	Hyperglycemia* N (%) =234 (22.7) N (%)	Hepatitis C# N (%) =153 (14.7) N (%)	HIV^ N (%) =39 (3.8) N (%)
Demographic				
Age				
Years, median (IQR)	35 (26 – 49)	42.5 (32 – 54)	42 (34 – 50)	40 (35 - 47)
Age Group				
16 – 40	642 (62.2)	107 (16.7)	72 (11.2)	21 (3.3)
41 – 65	347 (33.6)	109 (31.4)	76 (21.9)	18 (5.2)
66+	43 (4.2)	18 (41.9)	5 (11.6)	0 (0)
Male Gender	753 (73.0)	189 (25.1)	148 (19.7)	33 (4.4)
Ethnicity, Georgian	42 (91.3)	212 (22.5)	142 (15.1)	33 (3.5)
Baseline Clinical Information				
BMI				
Kg/m^2, median (IQR)	20.8 (19.1 – 22.9)	21.6 (19.9 – 24.8)	21.1 (19.4 – 23.2)	21.2 (18.4 – 22.0)
BMI Category				
Underweight (BMI <18.5)	170 (18.6)	24 (14.1)	1 (12.4)	10 (5.9)
Normal (BMI 18.5 – 24.9)	648 (70.8)	135 (20.8)	95 (14.7)	23 (3.6)
Overweight (BMI 25.0 – 29.0)	76 (8.3)	38 (50.0)	17 (22.4)	4 (5.3)
Obese (BMI ≥30)	21 (2.3)	13 (61.9)	2 (9.5)	0 (0.0)
Missing	117	24	18	2
Smear Positive	652 (66.6)	173 (26.4)	98 (15.0)	13 (2.0)
Culture Positive (n=883)	876 (99.2)	199 (22.7)	128 (14.6)	37 (4.2)
MDR/pre-XDR TB	761 (73.7)	170 (22.3)	118 (15.5)	32 (4.2)
Abnormal Chest X-ray Findings	975 (96.2)	213 (22.1)	149 (15.3)	34 (3.5)
Cavitary Disease	285 (27.6)	64 (22.5)	44 (15.4)	5 (1.8)
Infiltrate	755 (73.2)	169 (22.4)	113 (15.0)	22 (2.9)
Disseminated TB disease	251 (24.3)	60 (23.9)	45 (17.9)	10 (4.0)
Other Risk Factors				
Contact with DRTB patients	153 (14.8)	29 (19.0)	19 (12.4)	5 (3.3)

Characteristics	Total N=1032 N (%)	Hyperglycemia* N (%) =234 (22.7) N (%)	Hepatitis C# N (%) =153 (14.7) N (%)	HIV^ N (%) =39 (3.8) N (%)
History of internally displaced	36 (4.1)	5 (13.9)	6 (16.7)	2 (5.6)
Tobacco Use	455 (44.1)	104 (22.9)	97 (21.3)	18 (4.0)
Excessive Alcohol Intake	59 (5.9)	16 (27.1)	16 (27.1)	1 (1.7)
Other comorbidities				
Hepatitis B	32 (3.1)	9 (28.1)	8 (25.0)	3 (9.4)
CVD	12 (1.2)	7 (58.3)	5 (41.7)	0 (0.0)
Treatment Outcome				
Poor final treatment outcome	325 (33.2)	74 (22.8)	69 (21.2)	26 (8.0)
Return to care	95 (9.2)	32 (33.7)	18 (19.0)	6 (6.3)
Survival Status				
Alive	911 (88.3)	204 (22.6)	129 (14.2)	23 (2.5)
Mortality during treatment	34 (3.3)	3 (8.8)	6 (17.7)	7 (20.6)
Mortality post-TB treatment	87 (8.4)	27 (31.0)	18 (20.7)	9 (10.3)

*There were 220 with missing/unknown prediabetes/diabetes status
#There were 282 patients with missing/unknown Hepatitis C status
^There were 96 patients with missing/unknown HIV status
†P-values from Chi-square tests
‡P-values from Fisher's exact test
Abbreviations:
BMI – body mass index; CVD – cardiovascular diseases; DRTB – drug-resistant tuberculosis; MDR – multidrug resistant; PDR – polydrug resistant; RR – rifampicin resistant; TB – tuberculosis; XDR – extensively drug-resistant

Bold indicates that the finding is statistically significant at $\alpha=0.05$

Table 2.2 All-cause mortality post-tuberculosis among patients treated with second-line TB drugs with and without pre-existing comorbidities in Georgia, 2009 - 2017

Characteristics	All-cause mortality post-tuberculosis treatment					
	Death/Total (%)	Person time (Years)	Crude Rates (95%CI)	Median time to death post-TB treatment, months (IQR)	Hazard Rate Ratios	
					cHR (95%CI)	aHR (95%CI)*
All cohort	**87/998 (8.7)**	**4857**	**17.91 (14.43 – 21.99)**	**21 (7 – 39)**		
By comorbidity status						
Hyperglycemia						
Hyperglycemia						
No	41/558 (7.4)	2712	15.12 (11.13 – 20.53)	28 (9 – 43)	Reference	Reference
Yes	27/231 (11.7)	1129	23.91 (16.40 – 34.87)	19 (7 – 34)	1.60 (0.98 – 2.60)	1.19 (0.73 – 1.96)
Missing	19/209 (9.1)	1016	18.70 (11.93 – 29.32)	21 (6 – 40)	1.24 (0.72 – 2.14)	1.25 (0.73 – 2.16)
Diabetes Status						
No diabetes	41/558 (7.4)	2712	15.12 (11.13 – 20.53)	28 (9 – 43)	Reference	Reference
Pre-diabetes	13/119 (10.9)	593	21.92 (12.73 – 37.75)	17 (7 – 31)	1.47 (0.79 – 2.75)	1.44 (0.77 – 2.70)
Diabetes	14/112 (12.5)	536	26.12 (15.47 – 44.10)	22 (10 – 38)	1.73 (0.94 – 3.18)	1.01 (0.54 – 1.90)
Missing	19/209 (9.1)	1016	18.70 (11.93 – 29.32)	21 (6 – 40)	1.24 (0.72 – 2.14)	1.25 (0.73 – 2.16)
Hepatitis C						
No	46/580 (7.9)	2872	16.02 (12.00 – 21.38)	20 (9 – 39)	Reference	Reference
Yes	18/147 (12.2)	701	25.68 (16.18 – 40.76)	20 (8 – 38)	1.58 (0.92 – 2.73)	1.25 (0.71 – 2.19)
Missing	23/271 (8.5)	1284	17.91 (11.90 – 26.96)	22 (6 – 45)	1.12 (0.68 – 1.85)	1.01 (0.61 – 1.68)
HIV co-infection						
No	68/877 (7.7)	4195	16.12 (12.78 – 20.56)	22 (7 – 39)	Reference	Reference
Yes	9/32 (28.1)	130	69.23 (36.02 – 133.10)	7 (4 – 9)	**4.56 (2.27 – 9.15)**	**4.40 (2.17 – 8.93)**
Missing	10/88 (11.4)	532	18.80 (10.11 – 34.94)	23 (13 – 46)	1.11 (0.57 – 2.17)	1.12 (0.57 – 2.18)
Any Comorbidity†						
No	25/398 (6.3)	1953	12.80 (8.65 – 18.94)	30 (15 – 45)	Reference	Reference
Yes	62/600 (10.3)	2904	21.35 (16.65 – 27.38)	19 (7 – 38)	**1.67 (1.05 – 2.66)**	1.34 (0.84 – 2.15)

Characteristics	All-cause mortality post-tuberculosis treatment					
	Death/Total (%)	Person time (Years)	Crude Rates (95%CI)	Median time to death post-TB treatment, months (IQR)	Hazard Rate Ratios	
					cHR (95%CI)	aHR (95%CI)*
Number of Comorbidities†						
0	25/398 (6.3)	1953	12.80 (8.65 – 18.94)	30 (15 – 45)	Reference	Reference
1 – 2	40/374 (10.7)	1822	21.95 (16.10 – 29.93)	16 (6 – 34)	**1.71 (1.04 – 2.83)**	1.28 (0.77 – 2.13)
≥3	22/226 (9.7)	1082	20.33 (13.39 – 30.88)	21 (7 – 39)	1.60 (0.90 – 2.83)	1.46 (0.82 – 2.59)

*Model adjusted for age and gender (stand-alone models)

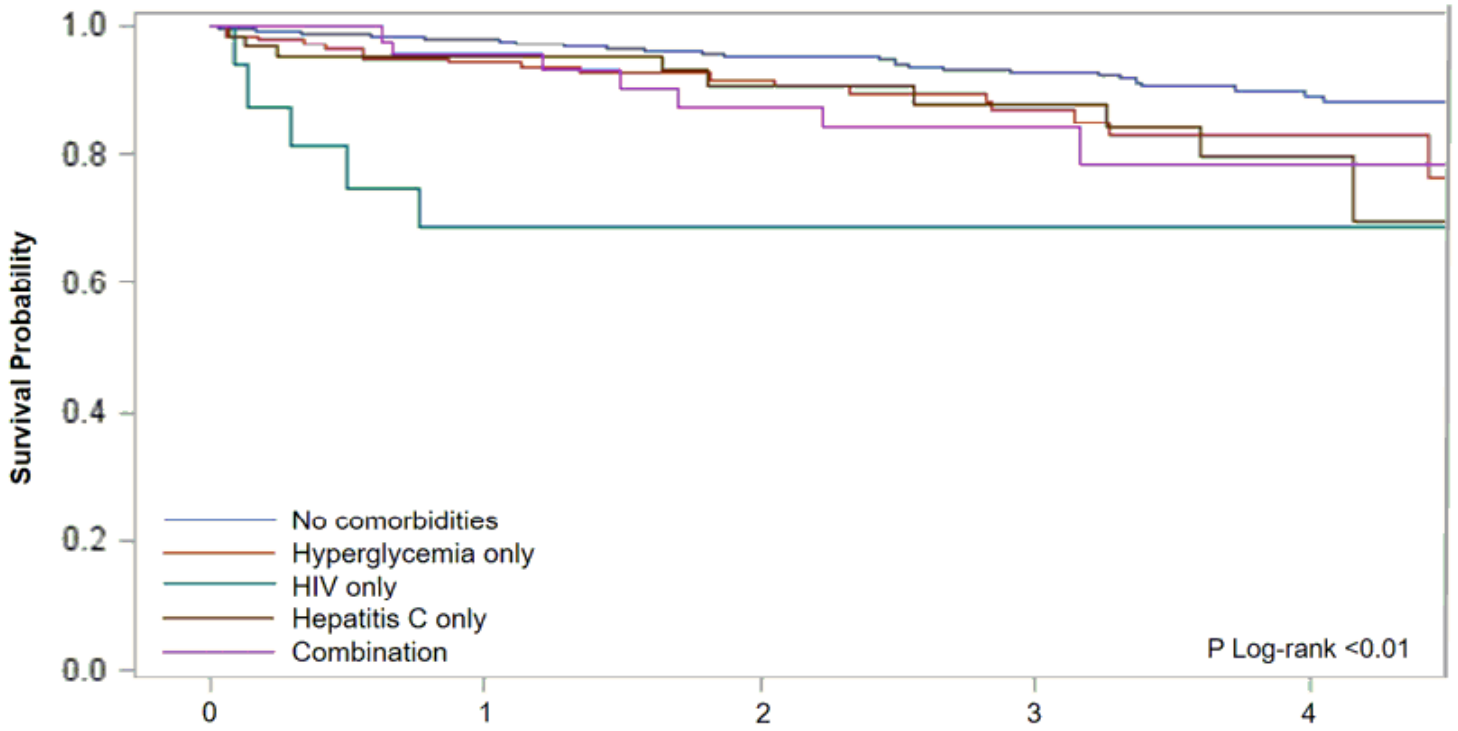
†Other comorbidities such as cardiovascular disease, renal disease, ulcer, pancreatitis, COPD, silicosis, sarcoidosis were included

Abbreviations:

aHR – adjusted hazard rate ratios; cHR – crude hazard rate ratios; CI – confidence interval; HIV – human immunodeficiency virus; RD – rate difference

Bold indicates that the finding is statistically significant at $\alpha=0.05$

Figure 2.2 Kaplan-Meier curve depicting the survival probability post-TB treatment among patients with and without pre-existing comorbidities (N=952)



Number at risk

No Comorbidities	602	550	457	344	237
Hyperglycemia only	186	169	145	104	70
Hepatitis C only	96	87	72	59	43
HIV only	18	13	12	9	7
Combination	50	47	36	26	15

Table 2.3 Sensitivity and subgroup analyses accounting for systematic errors on the association between various comorbidities and all-cause mortality post-tuberculosis among patients previously treated with second-line tuberculosis drugs in Georgia, 2009 – 2017

No	Various Bias/Sensitivity Analyses	Post-TB treatment Mortality Measure of Association	Comorbidities	Crude Estimates	Adjusted Estimates
1	**Model Specification**				
	Log binomial logistic regression (N=998)	Risk ratios	Hyperglycemia	**1.59 (1.00 – 2.52)**	1.19 (0.75 – 1.88) [+]
			Hepatitis C	1.54 (0.92 – 2.58)	1.19 (0.71 – 2.00) [+]
			HIV co-infection	**3.63 (1.99 – 6.61)**	**3.42 (1.90 – 6.16)[+]**
3	**Misinformation Bias**				
	Probabilistic (beta distribution) bias analysis to account for plausible **non-differential** misclassification of hyperglycemia status (1000 iterations)	Median risk ratio (2.5^{th} – 97.5^{th} percentile)	Systematic Error		**1.78 (1.65 – 2.17)**
			Total Error (random+systematic)		**1.84 (1.10 – 2.92)**
	Probabilistic (beta distribution) bias analysis to account for plausible **differential** misclassification of hyperglycemia status (1000 iterations)	Median risk ratio (2.5^{th} – 97.5^{th} percentile)	Systematic Error		**1.73 (1.21 – 2.37)**
			Total Error (random+systematic)		**1.75 (1.01 – 3.00)**
4	**Unknown Confounders**				
	Range of plausible true values adjusting for any unknown confounders using the E-value method[*]	Range of risk ratios	Hyperglycemia		0.30 – 1.19
			Hepatitis C		0.29 – 1.16
			HIV co-infection		0.69 – 2.72
	E-value (the magnitude of RR_{EU} and RR_{UD}) to explain away the observed estimates	Min (RR_{EU}, RR_{UD})	Hyperglycemia	≥ 2.56	
			Hepatitis C	≥ 2.43	
			HIV co-infection	≥ 6.72	

Footnote:
[+]adjusted for age and gender
[*]Simulated with various combination of HR_{EU} 2.00 – 10.00 and HR_{UD} 2.00 – 10.00 resulting in bias factor of 1.3 – 5.3
Abbreviations:
HIV – human immunodeficiency virus
RR_{EU} – risk ratio estimating the relationship between exposure of interest (i.e., pre-existing comorbidities) and any unknown confounders
RR_{UD} – risk rate ratio estimating the relationship between outcome of interest (i.e., post-TB all-cause mortality) and any unknown confounders

