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Diabetes mellitus and active tuberculosis disease: Clinical presentation and treatment outcomes in adult tuberculosis patients

By

Matthew J Magee

Doctor of Philosophy

Epidemiology

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Diabetes mellitus and active tuberculosis disease: Clinical presentation and treatment outcomes in adult tuberculosis patients

By

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MPH, University of Illinois at Chicago, 2006

BA, Grinnell College, 2001

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An abstract of

A dissertation submitted to the Faculty of the

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Abstract

Diabetes mellitus and active tuberculosis disease: Clinical presentation and treatment outcomes in adult tuberculosis patients

By Matthew J Magee

Objectives: Tuberculosis (TB) and diabetes mellitus (DM) contribute to extensive global morbidity and mortality. Although DM is an accepted risk factor for developing active TB disease, less is known about the relation between DM and TB clinical characteristics, including TB disease presentation and TB treatment outcomes. The overall goal of this dissertation was to estimate the association between DM and 1) TB disease severity at the time of TB diagnosis and 2) poor TB clinical outcomes.

Methods: This dissertation included three studies, each examined a subset of specific aims comparing TB disease in patients with and without DM. Study 1 was a cohort of new adult TB patients from Tbilisi, Georgia, screened for DM and impaired glucose tolerance (pre-DM) using a point-of-care hemoglobin A1c (HbA1c) test. We compared measures of TB severity at clinical presentation (including lung cavitary disease, sputum smear grade, and hemoptysis) in patients with and without DM. In study 2, we estimated the association between DM and time to sputum culture conversion in a cohort of multidrug-resistant (MDR) TB patients from the country of Georgia. Study 3 estimated the association between DM and time to all-cause mortality during TB treatment in a cohort of adult TB patients from the state of Georgia, United States.

Results: Study 1 demonstrated that patients with TB and DM were more likely to present with higher sputum smear grade (adjusted odds ratio 2.63, 95% confidence interval [CI] 1.14—6.06) compared to TB patients without DM. In study 2, the estimated hazard of sputum culture conversion was modestly, but non-significantly, lower in MDR TB patients with DM compared to those without DM (adjusted hazard ratio [aHR] 0.93, 95% CI 0.71—1.23). Compared to TB patients without DM, Study 3 reported TB-DM patients did not have significantly greater hazard of all-cause mortality during TB treatment (aHR 1.22, 95% CI 0.70—2.12).

Conclusion: Adult TB patients with DM may have more severe TB disease at clinical presentation. However, our findings did not suggest that DM has a clinically meaningful impact on time to TB culture conversion or all-cause mortality during TB treatment.

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CHAPTER 1: INTRODUCTION TO TUBERCULOSIS AND DIABETES MELLITUS EPIDEMIOLOGY

INTRODUCTION

The studies of this body of work address the inter-connection between two enormous global public health epidemics, tuberculosis (TB) and type 2 diabetes mellitus (DM). Tuberculosis causes vast morbidity and mortality globally; annually, more than 9 million people develop active TB and nearly 2 million die due to TB.[1] DM is also an escalating pandemic; globally, more than 371 million adults have DM and it is projected there will be 552 million persons with DM by 2030, with the majority of cases occurring in low- and middle- income countries.[2] While DM is recognized as a risk factor for TB, major gaps remain in our knowledge of the joint burden of these two diseases, including whether patients with DM and TB are more likely to present with severe TB disease, respond less well to anti-TB therapy, and have a higher risk of failure or mortality compared to TB patients without DM.

The overall dissertation goal was to investigate the relationship between DM and 1) TB disease severity at time of TB diagnosis and 2) poor TB clinical treatment outcomes. The overarching goals were addressed through three observational studies conducted among patients with both TB and DM (TB-DM). The three studies within this body of work each examined a subset of specific aims that compared TB disease characteristics in patients with DM to TB patients without DM. Our specific investigations included: 1) a study of DM prevalence and TB disease presentation in new TB patients from Tbilisi, Georgia; 2) a study of time to culture conversion among multi drug-resistant (MDR) TB patients with and without DM in the country of Georgia; and 3) a study to estimate the effect of DM on mortality during TB treatment in Georgia, USA.

Study 1 Aims and Overview

Beginning in September 2011, Emory University in collaboration with the National Center of TB and Lung Disease (NCTBLD) in Tbilisi implemented The *Hemoglobin A1c levels among tuberculosis patients in Tbilisi* (HALT) study. The specific aims of the HALT study were to:

1) Determine the prevalence of DM, pre-DM and normal blood glucose (using hemoglobin A1c [HbA1c] to measure DM status) in newly diagnosed active TB patients; and
2) Estimate the association between DM status (DM, pre-DM, and normal blood glucose) with differences in TB disease severity and clinical manifestations at the time of TB diagnosis;
3) Estimate the association between DM status and response to TB treatment including 2-month acid-fast bacilli (AFB) smear conversion and final TB treatment outcome.

Briefly, a cohort study was conducted at the NCTBLD in Tbilisi from September 2011 to June 2013. Newly diagnosed pulmonary TB patients were screened for eligibility to join the HALT study. Eligibility criteria included adult (aged >34 years) pulmonary TB patients who initiated directly observed treatment, short-course (DOTS) within the past 2 months. As per standard NCTBLD protocol, all patients received chest radiographs (CXR) and provided sputum samples at start of treatment. Physicians assessed clinical severity, collected data on patient demographics, and determined the duration of TB disease at the time of diagnosis. At enrollment, *Mycobacterium tuberculosis* isolates recovered from sputum/respiratory AFB smear and cultures were examined for drug susceptibility testing (DST), the current standard of care in Georgia. In addition, all HALT participants were screened for DM and pre-DM using a point-of-care HbA1c measurement from capillary

blood. Patients with HbA1c ≥6.5 were considered to have DM, 5.7-6.4% was defined as pre-DM, and those with HbA1c ≤5.6% were considered to have normal blood glucose. All patients enrolled in this cohort received standardized anti-TB treatment regimens according to the NCTBLD, which included 2 months of intensive phase and 4 months of continuation phase therapy under the DOTS model. To assess severity of clinical manifestations at diagnosis, we assessed four baseline measures of TB burden: 1) presence and of lung cavities, 2) AFB smear status and grade, 3) symptoms of cough, and 4) symptoms of cough with blood (hemoptysis). To assess response to anti-TB treatment, sputum specimens were be collected after 2 months and tested for AFB smear and culture conversion (from positive at diagnosis to negative after treatment). After 6 months of DOTS, standard World Health Organization TB treatment outcome (cure, complete, failure, default or death) was determined.

Study 2 aims and overview

Study 2 was also conducted in collaboration with NCTBLD in Tbilisi. The study followed all MDR TB patients treated with second-line anti-TB therapy between 2009 and 2012. The specific aims of the study were to estimate the following:

1) The association between DM status and time to *M. tuberculosis* sputum culture conversion (from positive to negative) among adult MDR TB patients;
2) The association between DM status and risk of default from second-line therapy among adult MDR TB patients.

Briefly, a cohort of all MDR TB patients between January 2009 and December 2012 was followed during second-line TB therapy at the NCTBLD in Tbilisi, Georgia. Eligible patients included adults (aged ≥18 years) with pulmonary MDR TB who initiated TB

treatment during the study period. In accordance with NCTBLD national treatment protocols, all MDR TB patients in the study received standard second-line anti-TB regimens. The primary study exposure of interest was DM status, and patients were categorized as TB-DM or TB only based on hospital admission forms completed by NCTBLD hospital TB physicians. Patients were not systematically screened for DM. The primary outcome of interest was time until *M. tuberculosis* complex sputum culture conversion. Time until culture conversion was defined as the number of days from MDR TB treatment initiation until the first of two consecutive negative cultures ≥30 days apart. The secondary outcome of interest was default, defined as a patient who had second-line TB therapy interrupted for ≥2 consecutive months (a standard treatment outcome category defined by the World Health Organization[3]). Cox proportion hazards models were used to estimate the association between DM status and time to sputum culture conversion. Log-binomial regression was used to estimate the association between DM and risk of TB second-line treatment failure.

Study 3 Aims and Overview

Study 3 was conducted in collaboration with the Georgia Department of Public Health (GDPH) in Georgia, USA. The study followed adult TB patients undergoing anti-TB treatment in the state of Georgia from January 2009 to September 2012. The specific aims of the study were to complete the following:

1) Compare the demographic and clinical presentation characteristics of adult TB patients with TB-DM to a) patients with TB-HIV and b) patients with TB only;
2) Estimate the association between DM and time to death (from any cause) during TB treatment;

3) Estimate the association between DM and specific site of extra pulmonary TB (EPTB).

To summarize the third study briefly, a cohort study of all adult TB patients in the state of Georgia was followed during standard TB treatment to compare the hazard of all-cause mortality during TB treatment among patients with and without DM. The primary study exposure of interest was DM status, abstracted from TB patients' medical records at the time of TB treatment initiation. Patients were classified as TB-DM patients, TB-HIV patients or TB-only patients based on self-reported DM or if the medical record contained details about previous DM diagnosis. The primary study outcome of interest was time until all-cause mortality during TB treatment. Time until death was measured as the number of days between TB treatment initiation and death date. The secondary study outcome, site of EPTB, was determined by tissue culture or radiograph. Patients with EPTB were classified as *lymphatic*, *pleural*, *central nervous system* (CNS), *bone/joint*, or *other* based on the primary site of EPTB. Cox proportional hazard rate ratios (HR) and 95% confidence intervals (CI) were used to estimate the association between DM and death. Polytomous logistic models were used to assess the association between DM and specific site of EPTB.

BACKGROUND

Public health interest has re-emerged regarding the increasing burden due to the co-occurrence of TB and DM in patients. The observed relation between TB and DM is not new. In 1689 Richard Morton's treatise on consumption "Phthisiologia" described DM symptoms as a consuming disease associated with what he characterized as TB.[4] Prior to the development of effective treatments for both diseases, TB was a leading cause of death among patients with DM.[5] Diabetes mellitus is an established risk factor for TB disease,

increasing the chance of developing active TB by approximately 3-fold.[6] The recent global explosion of DM, coupled with continued struggles to greatly reduce TB incidence has resulted in an increased burden of people experiencing concurrent TB and DM disease. The co-occurrence of TB and DM pandemics exemplifies a new epidemiologic transition where chronic diseases commonly occur simultaneously with infectious diseases, not simply in the same population, but in the same individual.[7]

Tuberculosis epidemiology

Tuberculosis (TB) is one of the most common infectious diseases in the world. The World Health Organization (WHO) estimates that there were 8.7 million (range 8.3—9.0 million) incident TB cases (125 per 100,000 persons) and 12 million prevalent TB cases in 2011.[1] Approximately 2 billion people, or 30% of the world, is infected with TB and has an estimated 10% lifetime risk of developing TB disease.[8, 9] Also in 2009, 1.7 million people died of TB, representing an estimated 3.0% of global deaths and 19.5% of global deaths due to infectious disease.[10] The majority of incident and prevalent TB cases occur in low- and middle-income countries (LMIC) with over 80% of cases occurring in 22 countries identified by WHO as high-burden countries.[11]

Although the 9.4 million incident cases of TB reported in 2009 was more than any other time in history, the worldwide TB incident rate peaked in 2004 and has dropped less than 1% each year since.[8, 12] In 1993 the WHO adopted the directly observed treatment, short-course (DOTS) strategy as the principal TB control program to be promoted worldwide. Between 1995 and 2008, 43 million people received treatment under DOTS averting an estimated 6 million deaths.[12] Nonetheless, major barriers impeding improvements to global TB control emerged with the HIV pandemic and multi-drug

resistant (MDR) TB. Worldwide case detection remains at 60%, indicating TB control efforts have not succeeded in many parts of the world, including sub-Saharan Africa and eastern Europe.[12]

Tuberculosis epidemiology in the country of Georgia

The country of Georgia has a population of 4.3 million, was a former republic of the Soviet Union, and is located in the Caucasus region where TB is widespread.[13] While TB incidence and control improved during the past two decades in parts of the world, Georgia and many other countries in Eastern Europe continue to struggle with reducing the TB incidence.[14] In 2010, the incidence rate of new TB cases in Georgia was 107 per 100,000 population (95% CI 95, 119), this figure has changed little since 1990 (Figure 1).[15] The mortality rate of non-HIV TB in Georgia fell from 14 per 100,000 in 1990 to 6.6 in 2000 but has remained stable for the past 10 years. The estimated prevalence of all forms of TB also decreased significantly between 1990 and 2000 but has leveled since 2000.[16] The 2009 estimated prevalence of TB in Georgia was 116 per 100,000 (95% CI 27, 205) or approximately 4,700 cases. In 2010 Georgia reported a total of 3,265 new TB cases and 1,409 retreatment TB cases.[15]

Of new TB cases reported in Georgia for 2009, the majority (3,174 or 71.2%) were pulmonary TB cases (Table 1) while 28.8% (1,283 cases) were extra-pulmonary. Most (64.7%) new pulmonary TB cases were acid-fast bacillus (AFB) smear positive, these patients are likely producing sputum or other droplet nuclei that are infectious agents.[17, 18] During 2009, most new pulmonary TB patients were male (74.4%). In addition, the majority of both smear positive (77.0%) and smear negative (69.9%) TB cases were male. Overall, new

pulmonary TB cases in Georgia were young; for example, 47.9% were between ages 15 and 34 years in 2009.

The NCTBLD provides free TB treatment for all Georgians. The TB treatment success rate for new smear-positive cases improved during 1999 to 2008 from an estimated 61% to 73%. The 2008 treatment success rate for new smear-negative and retreatment TB cases was 82% and 50%, respectively. The prevalence of MDR-TB is high in Georgia and is an important cause of TB treatment failure.[19] In Georgia, routine drug susceptibility testing for MDR-TB began in 2008. An estimated 10.3% of new TB cases and 31.1% of retreatment TB cases had MDR-TB in 2009. The prevalence of MDR-TB in many former Soviet republics is higher than most countries and the WHO recognizes Georgia as a high-burden MDR-TB country.[11, 19]

Tuberculosis epidemiology in the state of Georgia, USA

The incidence of active tuberculosis (TB) in the US has declined monotonically during the past two decades from 26,673 reported TB cases (10.4 per 100,000) in 1992 to 10,528 reported cases (3.4 cases per 100,000) in 2011.[20] The state of Georgia has also reported a large decrease in TB incidence in the last decade. In 2011 there were 347 (3.5 per 100,000) new TB cases reported in Georgia, a 62% decrease from 1991.[20, 21] Although the incidence of TB has decreased in Georgia, the state had the 19th highest incident rate in the US during 2011.[20]

Similar to national trends, the majority of 2011 active TB cases in the state of Georgia occurred among adults aged 25-64 (68%).[20] Of all 2011 TB cases in Georgia, 225 (65%) were among men and 122 (35%) were among women. In Georgia, more TB cases occurred among Black or African Americans (47.3%) compared to Hispanic or Latinos

(20.5%), Asians (17.6%), or Whites (14.7%). In the US, 37.8% of active TB cases during 2011 were among US-born persons while in Georgia 54.2% of TB cases were among US-born persons. Among foreign-born TB cases in Georgia, 25.8% were born in Mexico (41/159), 11.9% in India (19/159), and 8.8% in Guatemala (14/159).[20]

The metropolitan area of Atlanta accounted for 53% of all 2011 active TB cases in Georgia (DeKalb, Gwinnett, Fulton, and Cobb counties).[21] Co-infection with HIV is common in Georgia. For example, 10.0% of TB patients (who were tested for HIV) were HIV-positive during 2011.[21] Drug resistance, including MDR TB, is uncommon in Georgia. During 2011, 244 of 247 culture positive cases were tested for drug susceptibility pattern and only one (0.4%) case was MDR TB.

Diabetes mellitus epidemiology

Diabetes mellitus (DM) is recognized as one of the most common non-communicable diseases in the world. The International Diabetes Federation (IDF), using data from 91 countries, estimated that global prevalence of adult DM in 2012 was 8.3% or 371 million persons.[2] In 2011, impaired glucose intolerance, or pre-DM, was estimated to be prevalent in an additional 280 million (6.4%) adults.[22] Both DM and pre-DM prevalence are expected to increase rapidly in the next 20 years primarily in low- and middle-income countries. The regions with highest 2010 DM prevalence (age-adjusted) were North America, Eastern Mediterranean and Middle East, and South Asia. However, during the next two decades all world regions are expected to have increases in numbers of DM cases (estimated annual growth rate 2.2%) in excess of adult population growth.[23]

Estimating global mortality from DM is complicated because many countries do not collect mortality data and persons with DM frequently die from complications related to

cardiovascular disease or renal failure. [24-26] In 2012, the IDF estimated that 4.8 million people died from DM.[2] Another study used WHO life tables, country specific DM prevalence from IDF, and age-specific risk ratios for death among persons with DM from the United States (NHANES), to estimate the mortality attributable to DM in 2010.[24] In 2010 an estimated 3.96 million deaths among adults aged 20-79 years old were attributed to DM, which was approximately 6.8% of global all-cause mortality. The estimated proportion of all-cause mortality attributable to DM varied by region, the upper range was in North America (15.7%) and the lowest in Africa (6.0%). Table 2 presents the estimated number of DM-attributable deaths and proportion of adult all-cause mortality due to DM by region.

Diabetes mellitus epidemiology in the country of Georgia

Few published studies have investigated the prevalence of DM in the country of Georgia and little data estimating DM disease occurrence is available. The IDF Diabetes Atlas estimated that 287,100 Georgians or 9.2% of the adult population had DM in 2010.[27] In 2012, the IDF re-calculated estimates of DM in Georgia and the revised national estimate was 3.3% of adults (105,110 persons).[22] After adjusting the Georgian population to the age distribution of the world population, the national age adjusted prevalence of DM was estimated at 2.8% of adults.

In 2012, an estimated 55.6% of all DM prevalent cases were among Georgian females and 44.4% of cases were among adults aged 20-79 years. In the country of Georgia, a higher proportion of DM cases were estimated to be located in urban (59.4%) compared to rural (40.6%) settings. In 2012, an additional 338,240 Georgians were also estimated to have pre-DM, or 10.7% of the adult population (9.6% when age adjusted to the world population). The IDF Diabetes Atlas also estimated the prevalence of DM and pre-DM for

all nations in the year 2030. The Georgia national prevalence of DM in 2030 is estimated to increase to 4.0% and pre-DM will increase to 12.0%.[22] The age and sex distribution for 2030 estimates of DM and pre-DM in Georgia are expected to differ little from the 2012 prevalence estimates.

Diabetes mellitus epidemiology in the state of Georgia

According to the US Centers for Disease Control and Prevention's (CDC) Behavioral Risk Factor Surveillance System, large increases in the prevalence of DM occurred in the US and the state of Georgia in the past 10 years. In 2010 an estimated 703,289 adults had been diagnosed with DM in Georgia.[28] The prevalence of diagnosed DM among adults in Georgia increased from 4.0% in 1994 to 9.8% in 2010.[29] West Central (12.9%) and South (13.4%) regions of Georgia reported the highest prevalence of diagnosed DM in the state.[28] The incidence of newly diagnosed DM has also increased in the state of Georgia, from 5.6 per 100,000 adults (aged 18-76 years) in 1996 to 9.7 per 100,000 in 2010.[29] In addition, an estimated 5.6% of adults in Georgia had pre-DM in 2010 and consequently were at increased risk of developing DM.