Supplemental Materials Chapter 2

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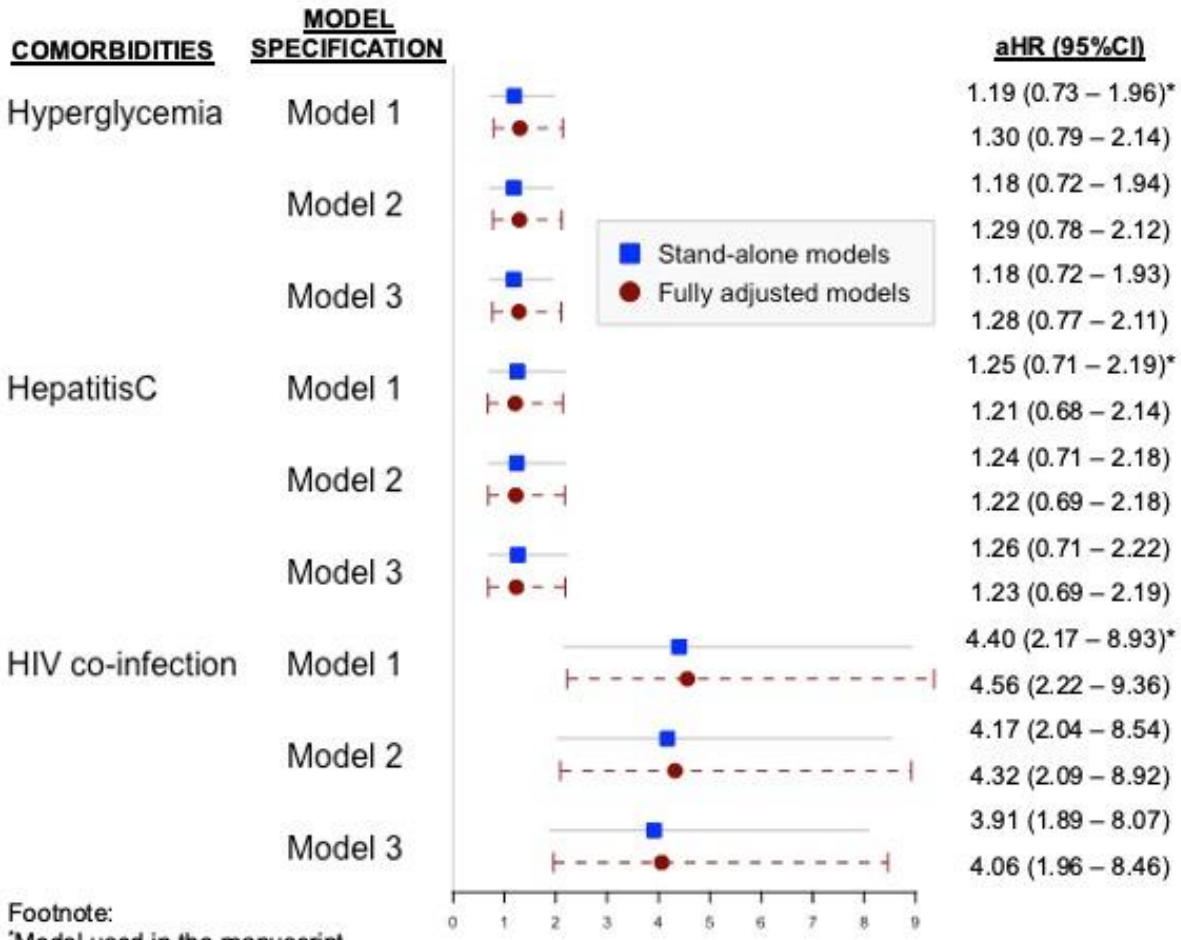
Table S2.1 Multiple multivariable models with different covariate specifications



Footnote:
*Model used in the manuscript
Model 1: adjusted for age and gender
Model 2: adjusted for age, gender, cavitary disease, and type of drug resistance
Model 3: adjusted for age, gender, cavitary disease, type of drug resistance, and smoking
- Stand alone models only contain corresponding pre-existing comorbidity factor and covariates
- Fully adjusted models included all pre-existing comorbidities in the model (i.e., estimates were also adjusted for other pre-existing comorbidity factors)

Table S2.2 Subgroup analyses to assess the association between pre-existing comorbidities and mortality post-tuberculosis treatment according to their final TB treatment outcomes among adult tuberculosis patients previously treated with second-line tuberculosis drugs in Georgia, 2009 – 2017 (N=952)

Pre-existing comorbidities	**aHR[*] (95%CI)**	
	Among those with favorable[†] treatment outcomes (n=640)	**Among those with poor[‡] treatment outcomes (n=312)**
Hyperglycemia		
No	Reference	Reference
Yes	1.08 (0.45 – 2.59)	1.04 (0.55 – 1.94)
Hepatitis-C		
Negative	Reference	Reference
Positive	1.78 (0.70 – 4.54)	0.94 (0.46 – 1.92)
HIV co-infection		
Negative	Reference	Reference
Positive	2.84 (0.37 – 21.87)	**3.49 (1.61 – 7.57)**

Abbreviations: aHR – adjusted hazard rate ratios; CI – confidence interval; HIV – human immunodeficiency virus

[*]Model adjusted for age and gender
[†]Including patients who were cured or completed the treatment
[‡]Including patients who were lost to follow up or failed the treatment

Table S2.3 Assessment of statistical interaction between pr pre-existing comorbidities and smoking among on mortality post-tuberculosis among adult tuberculosis patients previously treated with second-line tuberculosis drugs in Georgia, 2009 – 2017

Smoking Status	Comorbidities Status	Post-TB mortality (%)	cHR (95%CI)	aHR* (95%CI)
Hyperglycemia				
Non-smokers	No	7/74 (9.5)	Reference	Reference
	Yes	2/33 (6.1)	0.66 (0.14 – 3.17)	0.35 (0.07 – 1.78)
Smokers	No	19/256 (7.4)	Reference	Reference
	Yes	8/104 (7.7)	1.00 (0.44 – 2.29)	0.83 (0.36 – 1.93)
Hepatitis C				
Non-smokers	Negative	4/84 (4.8)	Reference	Reference
	Positive	1/9 (11.1)	2.55 (0.28 – 22.86)	3.12 (0.32 – 21.02)
Smokers	Negative	15/232 (6.5)	Reference	Reference
	Positive	11/97 (11.3)	1.96 (0.90 – 4.26)	1.70 (0.78 – 3.74)
HIV Co-infection				
Non-smokers	Negative	9/117 (7.7)	Reference	Reference
	Positive	1/2 (50.0)	**13.68 (1.63 – 114.64)**	**11.87 (1.14 – 124.18)**
Smokers	Negative	32/399 (8.0)	Reference	Reference
	Positive	4/18 (22.2)	**4.72 (1.65 – 12.49)**	**4.75 (1.65 – 13.65)**

Abbreviations: aHR – adjusted hazard rate ratios; cHR – crude hazard rate ratios; CI – confidence interval; HIV – human immunodeficiency virus

*Model adjusted for age and gender

CHAPTER 3

Paper 2: Metformin reduces the risk of poor treatment outcomes among drug-resistant tuberculosis patients with type-2 diabetes mellitus

Prepared according to manuscript instruction for *International Journal for Tuberculosis and Lung Diseases*

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SUMMARY

Introduction: The use of blood glucose-lowering agents (BGLA) among tuberculosis (TB) patients with type-2 diabetes mellitus (T2DM) may improve TB treatment outcomes. However, the use of BGLA among patients with drug-resistant TB (DRTB) is poorly described.

Objective: To describe BGLA use and estimate the association between BGLA use and treatment outcomes among DRTB patients with hyperglycemia.

Methods: We conducted a retrospective cohort study among newly diagnosed and bacteriologically-confirmed DRTB patients with hyperglycemia who initiated TB treatment in Tbilisi, Georgia, from 2009 – 2017. BGLA use was determined by medical chart review. Our study outcomes included: a) sputum culture conversion, b) final TB treatment outcomes, and c) all-cause mortality post-TB treatment. Log-binomial and Cox proportional hazard regression models were used to estimate the association between BGLA use and study outcomes.

Results: There were 128 DRTB patients with hyperglycemia that were included in the analyses. Of these, 60 had pre-diabetes, and 68 had T2DM. No BGLA use was reported among patients with pre-diabetes. Among DRTB patients with T2DM, metformin use was associated with a significantly lower risk of poor TB treatment outcomes after adjusting for age and gender (adjusted risk ratio 0.23, 95% confidence interval 0.05 – 0.97).

Conclusions: Our study findings underscore the potential benefits of incorporating metformin into TB treatment course among patients with DRTB and T2DM.

Keywords: metformin, diabetes, drug-resistant TB, second-line TB drugs, TB treatment outcomes

The convergence of tuberculosis (TB) and type-2 diabetes (T2DM) epidemics is a major barrier to achieve TB elimination goals (1). TB disproportionately affects developing countries in southeast Asia, Africa, and the Western Pacific area (2), regions that have reported high T2DM burden in the past few decades (3, 4). It is well established that T2DM increases the risk of TB infection (5-10), primary progression to TB disease, reactivation of latent infection to active TB disease (11-13), and poor TB treatment outcomes (14-19). With a >50% predicted rise of the global T2DM prevalence by 2045 (i.e., 451 million in 2017 to 693 million in 2045) (20), there is an alarming concern that the burden of TB and T2DM (TBDM) will also increase substantially. Thus, it is critical for the current TB strategy to include recommendations focused on attenuating the negative impact of T2DM on the public health burden associated with TB.

Emerging epidemiologic evidence suggests that the use of metformin may reverse the adverse effect of T2DM on TB infection or disease. Multiple observational studies have found that the use of metformin is associated with reduced rates of mortality during TB treatment (21, 22) and subsequent TB relapse (23). However, whether metformin is associated with improved sputum culture conversion (SCC) remains inconclusive (24). Interestingly, among patients with latent TB infection, the use of metformin was preventive of TB disease progression (25-27) even when compared to other oral hypoglycemic agents such as sulfonylureas (28). The biological pathways underlying metformin's potential properties (vs. other BGLA) in preventing TB disease progression or poor outcomes during TB treatment are unknown.

Metformin is a potential candidate of adjunctive host-directed therapy for TB disease (29, 30). However, there is a lack of data on whether metformin or other BGLAs have any impact on outcomes among patients with drug-resistant TB (DRTB). To date, the majority of clinical studies examining the potential benefits of metformin as host-directed therapy to improve treatment

outcomes were conducted among patient with drug-susceptible TB (22, 31). With the cure rates ~50% among patients with DRTB, it is critical to better understand the association between BGLA use and treatment outcomes among DRTB patients with T2DM. Thus, our study aimed to a) describe the use of BGLA among DRTB patients with hyperglycemia and b) estimate the association between BGLA use and TB treatment outcomes.

METHODS

Study Design and Population

We conducted a retrospective cohort study among adult patients (≥16 years old) who were newly diagnosed with bacteriologically-confirmed pulmonary TB and started their treatment using SLDs in National Center for Tuberculosis and Lung Diseases and two TB dispensaries located in Tbilisi, Georgia. Patients with pre-diabetes (i.e., fasting blood glucose [FBG] 5.7 – 6.9 mmol/L) or T2DM (i.e., FBG ≥7.0 mmol/L or previous diagnosis of T2DM or history of receiving T2DM treatment) at baseline were eligible. A list of potentially eligible patients was pooled from Georgia's TB surveillance system during the 2009 – 2017 period. Normoglycemic patients, pediatric cases, clinical cases (i.e., bacteriology confirmation was not available), and patients treated outside of Tbilisi were excluded from the final analyses.

Definitions and Study Measures

This study's primary exposure is BGLA use during TB treatment including metformin, sulfonylureas, and insulin. Patients with a record of BGLA use were assumed to take the medication as prescribed (i.e., intent-to-treat). According to Georgian TB treatment guidelines, TB patients with FBG 5.7 - 6.1 mmol/L and no prior history of T2DM diagnosis should receive a

follow-up glucose test every 2-3 months with a recommendation to modify patients' diet and lifestyle (32). BGLAs were recommended to be prescribed only among TB patients with FBG >6.1 mmol/L or anybody with a prior diagnosis of T2DM. Other study exposures included T2DM characteristics (e.g., T2DM status, years of living with T2DM). T2DM status was classified dichotomously as "pre-diabetes" and "T2DM". We also categorized T2DM status into three different levels (i.e., "pre-diabetes", "newly-diagnosed T2DM", and "known T2DM") to assess TB treatment outcomes among newly vs. previously diagnosed T2DM patients. Among patients with T2DM, we classify years of living with T2DM as "0-5 years", "6-10 years," and "≥11 years".

Our study outcomes, TB treatment outcomes, were measured in three metrics: a) time to SCC, b) final TB treatment outcome, and c) all-cause mortality post-TB treatment. Time to SCC was defined as the time (measured in days) from TB treatment initiation to the first of two consecutive negative cultures that were at least 30 days apart. We followed World Health Organization's guideline (33) in defining final TB treatment outcomes by grouping patients who received microbial cure status or completed their TB treatment in the "favorable outcome" category. Patients who were lost to follow-up, failed the treatment, or died during TB treatment were grouped in the "poor outcome" category. All-cause mortality post-TB treatment was defined by cross-referencing patients' vital information (first and last name, date of birth, and unique national identifiers) with the death registry managed by the National Statistics Office of Georgia on November 13th, 2019.

Demographic characteristics, case definition, baseline smear and culture results, TB history, behavioral risk factors including drug use, alcohol abuse, smoking, comorbidity factors, and final treatment outcomes were collected from the online surveillance system. Clinical information during TB follow-up visits (i.e., blood works, adverse event episodes) and T2DM

characteristics (i.e., T2DM status, type of T2DM, years of living with T2DM, and records of BGLA prescriptions) were abstracted from patients' medical chart.

Statistical Analyses

We used Chi-square or Fisher's exact tests to assess the association between patient characteristics and T2DM status. Log-binomial logistic regression models were used to estimate the association between T2DM characteristics and the risk of poor TB treatment outcomes. Cox proportional hazard models were used to determine whether T2DM characteristics were associated with a) time to achieve SCC and b) mortality post-TB treatment. We assessed the proportional hazard assumption for the Cox models using the log of negative log graph and goodness-of-fit test using Schoenfeld's residuals (34). Subset analyses were conducted among patients with T2DM to estimate the association between BGLA prescription and TB treatment outcomes. Covariates included in the multivariable models were purposively selected based on potential confounders identified in previously published studies, the observed bivariate associations, and directed acyclic graphs theory (35). All statistical analyses were performed using SAS version 9.4 (Cary, NC) with a p-value <0.05 considered statistically significant.

Sensitivity Analyses

We performed a sensitivity analysis to quantify systematic errors due to covariate misspecification in the multivariable models by running several multivariable models with different combinations of predictors. As part of this sensitivity analysis, we constructed a scoring metric to account for TB severity among patients with DRTB (Table S3.1). TB severity scores

(i.e., continuous, dichotomous [scored ">9.5" or "≤9.5"], and a 4-levels categorical variable [quartiles]) were then used to adjust the association between study exposures and outcomes.

RESULTS

During our study period, there were 1,416 newly diagnosed adults DRTB patients reported to Georgia's TB surveillance database, 234 (16.5%) of which had hyperglycemia according to blood glucose level at time of TB treatment initiation or a record of prior T2DM diagnosis (Figure 3.1). Among this group, 130 (55.6%) started their treatment either in NCTLD facilities or TB dispensaries in Tbilisi. Two of these patients had type-1 diabetes and were excluded from the analyses.

Of the 128 patients included in the final analyses, 60 (46.9%) had pre-diabetes, and 68 (52.1%) had T2DM. The majority of patients with T2DM had a record of BGLA prescription during TB treatment (60/68, 88.2%), 38.3% (23/60) of which had a record of receiving ≥2 classes of T2DM drugs during DRTB treatment (5 received metformin and sulfonylureas; 5 received metformin and insulin; 8 received sulfonylureas and insulin; 5 received all metformin, sulfonylureas, and insulin). Patients with newly diagnosed T2DM with a record of BLGA use (n=12) started their T2DM medication within 2 months after TB treatment initiation (range -3 – 61 days).

None of the DRTB patients with pre-diabetes had any records of BGLA prescription. In our cohort, patients with T2DM were older (median age=54, interquartile range [IQR] 42 – 60) compared to patients with pre-diabetes (median age=32, IQR 23 – 47) ($p<0.01$) (Table 3.1). Overweight/obesity was more common among patients with T2DM (25/56, 44.6%) compared to patients with pre-diabetes (5/57, 8.8%) ($p<0.01$). Patients with pre-diabetes and T2DM were

similar in terms of other characteristics, including reported symptoms at baseline, chest x-ray findings (Table 3.1), and adverse events reported during TB treatment (Table S3.2) (p>0.05).

Diabetes Characteristics and Tuberculosis Treatment Outcomes

Among 122 patients included in the SCC analyses, 109 (85.2%) converted their sputum to negative with a median time to conversion of 87 days (IQR 61 – 115) (Table 3.2). Although non-significant, the median time to achieve SCC among patients with T2DM was higher (median 91 days, IQR 61 – 121) compared to those with pre-diabetes (median 64, IQR 56 – 97) (p=0.15). After adjusting for age and gender, patients with T2DM had a non-significantly lower rate of sputum conversion when compared to patients with pre-diabetes (adjusted hazard rate [aHR] 0.81, 95% confidence interval [CI] 0.52 – 1.26) (Table 3.2). In the model where we differentiated between patients with pre-diabetes, newly-diagnosed, and known T2DM, the lowest hazard rate of SCC was reported among patients with newly-diagnosed T2DM (aHR 0.74, 95%CI 0.41 – 1.32).

Final TB treatment outcome status was available among 96.1% (123/128) of study participants. Poor TB treatment outcome was reported among 44 patients (35.8%) (Table 3.2), including 6 who failed the treatment (i.e., culture remained positive after 5 months after TB treatment initiation), 36 defaults/lost to follow-up, and 2 deaths during TB treatment. The proportion of poor TB treatment outcomes was similar among patients with pre-diabetes (36.2%) and T2DM (35.4%) (p=0.92). After adjusting for age and gender, the risk of poor TB treatment outcomes among patients with T2DM was 0.80 times the risk of those with pre-diabetes (95%CI 0.41 – 1.56). After adjusting for age and gender, patients with newly diagnosed T2DM had the lowest risk of poor TB treatment outcome (cumulative risk=30.3%; aRR 0.66, 95%CI 0.25 – 1.75).

Vital status post-TB treatment was obtained for 109 patients (85.2%). Of these, we reported 15 post-TB deaths during 352 person-years (crude post-TB mortality rate=4.26/100 person-years, 95%CI 2.48 – 6.87). After adjusting for age and gender, the hazard rate of all-cause mortality post-TB treatment among patients with T2DM was 0.72 times the hazard among patients with pre-diabetes (95%CI 0.21 – 2.44) (Table 3.3). Patients with newly diagnosed T2DM had the highest age and gender-adjusted hazard rate of all-cause mortality post-TB treatment (aHR 1.73, 95%CI 0.37 – 8.01).

BGLA Use and Tuberculosis Treatment Outcomes Among Patients With Diabetes

Among those with T2DM, the median time to achieve SCC was similar among patients with metformin use (median 93 days, IQR 61 – 121) compared to those without (median 92.5 days, IQR 62 – 142) (p=0.35). After adjusting for age and gender, the hazard rate of SCC among patients with a record of metformin use was 1.19 times the hazard rate among those without (95%CI 0.64 – 2.18) (Table 3.2). Similar trend was reported among patients with a record of sulfonylureas use (aHR 1.18, 95%CI 0.65 – 2.13) or insulin (aHR 1.13, 95%CI 0.65 – 1.97).

The risk of poor TB treatment outcomes was significantly lower among patients with a record of metformin use compared to those without after adjusting for age and gender (adjusted risk ratio [aRR] 0.23, 95%CI 0.05 – 0.97) (Table 3.2). In the same adjusted model, although non-significant, patients with a record of sulfonylureas use also had a lower risk of poor TB treatment outcomes compared to those without (aRR 0.74, 95%CI 0.32 – 1.66). The risk of poor TB treatment outcomes was similar among patients with or without a record of insulin use after adjusting for age and gender (aRR 1.08, 95%CI 0.57 – 2.04).

The hazard rate of all-cause mortality post-TB treatment among patients with a record of metformin use was 0.66 times the hazard rate among those without metformin use after adjusting for age and gender (95%CI 0.12 – 3.59). Interestingly, although non-significant, the hazard rate of all-cause mortality post-TB treatment was higher among patients with a record of sulfonylureas use (aHR 5.49, 95%CI 0.74 – 40.90). The hazard rates of all-cause mortality post-TB death were similar among patients with or without a record of insulin use (aHR 1.20, 95%CI 0.26 – 5.48).

Results From Sensitivity Analyses

Results from sensitivity analyses suggested that the association between T2DM and the three metrics of TB treatment outcomes were consistent across different multivariable models (range aHR for SCC was 0.58 – 0.84, range aRR for poor TB treatment outcomes was 0.70 – 0.80, and range aHR for all-cause mortality post-TB treatment was 0.35 – 0.67) (Table S3.3-5). In models assessing systematic errors due to covariate misspecification, the aHRs for SCC comparing patients with a record of metformin use to those without ranged from 0.81 – 1.20 (Table S3.3). Other BGLA, such as sulfonylureas and insulin, although non-significant, also improved the SCC. In models assessing systematic errors due to covariate misspecification, the aRRs for poor TB treatment outcomes comparing patients with a record of metformin use to those without ranged from 0.18 – 0.24 (Table S3.4), while the aHRs for all-cause mortality post-TB comparing patients with a record of metformin use to those without ranged from 0.58 – 1.69 (Table S3.5).

DISCUSSION

Among a cohort of patients with DRTB and hyperglycemia from the country of Georgia, BGLA was only prescribed among patients with T2DM and not among patients with pre-diabetes.

Our findings suggested that T2DM patients with metformin prescription had >75% lower risk of poor TB treatment outcomes than those without metformin prescription. Although larger studies are still needed, our results highlight the potential benefits of metformin in improving TB treatment outcomes among DRTB patients with T2DM.

Although non-significant, we reported a higher rate of sputum culture conversion among T2DM patients with metformin/sulfonylureas/insulin prescription. This finding is consistent with a previous study conducted among a cohort of pulmonary TB patients from South Korea that reported a higher sputum conversion rate at 2-month after TB treatment initiation among patients on metformin (60.9% vs. 55.6% among patients who were not on metformin) (p=0.06) (24). However, unlike this South Korean study that was conducted among drug-susceptible TB patients, our study was conducted among DRTB patients that may have more complicated TB clinical manifestations. Additionally, we utilized the survival analysis method, which introduces the time component to the SCC metric. Thus, our hazard ratios estimates may be more informative compared to the crude proportions and odds ratios reported by the South Korean study.

We also reported a significantly lower risk of poor TB treatment among T2DM patients with a record of metformin use than those without. Although non-significant, patients with a record of metformin use also had a lower rate of all-cause mortality post TB treatment. Our findings are consistent with previously published studies, although most were focused on mortality during TB treatment (21, 22). Interestingly, a strikingly increased hazard rate of all-cause mortality post-TB treatment was reported among patients with sulfonylureas prescription (i.e., >5 fold compared to patients without sulfonylureas prescription). Among T2DM patients (without TB), the use of sulfonylureas was associated with an increased risk of cardiovascular events and mortality (36).

However, we did not have any information whether post-TB mortality that we observed among our cohort of patients with sulfonylureas prescription is associated with any cardiovascular events.

The different trends we reported regarding metformin/sulfonylureas/insulin prescription and its association with TB treatment outcomes may be due to the distinctive mechanisms of action or safety levels across different BGLAs. For example, metformin works by reducing the level of hepatic glucose level released to the blood circulatory system as well as suppressing the glucose absorption in the gastrointestinal tract (37), while sulfonylureas promotes the insulin release by the pancreatic β-cells (38). In the face of TB, metformin's anti-inflammatory properties may help control the MTB growth and replication by increasing the mitochondrial reactive oxidative species and macrophage activation (37). However, whether these anti-inflammatory properties are also protective of poor TB treatment outcomes and mortality post-TB treatment is unknown. Whether similar properties are also introduced by other BGLAs such as sulfonylureas and insulin are unknown.

Our study is subject to several limitations. First, our study was conducted among a small cohort of DRTB patients with hyperglycemia indications from Tbilisi, Georgia. Thus, our results may not be generalizable in other parts of Georgia or other countries with a different burden of DRTB and/or T2DM. Second, our study may not be sufficiently powered with the small sample size. Third, all pre-diabetes patients in our cohort did not receive any BGLAs. Thus, we were not able to evaluate whether BGLA use among DRTB patients with pre-diabetes is also protective of poor outcomes during- or post-TB treatment. Fourth, we did not have detailed diabetes information (i.e., patients' adherence during the course of treatment with BGLA, blood glucose control during treatment), so our estimates were not accounted for these potential confounding factors. Lastly, we did not have any information regarding the cause of death post-TB treatment.

In conclusion, we reported a significantly lower risk of poor TB treatment among DRTB patients with T2DM who were on metformin. Our study findings underscore the importance of incorporating metformin (compared to other BGLA) as a candidate of host-adjunctive therapy during the DRTB treatment course (39-41). However, larger prospective studies to further understand the effect of metformin on TB treatment outcomes are still needed. Additionally, further studies aiming to assess whether metformin prescription can also improve TB outcomes among patients with pre-diabetes are still warranted.

Chapter 3 References

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TABLES AND FIGURES

Figure 3.1 Study flow

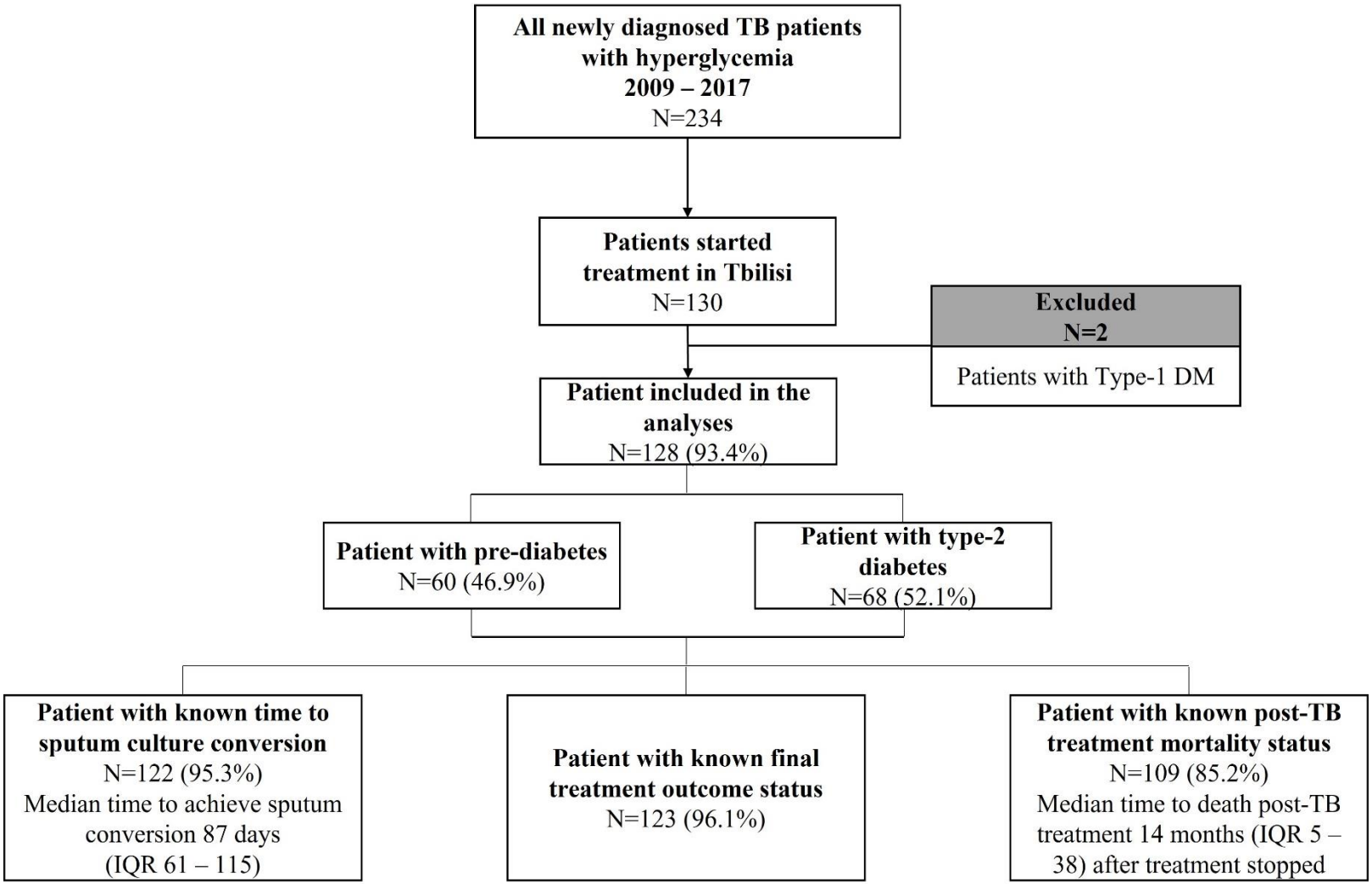

Table 3.1 Demographic and clinical characteristics of patients treated with second-line drugs according to hyperglycemia status, Georgia, 2009 – 2017 (N=128)

Characteristics	Total N=128 N (%)	Diabetes Status		p-values*
		Pre-diabetes N (%) = 60 (46.9)	Diabetes N (%) = 68 (52.1)	
Demographic				
Age, years, median (IQR)	45 (32 – 57)	32 (23 – 47)	54 (42 – 60)	**<0.01**†
Age Group				
16 – 40	53 (41.4)	40 (66.7)	13 (19.1)	**<0.01**‡
41 – 65	66 (51.6)	18 (30.0)	48 (70.6)	
65+	9 (7.0)	2 (3.3)	7 (10.3)	
Male gender	105 (82.0)	47 (78.3)	58 (85.3)	0.31
Reported Symptoms at Baseline				
Fever	78 (63.9)	35 (61.4)	43 (66.2)	0.59
Night sweats	66 (54.1)	31 (54.4)	35 (53.9)	0.95
Dry cough	34 (27.9)	18 (31.6)	16 (24.6)	0.39
Productive cough	78 (63.9)	35 (61.4)	43 (66.2)	0.59
Hemoptysis	13 (10.7)	10 (17.5)	3 (4.6)	**0.03**
Chest pain	19 (15.6)	11 (19.3)	8 (12.3)	0.29
Loss appetite	42 (34.4)	20 (35.1)	22 (33.9)	0.89
Weight loss	56 (45.9)	28 (49.1)	28 (43.1)	0.50
Body mass index at treatment initiation				
BMI, Kg/m^2, median (IQR)	21.3 (18.9 – 24.9)	19.9 (18.4 – 22.9)	22.9 (19.8 – 27.1)	**<0.01**†
BMI Category				
Underweight (BMI <18.5)	17 (15.0)	13 (22.8)	4 (7.1)	**<0.01**‡
Normal (BMI 18.5 – 24.9)	66 (58.4)	39 (68.4)	27 (48.2)	
Overweight (BMI 25.0 – 29.0)	23 (20.4)	3 (5.3)	20 (35.7)	
Obese (BMI ≥30)	7 (6.2)	2 (3.5)	5 (8.9)	
Missing	15	3	12	
Drug Resistance Type				

Characteristics	Total N=128 N (%)	Diabetes Status		p-values*
		Pre-diabetes N (%) = 60 (46.9)	Diabetes N (%) = 68 (52.1)	
RIF-resistance/polydrug resistance	27 (21.1)	10 (16.7)	17 (25.0)	0.49
MDR/pre-XDR TB	86 (67.2)	42 (70.0)	44 (64.7)	
XDR TB	15 (11.7)	8 (13.3)	7 (10.3)	
X-ray Findings at Baseline				
Cavitary Disease	45 (35.2)	19 (31.7)	26 (38.2)	0.44
Infiltrate	101 (78.9)	51 (85.0)	50 (73.5)	0.11
Miliary disease	42 (32.8)	18 (30.0)	24 (35.3)	0.52
Other Risk Factors				
Tobacco Use	52 (40.6)	22 (36.7)	30 (44.1)	0.46
Excessive Alcohol Intake	11 (8.7)	6 (10.3)	5 (7.4)	0.29
Comorbidities				
Hepatitis C	27 (22.9)	10 (18.9)	17 (26.1)	0.35
Hepatitis B	2 (1.7)	1 (1.9)	1 (1.6)	1.00‡
HIV co-infection	2 (1.7)	2 (3.5)	0 (0.0)	0.24‡
CVD	5 (6.9)	1 (8.3)	4 (6.6)	1.00‡
Blood Glucose Lowering Agents				
Taking any blood glucose lowering agents	60 (47.2)	0 (0.0)	60 (88.2)	**<0.01**
Type of glucose lowering agents				
Metformin	18	0	18	
Insulin	41	0	41	
Sulfonylureas	27	0	27	
Treatment Outcomes				
Sputum Culture Conversion (SCC)				
Time to SCC, median in days (IQR) (n=122)	90 (61 – 121)	74 (58 – 101)	93 (62 – 124)	0.15†
Converted to negative	109 (85.2)	51 (85.0)	58 (85.3)	0.96
Final TB treatment outcome (n=123)				
Favorable	79 (64.2)	37 (63.8)	42 (64.6)	0.92