In Georgia during 2010, risk factors for DM complications among patients with diagnosed DM were common. For example, after adjusting for age, smoking was prevalent in 19.5% of diagnosed DM cases, and a high proportion of persons with DM were obese (57.4%), physically inactive during leisure time (34.6%), had hypertension (71.7%), and had high blood cholesterol (63.2%).[29]

The association between tuberculosis and diabetes mellitus

The confluence of DM and TB was observed centuries ago. Perhaps the first to describe the TB-DM association was in the Indian siddhar Yugimahamuni who, 2000 years ago, depicted symptoms of both DM and TB his patients.[30] In 1689 Richard Morton's famous treatise on consumption "Phthisiologia" also described DM symptoms as a consuming disease associated with what he likely characterized as TB.[4] Prior to the development of effective treatments for both diseases, TB was a leading cause of death among patients with DM.[5] In 1934, the Massachusetts' physician Howard Root described the clinical TB-DM history:[5]

> "During the latter half of the nineteenth century the diabetic patient appeared doomed to die of pulmonary tuberculosis if he succeeded in escaping coma. In 1883 Bouchardat, the great French student of diabetes, stated in his text that at autopsy every case of diabetes had tubercles in the lungs."

Nonetheless, after the development of effective treatments for both DM and TB in the 1950s, (i.e., insulin and early antibiotics) the association between DM and TB received little additional attention in medical research.[31, 32] Only recently, with the rapidly expanding DM epidemic penetrating into low- and middle-income countries (LMIC) where TB burdens are greatest (Table 3), has the TB-DM epidemiology received renewed attention.

The increased prevalence of DM among patients with TB (compared to DM prevalence the general population) is likely due to a greater susceptibility to TB infection in persons with DM.[31] Diabetes mellitus has long been associated with infections, including group B streptococcus and soft tissue infections. Impaired innate and adaptive immune responses in persons with DM likely result from hyperglycemia and in turn lead in increased risk of infections, such as TB. A recent meta-analysis of cohort and case-control studies

estimated that the risk of developing active TB in persons with DM was 3.11 (95% CI 2.27, 4.26) times the risk among persons without DM.[6]

The hypothesis that infection with TB can lead to DM is not strongly supported by published epidemiologic literature. However evidence exists to suggest that infection (such as by TB) may lead to acute inflammation that may result in temporary hyperglycemia.[31, 33] Infections among humans may affect levels of glucose in the blood and consequently can result in glucose intolerance or hyperglycemia. Persistent inflammation caused by infection may also lead to hyperglycemia.[34, 35] Several studies have demonstrated a high prevalence of DM diagnosis in TB patients, however, whether these studies found incident DM or simply diagnosed an existing case is unclear.[31, 36] Increased glucose intolerance during TB infection may be temporary.[37] For example, a study in Nigeria reported high levels of newly diagnosed pre-DM among TB patients, however, glucose levels returned to normal in 87.5% of patients three months after TB treatment ended.[38] A similar study conducted in Turkey also demonstrated that abnormally high glucose levels in TB patients returned to normal after completing TB treatment.[39] Current published epidemiologic investigations of DM and TB suggest that new diagnosis of DM among TB patients is common. Some evidence exists to support the hypothesis that active TB disease may induce temporary hyperglycemia but more studies support the notion that the association between TB and DM primarily results from an increased risk of TB among persons with DM.

Clinical presentation at time of tuberculosis diagnosis

If DM increases the risk of acquiring active TB disease, the manner in which TB disease manifests in patients with DM may also result in a different clinical presentation. While standardized measures of TB disease severity are not widely used, determining

differences in TB disease severity and symptoms among TB patients with DM has important clinical implications.[40-43] For example, baseline TB characteristics can predict which patients might not respond to anti-TB therapy or have increased risk TB relapse.[44] Although TB severity scores are not widely used, examples of important measures of clinical severity at baseline include positive AFB smear status or grade,[45, 46] time (days to test becomes positive) to *M. tuberculosis* culture positivity,[44-47] extent of bacterial involvement in chest radiographs,[42, 45, 48, 49] frequency of patient symptoms (i.e., cough, fever, weight loss) and presence of TB drug resistance.[49]

Smear-positive (or AFB-positive) TB patients produce more bacteria in expectorated sputum, a symptom that indicates greater infectiousness and potentially greater quantity of TB in the lungs compared to TB patients who are smear-negative.[18] In addition to classification as AFB-positive or AFB-negative, smears are graded on a semi-quantitative scale; typically 3+ indicates more than 10 AFB viewed in each of 20 fields examined under microscope, 2+ indicates 1-10 AFB in each of 50 fields viewed, 1+ indicates 10-99 AFB total viewed in 100 fields, and scanty refers to less than 10 viewed in 100 fields.[50] Smear-positive patients with higher graded smears (i.e. 3+ and 2+) may also take longer to convert their sputum to AFB negative, an early indicator of TB treatment success.[51, 52]

Most studies that have examined AFB smear status and/or grade characteristics of TB patients with DM (at time of TB diagnosis) demonstrated an association between the more infectious, smear-positive forms of TB, and DM (Table 4).[53] For example, a recent study in Taiwan found that 88% of TB patients with DM had AFB smear-positive TB while only 59% of TB patients without DM were AFB smear-positive (p-value <0.01).[32] Similarly, in Texas 64.9% of TB-DM patients and 50.9% of TB patients without DM had positive AFB-smears at baseline.[54] After adjusting for age and sex, the association with AFB-

positivity remained elevated (AOR 1.8, 95% CI 1.3, 2.4) in the Texas study. Another study conducted in Saudi Arabia showed than having smears with "numerous AFB" was more common (65.2% versus 54.1%) among TB-DM patients.[55] Conversely, studies in Turkey,[56] Mexico,[54] and Indonesia[57] found lower proportions of AFB smear-positive sputum in patients with TB and DM compared to TB-only patients. While more studies of DM and TB demonstrated that DM is associated with smear-positive TB, others did not. Across studies, diagnosis of DM was inconsistent and did not use recommended measures of blood glucose to define DM. In addition, studies that assessed the relationship between smear status and DM did not control for other potential confounders, for example HIV status. Whether DM causes more TB bacterial burden and consequently higher or more positive smear grade remains under-investigated.

The amount of time it takes to grow *M. tuberculosis* bacteria in liquid culture media, or time to detection (TTD) is another indication of bacterial burden at baseline and predicts response to anti-TB treatment.[44, 58] In addition, TTD is associated with AFB smear grade, presence of cavitary disease, and number of cavities in the lung.[44, 45] A recent study demonstrated that patients with TTD <3 days were less likely to convert sputum cultures to negative by 2 months of anti-TB treatment, less likely to have a favorable TB treatment outcome after 6 months of therapy, and more likely to have TB relapse.[44] To our knowledge, no studies of TB patients with DM have examined TTD of cultures to assess baseline bacterial burden or to determine if the measure is associated with TB treatment outcomes.

Radiographs of the chest are commonly used to measure extent and severity of disease among patients with pulmonary TB.[59] Characteristics of a chest x-ray (CXR) that indicate severe disease are controversial and standardized measures do not exist.[41, 42] In addition, inter-observer readings of CXR have poor reliability.[60] Despite drawbacks, the

CXR are useful for determining the extent of TB infection and their clinical value is incontrovertible. A CXR among pulmonary TB patients may have the following indications: consolidation, cavitation (number and location), effusion, military, nodules, fibrosis, proportion of lung involved, disseminated, bilateral, location of involvement, infiltrate, tuberculoma, lymphadenopathy, et al.[42, 59, 61] Despite the wide range of CXR outcomes, the use of cavitary disease (number and location), bilateral disease (cavities in both lungs), location of involvement (upper lung versus lower lung), and a categorical combination describing extent of disease are most commonly used. Categorical CXR variables often dichotomize patients into "severe radiographic abnormalities" versus "normal" or use an ordinal scale such as mild, advanced, and far-advanced.[42, 57]

Early studies of DM and TB documented differences in radiographic findings among patients with both diseases.[31, 62] Studies have hypothesized that CXR among TB patients with DM have more atypical appearance, greater lower-lung involvement, and more cavities. If such findings are true, the resulting clinical implications are important. For example, pulmonary TB with lower-lung involvement in a CXR is often misdiagnosed as cancer or pneumonia and is less likely to produce a positive AFB smear result.[63] Whether pulmonary TB patients with DM have more lower lung involvement (and less positive AFB-smears) and more cavitary disease remains controversial. Many studies demonstrated that TB-DM patients had more lower lung involvement and more cavitary lesions, while others have found no difference (Table 5). Because CXR are important for TB diagnosis and may demonstrate the extent of TB disease, it is important to understand whether DM is associated with different or more severe radiographic findings at time of TB diagnosis.

Epidemiologic studies of TB-DM patients have demonstrated different distributions of common TB symptoms at the time of diagnosis including cough, hemoptysis, fever, and

weight loss. For example, previous studies comparing TB symptoms at time of presentation have reported more cough,[54, 55] hemoptysis,[54, 55, 64, 65] fever,[64, 65] and weight loss[64] among TB-DM patients. In another study that reported an unadjusted analysis of TB patients using Taiwanese hospital data during 2003-2006, DM was significantly associated with hemotypsis (OR 2.6, 95% CI 1.2–5.3) and fever (OR 2.2, 95% CI 1.2 – 4.0) at the time of TB treatment initiation.[64]

The emergence of drug resistant TB, including MDR TB, is a major threat to global TB control due to greatly increased risk of poor TB treatment outcomes in patients with MDR TB.[66] Tuberculosis patients can acquire resistance to anti-TB drugs by failing to complete treatment regimens, not having access to proper anti-TB regimens, taking regimens for inadequate time periods, or if absorption mechanisms are insufficient. Patients may also acquire an exogenous TB infection that is resistant (primary MDR TB). A recent meta-analysis of MDR-TB treatment outcomes estimated an overall success rate of TB treatment in less than 62% of patients, while an estimated 8% fail and 11% die.[67] Extensively drug resistant (XDR)-TB is defined as MDR TB that is also resistant to any member of the quinolone class of antibiotics plus resistant to at least one of the second-line injectable anti-TB drugs. Patients with XDR-TB have a high risk of poor TB treatment outcome—an estimated 20% of XDR-TB patients die due to the difficulty in successfully treating it.[68]

As with AFB-smear status and CXR results, the association between drug resistance and TB-DM is inadequately studied. Most studies have shown no association between DM and drug resistant TB,[32, 53, 55, 57] although there are important exceptions.[69, 70] The absorption of important anti-TB drugs, such as Rifampin, may be altered in TB-DM patients which could lead to increased development of drug resistance. Only two studies have examined differences in the absorption of Rifampin in TB patients with and without DM and they

reported conflicting results.[71, 72] If TB patients with DM are at increased risk of having resistance at the time of TB diagnosis or developing drug resistance during therapy, the consequences for clinical success are clearly important.

Response to anti-tuberculosis therapy

Because DM may effect clinical manifestations at baseline (including smear status, TTD, radiographic severity, and drug resistance), concomitant TB-DM is also is likely to impact response to anti-TB treatment. Unlike measures of TB severity at baseline, standard measures exist for TB treatment outcomes and therefore information across studies is generally easier to compare. Standardized treatment regimens, in the form of directly observed therapy, short-course (DOTS), are endorsed by the WHO and followed by most national TB programs.[73] Standard DOTS regimens for new, drug susceptible, pulmonary TB patients typically includes 2 months of intensive therapy with isoniazid, rifampicin, pyrazinamide and ethambutol followed by 4 months of continuation phase with isoniazid and rifampicin (2HRZE/4HR). The regimen can be given 3 times per week or daily depending on dose. Standard treatment outcomes for DOTS based on WHO recommendations include categories of cure, complete, failure, died, default, or transfer out (Table 6).[73] Additional TB treatment outcome measures frequently used in epidemiologic studies include 2-3 month sputum (smear or culture) conversion,[46] time-to sputum conversion,[51] development of drug resistance,[74] and relapse.[75] In reviewing the TB-DM literature, we found four outcomes that were utilized in studies that followed TB patients with DM during treatment: 1) sputum smear and culture conversion after 2-3 months of treatment, 2) time to sputum conversion, 3) relapse, and 4) mortality.

Sputum conversion and time to sputum conversion

Sputum culture conversion from positive to negative after 2 months (or between 2 and 3 months) of anti-TB treatment has been used as a measure of treatment efficacy and is strongly associated with TB treatment success.[46, 76-78] Sputum AFB smear conversion after 2 months of treatment is also used as a measure of treatment success and TB control programs frequently use this indicator to guide clinical decisions (e.g., intensive phase TB regimens are extended if patients do not convert AFB smears).[79] Among newly diagnosed pulmonary TB patients, factors that are associated with persistently positive *M. tuberculosis* sputum cultures after 2 months of treatment include older age, DM, HIV, upper lung lesions, bilateral disease, cavitation, and smear grade at baseline.[51] An increased number of pulmonary cavities and higher baseline AFB-smear grade are the strongest and most consistently reported factors that influence sputum conversion time.[51, 52, 78]

Most previously published studies evaluating the association between DM and 2-month culture conversion demonstrated a delayed response to treatment among TB-DM patients. A recent review paper found 9 studies that determined the proportion of TB-DM patients who converted sputum cultures by month three of TB treatment.[75] When comparing the proportion of TB-DM patients who converted 2-month sputum (from cultures culture positive to negative) to TB-only patients, 6 of the 9 studies found an increased risk of no culture conversion in TB-DM patients. The relative risk of failing to convert ranged from 0.79 (95% CI 0.3, 1.9)[80] to 3.3 (95% CI 1.7, 6.4).[81] One study conducted in Indonesia also examined sputum smear conversion after 2 months of treatment. Alisjahbana et al reported 71.3% (67/94) of TB-DM patients and 84.3% (455/540) of TB-only patients were AFB-sputum negative after the initial treatment phase.[57] Although several studies of TB-DM patients examined 2-month culture conversion, few used adequate

measures of DM, had sufficient sample size, or were conducted prospectively; none met all three of these criteria.

Sputum culture conversion is typically measured in days from initially positive to the first of two consecutive culture tests (≥30 days apart) that are negative. The time to AFB smear conversion may be measured similar to 2-month sputum culture conversion. Because most TB control programs measure sputum samples on a monthly basis, the measured time to sputum conversion is subject to the intervals chosen by the TB treatment management and when the patient provides follow-up sputum. However, for TB patients who do not convert their sputum by 2 months, the measure of time to conversion may be useful. Time to sputum conversion also permits the use of survival analysis for data regularly collected on sputum conversion status. Time to smear and culture conversion is theoretically affected by the same factors associated with persistent positive sputum after 2 months (older age, DM, HIV, upper lung lesions, bilateral disease, cavitation, and smear grade at baseline).

Six published studies have assessed time to sputum conversion among TB-DM patients and compared results to TB patients without DM.[32, 82-85] Five studies reported the time to sputum culture conversion among TB patients with DM was longer than for patients with TB only (Table 7). The greatest difference in median days to culture conversion between TB-DM patients and TB-only patients was 32 days and the shortest was 5 days.[32, 83] One study, among MDR TB patients from five countries, reported non-significant differences in time to culture conversion when comparing TB-DM patients to TB-only patients (HR 0.76, 95% CI 0.54, 1.06).[86] Most studies examining time to sputum conversion support the hypothesis that compared to TB-only patients, TB-DM patients tend to remain sputum positive for longer periods during anti-TB therapy, and consequently may be infectious for longer periods of time. However, the differences in time to sputum conversion

are observed early in TB treatment. Among TB patients who remain sputum positive after 2-3 months of anti-TB treatment, DM status no longer is associated with faster time to sputum conversion.

Relapse

Relapse among TB patients is defined by a patient who previously completed TB treatment successfully and subsequently is diagnosed with sputum smear or culture positive TB.[87] Recurrence is also used to describe a successful TB treatment followed by subsequent case requiring TB retreatment, but usually refers to a more general case in which molecular testing is not available to determine if the reoccurrence of TB is the same bacteria or a different infection. Globally, relapse of TB places a significant burden on the patient and on national TB control programs. For example, in 2007, an estimated 270,000 patients returned to TB treatment after relapse (5% of all TB notifications).[88] Additionally, relapse patients who return for a second TB treatment are at a greater risk of having or developing drug resistance and failing treatment.[89] However, unlike DOTS treatment outcomes (Table 6) WHO does not have surveillance systems in place to assess TB recurrence and few studies have conducted long-term cohort studies to determine factors influencing TB relapse.[90]

Whether DM increases the risk of TB relapse has been examined in few published studies. A retrospective cohort study conducted in China reported that 20% of 203 successfully treated TB-DM patients returned to treatment within 2 years of their first treatment completion, while among 1,938 TB patients without DM, only 5.3% relapsed to TB retreatment.[91] Baker et al developed a pooled risk ratio from 5 studies to estimate the risk of TB relapse comparing patients with and without DM -- the risk of relapse among TB-DM patients was 3.89 (95% CI 2.43, 6.23) times the risk of TB patients without DM.[75] The

pooled analysis for TB default did not examine differences in follow-up time for relapse assessment in the 5 studies. If TB-DM patients are at greater risk of treatment relapse, longer TB treatment regimens, improved DM monitoring during treatment, or more extensive end-of-treatment evaluations may be warranted for the TB-DM patient subgroup.

Mortality

The majority of previous studies that have examined mortality in TB patients with DM have reported an increased risk of death among TB-DM patients. For example, a 2011 systematic review by Baker et al. showed that 95.5% (21 of 23) of studies found an increased unadjusted risk of death among TB-DM patients when compared to TB patients without DM.[75] However, the analysis had important limitations. First, follow-up time and mortality measurement were inconsistent across studies, some followed patients to the end of TB treatment while others followed patients beyond TB treatment completion. Second, of the 21 studies that reported increased unadjusted mortality risk, only 9 were powered to detect a statistically significant difference in the comparison, and only 4 studies adjusted for age and other confounders.

Three studies in the US, all from Maryland, have estimated the effect of DM on mortality during TB treatment.[84, 92, 93] Only the study by Dooley et al.[84] examined the association between DM and mortality during TB treatment with a model that also adjusted for age and HIV status, but this study had low precision (aOR 6.70, 95% CI 1.11, 38.20). Two other studies from Maryland estimated the odds of death during TB treatment comparing patients with and without DM after adjusting only for age. Fielder et al.[92] estimated the odds of death among TB-DM was 3.80 (95% CI 1.42, 10.16) times the odds of

death among DM only patients, while Oursler et al.[93] estimated the same measure of effect at 6.70 (95% CI 1.57, 28.52).

Three previous studies reported the use of survival analysis to estimate the association between DM and time to death in TB patients. First, in a study that adjusted for age using a Cox proportional hazards model, Oursler et al reported that the hazard of death during TB treatment among TB-DM patients was significantly greater than TB patients without DM (aHR 4.7, 95% CI 1.9, 12.5).[93] Second, a Korean study that followed patients for one year after TB treatment initiation to determine time until death demonstrated a significantly increased hazard of mortality in TB-DM patients compared to TB patients without DM (aHR 2.18 95% CI 1.10, 4.34).[94] A third study was conducted in Tanzania that followed patients during the first 100 days after initiating TB treatment. The study found that the hazard of death among TB-DM patients was greater compared to TB-only patients (aHR 5.09 95% CI 2.36, 11.02).[95]

BIOLOGIC PLAUSIBILITY

The association between DM and TB is well established in both historical records[31] and in the medical literature beginning in the late 19th century.[5, 96] Nonetheless, the biologic and immunologic mechanisms that result in the increased co-occurrence of DM and TB are poorly understood.[97] Most hypotheses and observational data suggest that DM increases the risk of acquiring TB infection and/or transitioning from TB infection to TB disease. The hypothesis that TB infection increases the risk of DM is not implausible, but does not have as substantial evidence as the former assumption.[31] Murine and human studies have demonstrated that DM increases susceptibility to infection. Both innate and adaptive host immune responses are compromised by DM.[98-102] The primary immunologic mechanisms

that mediate the human response to TB infection (phagocytic macrophage and T-cell functions), which may be altered by DM, are reviewed below.