Characteristics	Total N=128 N (%)	Diabetes Status		p-values*
		Pre-diabetes N (%) = 60 (46.9)	Diabetes N (%) = 68 (52.1)	
Poor	44 (35.8)	21 (36.2)	23 (35.4)	
Missing	5	2	3	
Return to care	14 (10.9)	5 (8.3)	9 (13.2)	0.38
Survival Status				
Alive	93 (84.6)	44 (86.3)	49 (83.1)	0.89‡
Mortality during treatment	2 (1.8)	1 (2.0)	1 (1.7)	
Mortality post-TB treatment	15 (13.6)	6 (11.7)	9 (15.3)	
Missing	18	9	9	

Abbreviations:
BMI – body mass index; CVD – cardiovascular diseases; HIV – human immunodeficiency virus; IQR – interquartile range; MDR – multidrug resistant; RIF – rifampicin; SCC – sputum culture conversion; TB – tuberculosis; XDR – extensively drug resistant

Footnotes:
*p-values from Chi-square tests
†p-values from Wilcoxon rank sum tests
‡p-values from Fisher's exact test

Bold indicates that the finding is statistically significant at α=0.05

Table 3.2 Diabetes characteristics, sputum culture conversion, and risk of TB treatment outcomes among patients treated with second-line drugs with hyperglycemia indication, Georgia, 2009 - 2017

Characteristics	Sputum Culture Conversion			Poor Treatment Outcomes		
	Converted N (%)=109 (85.2)	Hazard Rate Ratios		Poor† N (%)=44 (35.8)	Risk Ratios	
	N (%)	cHR (95%CI)	aHR* (95%CI)	N (%)	cRR (95%CI)	aRR* (95%CI)
Pre-diabetes and Diabetes	***N=122***			***N=123***		
Diabetes Category						
Pre-diabetes	51 (91.1)	Reference	Reference	21 (36.2)	Reference	Reference
Diabetes	58 (87.9)	0.79 (0.54 – 1.15)	0.81 (0.52 – 1.26)	23 (35.4)	0.98 (0.61 – 1.57)	0.80 (0.41 – 1.56)
Diabetes						
Pre-diabetes	51 (91.1)	Reference	Reference	21 (36.2)	Reference	Reference
Newly diagnosed diabetes	18 (90.0)	0.72 (0.42 – 1.24)	0.74 (0.41 – 1.32)	6 (30.3)	0.83 (0.39 – 1.76)	0.66 (0.25 – 1.75)
Known diabetes	40 (87.0)	0.82 (0.54 – 1.24)	0.85 (0.53 – 1.37)	17 (37.8)	1.04 (0.63 – 1.73)	0.86 (0.50 – 1.49)
Diabetes	***N=66***			***N=65***		
Years with Diabetes						
0 – 5 years	27 (93.1)	Reference	Reference	9 (31.0)	Reference	Reference
6 – 10 years	11 (78.6)	0.72 (0.35 – 1.50)	0.76 (0.36 – 1.60)	5 (38.5)	1.24 (0.52 – 2.98)	1.21 (0.40 – 3.69)
≥11 years	7 (77.8)	0.87 (0.38 – 1.99)	0.78 (0.32 – 1.86)	4 (44.4)	1.43 (0.58 – 3.56)	1.43 (0.44 – 4.75)
Unknown	13			5		
Metformin prescription						
No	41 (85.4)	Reference	Reference	21 (43.8)	Reference	Reference
Yes	17 (94.4)	1.22 (0.69 – 2.15)	1.19 (0.64 – 2.18)	2 (11.8)	0.30 (0.08 – 1.11)	**0.23 (0.05 – 0.97)**
Sulfonylureas prescription						
No	35 (87.5)	Reference	Reference	15 (37.5)	Reference	Reference
Yes	23 (88.5)	1.07 (0.63 – 1.81)	1.18 (0.65 – 2.13)	8 (32.0)	0.87 (0.47 – 1.63)	0.74 (0.32 – 1.66)
Insulin prescription						
No	24 (88.9)	Reference	Reference	8 (29.6)	Reference	Reference
Yes	34 (87.2)	1.11 (0.66 – 1.88)	1.13 (0.65 – 1.97)	15 (39.5)	1.16 (0.71 – 1.89)	1.08 (0.57 – 2.04)
Abbreviations:						

Characteristics	Sputum Culture Conversion			Poor Treatment Outcomes		
	Converted N (%)=109 (85.2) N (%)	Hazard Rate Ratios		Poor† N (%)=44 (35.8) N (%)	Risk Ratios	
		cHR (95%CI)	aHR* (95%CI)		cRR (95%CI)	aRR* (95%CI)

aHR – adjusted hazard rate ratio; aRR – adjusted risk ratio; cHR – crude hazard rate ratio; CI – confidence interval; cRR – crude risk ratio

Footnotes:
*Model was adjusted for age and gender
†Including 6 who failed the treatment, 36 who stopped the treatment (i.e., defaulted), and 2 who died during TB treatment
Bold indicates that the finding is statistically significant at α=0.05

Table 3.3 Diabetes characteristics and the hazard rate of all-cause mortality post-TB treatment among patients treated with second-line drugs, Georgia, 2009 - 2017 (N=108)

Characteristics	Mortality post-TB treatment			
	Died N (%)=15 (13.9)	Median time to all-cause mortality post-TB treatment	Hazard Rate Ratios	
	N (%)	Median, month (IQR) 14 (5 – 38)	cHR (95%CI)	aHR* (95%CI)
Pre-diabetes and Diabetes		***N=108***		
Diabetes Category				
Pre-diabetes	6 (12.0)	14 (7 – 34)	Reference	Reference
Diabetes	9 (15.5)	19 (3 – 46)	1.23 (0.40 – 3.80)	0.72 (0.21 – 2.44)
Diabetes				
Pre-diabetes	6 (12.0)	14 (7 – 34)	Reference	Reference
Newly diagnosed diabetes	4 (25.0)	7 (3 – 25)	2.43 (0.65 – 9.08)	1.73 (0.37 – 8.01)
Known diabetes	5 (11.9)	40 (15 – 57)	0.82 (0.22 – 3.09)	0.49 (0.12 – 2.00)
Diabetes		***N=66***		
Years with Diabetes				
0 – 5 years	4 (15.4)	10 (1 – 53)	Reference	Reference
6 – 10 years	2 (14.3)	31 (2 – 60)	1.08 (0.18 – 6.55)	0.15 (0.01 – 3.92)
≥11 years	1 (14.3)	27 (27 – 27)	1.13 (0.12 – 10.92)	0.16 (0.00 – 5.97)
Unknown	2			
Metformin				
No	7 (16.7)	19 (4 – 39)	Reference	Reference
Yes	2 (12.5)	31 (2 – 60)	0.91 (0.18 – 4.51)	0.66 (0.12 – 3.59)
Taking sulfonylureas				
No	4 (11.8)	27 (10 – 39)	Reference	Reference
Yes	5 (20.8)	4 (2 – 53)	2.51 (0.60 – 10.58)	5.49 (0.74 – 40.90)
Taking insulin				
No	3 (13.0)	39 (4 – 53)	Reference	Reference

Characteristics	Mortality post-TB treatment			
	Died N (%)=15 (13.9)	Median time to all-cause mortality post-TB treatment	Hazard Rate Ratios	
	N (%)	Median, month (IQR) 14 (5 – 38)	cHR (95%CI)	aHR* (95%CI)
Yes	6 (17.1)	10 (2 – 27)	1.18 (0.28 – 4.96)	1.20 (0.26 – 5.48)

Abbreviations:
aHR – adjusted hazard rate ratio; cHR – crude hazard rate ratio; CI – confidence interval; IQR – interquartile range

Footnotes:
*Model was adjusted for age and gender

Bold indicates that the finding is statistically significant at α=0.05

Supplemental Materials Chapter 3

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Table S3.1 Scoring metrics used to quantify tuberculosis severity among patients treated with second-line drugs in Georgia

Characteristics	Parameters Evaluated	Score	Highest Score
TB clinical form	PTB/Smear+ EPTB PTB/Smear-	0 1 2	2
Case definition	Newly diagnosed Relapse Return after default Return after failed DOTS	0 1 2 3	3
Type of drug resistance	RIF-Resistance/PDR MDR /Pre-XDR TB XDR TB	0 1 2	2
BMI at TB treatment initiation	BMI >=18.5 kg/m2 Underweight (BMI <18.5kg/m2)	0 1	1
Symptoms reported at baseline	Cough (productive or dry) Dyspnea Chest Pain Night Sweats Hemoptysis	1 1 1 1 1	5
Chest findings at treatment initiation	Tuberculoma Infiltrate Cavitary disease Miliary disease	1 2 3 4	10
HIV Status	Negative Positive	0 3	3
Comorbidities	***Diabetes Status*** No diabetes Pre-diabetes Diabetes ***Other chronic comorbidities**** No Yes	 0 1 2 0 2	4
TOTAL POSSIBLE HIGHEST SCORE			30

Notes:
This scoring metric was adapted and modified from the Bandim tuberculosis score(42), prognostic score during TB treatment (43), and TB severity scoring system (44). This scoring metric was validated in the Georgian cohort consisting of all form TB patients treated with second-line drugs during the 2009 – 2017 period.
Case definition and ***diabetes status*** were not included when calculating the severity score in the present paper (conducted among a subset of the Georgian cohort consisting only patients with newly diagnosed drug-resistant TB and hyperglycemia indications)
*Assessment was based on the presence of other comorbidities such as hepatitis B/C, cardiovascular disease

Table S3.2 Adverse events reported during treatment among patients with hyperglycemia and type-2 diabetes in the country of Georgia, 2009 – 2017, N=128

Adverse events during TB treatment	**Total N (%)**	**Diabetes Status**		**p-value***
		Pre-diabetes N (%)	**Diabetes N (%)**	
Any adverse event	119 (94.4)	54 (91.5)	65 (97.0)	0.25#
Hearing loss	18 (14.1)	5 (8.3)	13 (19.1)	0.08
Peripheral neuropathy	14 (10.9)	6 (10.0)	8 (11.8)	0.75
Hypokalemia†	10 (7.8)	7 (11.7)	3 (4.4)	0.09#
Hepatotoxicity‡	25 (19.5)	12 (20.0)	13 (19.1)	0.90
Thyroid problem	15 (11.7)	6 (10.0)	9 (13.2)	0.57
Anemia§	20 (15.6)	11 (18.3)	9 (13.4)	0.43
QT_C prolongation¶	4 (3.1)	2 (3.3)	2 (2.9)	1.00#
Nausea	65 (50.8)	31 (51.7)	34 (50.0)	0.85
Rashes	23 (18.0)	10 (16.7)	13 (19.1)	0.72
Itchy	29 (22.7)	13 (21.7)	16 (23.5)	0.80
Joints pain	58 (45.3)	27 (45.0)	31 (45.6)	0.95
GIT disturbance	25 (19.5)	16 (26.7)	9 (13.2)	0.06
Creatine	27 (21.1)	10 (16.7)	17 (25.0)	0.25
Hemoptysis during treatment	21 (16.4)	13 (21.7)	8 (11.8)	0.13

Abbreviations: GIT – gastrointestinal tract; QT_C – corrected QT interval

Footnotes:
*p-values from Chi-square tests
#p-values from Fisher's exact tests
†Hypokalemia was defined as potassium level (K^+) <3.3mmol/dL
‡Hepatotoxicity was indicated by elevated liver enzymes (i.e., ALT >37 U/L or AST >42 U/L).
§Anemia was defined by the age-specific cut-off for hemoglobin count according to Georgian guidelines

Bold indicates that the finding is statistically significant at α=0.05

Table S3.3 Multivariable models with different set of covariates to estimate the adjusted association between diabetes characteristics and sputum culture conversion

Models	Diabetes Characteristics	Adjusted HR (95%CI)	AIC	Covariates included in the model
Model 1*	Pre-diabetes	Reference	871.18	Age, gender
	Diabetes	0.81 (0.52 – 1.26)		
	Diabetes - non-metformin users	Reference	*397.66*	
	Diabetes - metformin users	1.19 (0.64 – 2.18)		
Model 2	Pre-diabetes	Reference	871.66	Age, gender, baseline AFB
	Diabetes	0.82 (0.53 – 1.28)		
	Diabetes - non-metformin users	Reference	400.56	
	Diabetes - metformin users	1.17 (0.63 – 2.20)		
Model 3	Pre-diabetes	Reference	872.64	Age, gender, cavitary disease
	Diabetes	0.79 (0.51 – 1.24)		
	Diabetes - non-metformin users	Reference	399.66	
	Diabetes - metformin users	1.18 (0.64 – 2.18)		
Model 4	Pre-diabetes	Reference	874.53	Age, gender, BMI
	Diabetes	0.68 (0.42 – 1.13)		
	Diabetes - non-metformin users	Reference	401.81	
	Diabetes - metformin users	1.14 (0.61 – 2.10)		
Model 5	Pre-diabetes	Reference	*860.98*	Age, gender, type of drug resistance
	Diabetes	0.72 (0.46 – 1.12)		
	Diabetes - non-metformin users	Reference	399.47	
	Diabetes - metformin users	1.04 (0.54 – 1.97)		
Model 6	Pre-diabetes	Reference	864.54	Age, gender, BMI, type of drug resistance
	Diabetes	**0.58 (0.35 – 0.98)**		
	Diabetes - non-metformin users	Reference	402.44	
	Diabetes - metformin users	0.92 (0.47 – 1.80)		
Model 7	Pre-diabetes	Reference	877.54	Age, gender, BMI, hemoptysis
	Diabetes	0.66 (0.40 – 1.09)		

Models	Diabetes Characteristics	Adjusted HR (95%CI)	AIC	Covariates included in the model
	Diabetes - non-metformin users	Reference	404.13	
	Diabetes - metformin users	1.46 (0.69 – 3.09)		
Model 8	Pre-diabetes	Reference	867.25	Age, gender, BMI, type of drug resistance, hepatitis C co-infection
	Diabetes	0.61 (0.36 – 1.04)		
	Diabetes - non-metformin users	Reference	405.59	
	Diabetes - metformin users	0.87 (0.41 – 1.86)		
Model 9	Pre-diabetes	Reference	868.37	Age, gender, BMI, type of drug resistance, HIV co-infection
	Diabetes	**0.59 (0.35 – 1.00)**		
	Diabetes - non-metformin users	Reference	403.75	
	Diabetes - metformin users	0.85 (0.42 – 1.72)		
Model 10	Pre-diabetes	Reference	871.05	Age, gender, BMI, type of drug resistance, hepatitis C, and HIV co-infections
	Diabetes	0.62 (0.36 – 1.06)		
	Diabetes - non-metformin users	Reference	407.07	
	Diabetes - metformin users	0.81 (0.37 – 1.79)		
Model 11a	Pre-diabetes	Reference	873.15	Age, gender, TB severity score (continuous)
	Diabetes	0.81 (0.52 – 1.26)		
	Diabetes - non-metformin users	Reference	399.22	
	Diabetes - metformin users	1.18 (0.64 – 2.17)		
Model 11b	Pre-diabetes	Reference	873.18	Age, gender, TB severity score (dichotomous)
	Diabetes	0.81 (0.52 – 1.26)		
	Diabetes - non-metformin users	Reference	399.11	
	Diabetes - metformin users	1.16 (0.63 – 2.15)		
Model 11c	Pre-diabetes	Reference	875.85	Age, gender, TB severity score (quantiles)
	Diabetes	0.84 (0.54 – 1.31)		
	Diabetes - non-metformin users	Reference	399.95	
	Diabetes - metformin users	1.20 (0.65 – 2.22)		

Abbreviations:
AFB – acid fast bacilli; AIC – Akaike criterion information; BMI – body mass index; CI – confidence interval; HIV – human immunodeficiency virus; HR – hazard rate ratio; TB – tuberculosis

Models	Diabetes Characteristics	Adjusted HR (95%CI)	AIC	Covariates included in the model

Footnotes:
*Model used in the main manuscript

Bold indicates that the finding is statistically significant at α=0.05
Italic indicates the smallest AIC across 10 simulated multivariable models

Table S3.4 Multivariable models with different set of covariates to estimate the adjusted association between diabetes characteristics and final TB treatment outcome

Models	Diabetes Characteristics	Adjusted RR (95%CI)	AIC	Covariates included in the model
Model 1*	Pre-diabetes	Reference	*186.83*	Age, gender
	Diabetes	0.80 (0.41 – 1.56)		
	Diabetes - non-metformin users	Reference	*96.57*	
	Diabetes - metformin users	**0.22 (0.05 – 0.97)**		
Model 2	Pre-diabetes	Reference	189.78	Age, gender, baseline AFB
	Diabetes	0.78 (0.39 – 1.55)		
	Diabetes - non-metformin users	Reference	99.19	
	Diabetes - metformin users	**0.22 (0.05 – 0.97)**		
Model 3	Pre-diabetes	Reference	188.83	Age, gender, cavitary disease
	Diabetes	0.80 (0.41 – 1.57)		
	Diabetes - non-metformin users	Reference	97.98	
	Diabetes - metformin users	**0.22 (0.05 – 0.95)**		
Model 4	Pre-diabetes	Reference	194.37	Age, gender, BMI
	Diabetes	0.82 (0.39 – 1.75)		
	Diabetes - non-metformin users	Reference	103.39	
	Diabetes - metformin users	**0.20 (0.04 – 0.92)**		
Model 5	Pre-diabetes	Reference	188.25	Age, gender, type of drug resistance
	Diabetes	0.76 (0.38 – 1.52)		
	Diabetes - non-metformin users	Reference	99.83	
	Diabetes - metformin users	**0.22 (0.05 – 0.99)**		
Model 6	Pre-diabetes	Reference	195.54	Age, gender, BMI, type of drug resistance
	Diabetes	0.75 (0.34 – 1.64)		
	Diabetes - non-metformin users	Reference	106.32	
	Diabetes - metformin users	**0.19 (0.04 – 0.91)**		
Model 7	Pre-diabetes	Reference	197.46	Age, gender, BMI, hemoptysis
	Diabetes	0.80 (0.38 – 1.70)		

Models	Diabetes Characteristics	Adjusted RR (95%CI)	AIC	Covariates included in the model
	Diabetes - non-metformin users	Reference	104.28	
	Diabetes - metformin users	**0.18 (0.04 – 0.90)**		
Model 8	Pre-diabetes	Reference	197.71	Age, gender, BMI, type of drug resistance, hepatitis C co-infection
	Diabetes	0.70 (0.32 – 1.53)		
	Diabetes - non-metformin users	Reference	109.44	
	Diabetes - metformin users	0.22 (0.19 – 1.14)		
Model 9	Pre-diabetes	Reference	197.48	Age, gender, BMI, type of drug resistance, HIV C co-infection
	Diabetes	0.79 (0.35 – 1.78)		
	Diabetes - non-metformin users	Reference	107.88	
	Diabetes - metformin users	0.21 (0.04 – 1.01)		
Model 10	Pre-diabetes	Reference	198.78	Age, gender, BMI, type of drug resistance, hepatitis C, and HIV co-infections
	Diabetes	0.73 (0.33 – 1.63)		
	Diabetes - non-metformin users	Reference	119.51	
	Diabetes - metformin users	0.24 (0.05 – 1.24)		
Model 11a	Pre-diabetes	Reference	188.58	Age, gender, TB severity score (continuous)
	Diabetes	0.80 (0.41 – 1.55)		
	Diabetes - non-metformin users	Reference	97.27	
	Diabetes - metformin users	**0.22 (0.05 – 0.97)**		
Model 11b	Pre-diabetes	Reference	188.83	Age, gender, TB severity score (dichotomous)
	Diabetes	0.80 (0.41 – 1.56)		
	Diabetes - non-metformin users	Reference	98.18	
	Diabetes - metformin users	**0.20 (0.05 – 0.92)**		
Model 11c	Pre-diabetes	Reference	192.70	Age, gender, TB severity score (quantiles)
	Diabetes	0.80 (0.41 – 1.57)		
	Diabetes - non-metformin users	Reference	102.12	
	Diabetes - metformin users	**0.20 (0.04 – 0.93)**		

Abbreviations:
AFB – acid fast bacilli; AIC – Akaike criterion information; BMI – body mass index; CI – confidence interval; HIV – human immunodeficiency virus; HR – hazard rate ratio; TB – tuberculosis

Models	Diabetes Characteristics	Adjusted RR (95%CI)	AIC	Covariates included in the model

Footnotes:
*Model used in the main manuscript

Bold indicates that the finding is statistically significant at α=0.05
Italic indicates the smallest AIC across 10 simulated multivariable models

Table S3.5 Multivariable models with different set of covariates to estimate the adjusted association between diabetes characteristics and hazard rates of all-cause mortality post-TB treatment

Models	Diabetes Characteristics	Adjusted HR (95%CI)	AIC	Covariates included in the model
Model 1*	Pre-diabetes	Reference	125.24	Age, gender
	Diabetes	0.60 (0.19 – 1.89)		
	Diabetes - non-metformin users	Reference	62.45	
	Diabetes - metformin users	0.82 (0.16 – 4.15)		
Model 2	Pre-diabetes	Reference	126.91	Age, gender, baseline AFB
	Diabetes	0.76 (0.23 – 2.52)		
	Diabetes - non-metformin users	Reference	63.73	
	Diabetes - metformin users	0.95 (0.18 – 5.12)		
Model 3	Pre-diabetes	Reference	126.08	Age, gender, cavitary disease
	Diabetes	0.67 (0.21 – 2.12)		
	Diabetes - non-metformin users	Reference	64.38	
	Diabetes - metformin users	0.85 (0.16 – 4.41)		
Model 4	Pre-diabetes	Reference	131.75	Age, gender, BMI
	Diabetes	0.50 (0.13 – 1.96)		
	Diabetes - non-metformin users	Reference	69.29	
	Diabetes - metformin users	0.87 (0.17 – 4.54)		
Model 5	Pre-diabetes	Reference	*124.58*	Age, gender, type of drug resistance
	Diabetes	0.51 (0.15 – 1.74)		
	Diabetes - non-metformin users	Reference	61.47	
	Diabetes - metformin users	0.68 (0.11 – 4.13)		
Model 6	Pre-diabetes	Reference	130.71	Age, gender, BMI, type of drug resistance
	Diabetes	0.38 (0.09 – 1.64)		
	Diabetes - non-metformin users	Reference	68.17	
	Diabetes - metformin users	0.68 (0.10 – 4.78)		
Model 7	Pre-diabetes	Reference	135.02	Age, gender, BMI, hemoptysis
	Diabetes	0.51 (0.13 – 1.97)		

Models	Diabetes Characteristics	Adjusted HR (95%CI)	AIC	Covariates included in the model
	Diabetes - non-metformin users	Reference	70.80	
	Diabetes - metformin users	1.69 (0.24 – 12.00)		
Model 8	Pre-diabetes	Reference	132.73	Age, gender, BMI, type of drug resistance, hepatitis C co-infection
	Diabetes	0.35 (0.08 – 1.48)		
	Diabetes - non-metformin users	Reference	71.74	
	Diabetes - metformin users	0.82 (0.11 – 6.32)		
Model 9	Pre-diabetes	Reference	134.68	Age, gender, BMI, type of drug resistance, HIV co-infection
	Diabetes	0.38 (0.09 – 1.66)		
	Diabetes - non-metformin users	Reference	70.04	
	Diabetes - metformin users	0.58 (0.07 – 5.10)		
Model 10	Pre-diabetes	Reference	136.67	Age, gender, BMI, type of drug resistance, hepatitis C, and HIV co-infections
	Diabetes	0.35 (0.08 – 1.51)		
	Diabetes - non-metformin users	Reference	73.61	
	Diabetes - metformin users	0.71 (0.08 – 6.67)		
Model 11a	Pre-diabetes	Reference	127.00	Age, gender, TB severity score (continuous)
	Diabetes	0.61 (0.19 – 1.93)		
	Diabetes - non-metformin users	Reference	*61.32*	
	Diabetes - metformin users	0.94 (0.18 – 4.89)		
Model 11b	Pre-diabetes	Reference	127.14	Age, gender, TB severity score (dichotomous)
	Diabetes	0.60 (0.19 – 1.91)		
	Diabetes - non-metformin users	Reference	63.41	
	Diabetes - metformin users	0.68 (0.13 – 3.61)		
Model 11c	Pre-diabetes	Reference	131.12	Age, gender, TB severity score (quantiles)
	Diabetes	0.60 (0.18 – 1.95)		
	Diabetes - non-metformin users	Reference	65.78	
	Diabetes - metformin users	0.97 (0.17 – 5.51)		

Abbreviations:
AFB – acid fast bacilli; AIC – Akaike criterion information; BMI – body mass index; CI – confidence interval; HIV – human immunodeficiency virus; HR – hazard rate ratio; TB – tuberculosis

Models	Diabetes Characteristics	Adjusted HR (95%CI)	AIC	Covariates included in the model

Footnotes:
*Model used in the main manuscript

Bold indicates that the finding is statistically significant at α=0.05
Italic indicates the smallest AIC across 10 simulated multivariable models

CHAPTER 4

Paper 3: Metabolic syndrome and visceral adipose index after successful tuberculosis treatment: Preliminary findings from a post-tuberculosis cohort study in Georgia

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ABSTRACT

Background: Little is known about the impact of tuberculosis (TB) on chronic non-communicable disease risks. To address, we studied the prevalence of metabolic syndrome, an established risk factor for chronic diseases, and visceral adipose index (VAI) among TB patients with favorable treatment outcomes.

Methods: We utilized preliminary data from an ongoing prospective cohort study of patients successfully treated for pulmonary TB in the country of Georgia. Eligible participants were HIV-negative adults with newly diagnosed laboratory-confirmed pulmonary TB who completed TB treatment. The primary study exposure was drug-resistant status, i.e., drug-resistant (DRTB) vs. drug-susceptible (DSTB). The primary study outcome, metabolic syndrome, was defined as the prevalence of ≥3 of the following: a) elevated blood glucose (HbA1c ≥5.7%), b) low HDL (≤1.0mmol/L for male, ≤1.3mmol/L for female), c) elevated triglycerides (≥1.7mmol/L), d) elevated blood pressure (systolic ≥130mmHg or diastolic ≥80mmHg), and e) abdominal obesity (waist circumference ≥102cm for male, ≥88cm for female). The association between DRTB and study outcomes was estimated with log-binomial and general linear models.

Results: Among 105 participants (median age 34) enrolled at the end of TB treatment, metabolic syndrome was present in 7.8% (8/105); and median VAI was 1.23 (IQR 0.83-1.67). The risk of metabolic syndrome was 18.2% (4/22) among patients treated for DRTB and 4.8% (4/83) among patients treated for DSTB (p=0.06). After adjusting for age and gender, the prevalence ratio for metabolic syndrome comparing patients treated for DRTB vs. DSTB was 2.29 (95%CI 0.50, 10.37). After adjusting for confounders, on average, the mean VAI value was higher by 0.37 (95%CI -0.13, 0.86) points among patients treated for DRTB vs. DSTB.

Conclusions: Nearly 1 in 12 patients in our cohort had metabolic dysfunction after successful treatment for TB. These preliminary findings suggest that the prevalence of metabolic syndrome and VAI levels may be increased among patients formerly treated for DRTB.

Keywords: tuberculosis; post-TB health; metabolic disease; visceral adipose index

INTRODUCTION

Tuberculosis (TB) is a major global health problem with 10 million new cases and nearly 1.5 million deaths reported annually [1]. With the ambitious End TB strategy goals to eliminate the global TB epidemic by 2035 [2], a primary focus of the current TB research agenda focuses on successfully curing patients diagnosed with TB. However, despite global TB cure rates of approximately 87%, mounting evidence suggests that TB survivors may experience residual complications and have an increased risk of mortality post-TB treatment [3-6]. Identifying host characteristics associated with post-TB complications will be important to prevent premature deaths among the nearly 9 million annual TB survivors.

Studies assessing health after TB treatment are limited and there are no current recommendations or guidelines on patient care after TB treatment is completed. Previous studies indicate that a substantial proportion of patients with favorable final TB treatment outcomes (i.e., cured or completed TB treatment) have persistent lung lesions and scarring after TB treatment [3, 7, 8]. Persistent lesions and residual scarring can lead to chronic airflow limitation and impact mortality risk due to chronic obstructive pulmonary disease (COPD) and other pulmonary impairments. Recent findings also suggest that TB survivors may have an increased risk of developing chronic non-communicable diseases including diabetes, myocardial infarction, and stroke [9-12]. However, the biological pathways explaining how TB disease impacts chronic disease risk remains largely unknown. Moreover, existing studies are limited to the retrospective study designs as the progression of chronic non-communicable diseases could take years. Studies looking at intermediate risk factors for cardio-metabolic diseases such as metabolic syndrome may help explain how TB affects the risk of chronic non-communicable diseases.

Metabolic syndrome is a well-established risk factor for cardio-metabolic diseases such as diabetes, cardiovascular disease, stroke, and myocardial infarction [13]. Visceral adipose index

(VAI), an empirical-mathematical model, is a new gender-specific indicator of fat distribution and function [14]. VAI is a simple marker to detect adipose tissue dysfunction before someone develops overt metabolic syndrome [14, 15]. In previous studies, VAI had good predictive power for type-2 diabetes, hypertension [16], and all-cause mortality among hemodialysis patients [17]. Understanding the trend of metabolic syndrome and VAI after TB treatment may provide a better overview of cardio-metabolic disease risks post-TB.

Given the existing gaps regarding post-TB health, the present study aimed to determine the prevalence of metabolic syndrome and distribution of VAI at the end of successful TB treatment using preliminary data from the country of Georgia. We also determined the association between TB drug-susceptibility with metabolic syndrome and VAI. Lastly, we evaluated changes in metabolic syndrome and VAI from the end of TB treatment to 6-months post treatment. Results from our study will help identify and inform clinicians regarding host characteristics associated with increased cardio-metabolic risks post-TB treatment. We hypothesized that drug-resistant TB (DRTB) would be associated with increased risk of metabolic syndrome.

METHODS

Study Design and Setting

This study used preliminary data from a 1-year prospective cohort study entitled "*Pulmonary Impairment after Tuberculosis Treatment (PITT)"*. The PITT study aimed to evaluate pulmonary impairment after successful TB treatment. Adult pulmonary TB patients (≥16 years or older) with *M. tuberculosis* confirmed at baseline and favorable treatment outcomes (i.e., favorable TB outcome) [18] were eligible for study inclusion. Patients with a history of lung surgery or lung

cancer before the current TB episode, relapse or retreatment cases, and people living with HIV were excluded from the final analyses.

Briefly, we enrolled successfully treated TB patients at the end of treatment and followed-up 6-months after TB treatment completion. Anthropometric measurements (e.g., body weight, height, blood pressure, waist circumference), glycated hemoglobin [HbA1c], lipid physiology (i.e., high-density lipoprotein [HDL], low-density lipoprotein [LDL], total cholesterol, and triglycerides), and study questionnaire administration were done at each clinic visit. Blood works were performed at the NCTLD laboratory facility using BioSystem A25 Random Access Analyzer. Pulmonary function, exhaled nitric oxide (FeNO), and six-minute walking tests were measured at the end of TB treatment.

Definitions and Study Measures

Our primary exposure was phenotypic drug susceptibility test (DST) result, defined as drug-resistant or drug susceptible TB. Patients with TB susceptible to rifampicin were defined as "drug-susceptible TB [DSTB]," while patients with resistance to at least isoniazid and rifampicin were grouped as "drug-resistant TB [DRTB]." The primary study outcome was prevalence of metabolic syndrome at the end of TB treatment. Metabolic syndrome was defined by the presence of at least three of the following disorders: a) elevated plasma glucose level (i.e., glycated hemoglobin [HbA1c] ≥5.7%), b) elevated triglycerides (i.e., ≥1.69 mmol/L), c) lower high-density lipoprotein (HDL) (<1.03 mmol/L for male and <1.29 mmol/L for female), d) elevated blood pressure (i.e., ≥130/80mmHg), and e) central obesity (i.e., waist circumference ≥102 cm for men and ≥88 cm for women). Patients with prior diagnosis or a record of taking medication to manage any metabolic disorders mentioned above were considered as having metabolic syndrome [19]. A

secondary outcome at the end of TB treatment, VAI, was calculated using the formula described in previous studies (Figure S4.1) [14, 16, 20]. VAI was then categorized dichotomously with VAI <1.67 (third-quartile cut-off) considered as "lower VAI" and VAI ≥1.67 considered as "higher VAI."