Infection with *M. tuberculosis* occurs after a host inhales droplet nuclei which contain the TB bacilli. Following inhalation of the bacilli, macrophages and CD4+ T-cells determine the first critical human immune responses to TB infection.[34, 99, 103, 104] Phagocytic cells, in particular alveolar macrophages of the lungs, are the primary initial binding agents for *M. tuberculosis*.[105, 106] The presence of cholesterol is important for macrophage surface receptors to initially bind with *M. tuberculosis*. However, in patients with DM, a reduction in insulin and resulting hyperglycemia can cause an accumulation of cholesterol inside the macrophage, which may alter which receptors are utilized during the entry of *M. tuberculosis* into the phagocytic cell.[34] For example, macrophage ingestion via the CR3-mediated receptor hinders macrophage activation, a critical step in containing the infection and preventing disease.[105, 106] The CR3 receptor is dependent on the presence of cholesterol, a steroid that is more likely to have accumulated in the macrophage of patients with insulin deficiency.[34] Improper or impaired entry of *M. tuberculosis* into the phagocytic cell, potentially due to DM, compromises the host's immune mechanisms because it does not activate the macrophage, a process that signals the transfer of the bacilli to destructive lysosomes or promotes containment of the bacilli by encapsulating it within a granuloma.[106, 107]

Most *M. tuberculosis* infected hosts do not develop disease but instead harbor the bacteria within granulomas.[106] Once inside the macrophage, *M. tuberculosis* enters the phagosome, a harsh environment that has microbicidal properties. The granuloma is formed when T-cells and additional macrophage cells surround the macrophage containing *M. tuberculosis*. Activated macrophages within the granuloma present *M. tuberculosis* antigens to T-cells which subsequently release cytokines to signal important additional immune responses,

including the maintenance of the granuloma. The T-cells (CD4+ cells) exposed to TB antigens are classified into two principal types based on the pattern of cytokine expression by the cells: T helper 1 (Th1) and T helper 2 (Th2) cells. The Th1 response, compared to the Th2 response, is well recognized to be associated with containment of TB within the macrophage, a mechanism that prevents disease.[104, 108, 109] Importantly, patients with DM may have depressed Th1 responses and exhibit a greater tendency toward Th2 response, potentially explaining an increased risk of TB disease (Table 8).[99, 104, 110]

The Th1 response is partially characterized by the release of interferon-gamma (IFN-γ), an essential cytokine response for control of TB that may be altered in patients with DM.[97, 110-112] If the release of IFN-γ is altered in patients with DM, an increased risk of TB is plausible for at least four reasons. First, the activation of IFN-γ keeps the macrophages of the TB contained granuloma activated and signals for additional immune cells to the site of infection.[106] Second, IFN-γ is central to anti-mycobacterial activities within the macrophage such as the phagosomal maturation, an essential step in the formation of reactive oxygen intermediates (ROI).[105] Third, genetic studies demonstrated that mutations which result in IFN receptor or pathway damage frequently result in fatal disseminated TB.[113, 114] Fourth, the cytokine IFN-γ is used to effectively diagnose latent TB disease demonstrating its key role in persistent granuloma containment.[115, 116] Studies that have examined cytokine responses in patients with DM demonstrated altered IFN-γ levels, and this differential immune response may disrupt the cytokine pathway that is critical for containing TB infection.

As previously mentioned, IFN-γ pathways lead to phagosomal maturation, another immune process that is important for defense against *M. tuberculosis* infection and potentially interfered by DM. If phagosomes develop adequately, they fuse with lysosomes which have

anti-mycobacterial properties that can destroy the bacilli via ROI and reactive nitrogen intermediates (RNI).[34, 105, 106] However, in patients with DM, and especially those with chronic hyperglycemia, increased macrophage glycation occurs because of accumulated fructose and glucose.[34] The NADPH-oxidase enzyme in macrophages is necessary for the formation of nitric oxide and superoxide, two building blocks of both ROI and RNI. The enzyme NADPH is particularly subject to glycation which could result in subsequent ROI and RNI deficiencies.[34] Therefore the accumulated glucose and fructose that is more frequent in the macrophages of patients with DM may lead to enzyme glycation and ultimately reduced anti-mycobacterial ROI and RNI defenses.

A second cytokine signaling pathway involved in the Th1 response, IL-12, may be altered in patients with DM. The cytokine IL-12 has a reciprocal relationship with IFN-γ, each stimulating the creation of the other. Consequently IL-12 may have an important role in maintaining granulomas that prevent *M. tuberculosis* from activating to a disease state. For example, studies have demonstrated an increased presence of IL-12 in grulomatous lesions of TB,[104, 117] while other studies showed that genetic defects in the production of IL-12 are associated with both atypical and disseminated TB disease, emphasizing its role in the immune response against *M. tuberculosis*.[104] A study in diabetic mice found impaired production of IL-12[110], while studies of IL-12 among humans with DM have demonstrated both increased and decreased production of the cytokine.[118, 119] Nonetheless, an altered IL-12 response that influences an increased risk of TB among patients with DM is plausible.

The third Th1 cytokine that may be altered by DM status is IL-18. Like IL-12, IL-18 is important in differentiating the T-cell activation toward a Th1 response and mediates the production of INF-γ.[97] Patients with DM have increased levels of intracellular IL-18, but it has been hypothesized that the enhanced production of IL-18 is due to its ability to improve

insulin sensitivity to compensate for the effects of DM.[120-122] Therefore, the increased IL-18 circulating in patients with DM may be characterized as "IL-18 resistant" and lead to a lower rather than higher production of INF-γ and consequently lead to an increased risk of TB for patients with DM.[97]

In conclusion, patients with DM have depressed immune function, characterized by less effective macrophages and a reduced Th1 response, which places them at an increased risk of TB infection and disease. Patients with DM tend toward a reduced production of IFN-γ and macrophage activation, impaired phagosome maturation due to glycation, and altered IL-12 and IL-18 cytokine release. These immune responses are characteristic of a hindered response to *M. tuberculosis* infection and limit the host's ability to contain TB within the macrophage granuloma. The hypothesized immune irregularities in patients with DM may partially explain the increased risk of TB disease, more severe clinical presentation of disease, and poor response to anti-TB chemotherapy in these patients.

SUMMARY

The three studies contained within this body of work address the relation between two global public health epidemics—TB and DM. Each of the three observational cohort studies includes an estimate of the association between DM and 1) measures of TB clinical severity at the time of diagnosis and 2) TB treatment outcomes. The overarching goal of the three studies was to provide additional evidence to assess whether patients with TB-DM have more severe disease at the time of clinical presentation and are more likely to have poor TB treatment outcomes.

Figure 1.1

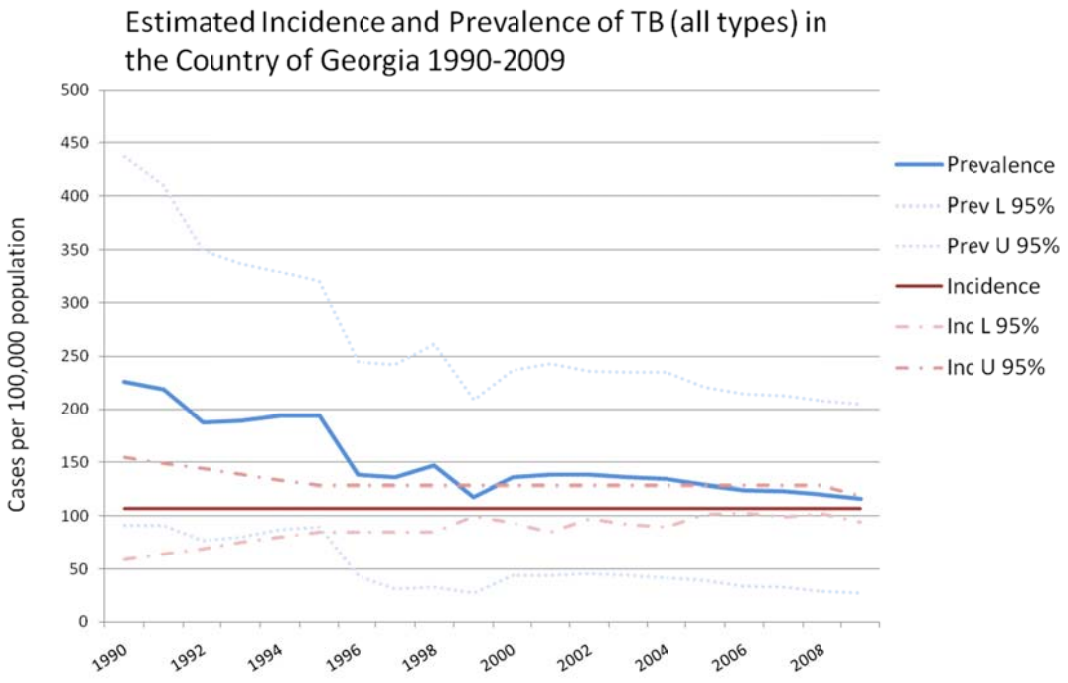
Estimated Incidence and Prevalence of TB (all types) in the Country of Georgia 1990-2009
Cases per 100,000 population
500
450
400
350
300
250
200
150
100
50
0
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
Prevalence
Prev L 95%
Prev U 95%
Incidence
Inc L 95%
Inc U 95%

Table 1.1 Age, sex, and smear status distribution of new pulmonary TB cases, country of Georgia, 2009

Age Group	0-14	15-24	25-34	35-44	45-54	55-64	64+	Total
New Pulmonary Smear (+)								
Male	2	327	435	310	284	135	89	1582
Female	5	124	134	74	60	30	46	473
New Pulmonary Smear (-)								
Male	15	140	170	141	145	73	98	782
Female	20	93	97	36	21	28	42	337
Total Pulmonary Smear (+) & (-)								
Male	17	467	605	451	429	208	187	2364
Female	25	217	231	110	81	58	88	810
Total	42	684	836	561	510	266	275	3174

Table 1.2. Estimated number of adult deaths attributable to diabetes, 2010†

Region	Number of deaths*	Regional percent (%) of all cause mortality*
Africa	332,584	6.0
Eastern Mediterranean and Middle East	294,037	11.5
Europe	634,054	11.0
North America	313,208	15.7
South and Central America	171,303	9.5
South-East Asia	1,142,914	14.3
Western Pacific	1,074,955	9.7

*In adults aged 20-79 years
†Data from Roglic and Unwin[24]

Table 1.3. Prevalence of tuberculosis (TB), diabetes mellitus (DM) and co-occurring TB-DM in selected high-burden countries

Country	2011 national TB incidence[A]	2011 national DM prevalence[B] (%)	DM prevalence in TB patients (%)	TB-DM prevalence reference
India	2200	61,258 (8.3)	1084/8109 (13.4)	TB-DM study group[123]
China	1000	90,045 (9.3)	1090/8886 (12.3)	Li et al[124]
Indonesia	450	7,293 (4.7)	94/634 (14.8)	Alisjahbana et al[57]
Pakistan	410	6,349 (6.7)	21/106 (19.8)	Jawad et al[37]
South Africa	500	1,947 (6.5)	NA	NA

A. Based on WHO 2012 report; in thousands (x1000)[1]
B. International Diabetes Federation 2011 unadjusted estimates in adults aged 20-79 years; in thousands (x1000)[2]

Table 1.4. Acid fast bacillus (AFB) smear positivity among tuberculosis patients with and without diabetes mellitus at time of tuberculosis diagnosis†

Study Location	Year*	DM-TB patients (n)	TB patients (n)	AFB-smear + DM-TB (%)	AFB-smear+ TB only (%)
Turkey[56]	2001	92	92	72.8	91.3
Saudi Arabia[55]	2006	187	505	65.2	54.1
Indonesia[57]	2007	94	540	29.8	38.9
Mexico[54]	2007	607	2804	96.8	94.9
Texas, USA[54]	2007	401	1040	64.9	50.9
Taiwan[64]	2009	74	143	68.9	53.8
Baltimore, USA[84]	2009	42	255	54.8	41.2
Taiwan[32]	2011	60	132	88.3	59.1
*Year published †Table modified and updated from Ruslami et al.[125]					

Table 1.5. Chest x-ray (CXR) findings among tuberculosis patients with and without diabetes mellitus at time of tuberculosis diagnosis†

Study Location	Year*	TB-DM patients (n)	TB-only patients (n)	More DM lower lobe involvement	More DM cavitary disease
Turkey[56]	2001	92	92	No	No
Mexico[61]	2003	192	130	Yes	Yes
Saudi Arabia[55]	2006	187	505	Yes	na
Indonesia[57]	2007	94	540	na	No
Texas, USA[54]	2007	401	1040	na	Yes
Saudi Arabia[63]	2009	57	78	na	No
Taiwan[64]	2009	74	143	Yes	Yes
Baltimore, USA[84]	2009	42	255	na	Yes
Taiwan[32]	2011	60	132	na	Yes

†Table modified and updated from Ruslami et al[125] and Dooley & Chaisson[84]

Table 1.6. Standard World Health Organization definitions for first-line TB treatment outcomes

Outcome	Description
Cure	A patient whose sputum smear or culture was positive at the beginning of the treatment but who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.
Treatment completed	A patient who completed treatment but who does not have a negative sputum smear or culture result in the last month of treatment and on at least one previous occasion.
Treatment failure	A patient whose sputum smear or culture is positive at 5 months or later during treatment. Also included in this definition are patients found to harbor a multidrug-resistant (MDR) strain at any point of time during the treatment, whether they are smear-negative or -positive.
Died	A patient who dies for any reason during the course of treatment.
Default	A patient whose treatment was interrupted for 2 consecutive months or more.
Transfer out	A patient who has been transferred to another recording and reporting unit and whose treatment outcome is unknown.
Treatment success	A sum of cured and completed treatment.
*For patients receiving standard DOTS regimens[73]	

Table 1.7. Median days to sputum *M. tuberculosis* culture conversion from positive to negative among TB patients with and without DM

Study location	Year	TB-DM time*	TB-only time*
Turkey[82]	2007	67	55
Texas, USA[83]	2008	42	37
Baltimore, USA[84]	2009	49	39
Tunisia[85]	2009	43	28
Taiwan[32]	2011	76	48
*Median days until culture conversion for TB patients with (DM-TB) and without DM.			

Table 1.8. Cell-mediated immunity CD4+T-Cell phenotypes: Variation in cytokine expression by diabetes status*

Phenotype	**Phenotype T helper type 1 (Th1)**	**Phenotype T helper type 2 (Th2)**
Cytokine expression	Interleukin-2 (IL-2) Lymphotoxin-α (TNF-β) †Interferon-gamma (IFN-γ) Interleukin-3 (IL-3) Interleukin-13 (IL-13) Granuloma-monocyte colony stimulating factor (GM-CSF) †Interleukin-12 (IL-12) †Interleukin-18 (IL-18) Interferon-gamma (IFN-γ)	Interleukin-4 (IL-4) Interleukin-5 (IL-5) Interleukin-6 (IL-6) Interleukin-10 (IL-10) Interleukin-3 (IL-3) Interleukin-13 (IL-13) Granuloma-monocyte colony stimulating factor (GM-CSF) Interleukin-4 (IL-4)
* In murine and human studies † Indicates cytokine response differentially observed in patients with DM and TB Table created from information provided in recent literature.[97, 99, 104, 110, 112]		

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CHAPTER 2: DIABETES MELLITUS AND TUBERCULOSIS SEVERITY IN THE COUNTRY OF GEORGIA

CHAPTER 2 ABSTRACT

Background: While diabetes mellitus (DM) and TB co-infection is an increasing global public health problem, there is limited knowledge regarding the association of DM and TB. The purpose of our study was to estimate the prevalence of DM and pre-DM among patients with TB in the country of Georgia, to determine if patients with TB-DM had more severe disease at the time of clinical presentation, and to estimate the association between DM and two-month smear conversion. Methods: All sputum culture positive TB patients >34 years old were eligible to participate. Patients were enrolled from October 2011 to February 2013 at the Georgia National Center for TB and Lung Disease in Tbilisi. Hemoglobin A1c from capillary blood was measured and used to define DM (≥6.5%), pre-DM (≥5.7%-6.4%), and normal glucose (<5.7%). Patient interviews along with medical chart and laboratory data abstraction were performed to measure clinical TB symptoms at presentation and to assess 2-month acid fast bacilli (AFB) status. Logistic regression analyses were used to determine associations between DM, pre-DM, and participant traits. Results: Of 393 eligible participants, 280 were enrolled. Prevalence of DM was 12.9% (95% confidence interval [CI] 9.3-17.2%) and pre-DM prevalence was 16.8% (95%CI 12.8-21.7%). Compared to participants with normal glucose, those with DM were more likely to be AFB smear positive (86.1% vs 67.0%), and more often reported cough (91.2% vs 73.9%) and hemoptysis (38.2% vs 21.9%) at the time of diagnosis. In multivariable analyses, patients with TB and DM were more likely to have cough (adjusted odds ratio [aOR] 3.51, 95%CI 0.98-12.61), hemoptysis (aOR 1.75, 95%CI 0.75-4.07), cavitary disease (aOR 2.38, 95%CI 1.02-5.57), and higher AFB smear grade (aOR 2.63, 95%CI 1.14-6.06) compared to patients with normal glucose. Of 194

participants with completed 2-month AFB sputum smear, 30.4% were positive, including 31.0% with DM and 28.9% without DM; the difference was not significantly different (OR 1.09, 95%CI 0.43, 2.74). Conclusions: Adults with newly diagnosed pulmonary TB in Tbilisi, Georgia with DM and pre-DM had more severe clinical disease at the time of presentation, but we did not detect a significant difference in 2-month AFB smear conversion. Additional longitudinal data is needed to determine if DM or pre-DM affects TB treatment outcomes.

INTRODUCTION

The relation between diabetes mellitus (DM) and tuberculosis (TB) is of increasing global public health importance due to recent rapid increases in DM prevalence and persistently high incidence of TB.[1, 2] In 2011 the estimated worldwide adult prevalence of DM was 366 million (8.3%)[3] and there were an estimated 9 million incident cases of active TB disease.[4] In addition, the global DM prevalence is expected to rise greatly (reaching 570 million adults by 2030), and the majority of the anticipated DM burden will affect low- and middle-income countries where TB is highly endemic.[5]

Previous observational studies have described the association between DM and TB. For example, a meta-analysis using data from 3 observational cohort studies estimated that persons with DM had 3.11 (95% confidence interval [CI] 2.27-4.26) times the risk of developing active TB compared to those without DM. In contrast to TB patients with normal glucose levels, TB patients with diabetes may require more time to convert sputum cultures from positive to negative,[6-8] may be at increased risk of TB treatment failure,[9] and may have higher rates of death during TB treatment.[10-12] However, most published studies to date relied on self-reported DM status or have not adjusted for important known

confounders. In addition, published information on the DM-TB relationship in Eastern Europe or the Caucasus region specifically is lacking.