Demographic characteristics, behavioral risk factors (i.e., smoking, alcohol, and drug use) were collected by administering a pre-structured study questionnaire at the time of study enrollment and follow up. Clinical information during TB treatment (e.g., time to achieve sputum conversion, chest findings, comorbidity factors) were abstracted from patients' medical charts. Study data were collected and managed using Research Electronic Data Capture (REDCap, Vanderbilt University, NC) electronic data capture tools hosted at Emory University [21, 22].

Statistical Analyses

We performed Chi-square/Fisher's exact test and Wilcoxon rank-sum test to assess the association between participant characteristics and metabolic outcomes at the end of TB treatment. Log-binomial logistic regression models were used to estimate the prevalence ratio (PR) and 95% confidence interval (CI) of metabolic syndrome at the end of TB treatment comparing patients treated for DRTB vs. DSTB. We used linear models to estimate the relationship between drug-resistant type and VAI at the end of TB treatment. Subgroup analyses were performed among patients with 6-month follow-up information available (n=62) to assess changes in study outcomes at the end of TB treatment and 6-month follow-up. For subgroup analyses, mixed models with a random intercept were used to estimate the association between DST status and changes in HbA1c, HDL, triglycerides, VAI, and body mass index (BMI) at the end of TB treatment and 6-month

follow-up. Covariates included in the multivariable models were identified by assessing bivariate associations, directed acyclic graph theory [23], and previously published literature.

Sensitivity Analyses

Sensitivity analyses were performed to account for systematic errors due to a) distribution assumptions used in the regression analyses, b) unmeasured confounders, and c) covariate misclassification in the multivariable models. In addition to the general linear models, we estimated prevalence ratios and 95%CI of having higher levels of VAI (VAI ≥1.67 vs. VAI <1.67) using log-binomial regression models. E-value was calculated to estimate the minimum strength of association between an unmeasured confounder with both exposure and outcome to explain away the observed association between DRTB and metabolic syndrome [24]. Last, we ran additional multivariable linear regression models to assess changes in the mean difference of VAI with different sets of covariates (the measure of association was presented in range).

Ethical Review

This study was submitted, reviewed, and approved by institutional review boards (IRBs) at Emory University, Atlanta, USA, and National Center for Tuberculosis and Lung Diseases, Tbilisi, Georgia.

RESULTS

We enrolled 105 patients from December 2019 – March 2021 who were successfully treated for TB disease. Among these, the majority were male (57.1%), Georgian (89.5%), and the median age was 34 (interquartile range [IQR] 26 – 51). Most (81/103, 78.6%) of study participants

were treated for DSTB. Compared to patients treated for DSTB, patients treated for DRTB were older and more likely to have a cavitary disease, but less likely to have pulmonary infiltration ($p<0.05$) (Table 4.1). Hepatitis C was reported among 3.2% (4/105, 95%CI 1.2 – 8.9) of enrolled participants, all of which were patients treated for DSTB.

The metabolic syndrome was present among 7.8% (95%CI 3.6 – 14.0) of participants at the end of TB treatment. The median VAI at the end of TB treatment was 1.23 (IQR 0.83 – 1.67). At the end of TB treatment, the prevalence of elevated blood glucose level was 3.9% (95%CI 1.3 – 9.1) (Table S4.1). The prevalence of lower HDL and elevated triglycerides was 37.9% (95%CI 28.2 – 48. 5) and 18. 5% (95%CI 11.8 – 26.8), respectively. Elevated blood pressure was common at the end of TB treatment (29.9%, 95%CI 21.4 – 39.6) vs. 9.6% (95%CI 5.0 – 16.5) at TB treatment initiation. Abdominal obesity was reported among 9.3% (95%CI 4.6 – 16.3) of patients treated for TB disease.

The Association Between Drug-Resistant Type and Risk Factors of Cardio-Metabolic Diseases at the End of Tuberculosis Treatment

Metabolic Syndrome

The prevalence of metabolic syndrome among patients treated for DRTB was 18.2% (4/22) and was 4.8% (4/83) among patients treated with DSTB (proportion difference 13.36 percentage points, 95% confidence interval [CI] -3.40, 30.12) (Table 4.2). After adjusting for age and gender, the proportion of metabolic syndrome at the end of TB treatment among patients formerly treated for DRTB was 2.29 times the proportion among patients formerly treated for DSTB (95%CI 0.50 – 10.37).

Visceral Adipose Index

At the end of TB treatment, the median VAI was non-significantly higher among patients treated for DRTB (median 1.46, IQR 0.74 – 2.21) compared to patients treated for DSTB (median 1.18, IQR 0.83 – 1.62) (p=0.34) (Table 4.1). In the model adjusted for age, gender, sputum conversion status at 2-month and cavitary disease, on average, the mean VAI value was higher by 0.37 (95%CI -0.13, 0.86) points among patients treated for DRTB vs. DSTB (Table 4.3).

Changes in Metabolic Syndrome and VAI From the End of Tuberculosis Treatment and at 6-month Follow-up

Among 105 study participants included in the analyses, 1 (1.0%) patient died before the 6-month follow-up. There were 62 (59.6%) patients with 6-month follow-up information available and included in the subgroup analyses. Among these, metabolic syndrome was reported among eight study participants (8/62, 12.9%), three of which were newly diagnosed, and five were persistent metabolic syndrome (i.e., study participants also had metabolic syndrome at the end of TB treatment). Accounting for the repeated measures, the odds of metabolic syndrome post-TB treatment was non-significantly higher among patients formerly treated for DRTB vs. DSTB after adjusting for age, gender, sputum conversion status at 2-month, cavitary disease (adjusted odds ratio [aOR] 2.03, 95%CI 0.20 – 20.34) (Table 4.4).

We observed an increasing trend of VAI, HbA1c, HDL, and LDL from the end of TB treatment vs. the 6-month follow-up (Figure 4.1). For example, the mean VAI among patients formerly treated for DRTB increased from 2.02 (SD 1.46) at the end of TB treatment to 2.44 (SD 2.15) at the 6-month follow-up (Table S4.2). The mean VAI among patients formerly treated for DSTB was similar at the end of TB treatment (mean=1.40, SD 0.68) vs. at 6-month follow-up

(mean=1.46, SD 1.05). On average, the mean VAI among patients formerly treated for DRTB was non-significantly higher by 0.60 (95%CI -0.20, 1.40) points when compared to participants formerly treated for DSTB after adjusting for age, gender, sputum conversion status at 2-month, cavitary disease, and the clustering of VAI at the individual level (Table 4.4).

The mean Hba1c among patients formerly treated for DRTB increased from 3.85 (SD 1.19) at the end of TB treatment to 5.22 (SD 1.49) at the 6-month follow-up. The mean HbA1c among patients formerly treated for DSTB increased from 4.37 (SD 1.13) at the end of TB treatment to 4.46 (SD 1.13) at the 6-month follow-up. On average, the mean HbA1c among patients treated for DRTB was non-significantly lower by 0.13 (95%CI -0.81, 0.55) percentage point when compared to participants treated for DSTB, after adjusting for age, gender, sputum conversion status at 2-month, cavitary disease, and the clustering of HbA1c at the individual level.

Results From Sensitivity Analyses

The crude risk ratio of having higher VAI at the end of TB treatment comparing patients treated for DRTB to DSTB was 1.69 (95%CI 0.77 – 3.69) (data not shown). After adjusting for age and gender, the risk of having higher VAI at the end of TB treatment among patients treated for DRTB was 1.46 (95%CI 0.53 – 4.05) times the risk of those treated for DSTB. With an observed crude prevalence ratio of 3.77, an unmeasured confounder that was associated with both DRTB and metabolic syndrome by a risk ratio of 7.00-fold each or greater would be needed to explain away the observed estimate. To move the 95% confidence interval to include the null value (RR=1), an unmeasured confounder that was associated with both DRTB and metabolic syndrome by a risk ratio of 1.16-fold each or greater could explain away the estimate, but weaker confounding could not. In the sensitivity analysis to quantify the systematic errors due to covariates

misclassification, the range of mean difference of VAI at the end of TB treatment comparing patients formerly treated for DRTB vs. DSTB was 0.30 – 0.43 (Table 4.3).

DISCUSSION

Among a well characterized prospective cohort of patients successfully treated for pulmonary TB, we found nearly 1 out of 12 participants had metabolic syndrome at the end of TB treatment. Although we had limited patients with DRTB, our intriguing finding suggest that participants treated for DRTB had twice the risk of metabolic syndrome compared to participants treated for DSTB. Consistently, the VAI levels were higher among patients treated for DRTB vs. DSTB. These preliminary findings may suggest that cardio-metabolic risks may be elevated among patients formerly treated for DRTB.

In our cohort, we reported a relatively high prevalence of metabolic syndrome considering the younger study population. We also reported higher proportion of metabolic syndrome among patients treated for DRTB. Previous studies also suggested that TB may influence post-TB metabolic health. A 2014 population-based cohort study with a maximum follow-up time of three years conducted in Taiwan reported a higher incidence of the acute coronary syndrome (ACS) among patients treated for TB vs. the general population (2.10 vs. 1.51 per 1000 person-years) [25]. However, unlike our study, the Taiwanese study did not compare the cardio-metabolic risk between patients by their drug-resistant type. A 2016 cross-sectional study conducted among human immunodeficiency virus (HIV)-infected patients in Ghana reported a higher prevalence of metabolic syndrome of those receiving highly active antiretroviral therapy (HAART) (29.6%) vs. HAART-naïve (13.7%) ($p<0.05$) [26]. Collectively, the Taiwanese and Ghanaian studies may

suggest that host response to infectious disease agents such as MTB and HIV can increase the risk of metabolic dysfunction.

We also reported a trend toward higher VAI levels among patients formerly treated for DRTB. While other reports of post-TB VAI have not been published, a 2020 cohort study conducted in India reported that a higher level of VAI (VAI >5.0) at TB treatment initiation was associated with poor TB treatment outcomes (OR 5.1, 95%CI 1.4 – 19.0) [20]. Further studies are needed to determine whether VAI measured during or post-TB treatment is predictive of later development of cardio-metabolic diseases after TB treatment completion. Importantly, established VAI values to indicate adverse risk of chronic non-communicable diseases are unavailable. Additional studies to evaluate VAI levels associated with cardio-metabolic disease risks are needed, especially across different populations and race/ethnicity groups.

Treating patients with DRTB has traditionally required longer, more toxic, and less effective regimens compared to DSTB treatment [27]. Patients with DRTB may experience prolonged systematic inflammation, which could increase the risk of metabolic syndrome over time [28]. Previous studies suggest that the immune dysregulation as a response to TB infection alone could induce metabolic reprogramming [29-31]. Moreover, TB disease is also characterized by wasting and significant loss of muscle mass, which likely can impact basal metabolic rate, another hallmark of metabolic syndrome [32, 33]. A 2012 animal model study reported a significant decrease of a few metabolites, namely lactate, nicotinamide, glutamine, choline, phosphocreatine, and ethanolamine after 30- and 60-days post- MTB infection compared to naïve controls [34]. Findings from the animal study were echoed in a recent cohort study conducted among patients with pulmonary and/or extrapulmonary TB disease in The Netherlands [35]. The 2020 Netherlands study reported a drastic metabolic reprogramming indicated by a significant

decreased in methionine, glutamine, threonine, tryptophan, histidine, citrulline, cysteine, and homoserine at the beginning of TB treatment. The Netherlands study also reported no significant changes of metabolic profile after six weeks of TB treatment [35].

Our study is subject to several limitations. First, we did not have information on individuals' physical activity, diet, family history, and other risk factors of metabolic syndrome. Thus, we were not able to control for these important confounders in our analyses. However, this was not a major concern as they would have to have been very strong confounders to eliminate the observed association. Second, we enrolled patients from one region in Georgia, and the majority were treated from the referral facility (i.e., NCTLD). Consequently, our findings may not be generalizable to other parts of Georgia or other countries with different TB burden. However, to our knowledge, the present study is the first prospective cohort study to characterize metabolic syndrome and VAI after a successful TB treatment. Third, we have yet completed the enrollment process (~80% as of March 2021), and less than 60% of patients enrolled had 6-month follow-up information available at the time of analyses. Fourth, we did not measure metabolic syndrome and VAI at the beginning of and during TB treatment. Thus, we could not present the complete trajectories of cardio-metabolic risks from the beginning of and after TB treatment.

In conclusion, we found that metabolic syndrome and VAI levels may be elevated among patients treated for DRTB. To date, there is no established guideline to follow-up patients after TB treatment completion. Although larger prospective studies with more granular and extensive follow-up time (i.e., including follow-ups during and after TB treatment) are needed, our results highlighted the need for clinicians to screen for cardio-metabolic risks post-TB treatment, especially among patients formerly treated for DRTB. Metabolic syndrome and VAI are two simple yet potential indicators to monitor metabolic profile and cardio-metabolic risks after TB

treatment. Incorporating these inexpensive methods, especially among target priority groups, may reduce premature deaths after successful TB treatment.

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TABLES AND FIGURES

Table 4.1 Demographic and clinical characteristics according to drug-resistance type of patients successfully treated for tuberculosis disease in the country of Georgia, N=105

Characteristics	Total	Drug resistance type		p-values*
		DSTB N (%) = 83 (79.1)	DRTB N (%) = 22 (21.0)	
Male gender	50 (57.1)	49 (59.0)	11 (50.0)	0.45
Age group (years)				
Median (IQR)	34 (26 – 51)	34 (26 – 49)	43 (28 – 60)	0.16‡
16 – 59	93 (88.6)	77 (92.8)	16 (72.7)	**0.02**
≥60	12 (11.4)	6 (7.2)	6 (27.3)	
Georgian	94 (89.5)	76 (91.6)	18 (81.8)	0.24
BMI at TB treatment initiation (n=91)				
Normal/Overweight (≥18.5kg/m^2)	66 (71.0)	54 (72.0)	12 (66.7)	0.65
Underweight (BMI <18.5kg/m^2)	27 (29.0)	21 (28.0)	6 (33.3)	
Missing	12	8	4	
X-ray findings at TB treatment initiation				
Cavitary disease	21 (20.0)	13 (15.7)	8 (36.4)	**0.04**†
Infiltration	101 (96.2)	82 (98.8)	19 (86.4)	**0.03**†
Fibrosis	58 (55.2)	45 (54.2)	13 (59.1)	0.68
Sputum culture conversion (n=91)				
Days (IQR)	60 (56 – 71)	61 (57 – 70)	56 (31 – 87)	0.19
2-month conversion	56 (55.4)	42 (53.2)	14 (63.6)	0.38
Missing/unknown	4	4	0	
Ever received fluoroquinolones	21 (20.0)	1 (4.8)	20 (95.2)	**<0.01**†
Comorbidities				
Current Smoker	34 (32.7)	29 (34.9)	5 (23.8)	0.33
Hypertension (n=102)				

Characteristics	Total	Drug resistance type		p-values*
		DSTB N (%) = 83 (79.1)	DRTB N (%) = 22 (21.0)	
Self-reported	5 (4.8)	2 (2.5)	3 (13.6)	0.06†
Elevated blood pressure measured at the time of TB treatment initiation	10 (9.6)	9 (11.0)	1 (4.5)	0.68†
Missing	1	1	0	
Self-reported pre-existing T2DM	10 (9.5)	6 (7.2)	4 (18.2)	0.21†
Self-reported pre-existing dyslipidemia	22 (20.9)	16 (19.3)	6 (27.3)	0.56†
Pulmonary Function at the end of TB treatment				
FeNO	10 (7 – 17)	11 (7 – 18)	9 (8 – 12)	0.23‡
Median %Prediction of FVC (IQR)	104 (96 – 114)	104 (96 – 114)	108 (94 – 115)	0.69‡
Median %Prediction of FEV1 (IQR)	99 (89 – 111)	100 (87 – 112)	98 (91 – 108)	0.73‡
Median %Prediction of FEV1/FVC (IQR)	99 (91 – 105)	99 (91 – 105)	95 (92 – 106)	0.47‡
Median %Prediction of TLC (IQR)	93 (85 – 101)	92 (84 – 100)	97 (91 – 104)	0.09‡
Median %Prediction of RV (IQR)	72 (43 – 100)	67 (40 – 93)	86 (56 – 110)	0.14‡
Median %Prediction of DLCO (IQR)	67 (57 – 78)	69 (57 – 79)	63 (57 – 68)	0.06‡
Laboratory				
HbA1c (n=103)				
Median (IQR)	4.3 (3.6 – 4.8)	4.3 (3.8 – 4.8)	4.1 (3.3 – 4.7)	0.38‡
Total Cholesterol (n=103)				
Median (IQR)	4.8 (4.0 – 5.7)	4.8 (4.1 – 5.7)	4.9 (3.9 – 5.2)	0.56‡
HDL (n=87)				
Median (IQR)	1.3 (1.0 – 1.6)	1.3 (1.0 – 1.6)	1.3 (0.9 – 1.6)	0.49‡
LDL				
Median (IQR)	2.0 (1.0 – 2.0)	2.0 (1.0 – 2.0)	2.0 (1.0 – 2.5)	0.79‡
Triglycerides				
Median (IQR)	1.0 (0.7 – 1.4)	1.0 (0.7 – 1.3)	1.3 (0.7 – 1.8)	0.13‡
Visceral Adipose Index (VAI) (n=79)				
Median (IQR)	1.23 (0.83 – 1.67)	1.18 (0.83 – 1.62)	1.46 (0.74 – 2.21)	0.34‡

*p-values from Chi-square tests (unless otherwise indicated)
†p-values from Fisher's exact tests

Characteristics	Total	Drug resistance type		p-values*
		DSTB	DRTB	
		N (%) = 83 (79.1)	N (%) = 22 (21.0)	

‡p-values from Wilcoxon rank sum tests

Abbreviations:
BMI – body mass index; DLCO – diffusing capacity; DRTB – drug-resistant tuberculosis; DSTB – drug-susceptible tuberculosis; FeNO – fractional exhaled nitric oxide; FEV – forced expiratory volume in one second; FVC – forced vital capacity; HbA1c – glycated hemoglobin; HDL – high-density lipoprotein; LDL – low-density lipoprotein; IQR – interquartile range; RV - residual volume; T2DM – type-2 diabetes mellitus; TLC – total lung capacity

Table 4.2 Unadjusted and adjusted prevalence of metabolic syndrome at the end of TB treatment among patients successfully treated for tuberculosis disease in the country of Georgia (n=105)

Patients' Characteristics	At the end of TB treatment (n=105)			
	Metabolic Syndrome N (%) = 8 (7.6)	Prevalence Ratios		
		Crude (95%CI)	Adjusted (95%CI)	
			Model 1*	Model 2†
Drug-resistance type				
DSTB	4/83 (4.8)	Reference	Reference	Reference
DRTB	4/22 (18.2)	**3.77 (1.02 – 13.90)**	2.29 (0.50 – 10.37)	2.42 (0.49 – 11.83)
Age group (years)				
16 – 59	4/93 (4.3)	Reference	Reference	Reference
60+	4/12 (33.3)	**7.75 (2.23 – 27.02)**	**5.70 (1.28 – 25.47)**	**5.73 (1.30 – 25.33)**
Gender				
Female	3/45 (6.7)	Reference	Reference	Reference
Male	5/60 (8.3)	1.25 (0.32 – 4.96)	1.23 (0.29 – 5.30)	1.24 (0.29 – 5.33)
Sputum culture conversion at 2-month (n=96)				
No	2/45 (4.4)	Reference		
Yes	6/56 (10.7)	2.41 (0.51 – 11.38)		
Cavitary disease				
No	6/84 (7.1)	Reference		Reference
Yes	2/21 (9.5)	1.33 (0.29 – 6.14)		0.84 (0.15 – 4.64)
Model Performance Metrics				
Akaike Information Criterion (AIC)			56.83	58.79
Bayesian Information Criterion (BIC)			67.45	72/06

Abbreviations:
CI – Confidence Interval; DRTB – drug-resistant tuberculosis; DSTB – drug-susceptible tuberculosis

*Model adjusted for drug-resistant type, age group, and gender
†Model adjusted for drug-resistant type, age group, gender, and cavitary disease

Bold indicates that the finding is statistically significant at α=0.05

Table 4.3 Results of fitting a taxonomy of multiple regression models to assess patients' characteristics associated with visceral adipose index at the end of tuberculosis treatment (N=79)

Demographic characteristics	**Parameter estimates (*se*)**				
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Intercept	1.322 (0.107)	1.282 (0.107)	1.228 (0.156)	1.093 (0.187)	1.158 (0.196)
Drug-resistance type					
DSTB	Reference	Reference	Reference	Reference	Reference
DRTB	0.426 (0.237)	0.308 (0.240)	0.321 (0.243)	0.303 (0.243)	0.365 (0.250)
Age group					
16 – 59		Reference	Reference	Reference	Reference
60+		0.633 (0.320)	0.611 (0.324)	0.684 (0.331)	0.678 (0.331)
Gender					
Female			Reference	Reference	Reference
Male			0.092 (0.193)	0.100 (0.196)	0.060 (0.199)
Sputum conversion at 2-month					
No				Reference	Reference
Yes				0.261 (0.195)	0.269 (0.195)
Cavitary disease					
No					Reference
Yes					-0.252 (0.236)
Root MSE	0.846	0.830	0.834	0.833	0.833
R^2	0.040	0.087	0.090	0.116	**0.130**
Model *F*-test	3.23	3.64*	2.48	1.92	1.79
(df_1, df_2)	(1, 77)	(2, 76)	(3, 75)	(5, 73)	(6, 72)

*$p<0.05$, **$p<0.01$, ***$p<0.001$

†

Abbreviations:
DRTB – drug-resistant tuberculosis; DSTB – drug-sensitive tuberculosis; MSE – mean square error; se – standard error

Figure 4.1 Changes in mean visceral adipose index [VAI] (a), glycated hemoglobin [HbA1c] (b), high-density lipoprotein [HDL] (c), and triglycerides [TGL] (d) at the end of TB treatment and 6-month post-TB treatment completion among patients successfully treated tuberculosis disease in the country of Georgia (n=62)

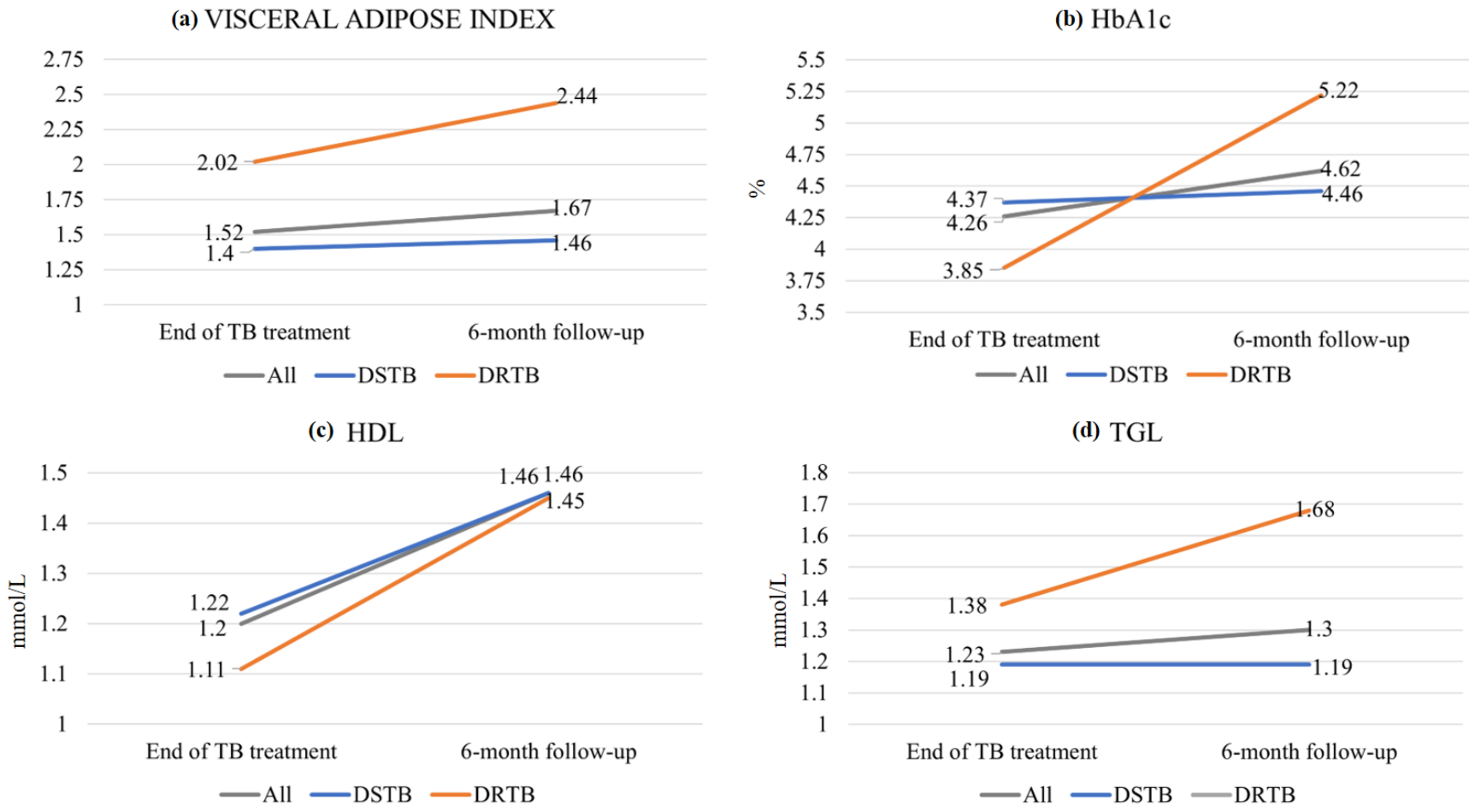

Table 4.4 Multilevel analysis for modeling glycated hemoglobin (HbA1c), high-density lipoprotein (HDL), triglycerides, body mass index (BMI), visceral adipose index (VAI), and metabolic syndrome as a function of type of drug resistance, age, gender, sputum conversion status at 2-month, and cavitary disease while accounting for repeated measures data (N=62)

Patients Characteristics	HbA1c Estimates (SE)	HDL Estimates (SE)	Triglycerides Estimates (SE)	BMI Estimates (SE)	VAI Estimates (SE)	Metabolic Syndrome aOR (95%CI)
Intercept	4.019 (0.288)***	1.330 (0.108)***	0.902 (0.191)***	21.724 (0.920)***	1.502 (0.305)***	
Type of resistance						
DSTB	Reference	Reference	Reference	Reference	Reference	Reference
DRTB	-0.128 (0.347)	<0.001 (0.130)	0.387 (0.252)	0.402 (1.539)	0.598 (0.408)	2.03 (0.20 – 20.34)
Age group						
16 – 45	Reference	Reference	Reference	Reference	Reference	Reference
46+	0.613 (0.392)	-0.167 (0.147)	0.111 (0.286)	4.724 (1.760)*	0.775 (0.463)	13.49 (1.24 – 146.40)*
Gender						
Female	Reference	Reference	Reference	Reference	Reference	Reference
Male	0.089 (0.250)	-0.118 (0.095)	0.296 (0.182)	-0.537 (1.120)	-0.012 (0.297)	0.62 (0.10 – 3.98)
Sputum conversion at 2-month						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.211 (0.308)	-0.086 (0.119)	0.128 (0.163)	0.483 (0.368)	0.084 (0.302)	2.68 (0.23 – 38.41)
Cavitary disease						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.109 (0.344)	-0.057 (0.130)	-0.411 (0.209)	0.003 (0.410)	-0.397 (0.329)	0.96 (0.06 – 16.01)

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Abbreviations:
aOR – adjusted odds ratio; BMI – body mass index; CI – confidence interval; DRTB – drug-resistant tuberculosis; DSTB – drug-susceptible tuberculosis; HbA1c – glycated hemoglobin; HDL – high-density lipoprotein; SD – standard error; VAI – visceral adipose index

Supplemental Materials

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Figure S4.1 Formulas to calculate gender-specific visceral adipose index

$$VAI_{Male} = \left(\frac{WC}{39.68 + (1.88 \times BMI)}\right) \times \left(\frac{TG}{1.03}\right) \times \left(\frac{1.31}{HDL}\right)$$

$$VAI_{Female} = \left(\frac{WC}{36.58 + (1.89 \times BMI)}\right) \times \left(\frac{TG}{0.81}\right) \times \left(\frac{1.52}{HDL}\right)$$

Abbreviations:

BMI – body mass index; HDL – high-density lipoprotein; TG – triglycerides; VAI – visceral adipose index; WC – waist circumference

Table S4.1 Prevalence of metabolic syndrome at the end of tuberculosis treatment among patients successfully treated for tuberculosis disease in the country of Georgia (n=105)

Patients' Characteristics	Prevalence of metabolic syndrome measured at the end of TB treatment				
	Elevated HbA1c (n=103)	**Lower HDL (n=87)**	**Elevated triglyceride (n=103)**	**Elevated Blood Pressure (n=97)**	**Abdominal Obesity (n=97)**
	N (%) = 4 (3.9)	**N (%) = 33 (37.9)**	**N (%) = 19 (18.5)**	**N (%) = 29 (29.9)**	**N (%) = 9 (9.3)**
Drug-resistance type					
DSTB	3/81 (3.7)	24/69 (34.8)	13/81 (16.1)	21/77 (27.3)	5/77 (6.5)
DRTB	1/22 (4.6)	9/18 (50.0)	6/22 (27.3)	8/20 (40.0)	4/20 (20.0)
Age group					
16 – 59	2/91 (2.2)	28/78 (35.9)	16/91 (17.6)	23/86 (26.7)	6/86 (7.0)
60+	2/12 (16.7)	5/9 (55.6)	3/12 (25.0)	6/11 (54.6)	3/11 (27.3)
Gender					
Female	1/43 (2.3)	14/37 (37.8)	4/43 (9.3)	5/40 (12.5)	6/39 (15.4)
Male	3/60 (5.0)	19/50 (38.0)	15/60 (25.0)	24/57 (42.1)	3/58 (5.2)
Sputum conversion at 2-month					
No	1/44 (2.3)	12/39 (30.8)	5/44 (11.4)	13/42 (31.0)	3/42 (7.1)
Yes	3/56 (5.4)	20/45 (44.4)	13/56 (23.2)	10/52 (28.9)	6/52 (11.5)
Cavitary disease					
No	3/82 (3.7)	25/69 (36.2)	17/82 (20.7)	24/76 (31.6)	6/76 (7.9)
Yes	1/21 (4.8)	8/18 (44.4)	2/21 (9.5)	5/21 (23.8)	3/21 (14.3)

Abbreviations:
DRTB – drug-resistant tuberculosis; DSTB – drug-susceptible tuberculosis; HDL – high-density lipoprotein; TB - tuberculosis

Table S4.2 Anthropometry and biochemical measures at the end of TB treatment and 6-month follow-up of patients successfully treated for tuberculosis disease in the country of Georgia