The country of Georgia, a former republic of the Soviet Union located in Caucasus region, has a population of 4.3 million people and has a high burden of TB (2010 incidence 107/100,000) and multi-drug resistant (MDR) TB (9.5% of new TB cases).[4] The 2009 prevalence of DM in Georgia was estimated at 9.2% among adults, and an additional 7.2% were estimated to have pre-DM (i.e., at high risk of developing DM).[13] In Georgia, the prevalence of DM among new adult TB patients and the association between DM and TB clinical characteristics has not been carefully described. Consequently, the objectives of this study were to estimate the prevalence of DM and pre-DM using a rapid hemoglobin A1c (HbA1c) test among new adult TB patients in Tbilisi, Georgia, and to estimate the association between DM status and TB patient characteristics including measures of TB disease severity. We also aimed to estimate the association between DM status and TB treatment outcomes including two-month acid fast bacilli (AFB) smear.

METHODS

Setting and Participants

Between October 2011 and February 2013 a prospective cohort study was conducted at the National Center for TB and Lung Disease (NCTBLD) in Tbilisi, Georgia. Eligible participants included new pulmonary TB patients aged 35 years or older that were *Mycobacterium tuberculosis* sputum culture positive. Trained TB physicians and study staff recruited eligible participants from inpatient and ambulatory clinics at the NCTBLD. Participants were followed throughout standard WHO recommended anti-TB treatment regimens[14] and were monitored for study outcomes after two months of treatment.

Variables and Study Measures

Diabetes mellitus, pre-DM, and normal glucose were measured by a rapid, point-of-care HbA1c device (Afinion, Axis Shield, Oslo, Norway) administered to all study participants using capillary blood collected within 60 days of TB treatment initiation. Sterilized lancets were used to obtain capillary blood samples from participants' fingers that were first cleaned with alcohol swabs. All capillary blood samples were analyzed for HbA1c within 20 seconds of collection. Participants' DM status was categorized according to American Diabetes Association recommended HbA1c scale: DM ≥6.5%, pre-DM 5.7-6.4%, and normal glucose <5.7%.[15] Participants with HbA1c <6.5% who reported previous diagnosis of DM by a physician or health-care worker and had documented use of DM medication were also defined as DM.

Clinical TB characteristics at the time of TB diagnosis were abstracted from participants' medical records and sputum microscopy information was obtained from the National TB Reference Laboratory in Tbilisi. Radiographic information (presence and location of lung infiltrates and cavitary disease), body mass index (BMI), and HIV status information were obtained from medical records. The Reference Laboratory conducted Ziehl-Neelsen staining for sputum smear AFB grade, Lowenstein-Jensen (LJ) and BACTEC MGIT broth for *M. tuberculosis* culture, and the LJ absolute concentration method for TB drug susceptibility (DST), as previously described.[16] Multi-drug resistant TB was defined as resistance to at least isoniazid (INH) and rifampicin (RIF). Extremely drug resistant (XDR) TB was defined as MDR plus resistance to any quinolone (ofloxicin or levofloxicin) and one second-line injectable drug (kanamycin, capromycin, or amikacin). Rapid HIV and Western Blot confirmatory tests were performed on venous blood for all participants.

At enrollment, patients were interviewed in Kartuli or Russian for information on socio-demographics, smoking and alcohol use, TB symptom history, and previous DM diagnosis. Alcohol use was defined as heavy (≥5 drinks per setting), intermediate (≤4 drinks per setting), frequent (≥3 days per week), and infrequent (≤2 days per week). Symptom to TB treatment time was calculated as the difference in days from first reported TB cough symptoms until TB treatment initiation date. Seeking care to TB treatment time was calculated as the difference in days from first reported seeking care for TB symptoms until TB treatment initiation date.

Sputum smear AFB and *M. tuberculosis* sputum cultures were performed after two months of anti-TB treatment when participants visited the NCTBLD directly observed therapy short-course (DOTS) clinic or in the hospital for admitted patients. Six months after TB treatment initiation, treatment outcomes were assessed using the NCTLD treatment database. Outcomes were categorized according to WHO standard definitions: cured, completed, defaulted, failed, died, or transferred.[14] An additional category was created for participants who remained on treatment at the end of the study period. Favorable outcome was defined as participants who were cured or completed after six months of treatment and poor outcome included participants who defaulted, failed, or died.

Data analyses

All collected data were entered into a REDCap (Vanderbuilt University, Nashville, TN, USA) electronic database. Analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). The $\chi 2$ tests and Fisher exact tests (for expected cell counts <5) were used to calculate p-values for categorical variables. Student's t-tests or ANOVA F-tests were used to compare differences in normally distributed continuous variables (means) and

the Kruskal-Wallis test was used for comparison of non-normally distributed variables (medians). A two-sided p-value less than 0.05 was considered statistically significant throughout the analyses. Associations between baseline patient characteristics and DM status were examined in bivariate analyses. Logistic regression models were used to calculate adjusted odds ratios (aOR) for DM status and 1) baseline TB clinical severity symptoms and 2) two-month AFB sputum culture result. Confounding variables included in the models were determined based on previous literature, bivariate associations in the data, and directed-acyclic graph theory.[17]

Ethical approval

The study protocol and study materials were reviewed and approved by Institutional Review Boards (IRBs) at the NCTBLD, Tbilisi, Georgia and Emory University, Atlanta, USA.

RESULTS

Of 393 eligible TB patients seeking treatment at the NCTBLD in Tbilisi during the study period, 280 enrolled. All enrolled patients received HbA1c screening and were included in baseline disease severity analyses. Of 280 enrolled participants, 194 (69.2%) had complete two-month AFB smear follow-up data.

The median age of participants was 49 years (inter-quartile range 42-59) and 73.9% were male (Table 1). Most participants received a high school education (58.4%), the median income was 200 Georgian Lari (GEL, $1USD ≈1.65 GEL) per month, and few were internally placed citizens (8.6%) or had ever been imprisoned (12.5%). Current or past smoking was frequently reported (75.0%) and 43.1% indicated heavy alcohol use. The mean

BMI was 21.7 (standard deviation 3.5), and most participants were HIV negative (93.6.2%), AFB smear positive (70.6%), and did not have cavitary disease (75.1%). Prevalence of MDR TB was high (20.9%).

The prevalence of HbA1c ≥6.5% was 11.1% (31 of 280), an additional five participants with HbA1c <6.5% had previously diagnosed DM, therefore the overall estimated prevalence was 12.9% (95% CI 9.3-17.2%). Among 31 participants with HbA1c ≥6.5%, 22 had previously been diagnosed with DM, and 29.0% (9 of 31) were newly diagnosed with DM. Prevalence of pre-DM was 16.8% (95%CI 12.8-21.7%) and any hyperglycemia prevalence was 29.6% (95% CI 24.5-35.2%).

Clinical presentation and TB severity

Compared to TB participants with normal glucose levels (HbA1c <5.7%), participants with DM more frequently reported symptoms of cough (91.2 vs. 73.9) at the time of TB treatment initiation (p-value <0.05). After adjusting for age, sex, smoking status, HIV status, BMI, the odds of reporting cough among participants with DM was 3.51 (95% CI 0.98-12.61) times the odds of cough among participants without DM (Table 2). In similar models adjusted for the same confounders, the odds of prevalent cavitary disease (aOR 2.38, 95% CI 1.02-5.57) and higher AFB smear grade (aOR 2.63, 95% CI 1.14, 6.06) at TB treatment initiation was statistically significantly greater among participants with DM compared to those with normal glucose levels.

Patients with TB with any hyperglycemia (DM or pre-DM) were also compared to those TB participants with normal glucose levels (Table 3). In models adjusted for age, sex, smoking status, HIV status, and BMI, cough (aOR 2.46, 95% CI 1.11-5.43) and higher AFB grade (aOR 2.00, 1.11-3.59) were statistically significantly associated with any hyperglycemia.

Two-month AFB-smear status and six-month treatment outcome

After two months of follow-up during TB treatment, 30.4% (59 of 194) of participants remained AFB sputum smear positive (Table 4). The risk of remaining smear positive after two months was higher for participants with pre-DM (risk difference [RD] 6.2%, 95% CI -11.0-23.5%) and DM (RD 2.1%, 95% CI -16.5-20.7%) compared to participants with HbA1c <5.7%. Compared to participants with normal blood glucose, those with DM had increased odds (OR 1.11, 95% CI 0.46-2.65) of remaining smear positive after two months of treatment. Other characteristics associated with positive two month AFB smear included current smoker (OR 2.60, 95% CI 1.04-6.50) and 3+ or 4+ baseline AFB grade (OR 6.54, 95% CI 2.44-17.50). After adjusting for sex, smoking status, baseline AFB grade, cavitary disease, and MDR status, those with DM compared to those with normal blood glucose were modestly more likely to be AFB sputum smear positive after two months, but this difference was not statistically significant (aOR 1.09, 95% CI 0.43-2.74).

DISCUSSION

At the time of TB treatment initiation, we found that adult participants with DM in Georgia presented with clinical TB characteristics consistent with more severe pulmonary disease. In models adjusting for known confounders, new TB patients with DM had more cough, hemoptysis, and lung cavitation compared to TB patients with normal glucose tolerance. We also demonstrated that in comparison to patients with normal glucose levels, those with any hyperglycemia (combined DM or pre-DM) had statistically significant associations with cough and AFB sputum smear grade. Despite increased severity at the time of TB treatment initiation among patients with DM and TB, we did not detect a statistically

significant difference in response to TB therapy as measured by two-month AFB sputum smear conversion.

Innate and adaptive immune responses, including those specific to TB infection, are likely altered in patients with DM,[18-21] these immune differences may partially explain increased severity at clinical presentation among patients with concomitant DM and TB. Patients with DM and TB exhibit a reduced production of interferon-γ[22, 23] and alveolar macrophage activation,[24, 25] impaired phagosome maturation due to glycation,[26] and altered interleukin (IL)-12[27, 28] and IL-18[29-31] cytokine release. These immune responses are characteristic of a hindered response to *M. tuberculosis* infection, including fewer circulating anti-mycobacterial reactive oxygen species,[26] and may limit the host's ability to contain of TB infection. Inhibited containment of TB and greater bacterial growth is one plausible explanation for increased burden of TB disease (including more symptoms, smear grade, and cavitation) at the time of clinical presentation among patients with DM.

Most studies that examined sputum smear results among TB patients with and without DM reported a greater proportion AFB-positive[8, 12, 32-35] and higher smear grade[34] among patients with DM, but some studies found no difference,[33] or more AFB-positive results among patients without DM.[10, 36] Consistent with our results, a cross-sectional analysis of TB among patients in Texas from 1996 to 2002 reported that patients with DM (compared to those without DM) had increased odds of being positive AFB smear positive at the time of TB diagnosis after adjusting for age and sex (aOR 1.8, 95% CI 1.3-2.4).[33] Similar to our results, previous studies comparing TB symptoms at time of presentation have also reported more cough[32, 33] and hemoptysis[12, 32, 33] among patients with DM. For example, an unadjusted analysis of DM and TB from Taiwanese hospital data during 2003-2006 demonstrated that DM was significantly associated with hemotypsis at the time of TB

treatment initiation (OR 2.6, 95% CI 1.2–5.3).[12] Our findings were also consistent with previous studies that reported more frequent lung cavitation[12, 33-35] among TB patients with DM compared to TB patients without DM. However, unlike previous studies that demonstrated lower lung infiltrate involvement[37] and slower conversion time until AFB smear negative[38] in patients with DM and TB, we did not detect statistically significant differences in infiltrate location or two month smear conversion.

Our study is subject to several limitations. Patients were enrolled at a single site and therefore results may not reflect findings of all patients in Georgia. Second, while AFB cultures were performed at baseline on all patients, they were not routinely performed after two months of therapy. Because AFB smear microscopy data was available from a much higher proportion of patients (compared to culture) after two months therapy, two month treatment outcomes were assessed based on smear microscopy. Further studies using two month culture conversion or time to culture conversion will be needed to assess whether patients with DM or pre-DM respond less well to therapy than patients with TB who are euglycemic a will likely require large sample size to ensure they are not underpowered. Use of AFB smear as an early measurement of response to TB therapy is subject to low sensitivity and specificity, which may have resulted in misclassification of the outcome. Nonetheless, misclassification was likely non-differential with respect to DM status and resulting biases should not affect internal validity. Thirdly we only performed one measure of HbA1c to classify participants as DM and pre-DM. Because the immune response to TB infection may cause prolonged inflammation,[2] hyperglycemia at the time of TB treatment initiation may be transient for some participants, this temporary abnormal glucose level may introduce misclassification of DM or pre-DM status. If participants were misclassified as DM (and later resolved due to transient TB induced hyperglycemia) our prevalence estimates

of DM and pre-DM may be overestimated. However, the relationship between baseline blood glucose levels and TB severity and TB outcomes is of clinical importance regardless of DM status, consequently bias due to misclassification of DM status is of minimal concern because reported measures of association between HbA1c levels and study outcomes are unaffected.

The present study prospectively screened new adult TB patients for DM and pre-DM using a point-of-care HbA1c capillary blood test, a great strength to our study. Compared to most previous studies of DM and TB that have relied on self-reported DM and did not examine pre-DM, our study classified TB patients using HbA1c, a valid quantitative method that provided an average level of blood glucose among patients during the previous three months. Another strength of our study was the ability to control for multiple known confounders age, sex, HIV status and smoking status.

Conclusions

Currently few published studies have examined the relationship between DM and pre-DM with measures of TB severity at the time of presentation, adjusted for important confounding factors including HIV status, smoking status, and BMI. A strength of this study is that it examined DM status directly using HbA1c and prospectively followed TB patients during two months of TB treatment. We found a high prevalence of DM in new adult TB patients in Tbilisi, and we reported that these DM-TB patients had more severe symptoms at the time of TB treatment initiation. Our findings highlight the importance of linking TB and DM diagnostic and treatment services in Georgia.

Table 2.1. Distribution of hemoglobin A1c blood glucose levels and baseline characteristics of culture positive adult pulmonary TB patients in Tbilisi, Georgia, 2011-2012

TABLE 2.1 **Baseline patient characteristic**	**Normal glucose** **HbA1c ≤ 5.6%** **N=197 (70.3)** **N (%)**	**Pre diabetes** **HbA1c 5.7-6.4%** **N=47 (16.8)** **N (%)**	**Diabetes[A]** **HbA1c ≥ 6.5%** **N=36 (12.9)** **N (%)**	**Total** **N=280** **N (%)**	**P-value[B]**
Demographics					
Age (years)					
Mean (STD)	50.7 (11.0)	53.0 (10.4)	50.6 (11.1)	51.1 (11.0)	
Median (IQR)	49.0 (42-58)	53.0 (44-61)	49.5 (41-59)	49.0 (42-59)	0.30
35-44	67 (34.0)	12 (25.5)	14 (38.9)	93 (33.2)	0.10
45-54	69 (35.0)	12 (25.5)	9 (25.0)	90 (32.1)	
55-64	34 (17.3)	17 (36.2)	10 (27.8)	61 (21.8)	
≥65	27 (13.7)	6 (12.8)	3 (8.3)	36 (12.9)	
Sex					
Female	55 (27.9)	7 (14.9)	11 (30.6)	73 (26.1)	0.15
Male	142 (72.1)	40 (85.1)	25 (69.4)	207 (73.9)	
Education (formal years)					
Mean (STD)	11.4 (2.6)	11.3 (2.4)	11.9 (2.6)	11.4 (2.6)	
Median (IQR)	11.0 (10-13)	10.0 (10-13)	11.0 (10-14)	11.0 (10-13)	0.61
< High school completed (≤9)	26 (13.2)	5 (10.6)	0	31 (11.1)	0.24
High school (10-11)	111 (56.4)	28 (59.6)	24 (68.6)	163 (58.4)	
> High school (≥12)	60 (30.5)	14 (29.8)	11 (31.4)	85 (30.5)	

TABLE 2.1 **Baseline patient characteristic**	**Normal glucose** **HbA1c ≤ 5.6%** **N=197 (70.3)** **N (%)**	**Pre diabetes** **HbA1c 5.7-6.4%** **N=47 (16.8)** **N (%)**	**Diabetes[A]** **HbA1c ≥ 6.5%** **N=36 (12.9)** **N (%)**	**Total** **N=280** **N (%)**	**P-value[B]**
Income, household GEL/Month*					
Mean (STD)	518 (660)	352 (552)	408 (356)	476 (649)	
Median (IQR)	225 (70-700)	150 (10-300)	300 (145-665)	200 (70-600)	0.22
≤ 100	63 (32.5)	17 (37.0)	8 (22.2)	88 (31.9)	0.11
101-300	55 (28.4)	19 (41.3)	11 (30.6)	85 (30.8)	
≥ 301	76 (39.2)	10 (21.7)	17 (47.2)	103 (37.3)	
Internally displaced					
No	178 (90.4)	43 (93.5)	34 (94.4)	255 (91.4)	0.62
Yes	19 (9.6)	3 (6.5)	2 (5.6)	24 (8.6)	
Ever imprisoned					
No	166 (86.5)	39 (86.7)	34 (94.4)	239 (87.6)	0.40
Yes	26 (13.5)	6 (13.3)	2 (5.6)	34 (12.5)	
Smoking Status					
Never smoker	50 (25.5)	9 (19.2)	11 (30.6)	70 (25.1)	0.29
Past smoker	47 (24.0)	13 (27.7)	13 (36.1)	73 (26.2)	
Current smoker	99 (50.5)	25 (53.2)	12 (33.3)	136 (48.8)	
Alcohol use**					
Frequent heavy	33 (16.8)	9 (19.2)	2 (5.7)	44 (15.8)	0.41
Infrequent heavy	52 (26.5)	15 (31.9)	9 (25.7)	76 (27.3)	
Frequent/infrequent intermediate	51 (26.0)	11 (23.4)	8 (22.9)	70 (25.2)	
Never	60 (30.6)	12 (25.5)	16 (45.7)	88 (31.7)	
Self-reported symptoms					
Cough					
No	49 (26.1)	6 (14.3)	3 (8.8)	58 (22.0)	**0.04**
Yes	139 (73.9)	36 (85.7)	31 (91.2)	206 (78.0)	

TABLE 2.1 **Baseline patient characteristic**	**Normal glucose** **HbA1c ≤ 5.6%** **N=197 (70.3)** **N (%)**	**Pre diabetes** **HbA1c 5.7-6.4%** **N=47 (16.8)** **N (%)**	**Diabetes[A]** **HbA1c ≥ 6.5%** **N=36 (12.9)** **N (%)**	**Total** **N=280** **N (%)**	**P-value[B]**
Hemoptysis[C]					
No	146 (78.1)	34 (81.0)	21 (61.8)	201 (76.4)	0.09
Yes	41 (21.9)	8 (19.0)	13 (38.2)	62 (23.6)	
Chest pain					
No	123 (65.8)	22 (53.7)	20 (58.8)	165 (63.0)	0.30
Yes	64 (34.2)	19 (46.3)	14 (41.2)	97 (37.0)	
Fever					
No	42 (35.6)	8 (34.8)	5 (29.4)	55 (34.8)	0.88
Yes	76 (64.4)	15 (65.2)	12 (70.6)	103 (65.2)	
Missing	79	24	19	122	
Weight loss					
No	45 (39.1)	5 (21.7)	3 (17.7)	53 (34.2)	0.10
Yes	70 (60.9)	18 (78.3)	14 (82.4)	102 (65.8)	
Missing	82	24	19	125	
Night sweats					
No	40 (35.4)	6 (26.1)	6 (35.3)	52 (34.0)	0.72
Yes	73 (64.6)	17 (73.9)	11 (64.7)	101 (66.0)	
Missing	84	24	19	127	
Weakness					
No	32 (27.8)	3 (13.0)	4 (23.5)	39 (25.2)	0.34
Yes	83 (72.2)	20 (87.0)	13 (76.5)	116 (74.8)	
Missing	82	24	19	125	
Clinical information					