Variables	Unit	Study Baseline (n=105)			Among patients with 6-month follow up information available (n=62)					
					Study Baseline			6-month follow-up		
		All	DSTB	DRTB	All	DSTB	DRTB	All	DSTB	DRTB
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Anthropometry										
Weight	kg	64.2 (13.3)	64.0 (117)	64.9 (18.5)	64.9 (14.2)	64.5 (11.3)	66.5 (22.5)	67.4 (14.4)	67.0 (11.6)	68.5 (23.0)
BMI	kg/m^2	22.2 (4.1)	21.9 (3.3)	23.3 (6.4)	22.5 (4.4)	22.0 (3.1)	24.4 (7.4)	23.3 (4.7)	22.8 (3.4)	25.2 (7.7)
Waist	cm	84.2 (15.8)	83.6 (15.5)	86.4 (17.4)	84.2 (13.3)	83.1 (10.6)	88.5 (20.6)	85.1 (12.6)	84.2 (9.8)	88.2 (20.3)
SBP	mmHg	119.8 (16.0)	118 (13.0)	126.9 (23.5)	119.6 (16.9)	117.3 (11.8)	128.2 (28.1)	122.4 (16.9)	122.2 (16.8)	120.6 (16.1)
DBP	mmHg	70.9 (11.4)	70.4 (11.5)	73 (11.4)	70.0 (9.3)	69.1 (8.3)	73.1 (12.1)	75.9 (10.9)	76.1 (10.8)	75.0 (12.2)
Biochemistry										
HbA1c	%	4.2 (1.0)	4.3 (1.0)	4.0 (1.0)	4.3 (1.1)	4.4 (1.1)	3.9 (1.2)	4.6 (1.2)	4.5 (1.1)	5.2 (1.5)
Cholesterol	mmol/L	4.9 (1.3)	5.0 (1.4)	4.7 (1.1)	5.0 (1.2)	5.0 (1.2)	4.9 (1.1)	5.0 (1.4)	5.0 (1.5)	5.1 (1.1)
HDL	mmol/L	1.3 (0.5)	1.4 (0.5)	1.3 (0.4)	1.2 (0.4)	1.2 (0.4)	1.1 (0.3)	1.5 (0.4)	1.5 (0.4)	1.5 (0.5)
LDL	mmol/L	1.9 (0.9)	1.9 (0.9)	1.9 (1.1)	1.7 (0.8)	1.8 (0.9)	1.6 (0.7)	2.2 (1.0)	2.2 (0.8)	2.3 (1.6)
Triglycerides	mmol/L	1.2 (0.9)	1.2 (0.9)	1.4 (0.7)	1.2 (1.0)	1.2 (1.0)	1.4 (0.8)	1.3 (0.7)	1.2 (0.6)	1.7 (0.8)
Metabolic syndrome indicator										
VAI		1.4 (0.9)	1.3 (0.7)	1.7 (1.2)	1.5 (0.9)	1.4 (0.7)	2.0 (1.5)	1.7 (1.4)	1.5 (1.1)	2.4 (2.2)

Abbreviations:
BMI – body mass index; DBP – diastolic blood pressure; DRTB – drug-resistant tuberculosis; DSTB – drug-susceptible tuberculosis; HbA1c – glycated hemoglobin; HDL – high-density lipoprotein; LDL – low-density lipoprotein; SD – standard deviation; SBP – systolic blood pressure; VAI – visceral adipose index

CHAPTER 5

Summary and Future Directions in Research

5.1 Overview of Findings

From **Study 1**, we found that HIV is a strong predictor of mortality post-TB treatment. Although not statistically significant, the hazard rate of post-TB mortality was slightly higher among TB survivors with hyperglycemia or hepatitis C co-infection compared to those without. Study 1 was conducted using TB surveillance records of 1,032 patients treated with SLDs in the country of Georgia between 2009 – 2017. Post-TB mortality was determined by cross-validating vital status from Georgia's death registry. The median age of patients in our cohort was 35 years old (IQR 26 – 9), and the majority were male (73%). The prevalence of MDR TB was 44.7%, and pre-XDR TB was 29.1%. We reported an overall post-TB mortality risk of 8.7%, with a median time to death of 21 months (IQR 7 – 39) after TB treatment was completed or stopped. We observed 27 deaths during 1,129 person-years among 231 TB survivors with hyperglycemia (post-TB mortality $rate_{hyperglycemia}$=23.91, 95%CI 16.40 – 34.87). There were 18 deaths during 701 person-years among 147 TB survivors with hepatitis C (post-TB mortality $rate_{hepatitis\ C}$=25.68, 95%CI 16.18 – 40.76). Among 32 TB survivors with HIV co-infection, we observed 9 deaths during 130 person-years (post-TB mortality $rate_{HIV}$=69.23, 95%CI 36.02 – 133.10). After adjusting for age and gender, the hazard rate of post-TB mortality was higher among TB survivors with hyperglycemia (aHR 1.19, 95%CI 0.73 – 1.96), hepatitis C (aHR 1.25, 95%CI 0.71 – 2.19) or HIV co-infection (aHR 4.40, 95%CI 2.17 – 8.93) compared to those without comorbidities.

From **Study 2**, we reported a statistically significantly lower risk of poor TB treatment outcome among TBDM patients with a record of metformin use compared to those with no record of metformin use. In study 2, we included 128 DRTB patients with hyperglycemia (with or without

T2DM) who started treatment at the National Center for Tuberculosis and Lung Diseases and two TB dispensaries in Tbilisi, Georgia. In our cohort of patients with hyperglycemia, the majority were male (82.0%), with a median age of 45 years (IQR 32 – 57). The prevalence of MDR TB or pre-XDR TB was 67.2%. Hepatitis C was reported among 22.9%, and HIV was 1.7% among our cohort of DRTB patients with hyperglycemia. More than half of our cohort (52.1%) had T2DM according to the fasting blood glucose level or self-reported previous diagnosis of T2DM at TB treatment initiation. Compared to patients with pre-diabetes, patients with T2DM had a non-significantly lower rate of sputum culture conversion (aHR 0.81, 95%CI 0.52 – 1.26) after adjusting for age and gender. Surprisingly, DRTB patients with T2DM had lower risk of poor TB treatment outcomes (aRR 0.80, 95%CI 0.41 – 1.56) or post-TB mortality (aHR 0.72, 95% CI 0.21 – 2.44). Among DRTB patients with T2DM (n=66), metformin use was associated with a significantly lower risk of poor TB treatment outcomes (aRR 0.23, 95%CI 0.05 – 0.97). Although non-significant, T2DM patients with a record of metformin use had a higher hazard rate of sputum culture conversion (aHR 1.19, 95%CI 0.64 – 2.18) and lower hazard rate of post-TB mortality (aHR 0.66, 95%CI 0.12 – 3.59) after adjusting for age and gender.

From **Study 3**, we found that the prevalence of metabolic syndrome and VAI at the end of TB treatment was higher among patients treated for DRTB. In study 3, we included 105 patients with favorable pulmonary TB treatment outcomes we enrolled from December 2019 until March 2021. The median age of patients in this successfully treated TB cohort was 34 years (IQR 26 – 51), and the majority were male (57.1%). The proportion of patients treated for DRTB was 27.4%. At the end of TB treatment (i.e., study baseline), the prevalence of metabolic syndrome was 7.8% (95%CI 3.6 – 14.0), and the median VAI was 1.23 (IQR 0.83 – 1.67). Although non-significant, the adjusted prevalence of metabolic syndrome (adjusted prevalence ratio [aPR] 2.29, 95%CI 0.50

– 10.37) and the mean of VAI levels (mean difference 0.37, 95%CI -0.13 – 0.86) were higher among patients treated for DRTB vs. DSTB. Importantly, we observed a trend toward an increase in the VAI and HbA1c levels when comparing levels at the end of TB treatment vs. 6-month post-TB treatment completion. Results from the mixed model suggested that, although non-significant, on average, patients treated for DRTB may have slightly higher VAI (adjusted mean difference 0.60, 95%CI -0.20, - 1.40) but lower HbA1c (adjusted mean difference -0.13, 95%CI -0.81 – 0.55) when compared to patients treated for DSTB.

Collectively, findings from the three studies highlight the important role of continued health complications after TB treatment completion. More specifically, we found that pre-existing comorbidities, specifically HIV co-infection, may increase the risk of post-TB mortality. We also found that metformin use among DRTB patients with T2DM may reduce the risk of poor TB treatment outcomes and post-TB mortality. Moreover, our preliminary findings also suggest that patients successfully treated for TB, specifically patients treated for DRTB, may be at greater risk of post-TB metabolic syndrome.

5.2 Public Health and Clinical Implications

To date, most TB research has focused on TB diagnosis and treatment. Moreover, the WHO END TB strategy clearly stated that the primary target to curb the global TB epidemic by 2035 is to reduce 95% tuberculosis deaths (compared to 2015 figures) and 90% TB incidence rate (equivalent to <10 TB cases per 100,000 population). However, results from the three studies in this dissertation highlight an urgent need for a life-course approach in tuberculosis research. Our findings echoed the mounting evidence that patients treated for TB disease may have residual complications which may increase the risk of chronic non-communicable disease progression or

mortality post-TB treatment. Therefore, results from the three dissertation studies will have a substantial impact on TB epidemiology and care, especially with the absence of guidelines to follow-up patients after TB treatment completion. These dissertation findings will support future efforts to understand the total and lifetime burden of TB disease as presented in the post-TB lung health symposium (22 – 23 July 2019, Stellenbosch University, South Africa) and workshop (30 October – 2 November 2019, 50th Union World Conference on Lung Health in Hyderabad, India).

Results from **Study 1** suggested that DRTB patients with pre-existing HIV co-infection had the greatest risk of post-TB mortality. Although it has been well documented that patients treated for TB disease have an increased risk of post-TB mortality, the role of pre-existing comorbidities has not been well characterized in different settings. Our first study provides a more thorough identification of comorbidities risk factors of post-TB mortality from a country with high MDR TB burden. For resource-limited settings like Georgia, it may be challenging to provide TB patients with an extensive TB follow-up care. Thus, strengthening care among patients with pre-existing comorbidities (e.g., by providing CD4 count test for patients with HIV co-infection, blood glucose level for patients with hyperglycemia, and viral load test for those with HCV co-infection) may help reduce the rates of post-TB mortality. Linkage to other health clinics after TB treatment completion to continue care for pre-existing comorbidities reported at the beginning of TB treatment may help reduce premature deaths post-TB treatment. For example, linking TB-HIV patients to the "Infectious Diseases, AIDS and Clinical Immunology Research Center (National AIDS Center)" which is the primary HIV/AIDS clinical services provider in Georgia, after TB treatment may reduce post-TB treatment deaths associated with HIV/AIDS.

Results from **Study 2** highlighted the role of metformin (vs. other blood glucose-lowering agents) in preventing poor TB treatment outcomes. Despite the small sample size, our findings are

consistent with previous studies that suggested metformin could be used as adjuvant TB therapy. Results from study 2 also expanded the current knowledge regarding the potential role of metformin to reduce post-TB mortality among patients with TBDM. Although non-significant, we reported lower hazard rates of post-TB mortality among patients with a record of metformin use vs. patients without metformin use. Our findings may need to be interpreted with cautions as there could be other clinical considerations why endocrinologists prescribed insulin/sulfonylureas over metformin, which was not captured in our cohort. According to our study findings, clinicians should consider incorporating metformin (over other BGLAs) into TB treatment regimen, especially among those with diabetes, to reduce the risk of poor TB treatment outcomes.

Results from **Study 3** demonstrated important implications for metabolic disease post-TB. Among patients successfully treated for TB disease, we reported nearly 10% had metabolic syndrome at the end of TB treatment. Study 3 introduced two simple, inexpensive, and routinely applicable markers to measure cardio-metabolic diseases, namely metabolic syndrome, and visceral adipose index. In addition to the routine follow-up post-TB treatment implemented in Georgia (i.e., TB symptoms screening every 6-month for the first two years post-TB treatment), the National TB control program should consider adding either screening for metabolic syndrome or VAI measurement to the post-TB treatment standard of care. Other settings, especially those with high TB and comorbidities burden, should implement post-TB care and include simple tools to measure cardio-metabolic disease risks. This will allow clinicians to capture the early detection of cardio-metabolic diseases and make sure TB survivors receive proper care after TB treatment completion.

Altogether, findings from the three studies underscore the importance of continued care even after a successful TB treatment. The three studies provided epidemiologic evidence to help

set priorities to address gaps and barriers regarding post-TB health. Ultimately, we hope that findings from the three dissertation studies would advocate public health experts and TB clinicians to formulate new global recommendations and guidelines on post-TB treatment care.

5.3 Future Direction and Research

This dissertation was subject to limitations and future studies should be implemented to overcome these drawbacks. One of the major limitations of our studies was the lack of data on cause of death post-TB treatment. Thus, we could not separate post-TB deaths associated with pre-existing comorbidities from other common causes of death such as cancer or injury. Moreover, we determined HCV and HIV co-infection status at TB treatment initiation. Thus, we did not have information on the chronic manifestation of the two diseases or any complications during TB treatment. Therefore, our post-TB mortality hazard rates may be confounded by pre-existing comorbidity factors such as CD4 count, ART use, blood glucose control, or viral load during TB treatment. In our study, we addressed this issue by estimating the E-values. However, further studies with prospective study designs following TB patients from treatment initiation until after treatment completion with detailed cause of post-TB deaths are still warranted to fully understand the impacts of pre-existing comorbidities on post-TB mortality risks.

In the future, plasma glycated hemoglobin (HbA1c) may be a better option to determine hyperglycemia status. In our study, we identified misclassification of hyperglycemia as one of the strongest sources of systematic errors. In studies 1 and 2, hyperglycemia was determined either by fasting blood glucose levels or a self-reported prior diabetes diagnosis by a healthcare provider. There are two possible errors here: measurement error and recall bias. In our cohort, patients may not follow doctors' instructions to fast before coming to the clinic. Thus, instead of fasting plasma

glucose level, results recorded in the TB surveillance database could be random blood glucose levels. Furthermore, some patients may not remember or recall a prior diabetes diagnosis. We addressed this misclassification issue in study 1 using probabilistic bias analysis to estimate the adjusted association between pre-existing comorbidities and post-TB mortality after accounting for both random and systematic errors and found that hyperglycemia misclassification may result in bias towards the null. Thus, we believe that plasma glycated hemoglobin (HbA1c) level is a much better measure as it can provide us a summary of a three-month average of patients' blood sugar levels and patients do not need to fast before taking the test.

Further and larger studies, including patients with pre-diabetes, diabetes, and normoglycemia, are still needed to determine whether metformin could be a candidate for TB adjuvant therapy. Studies 2 and 3 may not be sufficiently powered to observe statistically significant results with the small sample size included. Moreover, none of the patients with pre-diabetes in study 2 cohort had a record of BGLA use. Thus, we could not assess the potential role of metformin in preventing poor TB treatment outcomes among patients with pre-diabetes. Furthermore, in our study 2 cohort, the key driving factor of poor TB treatment outcomes is lost to follow-up. Further studies should evaluate whether these patients who were lost to follow-up ever returned to care and whether returning to care affects the association between the use of BGLA or other diabetes characteristics and TB treatment outcomes. Lastly, it is still unknown whether metformin works synergistically better with SLDs compared to other BGLA such as sulfonylureas and insulin. More clinical studies to investigate the drug-drug interactions between BGLA and anti-TB drugs are needed.

Specific for study 3, the study enrollment was incomplete when we performed the analyses. Thus, the full interpretation of study findings is contingent upon the completion of the study and

inclusion of data from both follow-up visits (at 6- and 12-months post TB treatment). However, one major limitation from study 3 is we did not have any baseline risk or measurement of metabolic syndrome nor VAI at the beginning of TB treatment. Future studies should include measurement of both metabolic syndrome and VAI at the beginning, during, at the end, and after TB treatment to provide a complete cardio-metabolic risk trajectory associated with TB disease.

Last, the three dissertation studies only looked at the clinical implications of TB disease after treatment is complete. However, post-TB complications may include financial and economic hardship as well as psychosocial implications. Future studies should also measure the general quality of life and mental health after TB treatment completion to address these gaps.

5.4 Conclusions

Our preliminary findings highlight an urgent need to continue healthcare after TB treatment completion. From the three dissertation studies, we concluded that 1) DRTB patients with HIV co-infection are at the greatest risk of post-TB mortality; 2) DRTB and T2DM patients with a history of metformin use during TB treatment had a lower risk of poor TB treatment outcomes; and 3) the post-TB prevalence of metabolic syndrome and VAI levels may be elevated among patients with DRTB. Our study findings emphasized that patients with pre-existing comorbidities and DRTB should be a target priority group to receive continued care after TB treatment completion. Collectively, this dissertation highlights the importance of a life-course approach in understanding the total burden of TB disease on human health.