TABLE 2.1 **Baseline patient characteristic**	**Normal glucose** **HbA1c ≤ 5.6%** **N=197 (70.3)** **N (%)**	**Pre diabetes** **HbA1c 5.7-6.4%** **N=47 (16.8)** **N (%)**	**Diabetes[A]** **HbA1c ≥ 6.5%** **N=36 (12.9)** **N (%)**	**Total** **N=280** **N (%)**	**P-value[B]**
Symptom to TB treatment time (days)	83 (117)	89 (108)	77 (89)	83 (112)	
Mean (STD)	35 (19-108)	40 (18-131)	37 (17-102)	35 (19-110)	0.99
Median (IQR)					
	43 (32.1)	11 (34.4)	11 (37.9)	65 (33.3)	0.47
0-21	50 (37.3)	9 (28.1)	6 (20.7)	65 (33.3)	
22-70	41 (30.6)	12 (37.5)	12 (41.4)	65 (33.3)	
≥ 71	63	15	7	85	
Missing					
Seek care to TB treatment time (days)	42 (100)	47 (66)	24 (29)	41 (90)	
Mean (STD)	17 (4-37)	15 (4-56)	15 (4-28)	16 (4-39)	0.37
Median (IQR)					
	81 (46.8)	17 (46.0)	13 (46.4)	111 (46.6)	0.90
0-14	48 (27.8)	8 (21.6)	8 (28.6)	64 (26.9)	
15-35	44 (25.4)	12 (32.4)	7 (25.0)	63 (26.5)	
≥ 36	24	10	8	42	
Missing					
BMI					
Mean (STD)	21.3 (3.4)	21.4 (3.0)	24.1 (4.1)	21.7 (3.5)	
Median (IQR)	21.1 (19.1-23.4)	21.2 (19.2-23.1)	23.3 (21.3-26.0)	21.3 (19.4-23.7)	**<0.01**
<18.5	38 (19.8)	7 (15.6)	2 (5.9)	47 (17.3)	0.08
18.5-24.9	127 (66.2)	33 (73.3)	22 (64.7)	182 (67.2)	
≥25	27(14.1)	5 (11.1)	10 (29.4)	42 (15.5)	

TABLE 2.1 **Baseline patient characteristic**	**Normal glucose** **HbA1c ≤ 5.6%** **N=197 (70.3)** **N (%)**	**Pre diabetes** **HbA1c 5.7-6.4%** **N=47 (16.8)** **N (%)**	**Diabetes[A]** **HbA1c ≥ 6.5%** **N=36 (12.9)** **N (%)**	**Total** **N=280** **N (%)**	**P-value[B]**
HIV status					
Positive	9 (4.6)	0	1 (2.8)	10 (3.6)	0.61
Negative	183 (92.9)	45 (95.7)	34 (94.4)	262 (93.6)	
Unknown	5 (2.5)	2 (4.3)	1 (2.8)	8 (2.9)	
AFB smear					
Negative[C]	65 (33.0)	12 (26.1)	5 (13.9)	82 (29.4)	0.06
Positive	132 (67.0)	34 (73.9)	31 (86.1)	197 (70.6)	
1+ (Among AFB positive)	34 (25.8)	7 (21.2)	8 (25.8)	49 (25.0)	0.77
2+	47 (35.6)	9 (27.3)	8 (25.8)	64 (32.6)	
3+	26 (19.7)	10 (30.3)	9 (29.0)	45 (23.0)	
4+	25 (18.9)	7 (21.2)	6 (19.4)	38 (19.4)	
Drug susceptibility					
XDR	2 (1.4)	0	0	2 (0.9)	0.25
MDR	27 (18.4)	6 (17.1)	11 (35.4)	44 (20.7)	
First-line therapy	118 (80.3)	29 (82.9)	20 (64.5)	167 (78.4)	
Missing	50	12	5	67	
MDR[C]	27 (18.6)	6 (17.1)	11 (35.5)	44 (20.9)	0.09
First-line therapy	118 (81.4)	29 (82.9)	20 (64.5)	167 (79.1)	

TABLE 2.1 **Baseline patient characteristic**	**Normal glucose** **HbA1c ≤ 5.6%** **N=197 (70.3)** **N (%)**	**Pre diabetes** **HbA1c 5.7-6.4%** **N=47 (16.8)** **N (%)**	**Diabetes[A]** **HbA1c ≥ 6.5%** **N=36 (12.9)** **N (%)**	**Total** **N=280** **N (%)**	**P-value[B]**
Cavitary disease					
None	147 (77.4)	33 (75.0)	22 (62.9)	202 (75.1)	0.19
Any cavity	43 (22.6)	11 (25.0)	13 (37.1)	67 (24.9)	
Any cavity, Bilateral	11 (27.9)	2 (18.2)	3 (23.1)	17 (25.4)	0.79
Any cavity, Unilateral	31 (72.1)	9 (81.8)	10 (76.9)	50 (74.6)	
Unilateral, Right side only	15 (48.4)	5 (55.6)	6 (60.0)	26 (52.0)	0.79
Unilateral, Left side only	16 (51.6)	4 (44.4)	4 (40.0)	24 (48.0)	
Infiltrate, upper left side					
No	72 (37.7)	20 (45.5)	19 (54.3)	111 (41.1)	0.15
Yes	119 (62.3)	24 (54.6)	16 (45.7)	159 (58.9)	
Infiltrate, lower left side					
No	126 (66.0)	30 (69.8)	25 (71.4)	181 (67.3)	0.76
Yes	65 (34.0)	13 (30.2)	10 (28.6)	88 (32.7)	
Infiltrate, upper right side[C]					
No	48 (25.0)	12 (26.7)	15 (42.9)	75 (27.6)	0.09
Yes	144 (75.0)	33 (73.3)	20 (57.1)	197 (72.4)	
Infiltrate, lower right side					
No	119 (62.3)	31 (72.1)	28 (80.0)	178 (66.2)	0.08
Yes	72 (37.7)	12 (27.9)	7 (20.0)	91 (33.8)	

Abbreviations: HbA1c-hemoglobin A1c; STD-standard deviation; IQR-interquartile range; GEL- Georgian Lari; BMI-body mass index; AFB-acid-fast bacilli; XDR-extremely drug resistant; MDR-multi-drug resistant

A. Diabetes mellitus defined by HbA1c ≥ 6.5%, and 5 patients with HbA1c <6.5% who self-reported physician diagnosed diabetes and current use of diabetes medications.
B. Two sided p-value, chi-square tests for categorical variables and Kruskal-Wallis tests for continuous variables
C. Statisticallly significant, two sided p-value <0.05 when diabetes mellitus status was categorized as dichotomous (Yes/No).
D. GEL: Georgian Lari (1 USD ≈ 1.65 GEL)
E. Alcohol use: Heavy ≥5 drinks/setting, intermediate ≤4 drinks/setting, frequent ≥3 days/week, infrequent ≤2 days/week.

Statistical tests: *Categorical*: Chi-square, Fishers exact for expected cell counts <5; *Continuous*: Kruskal-Wallis test for comparison of medians (non-normally distributed), ANOVA F-test for comparison of means (normally distributed); *Missing*: If >10% of a characteristic was missing, the category *Missing* is included as a response

Table 2.2. Multivariable analyses for self-reported tuberculosis (TB) severity symptoms at the time of TB presentation among new adult TB patients with diabetes mellitus in Tbilisi, Georgia, 2011-2012

	Cough		**Hemoptysis**		**Chest pain**	
DM status[A]	**OR (95% CI)**	**AOR (95% CI)**	**OR (95% CI)**	**AOR (95% CI)**	**OR (95% CI)**	**AOR (95% CI)**
DM	**3.64 (1.07, 12.44)**	3.51 (0.98, 12.61)	**2.20 (1.02, 4.78)**	1.75 (0.75, 4.07)	1.35 (0.64, 2.84)	0.99 (0.42, 2.27)
Pre-DM	2.12 (0.84, 5.33)	1.98 (0.77, 5.07)	0.84 (0.36, 1.95)	0.81 (0.34, 1.92)	1.66 (0.84, 3.29)	1.53 (0.75, 3.10)
NGT	1	1	1	1	1	1
DM/Pre-DM	**2.62 (1.22, 5.66)**	**2.46 (1.11, 5.43)**	1.36 (0.74, 2.50)	1.17 (0.62, 2.22)	1.51 (0.87, 2.61)	1.27 (0.71, 2.27)
NGT	1	1	1	1	1	1
DM	3.25 (0.96, 11.03)	3.17 (0.89, 11.33)	**2.27 (1.06, 4.86)**	1.81 (0.79, 4.17)	1.22 (0.59, 2.55)	0.91 (0.40, 2.07)
Pre-DM/NGT	1	1	1	1	1	1
HbA1c, per 1% increase	1.50 (0.99, 2.26)	1.43 (0.96, 2.14)	1.03 (0.81, 1.29)	0.95 (0.74, 1.22)	1.14 (0.93, 1.39)	1.06 (0.85, 1.32)

Abbreviations: DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; AOR, multivariable adjusted odds ratio; Pre-DM, pre-diabetes mellitus; NGT, normal glucose tolerance; HbA1c, hemoglobin A1c

A. In addition to diabetes status, adjusted models included age, sex, HIV status, smoking status, BMI

Table 2.3. Multivariable analyses for measures of clinical tuberculosis (TB) severity symptoms at the time of TB presentation among new adult TB patients with diabetes mellitus in Tbilisi, Georgia, 2011-2012

	Cavity[A]		**High AFB[B]**		**MDR TB[C]**	
DM status[D]	**OR (95% CI)**	**AOR (95% CI)**	**OR (95% CI)**	**AOR (95% CI)**	**OR (95% CI)**	**AOR (95% CI)**
DM	2.02 (0.94, 4.34)	**2.38 (1.02, 5.57)**	2.05 (0.98, 4.27)	**2.63 (1.14, 6.06)**	2.24 (0.97, 5.19)	2.44 (0.97, 6.17)
Pre-DM	1.14 (0.53, 2.44)	1.21 (0.55, 2.65)	1.74 (0.88, 3.44)	1.67 (0.82, 3.39)	0.84 (0.32, 2.22)	0.87 (0.32, 2.32)
NGT	1	1	1	1	1	1
DM/Pre-DM	1.49 (0.83, 2.69)	1.61 (0.87, 3.01)	1.87 (1.08, 3.23)	**2.00 (1.11, 3.59)**	1.41 (0.71, 2.80)	1.44 (0.70 2.96)
NGT	1	1	1	1	1	1
DM	1.97 (0.93, 4.17)	**2.30 (1.00, 5.30)**	1.83 (0.89, 3.75)	**2.36 (1.04, 5.36)**	2.31 (1.01, 5.26)	**2.51 (1.01, 6.23)**
Pre-DM/NGT	1	1	1	1	1	1
HbA1c, per 1% increase	1.10 (0.89, 1.36)	1.13 (0.910, 1.42)	**1.22 (1.00, 1.49)**	**1.28 (1.02, 1.59)**	1.15 (0.92, 1.44)	1.18 (0.93, 1.50)

Abbreviations: DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; AOR, multivariable adjusted odds ratio; Pre-DM, pre-diabetes mellitus; NGT, normal glucose tolerance; HbA1c, hemoglobin A1c

A. Any cavitary disease
B. High grade is defined as 4+ or 3+ vs. 2+, 1+, or negative
C. Any resistance pattern that includes resistance to both rifampin and isoniazid
D. In addition to diabetes status, adjusted models included age, sex, HIV status, smoking status, BMI

Table 2.4. Patient characteristics associated with two-month acid-fast bacilli (AFB) sputum smear positive results among adult pulmonary TB patients in Tbilisi, Georgia, 2011-2012

Table 2.4. **Baseline patient characteristic**	**2-month AFB Positive** **59/194 (30.4)** **Positive/Total (%)**	**Odds ratio** **OR (95% CI)**	**Adjusted odds ratio** **AOR (95% CI)[A]**
DM status			
NGT	37/128 (28.9)	1	1
Pre-DM	13/37 (35.1)	1.33 (0.61, 2.89)	1.37 (0.61, 3.08)
DM	9/29 (31.0)	1.11 (0.46, 2.65)	1.09 (0.43, 2.74)
Age (years)			
35-44	22/71 (31.0)	1	
45-54	19/57 (33.3)	1.11 (0.53, 2.35)	
≥ 55-64	18/66 (27.3)	0.84 (0.40, 1.75)	
Sex			
Female	11/46 (23.9)	1	1
Male	48/148 (32.4)	1.53 (0.71, 3.27)	0.71 (0.25, 2.01)
Smoking Status			
Never smoker	7/44 (15.9)	1	1
Past smoker	21/56 (37.5)	3.17 (1.20, 8.39)	4.30 (1.23, 15.01)
Current smoker	31/94 (33.0)	2.60 (1.04, 6.50)	3.87 (1.11, 13.49)
Cough			
No	9/39 (23.1)	1	1.00 (0.97, 1.03)[B]
Yes	43/141 (30.5)	1.46 (0.64, 3.34)	
Hemoptysis			
No	40/137 (29.2)	1	
Yes	12/42 (28.6)	0.97 (0.45, 2.08)	

Table 2.4. **Baseline patient characteristic**	**2-month AFB Positive** **59/194 (30.4)** **Positive/Total (%)**	**Odds ratio** **OR (95% CI)**	**Adjusted odds ratio** **AOR (95% CI)[A]**
BMI			
<18.5	9/35 (25.7)	0.81 (0.34, 1.90)	
18.5-24.9	36/120 (30.0)	1	
≥25	10/30 (30.0)	1.17 (0.50, 2.74)	
HIV status			
Positive	2/8 (25.0)	0.79 (0.16, 4.10)	
Negative	53/179 (29.6)	1	
Unknown	4/7 (57.1)	3.17 (0.69, 14.65)	
AFB smear			
Negative	6/52 (11.5)	1	
1 or 2+	24/78 (30.8)	3.41 (1.28, 9.05)	
3 or 4+	29/63 (46.0)	6.54 (2.44, 17.50)	
Drug susceptibility			
Drug susceptible	41/154 (26.6)	1	1
XDR/MDR	18/40 (45.0)	2.26 (1.10, 4.63)	2.58 (1.20, 5.52)
Cavitary Disease			
None	31/121 (25.6)	1	
Any	23/64 (35.9)	1.63 (0.85, 3.13)	

Abbreviations: OR-odds ratio; AOR-adjusted odds ratio; HbA1c-hemoglobin A1c; BMI-body mass index; AFB-acid-fast bacilli; MDR-multi-drug resistant; XDR-extremely drug resistant

A. Adjusted binomial logistic model included all variables that have AOR reported in the table.
B. Age was also included in the multivariable model as a continuous variable

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CHAPTER 3: CULTURE CONVERSION AMONG MULITDRUG-RESISTANT TUBERCULOSIS PATIENTS WITH DIABETES MELLITUS

CHAPTER 3 ABSTRACT

Background: Diabetes mellitus (DM) is an established risk factor for developing active tuberculosis (TB) but little is known about the effect of DM on sputum culture conversion among multidrug-resistant (MDR) TB patients. We aimed to estimate the effect of DM on time to *Mycobacterium tuberculosis* culture sputum culture conversion (from positive to negative) among adult pulmonary MDR TB patients on second-line therapy in the country of Georgia. We also sought to estimate the effect of DM on risk of default from second-line TB therapy in the same cohort. Methods: A retrospective cohort of all MDR TB patients between January 2009 and December 2012 was followed during second-line TB therapy at the National Center of TB and Lung Disease in Tbilisi, Georgia. Eligible patients included adults (aged ≥18 years) with pulmonary MDR TB who initiated TB treatment during the study period. Cox proportion hazards models were used to estimate the association between DM status and time to sputum culture conversion. Log-binomial regression was used to estimate the association between DM and risk of TB second-line treatment failure. Results: Of 2,445 included MDR TB patients, 122 (5.0%) had DM. Compared to patients without DM, those with DM were older (median age 34.9 vs. 48.7 years), less likely to have been imprisoned (42.6% vs. 17.5%), less likely to be a current smoker (48.0% vs. 36.9%), and heavier (median body mass index [BMI] 20.4 vs. 23.4). Of 1,467 patients with culture conversion information, 1,000 (68.2%) converted sputum cultures from positive to negative. The estimated rate of culture conversion was modestly but non-significantly lower in patients with MDR TB and DM (adjusted hazard ratio [aHR] 0.93, 95% CI 0.71, 1.23) than

in MDR TB patients without DM. The adjusted risk of default from MDR TB therapy among patients with DM was 1.14 (95% CI 0.90, 1.44) times the risk in patients without DM. Conclusions: After adjusting for confounding variables, DM was associated with slower culture conversion and increased risk of treatment default, but the differences were not clinically meaningful.

INTRODUCTION

In the past 5 years, the need to better understand the relationship between type 2 diabetes mellitus (DM) and tuberculosis (TB) has re-emerged as a global public health priority.[1,2] Currently estimated at 366 million and expected to reach 570 million by 2030,[3] DM prevalence is increasing rapidly worldwide. Prevalence of DM is expected to increase most in low- and middle-income countries (LMIC) where TB is endemic and the burden remains the greatest.[4] Each year there are nearly 9 million new cases of TB,[5] and an estimated 82% of these occur in 22 high-burden countries all of which are LMIC.[6] The concern over co-occurring DM-TB epidemics is also supported by previous literature reviews[2,7,8] and meta-analyses[9,10] which suggest that patients with DM, compared to those without DM, have approximately a 3-fold increased risk of developing active TB disease.

Global increases in multidrug-resistant (MDR) TB have brought additional challenges to TB control efforts. In 2012 the World Health Organization (WHO) estimated that 3.7% of new cases and 20.0% of previously treated cases were MDR TB.[6] Second-line anti-TB drugs are costly,[11] treatment regimens must be extended for long periods of time (typically 20 months or longer), and the proportion of patients achieving treatment success is low (48% in 2012).[6] Consequently, management of MDR TB treatment is difficult and requires national TB programs to use extensive financial resources.[12,13]

Little is known about the relationship between DM and MDR TB. Several studies have reported a high prevalence of DM among MDR TB patients,[14-17] including an association between DM and prevalent MDR TB after adjusting for confounding factors.[18,19] While DM has been associated with poor TB outcomes (including slower sputum culture conversion and higher risk of death and relapse[9]) among patients receiving first-line anti-TB therapy, whether DM increases the time to culture conversion or increases the risk of poor outcomes among MDR TB is an area of research that needs further longitudinal epidemiologic evidence.[1]

Georgia is a former Soviet Republic of 4.3 million people located in the Caucasus region. In 2010 the TB incidence in Georgia was 107 per 100,000.[5] In addition, the WHO classifies Georgia as one of the world's high-burden MDR TB nations—9.5% of new TB cases and 31.0% of previously treated TB cases had MDR TB in 2011.[6,20] In 2009, DM in Georgia was estimated to be prevalent in 9.2% of adults, and an additional 7.2% were estimated to have pre-DM (i.e., at high risk of developing DM).[21] In Georgia, the relationship between DM and MDR TB treatment outcomes, including culture conversion and default, have not been previously published. Therefore, the primary objective of this study was to estimate the association between DM and time to sputum culture conversion among adult pulmonary TB patients receiving MDR TB second-line therapy in Georgia. Secondarily, we also sought to estimate the association between DM and the risk of defaulting from MDR TB second-line therapy in the same patient population.

METHODS

Setting and Participants

A retrospective cohort of all MDR TB patients starting second-line therapy between January 2009 and December 2012 was conducted at the National Center for TB and Lung Disease (NCTBLD) in Tbilisi, Georgia. Treatment of MDR TB at the NCTBLD and its clinics represents virtually all treated MDR TB cases in the country of Georgia. Baseline data was collected on all MDR TB patients who were then followed during second-line treatment for up to three years, until therapy was completed, or until December 2012, which ever occurred first. Eligible patients included all adult (aged 18 years or greater) pulmonary confirmed MDR TB cases who initiated second-line therapy during the study period. In Georgia, confirmation of pulmonary MDR TB is defined by a positive sputum culture that is resistant to at least isoniazid (INH) and rifampicin (RIF).

Study measures and data collection

The primary study outcome, *culture conversion time*, was defined as time (in days) from MDR TB treatment initiation until the first of two consecutive negative sputum cultures ≥30 days apart. The date of culture conversion was abstracted from patients' medical records and entered into the NCTBLD treatment database. The study's secondary outcome, *default*, was defined as a patient whose treatment was interrupted for ≥2 consecutive months. Default is one of six final treatment outcomes defined by the World Health Organization (WHO); other WHO outcomes include cured, completed, failed, defaulted, died, or transferred).[22,23] Treatment outcomes were assigned one of the WHO definitions at the end of therapy (date of treatment completion) and were abstracted from the MDR TB treatment registry.

The primary study exposure of interest was DM status. At the time of MDR TB treatment initiation, NCTBLD physicians completed a hospital admission form for all study patients. Physicians indicated if patients had previously been diagnosed with DM—either

from hospital medical records or self-reported by the patient. Patients DM status (DM or no history of DM) was abstracted from the hospital admission form and entered into the NCTBLD treatment database.

Additional patient characteristics collected in the study included demographic and socio-behavioral information, concomitant infectious diseases, and TB clinical features. Body mass index (BMI) was calculated (kg/m^2) from patient height and weight at the time of MDR TB treatment initiation. Hepatitis co-infection status was classified as positive if the patient had hepatitis A, B, or C. All patients were screened for HIV co-infection by rapid test and positive tests were confirmed with Western Blot following national protocols.[24] Patient information regarding history of previous TB treatment, chest radiographic findings (presence of any lung cavity, dissemination), and presence of extra-pulmonary TB were abstracted from patient medical records.

All laboratory measures were conducted at the Georgia National TB Reference Laboratory in Tbilisi, which has received annual external quality assessment from Antwerp WHO Supranational TB Reference Laboratory since 2005.[25] Sputum smear acid fast bacilli (AFB) microscopy was performed with Ziehl-Neelsen methods and a standard semi-quantitative scale was used to classify the number of organisms present (negative through 4+).[26] Sputum cultures for *M. tuberculosis* were measured by smear light microscopy, Lowenstein-Jensen based solid medium, and the BACTEC MGIT 960 as previously described.[25] First-line drug susceptibility testing (DST) was performed using the absolute concentration method or using the BACTEC, DST to second-line therapy was performed using the proportion method. Patients with resistance to a fluoroquinolone (levofloxacin or ofloxacin) or an injectable (capreomycin, kanamycin, or amikacin) were classified as having any second-line resistance.

Data analyses

Laboratory results and data from patient medical records were entered into an electronic database and analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC). The association between patient characteristics and DM status was analyzed using bivariate analysis. The $\chi 2$ test was used to calculate p-values for categorical variables, the Student's t-test was used to compare differences in normally distributed continuous variables (means), and the Kruskal-Wallis test was used for comparison of non-normally distributed variables (medians). A two-sided p-value less than 0.05 was considered statistically significant throughout the analyses. Cox proportional hazards regression models were used to estimate the hazard rate ratios (HR) and 95% confidence intervals (CI) for time to culture conversion. Patients were censored if at the time of treatment completion they did not have a prior documented sputum conversion. Treatment completion was defined as the outcomes completed, defaulted, died, or transferred. Proportional hazard assumptions were assessed graphically (log negative log curves), with goodness-of-fit (Schoenfeld residuals) and using time-dependent models.[27] Adjusted survival curves were used to graphically represent the time until sputum culture conversion. The cumulative risk ratio (RR) and 95% CI for default from MDR TB therapy was modeled using log-binomial regression models. Selected covariates considered to be known confounders were included in Cox and log-binomial regression models based on significant bivariate associations with the primary exposure and outcomes, previous literature, or directed acyclic graph theory.[28] Sensitivity analyses were performed to determine if patients with missing outcome information had significantly different demographic or clinical characteristics compared to patients with complete follow up information.

RESULTS

During the study period (January 2009 to December 2012) 2,531 MDR TB confirmed pulmonary patients began second-line therapy at the NCTBLD in Georgia. Of all new pulmonary MDR patients 96.6% (2,445 of 2,531) were adults and included in the study. Culture conversion and censorship follow-up information was available for 60% (1,467 of 2,445) of eligible patients and 62% (1,524 of 2,445) had WHO defined treatment outcome information available (Figure 1). An additional 916 MDR TB patients remained on treatment, were missing culture conversion data, or had no treatment outcome information.

Of enrolled patients, most were male (81.3%), current smoking was common (47.4%), and the median age was 35.5 years (Table 1). Diabetes mellitus was prevalent in 5.0% of MDR TB patients starting second-line therapy. Compared to patients without DM, those with DM were older (median age 34.9 vs. 48.7 years), less likely to have been imprisoned (42.6% vs. 17.5%) or be a current smoker (48.0% vs. 36.9%), and heavier (median BMI 20.4 vs. 23.4) (p-value <0.05 for all comparisons). The median fasting blood glucose (FBG) among MDR TB patients with DM was 6.8 mmol/L (IQR 5.1) compared to 5.0 mmol/L (IQR 1.7) among patients without DM (p-value <0.05). HIV infection was prevalent in 4.8% of MDR TB patients without DM, but no patients with DM had HIV infection (p-value <0.05). Clinical TB characteristics at the time of second-line treatment initiation were similar in patients with and without DM. However, patients with DM were less likely to have received previous TB treatment (57.1% vs. 65.9%) and more likely to have an AFB sputum smear grade ≥ 2 (54.6% vs. 43.4%) compared to MDR TB patients without DM (p-value <0.05 for all comparisons).

Among MDR TB patients with complete sputum culture and censorship follow-up information, 68.2% (1000 of 1467) converted sputum cultures from positive to negative

during second-line therapy (Table 2). The median time to culture conversion from positive to negative was 69 (IQR 70.0) days among MDR TB patients. In unadjusted analysis, the proportion of patients with MDR TB and DM who converted sputum cultures during treatment was greater than MDR TB patients without DM (RR 1.08 95% CI 0.94, 1.24). Among patients with WHO treatment outcome information, those with DM had greater risk of poor treatment outcome (failed, defaulted, died, or transferred) compared to patients without DM (RR 1.07 95% CI 0.87, 1.30).

In an unadjusted Cox proportional model, the hazard rate of sputum culture conversion (from positive to negative) was greater (HR 1.11 95% CI 0.85, 1.45) in MDR TB patients with DM compared to patients without DM (Table 3). After adjusting for confounding covariates, the estimated rate of culture conversion was modestly but non-significantly lower in MDR TB patients with DM (adjusted hazard ratio [aHR] 0.93 95% CI 0.71, 1.23) compared to those without DM. Selected covariates associated with lowest sputum culture conversion rates in unadjusted models included heavy alcohol use (HR 0.62 95% CI 0.46, 0.83), BMI <18.5 (HR 0.64 95% CI 0.54, 0.76), any second-line anti-TB therapy resistance (HR 0.44 95% CI 0.34, 0.56), and presence of lung cavity (HR 0.62 95% CI 0.54, 0.72).

The risk of defaulting from second-line therapy among MDR TB patients with DM was 1.14 (95% CI 0.90, 1.45) times the risk of default among MDR TB patients without DM (Table 4). After adjusting for multiple confounders in a log-binomial model, the estimated effect of DM on risk of default from second-line therapy was unchanged (adjusted risk ratio [aRR] 1.14 95% CI 0.90, 1.44). Other selected covariates that were strongly associated with increased risk (unadjusted) of default included male sex (RR 1.52 95% CI 1.26, 1.85), any

second-line anti-TB therapy resistance (RR 1.49 95% CI 1.28 1.74), and 4+ sputum AFB smear grade at treatment initiation (RR 1.40 95% CI 1.14, 1.70).

DISCUSSION

This retrospective cohort study of adult pulmonary MDR TB patients from the country of Georgia found that after adjusting for important confounding factors, MDR TB patients with concurrent DM did convert sputum cultures from *M. tuberculosis* positive to negative at a slightly slower rate than MDR TB patients without DM (aHR 0.95 95% CI 0.72, 1.25); however, the estimated difference was not clinically meaningful (p-value >0.05). We also reported that although MDR TB patients with DM are at greater risk of default from second-line therapy than patients without DM, the increased risk was not statistically significant in adjusted estimates (aRR 1.14 95% CI 0.90, 1.44).

Hypotheses of biologic mechanisms exist which may partially explain the delay in sputum culture conversion among TB patients with DM. First, TB patients with DM may have a greater bacterial burden (and higher AFB sputum smear grade) at the time of TB treatment initiation, consequently additional exposure to anti-TB regimens would be required in order for clearance of *M. tuberculosis* in the sputum. Second, early release of interferon (IFN)-γ is an important marker of immune repose to TB infection[29] and may be delayed in TB patients with DM.[30-32] Third, chronic hyperglycemia that leads to glycation of alveolar macrophage proteins and binding sites may reduce macrophage containment of TB,[33,34] also leading to increased bacterial burden and longer sputum culture conversion time. However, the extent to which these postulated biologic mechanisms are relevant to MDR TB patients with DM on second-line therapy is unclear. Drug susceptibility patterns, previous TB treatment, TB treatment adherence, HIV status, and tobacco use may impair the host response to sputum culture clearance such that in the context of second line

regimens the effect of DM is modest. Among MDR TB patients with DM, glucose control and active DM care during TB treatment have the greatest effect on culture conversion.[35]

Few other studies have examined the effect of DM status on time until sputum culture conversion or risk of TB treatment default among patients with MDR TB. Consistent with our adjusted HR estimate, a large multi-site cohort study of MDR TB patients from Peru, Latvia, Estonia, Russia, and Manila published in 2012 reported that the unadjusted HR of sputum culture conversion among patients with DM was 0.76 (95% CI 0.54, 1.06) times the HR of MDR TB patients without DM, but the study did not report an adjusted estimate of DM on culture conversion time.[36] Among previous studies of sputum culture conversion in TB patients on first-line regimens, most reported that patients with DM had delayed culture conversion time. For example, a study from Baltimore reported that DM was associated with an unadjusted increased time to culture conversion—the median time to conversion was 49 days in patients with DM vs. 39 days (p-value 0.09) among TB patients without DM.[37] After adjusting for age and sex, a study from Texas estimated the HR of conversion among patients with DM was 0.75 (95% CI 0.59, 0.96) times the rate among TB patients without DM.[38] A similar study in Taiwan reported that compared to TB patients without DM, those with DM had a slower rate of sputum culture conversion (unadjusted HR 0.78 95% CI 0.61, 1.00).[39] Although unadjusted for age and other important confounders, a study published in 2013 from Mexico reported the proportion of TB patients on first line TB therapy who converted sputum cultures to negative after ≥ 60 days of treatment was significantly greater in patients with DM (45.9%) compared to those without DM (37.2%).[40]

This study was subject to several limitations, including those inherent in a retrospective cohort design. First, DM status was not systematically measured for all MDR

TB patients at the time of second-line TB treatment initiation, which may have resulted in misclassification of the primary study exposure. However, TB patients previously diagnosed with DM or who were taking drugs for glycemic control likely knew their DM status. Therefore, the specificity of our measured DM status was likely high, but the prevalence of DM among MDR TB patients in Georgia is likely greater than we reported. The misclassification of DM status was unlikely differential with respect to sputum culture conversion and therefore our estimated effect of the dichotomous exposure (DM) on the outcome (culture conversion) is plausibly biased toward the null.[41] Second, the measurement of DM in this study did not include a comprehensive assessment of glucose control (e.g., fasting blood glucose or hemoglobin A1c) and consequently we were unable to determine the effect of DM control on sputum culture conversion. Third, 37.5% of patients who began MDR TB treatment did not have treatment or culture conversion information available at the end of the study and as a result, the generalizability of the study findings to all MDR TB patients in Georgia may be limited. Missing treatment outcome information for MDR TB patients is high in most national TB programs—globally, 28% of MDR TB patients on second-line therapy were lost to follow up or did not have treatment outcome information reported in 2011.[6] In descriptive sensitivity analyses, patients with missing outcome information were similar to MDR TB patients who had complete follow-up outcome data (Table 5) with respect to DM status, demographic characteristics, and clinical presentation. Fourth, we were unable to assess the effect of DM on mortality in this cohort of MDR TB patients. The risk of death during second-line TB was 10.1% in this study, lower than is typically reported in studies of MDR patients in LMIC.[6,42] The proportion of patients who defaulted (i.e., as a result of severe illness or inability to tolerate second-line regimens) and then died due to TB disease is unknown in our study. Similarly, death before MDR TB

diagnosis or treatment initiation was also possible in this cohort and not captured in our data. If death from TB disease before MDR TB treatment initiation was differential by DM status, we would be unable to predict the direction in which this bias would affect our estimate of DM on MDR TB culture conversion time. Fifth, we did not have detailed measurements of patients' specific second-line regimen or their drug susceptibility profile. Consequently, we were unable to assess if patients were on appropriate TB regimens that may result in confounding caused by the strong association between drug resistance profile and culture conversion. Nonetheless, we do not have a reason to believe that the distribution of appropriate TB regimens would be different by DM status. We did assess the proportion of patients with any resistance to second-line regimens, and this proportion did not vary significantly among those with DM (12.6%) or without DM (11.7%).

Strengths of our study include a large sample from a well-described retrospective cohort of MDR TB patients with information on DM status, demographics, and clinical characteristics. While few other studies have examined the effect of DM on time to sputum culture conversion in drug-susceptible TB cohorts, we present the first analysis of the effect of DM on culture conversion among TB patients with confirmed MDR. In addition, our study was able to control for multiple confounding factors not accounted for in previous studies of culture conversion among TB patients with DM, including alcohol use, smoking status, and cavitary lung disease.

Conclusions

To our knowledge, this is the largest study to estimate the effect of DM status on time to sputum culture conversion in a cohort of confirmed MDR TB patients. We also estimated the effect of DM on risk of default during TB second-line treatment regimens.

Although previous studies suggest DM may increase the time to sputum culture conversion and default among drug susceptible TB patients, our results did not detect a clinically meaningful difference in time to conversion or risk of treatment default in MDR TB patients from the country of Georgia.

Table 3.1. Diabetes mellitus and baseline characteristics of adult pulmonary MDR TB patients in Georgia 2009-2012

Table 3.1 Patient characteristic at MDR TB treatment start	**No diabetes N=2323 (95.0) N (%)**	**Diabetes mellitus[A] N=122 (5.0) N (%)**	**Total N=2445 N (%)**
Age (years)			
Mean (STD)	37.0 (12.3)	47.4 (12.8)	37.5 (12.6)
Median (IQR)[B]	34.9 (18.0)	48.7 (17.8)	35.5 (18.3)
18-34[B]	1166 (50.2)	23 (18.9)	1189 (48.6)
35-44	569 (24.5)	29 (23.8)	598 (24.5)
45-54	371 (16.0)	32 (26.2)	403 (16.5)
≥55	217 (9.3)	38 (31.2)	255 (10.4)
Sex			
Female	435 (18.7)	23 (18.8)	458 (18.7)
Male	1888 (81.3)	99 (81.2)	1987 (81.3)
Ever imprisoned[B]			
No	1307 (57.4)	99 (82.5)	1406 (58.7)
Yes	969 (42.6)	21 (17.5)	990 (41.3)
Current smoker[B]			
No	1209 (52.0)	77 (63.1)	1286 (52.6)
Yes	1114 (48.0)	45 (36.9)	1159 (47.4)
Alcohol use			
None	1382 (64.7)	69 (58.0)	1451 (64.4)
Moderate	626 (29.3)	44 (37.0)	670 (29.7)
Heavy	128 (6.0)	6 (5.0)	134 (5.9)
Body mass index			
Mean (STD)	20.6 (2.9)	23.8 (3.8)	20.7 (3.1)
Median (IQR)[B]	20.4 (3.5)	23.4 (4.9)	20.5 (3.7)
<18.5[B]	495 (22.9)	5 (4.4)	500 (22.0)
18.5-24.9	1531 (70.8)	72 (63.2)	1603 (70.4)
25-29.9	118 (5.5)	32 (28.1)	150 (6.6)
≥30	19 (0.9)	5 (4.4)	24 (1.0)
Random blood glucose			
Mean (STD)	5.2 (1.4)	6.4 (2.8)	5.2 (1.5)
Median (IQR)[B]	5.0 (1.7)	6.8 (5.1)	5.0 (1.8)
<7.7 mmol/l	1246 (94.8)	28 (70.0)	1274 (94.1)
≥7.7 mmol/l	68 (5.2)	12 (30.0)	80 (5.9)
Missing	1009	82	1091

Table 3.1 Patient characteristic at MDR TB treatment start	**No diabetes N=2323 (95.0) N (%)**	**Diabetes mellitus[A] N=122 (5.0) N (%)**	**Total N=2445 N (%)**
HIV status[B]			
Negative	1842 (95.2)	94 (100.0)	1936 (95.4)
Positive	93 (4.8)	0	93 (4.6)
Missing	388	28	416
Hepatitis (A, B, or C)			
Negative	2105 (90.6)	116 (95.1)	2221 (90.8)
Positive	218 (9.4)	6 (4.9)	224 (9.2)
Previous TB treatment[B]			
No	781 (34.1)	51 (42.9)	832 (34.5)
Yes	1513 (65.9)	68 (57.1)	1581 (65.5)
Any 2nd-line resistance			
No	2025 (88.3)	104 (87.4)	2129 (88.2)
Yes	269 (11.7)	15 (12.6)	284 (11.8)
AFB smear grade[B]			
Negative	610 (27.3)	11 (9.1)	621 (26.4)
1+	656 (29.4)	44 (36.4)	700 (29.7)
2+	360 (16.1)	31 (25.6)	391 (16.6)
3+	312 (14.0)	18 (14.9)	330 (14.0)
4+	297 (13.3)	17 (14.1)	314 (13.3)
Any lung cavity			
No	1616 (75.7)	95 (79.8)	1711 (75.9)
Yes	520 (24.3)	24 (20.2)	544 (24.1)
Disseminated TB			
No	1781 (83.4)	101 (84.9)	1882 (83.5)
Yes	355 (16.6)	18 (15.1)	373 (16.5)
Extra-pulmonary TB[C]			
No	2190 (94.3)	117 (95.9)	2307 (94.4)
Yes	133 (5.7)	5 (4.1)	138 (5.6)

Abbreviations: MDR-multi-drug resistant; STD-standard deviation; IQR-interquartile range; AFB-acid fast bacilli

A. Based on medical records or self-reported by MDR TB patients

B. Statistically significant, two-sided p-value <0.05

C. All patients were pulmonary; extra-pulmonary includes those with both pulmonary and extra-pulmonary

Table 3.2. Diabetes mellitus and treatment outcomes among adult pulmonary MDR TB patients in Georgia 2009-2012

Outcome	No DM N/Total (%)	DM[A] N/Total (%)	RR (95% CI)[B]
WHO Defined Treatment, N=1524	**N=1442**	**N=82**	
	683/1442	*36/82*	*0.93 (0.72, 1.19)*
Favorable outcome	406 (28.2)	26 (31.7)	1.13 (0.81, 1.56)
Cured	277 (19.2)	10 (12.2)	0.64 (0.35, 1.15)
Completed			
	759/1442 (52.6)	*46/82 (56.1)*	1.07 (0.87, 1.30)
Poor Outcome	62 (4.3)	3 (3.7)	0.86 (0.27, 2.65)
Failed	535 (37.1)	36 (43.9)	1.18 (0.92, 1.53)
Defaulted	150 (10.4)	4 (4.9)	0.47 (0.18, 1.23)
Died	12 (0.8)	3 (3.7)	**4.63 (1.27, 15.27)**
Transferred			
Sputum Culture Result N=1467	**N=1388**	**N=79**	
Converted	942 (67.9)	58 (73.4)	1.08 (0.94, 1.24)
Time to conversion (days)			
Mean (STD)	94.1 (84.9)	92.2 (79.4)	
Median (IQR)[C]	69.0 (71.0)	63.5 (58.0)	

Abbreviations: DM-diabetes mellitus; MDR-multi-drug resistant; STD-standard deviation; IQR-interquartile range; AFB-acid fast bacilli

A. Self-reported by MDR TB patients

B. No diabetes was considered as the referent group

C. For comparison of medians, two-sided Wilcoxon p-value=0.86

Table 3.3. Bivariate and multivariable hazard rate ratios for patient characteristics associated with sputum TB culture conversion among MDR TB patients in Georgia, 2009-2012

Table 3.3 Patient characteristic	**Converted 1000/1467 (68.2) N/Total (%)**	**cHR (95% CI)**	**aHR (95% CI)[A]**
Diabetes Mellitus			
No	942/1388 (67.9)	1.00	1.00
Yes	58/79 (73.4)	1.11 (0.85, 1.45)	0.93 (0.71, 1.23)
Age (years)			
18-34	522/721 (72.4)	1.00	1.00
35-44	222/355 (62.5)	0.82 (0.70, 0.96)	**0.84 (0.71, 0.99)**
45-54	147/233 (63.1)	**0.82 (0.68, 0.98**)	0.87 (0.72, 1.05)
≥55	109/158 (69.0)	1.07 (0.87, 1.31)	1.03 (0.83, 1.27)
Sex			
Female	209/286 (73.1)	1.00	1.00
Male	791/1181 (67.0)	**0.82 (0.70, 0.96)**	1.05 (0.88, 1.24)
Ever imprisoned[B]			
No	652/927 (70.3)	1.00	
Yes	348/540 (64.4)	**0.85 (0.74, 0.97)**	
Current Smoker			
No	575/790 (72.8)	1.00	1.00
Yes	425/677 (62.8)	**0.77 (0.67, 0.87)**	**0.82 (0.71, 0.94)**
Alcohol use			
None	698/995 (70.2)	1.00	1.00
Moderate	257/387 (66.4)	0.89 (0.77, 1.02)	0.99 (0.84, 1.16)
Heavy	45/85 (52.9)	**0.62 (0.46, 0.83)**	0.74 (0.54, 1.01)
Body mass index			
<18.5	168/321 (52.3)	**0.64 (0.54, 0.76)**	**0.64 (0.54, 0.76)**
18.5-24.9	661/929 (71.2)	1.00	1.00
≥25	95/111 (85.6)	**1.36 (1.10, 1.69)**	**1.41 (1.13, 1.76)**
Missing	76/106 (71.7)	**0.97 (0.77, 1.23)**	0.95 (0.75, 1.20)
Random blood glucose			
<7.7 mmol/l	433/625 (69.3)	1.00	
≥7.7 mmol/l	34/49 (69.4)	0.90 (0.63, 1.28)	
Missing	533/793 (67.2)	**0.86 (0.76, 0.97)**	
HIV status			
Negative	797/1152 (69.2)	1.00	
Positive	29/54 (53.7)	0.80 (0.55, 1.16)	
Missing	174/261 (66.7)	0.99 (0.84, 1.17)	
Hepatitis (A, B, or C)			
Negative	909/1317 (69.0)	1.00	
Positive	91/150 (60.7)	0.92 (0.74, 1.14)	
Previous TB treatment			
No	358/468 (76.5)	1.00	1.00
Yes	642/999 (64.3)	**0.76 (0.66, 0.86)**	**0.80 (0.70, 0.91)**

Table 3.3 Patient characteristic	**Converted 1000/1467 (68.2) N/Total (%)**	**cHR (95% CI)**	**aHR (95% CI)[A]**
Any 2nd-line resistance			
No	934/1289 (72.5)	1.00	
Yes	66/178 (37.1)	**0.44 (0.34, 0.56)**	
AFB smear grade			
Negative	284/409 (69.4)	1.00	
1+	311/433 (71.8)	0.87 (0.74, 1.02**)**	
2+	165/230 (71.7)	0.84 (0.70, 1.02)	
3+	137/206 (66.5)	**0.71 (0.58, 0.87)**	
4+	103/189 (54.5)	**0.51 (0.41, 0.64)**	
AFB smear grade			
Negative/1+/2+	760/1072 (70.9)	**1.00**	
3+/4+	240/395 (60.8)	**0.67 (0.58, 0.77)**	
Any lung cavity			
No	779/1078 (72.3)	1.00	1.00
Yes	221/389 (56.8)	**0.62 (0.54, 0.72)**	**0.73 (0.62, 0.85)**
Disseminated TB			
No	849/1224 (69.4)	1.00	
Yes	151/243 (62.1)	**0.83 (0.69, 0.98)**	
Extra-pulmonary TB[C]			
No	954/1376 (69.3)	1.00	1.00
Yes	46/91 (50.6)	0.87 (0.75, 1.01)	**0.86 (0.74, 1.00)**

Table 3 Abbreviations: MDR-multi-drug resistant; cHR-crude hazard rate ratio; aHR-adjusted hazard rate ratio; AFB-acid fast bacilli; **Bold** indicates statistically significant, two sided p-value <0.05

A. The adjusted stratified model included all variables with estimates in the aHR column: age, sex, smoking status, alcohol use, body mass index, and previous TB treatment
B. Missing data for this variable was recoded into no/null category
C. All patients were pulmonary, extra-pulmonary includes those with both pulmonary and extra-pulmonary TB

Table 3.4. Bivariate and multivariable analyses of patient characteristics associated with cumulative risk of default during TB treatment among adult pulmonary MDR TB patients in Georgia 2009-2012

Table 3.4 **Patient characteristic**	**Default[A]** **571/1290 (44.3)** **N/Total (%)**	**cRR** **(95% CI) for default**	**aRR[B]** **(95% CI) for default**
Diabetes Mellitus			
No	535/1218 (43.9)	1.00	1.00
Yes	36/72 (50.0)	1.14 (0.90, 1.45)	1.14 (0.90, 1.44)
Age (years)			
18-34	291/667 (43.6)	1.00	--
35-44	138/305 (45.3)	1.04 (0.90, 1.21)	
45-54	90/188 (47.9)	1.10 (0.92, 1.30)	
≥55	52/130 (40.0)	0.92 (0.73, 1.15)	
Sex			
Female	82/263 (31.2)	1.00	1.00
Male	489/1027 (47.6)	**1.52 (1.26, 1.85)**	1.35 (1.10, 1.65)
Ever imprisoned[C]			
No	333/844 (39.2)	1.00	--
Yes	238/446 (53.4)	**1.35 (1.20, 1.53)**	
Current Smoker			
No	274/708 (38.7)	1.00	1.00
Yes	297/582 (51.0)	**1.32 (1.17, 1.49)**	**1.22 (1.07, 1.38)**
Alcohol use[C]			
None	372/888 (41.9)	1.00	--
Moderate	164/338 (48.5)	**1.16 (1.01, 1.32)**	
Heavy	35/64 (54.7)	**1.31 (1.03, 1.65)**	
Body mass index			
<18.5	121/233 (51.9)	**1.18 (1.02, 1.36)**	**1.19 (1.03, 1.36)**
18.5-24.9	380/861 (44.1)	1.00	1.00
≥25	40/103 (38.8)	0.88 (0.68, 1.13)	0.86 (0.67, 1.11)
Missing	30/93 (32.3)	**0.73 (0.54, 0.99)**	0.77 (0.57, 1.04)
Random blood glucose			
<7.7 mmol/l	265/552 (48.0)	1.00	--
≥7.7 mmol/l	16/39 (41.0)	0.85 (0.58, 1.26)	
Missing	290/699 (41.5)	**0.86 (0.76, 0.98)**	
HIV status			
Negative	454/1019 (44.6)	1.00	--
Positive	15/33 (45.5)	1.02 (0.70, 1.49)	
Missing	102/238 (42.9)	0.96 (0.82, 1.13)	
Hepatitis (A, B, or C)			
Negative	504/1167 (43.2)	1.00	--
Positive	67/123 (54.5)	**1.26 (1.06, 1.50)**	

Table 3.4 Patient characteristic	**Default[A] 571/1290 (44.3) N/Total (%)**	**cRR (95% CI) for default**	**aRR[B] (95% CI) for default**
Previous TB treatment[C]			
No	180/446 (40.4)	1.00	1.00
Yes	391/844 (46.3)	**1.15 (1.00, 1.31)**	1.10 (0.96, 1.25)
Any 2nd-line resistance[B]			
No	494/1168 (42.1)	1.00	
Yes	77/122 (63.1)	**1.49 (1.28, 1.74)**	--
AFB smear grade[B]			
Negative	155/408 (38.0)	1.00	1.00
1+	179/380 (47.1)	**1.24 (1.05, 1.46)**	1.17 (0.99, 1.37)
2+	85/193 (44.0)	1.16 (0.95, 1.42)	1.02 (0.83, 1.25)
3+	77/167 (46.1)	1.21 (0.99, 1.49)	1.07 (0.87, 1.31)
4+	75/142 (52.8)	**1.40 (1.14, 1.70)**	**1.29 (1.06, 1.57)**
Any lung cavity[C]			
No	427/992 (43.0)	1.00	--
Yes	144/298 (48.3)	1.12 (0.98, 1.29)	
Disseminated TB[C]			
No	476/1089 (43.7)	1.00	--
Yes	95/201 (47.3)	1.08 (0.92, 1.27)	
Extra-pulmonary TB[D]			
No	544/1222 (44.5)	1.00	--
Yes	27/68 (39.7)	0.89 (0.66, 1.20)	

Abbreviations: MDR-multi-drug resistant; cRR-crude risk ratio; aRR-adjusted risk ratio; AFB-acid fast bacilli; **Bold** indicates statistically significant, two sided p-value <0.05

A. Defaulted from therapy vs. successful (cured or completed by WHO definition) treatment outcome
B. Variables in the adjusted model included all those with reported aOR estimates.
C. Missing values were coded as no, none, or negative.
D. All patients were pulmonary, extra-pulmonary includes those with both pulmonary and extra-pulmonary TB

Figure 3.1. Study flow diagram of MDR TB patients in Georgia, 2009-2012

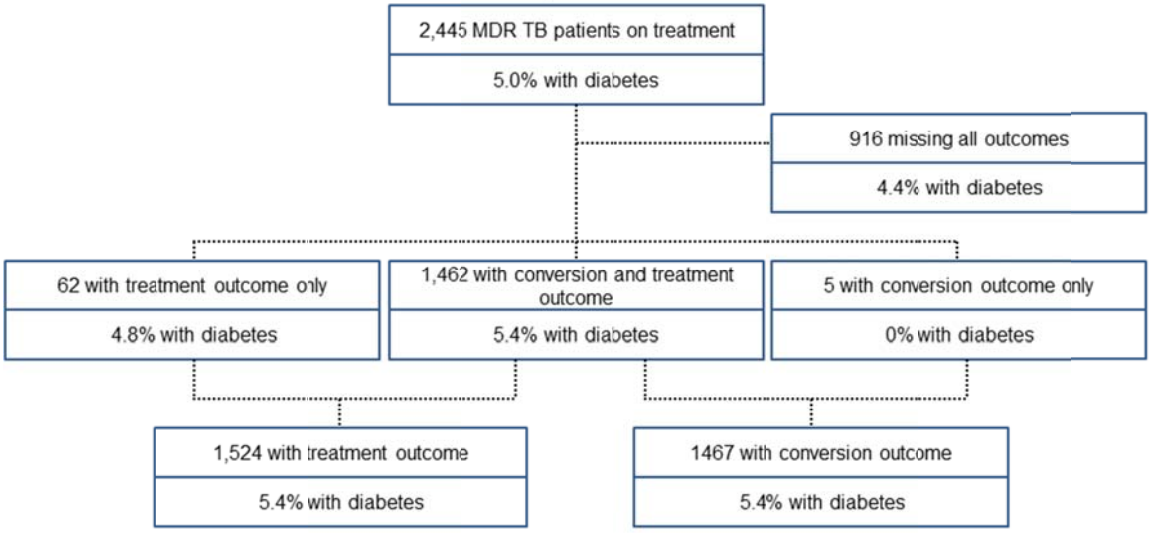

Table 3.5. Sensitivity analysis comparing baseline characteristics of patients with and without treatment outcome information among of adult pulmonary MDR TB patients in Georgia 2009-2012

Table 3.5 Patient characteristic at MDR TB treatment start	**Treatment outcome missing N=916 (37.5) N (%)**	**Treatment outcome recorded N=1529 (62.5) N (%)**	**Total N=2445 N (%)**	**P-value**
Diabetes				
No	876 (95.6)	1447 (94.6)	2323 (95.0)	0.27
Yes	40 (4.4)	82 (5.4)	122 (5.0)	
Age (years)				
18-34	436 (47.3)	753 (49.4)	1189 (48.6)	0.70
35-44	235 (25.5)	363 (23.8)	598 (24.5)	
45-54	156 (16.9)	247 (16.2)	403 (16.5)	
≥55	94 (10.2)	161 (10.6)	255 (10.4)	
Sex				
Female	160 (17.5)	298 (19.5)	458 (18.7)	0.21
Male	756 (82.5)	1231 (80.5)	1987 (81.3)	
Ever imprisoned				
No	488 (53.3)	967 (63.2)	1455 (59.5)	<0.01
Yes	428 (46.7)	562 (36.8)	990 (40.5)	
Current smoker				
No	462 (50.4)	824 (53.9)	1286 (52.6)	0.10
Yes	454 (49.6)	705 (46.1)	1159 (47.4)	
Alcohol use				
None	610 (66.6)	1031 (67.4)	1641 (67.1)	0.19
Moderate	264 (28.8)	406 (26.6)	670 (27.4)	
Heavy	42 (4.6)	92 (6.0)	134 (5.5)	
Body mass index				
<18.5	166 (19.4)	334 (23.5)	500 (22.0)	0.05
18.5-24.9	632 (73.7)	971 (68.4)	1603 (70.4)	
25-29.9	50 (5.8)	100 (7.1)	150 (6.6)	
≥30	10 (1.2)	14 (1.0)	24 (1.0)	
HIV status				
Negative	742 (81.0)	1194 (78.1)	1936 (79.2)	0.14
Positive	36 (3.9)	57 (3.7)	93 (3.8)	
Missing	138 (15.1)	278 (18.2)	416 (17.0)	
Previous TB treatment				
No	381 (41.6)	483 (31.6)	864 (35.3)	<0.01
Yes	535 (58.4)	1046 (68.4)	1581 (64.6)	

Table 3.5 Patient characteristic at MDR TB treatment start	**Treatment outcome missing N=916 (37.5) N (%)**	**Treatment outcome recorded N=1529 (62.5) N (%)**	**Total N=2445 N (%)**	**P-value**
Any 2nd-line resistance				
No	816 (89.1)	1345 (88.0)	2161 (88.4)	0.40
Yes	100 (10.9)	184 (12.0)	284 (11.6)	
AFB smear grade				
Negative/1+/2+	675 (73.7)	1126 (73.6)	1801 (73.7)	0.98
3+/4+	241 (26.3)	403 (26.4)	644 (26.3)	
Any lung cavity				
No	774 (84.5)	1127 (73.7)	1901 (77.8)	<0.01
Yes	142 (15.5)	402 (26.3)	544 (22.2)	
Disseminated TB				
No	792 (86.35)	1280 (83.7)	2072 (84.7)	0.07
Yes	124 (13.5)	249 (16.3)	373 (15.3)	

Abbreviations: MDR-multi-drug resistant; STD-standard deviation; IQR-interquartile range; AFB-acid fast bacilli

A. Based on medical records or self-reported by MDR TB patients

B. Statistically significant, two-sided p-value <0.05

C. All patients were pulmonary; extra-pulmonary includes those with both pulmonary and extra-pulmonary

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CHAPTER 4: MORTALITY DURING TUBERCULOSIS TREATMENT AMONG PATIENTS WITH DIABETES MELLITUS IN THE STATE OF GEORGIA

CHAPTER 4 ABSTRACT

Background: Little is known about the relation between diabetes mellitus (DM) and time to death among patients receiving TB treatment in the United States (US). The primary objectives of this study were to 1) compare the demographic and clinical presentation characteristics of adult patients with TB and DM, TB and HIV, and TB without HIV or DM; 2) estimate the association between DM and time until death during TB treatment.

Methods: A retrospective cohort study of consecutively reported TB cases in the state of Georgia was conducted between 2009 and 2012. Patients were classified by DM and HIV status at time of TB diagnosis and followed during TB treatment to determine mortality status. Cox proportional hazard ratios (HR) and 95% confidence intervals (CI) were used to estimate the association between DM and death. Logistic models were used to assess the association between DM and site of EPTB.

Results: Among 1,325 TB patients, 151 (11.5%) also had DM, 147 (11.2%) had HIV, and 7 (0.5%) had both DM and HIV. Compared to patients with TB only, those with TB and DM were more likely to have lung cavitation (51.0% vs. 34.7%), while patients with TB and HIV were more likely to have milliary TB (12.9% vs. 3.4%) and resistance to rifampin or isoniazid (21.8% vs. 9.0%) (p-value <0.01). Overall 83 (6.4%) TB patients died. Compared to TB patients without DM, the hazard of death during TB treatment was greater among TB patients with DM (crude HR 1.88, 95% CI 1.10, 3.20). After adjusting for potential confounders, DM was not statistically significantly associated with time until death during TB treatment (adjusted HR 1.22, 95% CI 0.70, 2.12).

Conclusions: Our findings suggest TB patients with DM may not have increased risk of all-cause mortality during TB treatment. Current TB treatment guidelines may not require modification to improve treatment outcomes in TB patients with DM.

INTRODUCTION

The incidence of active tuberculosis (TB) in the US has declined monotonically during the past two decades from 26,673 reported TB cases (10.4 per 100,000) in 1992 to 10,528 reported cases (3.4 cases per 100,000) in 2011.[1] Mortality among TB patients in the US has also decreased. In 1992 the TB-specific mortality rate was 0.7 per 100,000 and by 2011 the mortality rate decreased to 0.2 per 100,000.[1] Despite decreases in active TB cases and TB mortality, subgroups of TB patients remain at higher risk of death. Increased TB mortality has consistently been reported among patients with multidrug-resistant (MDR) TB,[2-4] HIV,[5,6] extrapulmonary TB,[7,8] substance abuse,[9,10] and concurrent chronic non-communicable diseases.[11,12] Diabetes mellitus (DM), a non-communicable disease with high and rapidly expanding prevalence in the US,[13] increases the risk of active TB approximately 3-fold,[14] and may affect clinical outcomes including TB mortality.[15] A 2011 meta-analysis of 23 studies estimated that mortality was more likely among TB patients with DM (TB-DM) compared to those without DM (unadjusted risk ratio [RR] 1.89, 95% CI 1.53, 2.36).[16]

The prevalence of DM among new TB patients in the US has not been historically reported by national surveillance systems but regional studies have reported DM prevalence between 14—28% among US adults with TB.[17-19] Studies in the US have also reported that compared to TB patients without DM, TB patients with DM were more likely to have cavitary disease and required longer time to convert sputum cultures from positive to

negative.[18,20] However, few studies have estimated the adjusted effect of DM on TB mortality in the US.

There were two primary objectives of this study. First we aimed to compare the demographic and clinical presentation characteristics of adult patients with TB and DM, TB and HIV, and TB without HIV or DM. The second aim was to estimate the association between DM and time until death during TB treatment.

METHODS

Setting and Participants

A retrospective cohort of all TB cases reported between January 2009 and September 2012 in the state of Georgia, USA, was created. In Georgia, TB is a reportable disease. All physicians, laboratories, and other health care providers are required by law to report clinical and laboratory confirmed TB cases to the public health department.[21] Eligible study patients included all confirmed TB patients aged ≥16 years reported to the Georgia state registry during the study period. Baseline data was collected on all TB patients who were then followed during TB treatment until the date of therapy completion, death, loss to follow-up, or until March 2013, whichever occurred first.

Study Measures and Data Collection

The Georgia Department of Public Health (GDPH) is responsible for systematic collection of all patient information for reported TB cases in the state. The GDPH verified reported TB cases, administered directly observed therapy (DOT), monitored patients until therapy completion, and entered data into electronic databases. Standardized TB reporting forms documented TB diagnosis, patient demographic and clinical characteristics, and treatment outcomes. All TB case information was entered into the State Electronic Notifiable Disease Surveillance System (SendSS), a secure GDPH web-based software tool.

The primary study outcome was time until death, measured among patients who died from any cause during TB treatment. Patients with a date of death (determined from reported date of death in SendSS) prior to TB treatment completion date were defined as a death during TB treatment. Time to death was calculated as the number of days between TB treatment start and death date.

The primary exposures of interest in this study were DM status and HIV status. Medical records and patient charts were reviewed to determine DM status. All patients were asked if they had ever been diagnosed with DM, patients who self-reported having DM were categorized as DM patients. Patients were not systematically screened for DM. Standard state TB protocols included offering all TB patients HIV screening for with an Enzyme-Linked Immunoabsorbent Assay (ELISA) test, those screened ELISA positive were confirmed by Western Blot.

Additional TB patient covariates of interest measured by interview in the cohort included demographic information, socio-behavioral characteristics, concomitant disease, and TB clinical features. Multidrug-resistant (MDR) TB was defined as at least resistance to rifampin and isoniazid. Occupation, country of birth, history of homelessness, correctional facility history, excess alcohol use in the past year, and illicit drug use were self-reported during patient interviews conducted by physicians or public health staff. End stage renal disease (ESRD) was determined from medical records and patient charts. Sputum acid fast bacilli (AFB) smear status, *Mycobacterium tuberculosis* complex culture, previous history of TB, tuberculin skin test (TST) result, chest radiograph information (presence of lung cavity), and TB drug susceptibility information was abstracted from GDPH laboratory and medical records in SendSS.

Data Analyses

All abstracted data from GDPH's SendSS database were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC). The association between patient characteristics with TB-DM, TB-HIV, and TB only was analyzed using bivariate analyses. The $\chi 2$ test was used to calculate p-values for categorical variables, ANOVA procedures were used to compare differences in normally distributed continuous variables (means), and the Kruskal-Wallis test was used for comparison of non-normally distributed variables (medians). A two-sided p-value less than 0.05 was considered statistically significant throughout the analyses. Cox proportional hazards regression models were used to estimate the hazard rate ratios (HR) and 95% confidence intervals (CI) for time to mortality during TB treatment. Patients were censored at the time of treatment completion or last documented clinical visit date if death did not occur on or before either date of completion or last visit date. Proportional hazard assumptions were assessed graphically (log negative log curves), with goodness-of-fit tests (Schoenfeld residuals) and using time-dependent models.[22] Selected covariates considered to be known confounders were included in Cox regression models based on significant bivariate associations with the primary exposure and outcomes, previous literature, or directed acyclic graph theory.[23] Statistical interaction was assessed between DM and all covariates included in the final Cox model.

Ethical approval

The study was reviewed by the Georgia Department of Public Health Institutional Review Board (IRB) and was determined to be exempt from full review.

RESULTS

A total of 1,428 TB patients were reported to the state of Georgia during the study period and 103 patients <16 years of age were excluded. A total of 1,325 were included in baseline analyses. Among all patients, 1,238 (93.4%) had TB treatment follow-up information available and were used in longitudinal analyses. Patients who died before TB treatment initiation (N=34) or who had no TB treatment follow-up information (N=53) were excluded from longitudinal analyses.

Among the 1,325 TB patients, DM was prevalent in 151 (11.4%), 147 patients had HIV (11.1%), and seven (0.5%) patients had both DM and HIV. Most patients were male (66.5%), Non-Hispanic black (48.2%), US born (54.6%) and the median age was 45 years (Table 1). Nearly 10.0% of TB patients were recently homeless, 8.4% were diagnosed with TB in a correctional facility, 9.7% used illicit drugs, and 2.6% had ESRD. Compared to TB patients without DM and without HIV (TB only), TB-DM patients had higher prevalence of ESRD (6.6% vs. 1.6%) but were less likely to be diagnosed with TB in a correctional facility (2.0% vs. 9.4%) (p-value <0.01). Patients with TB and HIV were more likely to report heavy alcohol use (25.2% vs. 14.5%), illicit drug use (28.0% vs. 7.7%), and recent homelessness (29.2% vs. 7.1%) than TB only patients (p-value <0.01).

Clinical TB characteristics at baseline differed among TB-DM patients, TB-HIV patients, and TB patients without DM and without HIV (Table 1). Any EPTB was more common among TB-HIV patients (38.1%) than TB-DM (25.9%) or TB only (26.6%) patients (p-value <0.01). Patients with TB and DM had the highest prevalence of lung cavitation (51.0%) compared to patients with TB and HIV (19.9%) and patients with TB only (34.7%) (p-value <0.01). Compared to TB only patients, TB-HIV patients had significantly higher prevalence of milliary TB (12.9% vs. 3.4%). More TB-DM patients

(52.2%) were AFB smear positive compared to TB-HIV patients (43.7%) and TB only (41.4%) patients (p-value =0.06). The overall prevalence of MDR was low (0.6%), but more TB-HIV patients had resistance to rifampin or isoniazid (21.8%) compared to TB-DM patients (8.3%) or TB only (9.0%) patients (p-value <0.01).

During TB treatment, 83 (6.4%) patients died including 5.2% (52/1020) of TB only patients, 10.8% (16/151) of TB-DM, 10.1% (14/147) of TB-HIV, and 14.3% (1/7) of patients with DM, HIV, and TB, and (p-value <0.01). Among TB patients who completed treatment and did not die, the median treatment time was 212 days (interquartile range [IQR] 99 days). Compared to TB only patients, the unadjusted hazard of time to death during treatment was greater among TB-DM (crude hazard rate ratio [cHR] 1.88, 95% CI 1.10, 3.20) (Table 2 and Figure 1). After adjusting for covariates significantly associated with both mortality during TB treatment and DM status (age, sex, race, occupation, birthplace, alcohol use, HIV status, and culture status) the adjusted hazard of death among TB-DM patients was 1.22 (95% CI 0.70, 2.12) times the hazard of death among TB patients without DM.

DISCUSSION

In this retrospective cohort of adult TB patients from the US state of Georgia, we estimated DM to be associated with a nearly two-fold unadjusted hazard rate of mortality during TB treatment. After adjusting for age and other important confounders, TB-DM patients, compared to TB patients without DM, did not have significantly higher hazard of death during TB treatment (aHR 1.15, 95% CI 0.66, 2.02). We also found that 27.9% of all TB patients in this cohort had at least one EPTB site, including among 26.6% of TB-DM patients.

The majority of previous studies that have examined mortality in TB patients with DM have reported an increased risk of death among TB-DM patients. For example, a 2011 systematic review by Baker et al. showed that 95.5% (21 of 23) of studies found an increased unadjusted risk of death among TB-DM patients when compared to TB patients without DM.[16] However, the analysis had important limitations. First, follow-up time and mortality measurement was inconsistent across studies, some followed patients to the end of TB treatment while others followed patients beyond TB treatment completion. Second, of the 21 studies that reported increased unadjusted mortality risk, only 9 were powered to detect a statistically significant difference in the comparison, and only 4 studies adjusted for age and other confounders. Our unadjusted estimate (cHR 1.88, 95% CI 1.10, 3.20) for death during treatment was remarkably similar to the unadjusted pooled risk ratio (RR) in the systematic analysis (RR 1.89, 95% CI 1.52, 2.36).

In the US only three studies, all from Maryland, have estimated the effect of DM on mortality during TB treatment.[17,18,24] A study by Dooley et al.[18] was the only work to estimate the association between DM and mortality during TB treatment with a model that also adjusted for age and HIV status, but this study had low precision (aOR 6.70, 95% CI 1.11, 38.20). Two other studies from Maryland estimated the odds of death during TB treatment comparing patients with and without DM after adjusting only for age. Fielder et al.[24] estimated the odds of death among TB-DM was 3.80 (95% CI 1.42, 10.16) times the odds of death among DM only patients, while Oursler et al.[17] estimated the same measure of effect at 6.70 (95% CI 1.57, 28.52).

Only three previous studies reported the use of survival analysis to estimate the association between DM and time to death in TB patients. First, in a study that adjusted for age in a Cox proportional hazards model, Oursler et al reported that the hazard of death in

TB-DM patients was greater than among TB only patients (aHR 4.7, 95% CI 1.9, 12.5).[17] Second, a Korean study that followed patients one year after TB treatment initiation to determine time until death demonstrated a significant increased hazard of TB-DM mortality compared to TB patients without DM (aHR 2.18 95% CI 1.10, 4.34).[25] Third, during the first 100 days after initiating treatment, a Tanzanian study reported that the hazard of death among TB-DM patients was greater than in TB patients without DM (aHR 5.09 95% CI 2.36, 11.02).[26] Our findings differed from these two studies. After adjusting for age, HIV, and other important confounders, our estimated HR for the effect of DM on time to death during TB treatment was no longer significantly different than TB patients without DM.

Several hypotheses have been postulated to explain mechanisms that lead to increased risk of mortality in patients with TB and DM. Both mouse and animal models have demonstrated that DM alters both adaptive and cell-mediated immune responses.[27-29] Impaired alveolar macrophage activation and subsequent granuloma formation may occur in TB-DM patients due to glycation of binding sites.[30] In addition, altered T-helper 1 and T-helper 2 cytokine responses have been demonstrated among patients with TB-DM.[28] Chronic hyperglycemia may disrupt the regulation of key cytokines, such as interferon-gamma,[29,31] which in turn may increase the *M. tuberculosis* bacterial burden and subsequent risk of death in TB-DM patients. However, whether observed differences in immune responses to TB among patients with DM cause increased clinical severity or directly increase risk of TB death remains understudied.

There are important limitations to note in our study. First, we relied on self-report and medical chart abstraction to determine whether TB patients had DM. Because we did not systematically measure DM status, the primary exposure of interest was subject to misclassification due to TB patients who did not know they had DM or who had never been

screened. However, patients who were classified with DM were unlikely to be non-DM patients, consequently the specificity of DM measurement in our cohort was likely high. Moreover, we do not have reason to believe that the misclassification of DM status was differential with respect to mortality during TB treatment and therefore our estimated effect of the dichotomous exposure (DM) on the outcome (death) is plausibly biased toward the null.[32] Second, our study did not have measures of glucose control or DM duration and therefore we could not estimate the effect of hyperglycemia or chronic DM (vs. acute hyperglycemia). If the effect of DM on mortality during TB treatment is modified by blood glucose level, our estimated null effect could be due to a mixing of the DM patients with higher blood glucose (and higher mortality risk) and those with controlled blood glucose (and lower mortality risk). Third, we did not have any measurements of body mass index (BMI) or anthropometry of TB patients. Similar to measures of glucose control, the effect of DM on mortality during TB treatment may be modified by BMI and our study was unable to assess this potential relationship. Finally, although all-cause mortality in this study was well documented during TB treatment, we were unable to determine if the cause of death was specific to TB disease. Similarly, we did not assess mortality after patients completed TB treatment and were therefore unable to estimate the association between DM and mortality in patients who may have died from TB disease after treatment ended.

Our study had excellent follow-up information from a large, well-characterized cohort of TB patients from US, a major strength of this study. Unlike previously published studies estimating the association between DM and mortality during TB treatment, we were able to estimate the association between DM and time to death adjusting for age, HIV status, and other potentially important confounders. The scope of our study was also innovative. Only one previous study has examined the association between DM and time to death

during TB treatment in the US. To our knowledge, no previous studies have compared the clinical presentation of TB-DM patients to clinical characteristics of TB-HIV patients.

Conclusion

With an increasing prevalence of DM across the world, it is critical to improve knowledge regarding the effects of the disease on public health, including co-morbidity with infectious diseases. Our results suggest that patients with DM and TB have more severe clinical symptoms at the beginning of TB treatment. However, this study challenges previous studies that reported unadjusted measures of association between DM and mortality. Earlier studies concluded that DM influences risk of death in TB patients, but we did not find a clinically meaningful association between DM and the hazard of all-cause mortality after adjusting for age, HIV, and other potential confounding factors. Nonetheless, more studies are needed to determine if well-controlled blood glucose in patients with TB-DM reduces the risk of mortality during TB treatment.

Table 4.1. Diabetes mellitus, HIV, and baseline characteristics of adult TB patients in the state of Georgia 2009-2012

TABLE 4.1 Patient characteristic (at TB treatment start)	**No HIV/DM N=1020 (77.4) N (%)**	**HIV N=147 (11.2) N (%)**	**DM[A] N=151 (11.5) N (%)**	**Total[B] N=1318 N (%)**	**P-value**
Age (years)					
Mean (STD)	44.3 (17.8)	41.2 (10.0)	57.4 (14.4)	45.5 (17.3)	<0.01
Median (IQR)[C]	43.0 (27.0)	43.0 (16.0)	56.0 (19.0)	45.0 (26.0)	
16-24[B]	151 (14.8)	9 (6.1)	0	160 (12.1)	<0.01
25-34	217 (21.3)	33 (22.5)	12 (8.0)	262 (19.9)	
35-44	164 (16.1)	46 (31.3)	11 (7.3)	221 (16.8)	
45-54	195 (19.1)	51 (34.7)	48 (31.8)	294 (22.3)	
55-64	144 (14.1)	7 (4.8)	35 (23.2)	186 (14.1)	
≥65	149 (14.6)	1 (0.7)	45 (29.8)	195 (14.8)	
Sex					
Female	353 (34.6)	40 (27.2)	48 (31.8)	441 (33.5)	0.19
Male	667 (65.4)	107 (72.8)	103 (68.2)	877 (66.5)	
Race/Ethnicity					
NH Black[C]	447 (43.9)	114 (77.5)	73 (48.3)	634 (48.2)	<0.01
NH Asian	206 (20.2)	6 (4.1)	22 (14.6)	234 (17.8)	
NH White	171 (16.8)	7 (4.8)	26 (17.2)	204 (15.5)	
Hispanic	194 (19.1)	20 (13.6)	30 (19.9)	244 (18.5)	
Occupation[C]					
Employed	426 (41.8)	44 (29.9)	48 (31.8)	518 (39.3)	<0.01
Unemployed	347 (34.0)	94 (64.0)	53 (35.1)	494 (37.5)	
Retired	108 (10.6)	1 (0.7)	33 (21.9)	142 (10.8)	
Other/unknown[D]	139 (13.6)	8 (5.4)	17 (11.3)	164 (12.4)	
Foreign born[C]					
No	516 (50.8)	109 (74.2)	92 (60.9)	717 (54.6)	<0.01
Yes	500 (49.2)	38 (25.9)	59 (39.1)	597 (45.4)	
Recent homelessness					
No	942 (92.9)	102 (70.8)	139 (92.7)	1183 (90.4)	<0.01
Yes	72 (7.1)	42 (29.2)	11 (7.3)	125 (9.6)	
In correctional facility when TB diagnosed[B]					
No	920 (90.6)	133 (91.7)	147 (98.0)	1200 (91.6)	0.01
Yes	95 (9.4)	12 (8.3)	3 (2.0)	110 (8.4)	
Heavy alcohol use					
No	858 (85.5)	107 (74.8)	129 (86.0)	1094 (84.4)	<0.01
Yes	146 (14.5)	36 (25.2)	21 (14.0)	203 (15.7)	

TABLE 4.1 Patient characteristic (at TB treatment start)	**No HIV/DM N=1020 (77.4) N (%)**	**HIV N=147 (11.2) N (%)**	**DM[A] N=151 (11.5) N (%)**	**Total[B] N=1318 N (%)**	**P-value**
Illicit drug use					
No	928 (92.3)	103 (72.0)	142 (94.0)	1173 (90.3)	<0.01
Yes	77 (7.7)	40 (28.0)	9 (6.0)	126 (9.7)	
End stage renal disease[C]					
No	1004 (98.4)	139 (94.6)	141 (93.4)	1284 (97.4)	<0.01
Yes	16 (1.6)	8 (5.4)	10 (6.6)	34 (2.6)	
TB Characteristics					
AFB smear status					
Negative	542 (58.6)	76 (56.3)	64 (47.8)	682 (57.1)	0.06
Positive	383 (41.4)	59 (43.7)	70 (52.2)	512 (42.9)	
Unavailable	95	12	17	124	
Baseline culture					
Negative	177 (17.4)	26 (17.7)	18 (11.9)	221 (16.8)	0.53
Pulm TB positive	576 (56.5)	89 (60.5)	89 (58.9)	754 (57.2)	
EPTB positive	192 (18.8)	24 (16.3)	34 (22.5)	250 (19.0)	
Unavailable	75 (7.4)	8 (5.4)	10 (6.6)	93 (7.1)	
TB site of disease					
Pulm only	749 (73.4)	91 (61.9)	112 (74.2)	952 (72.2)	<0.01
Pulm and EPTB	66 (6.5)	30 (20.4)	14 (9.3)	110 (8.4)	
EPTB only	205 (20.1)	26 (17.7)	25 (16.6)	256 (19.4)	
Previous TB treatment					
No	959 (94.5)	135 (91.8)	142 (94.0)	1236 (94.1)	0.44
Yes	56 (5.5)	12 (8.2)	9 (6.0)	77 (5.9)	
TST status					
Negative	175 (23.5)	43 (55.8)	42 (42.4)	260 (28.3)	<0.01
Positive	569 (76.5)	34 (44.2)	57 (57.6)	660 (71.7)	
Not done/unknown	276	70	52	398	
Any lung cavity					
No	643 (65.3)	113 (80.1)	72 (49.0)	828 (65.0)	<0.01
Yes	342 (34.7)	28 (19.9)	75 (51.0)	445 (35.0)	
Milliary TB					
No	933 (96.6)	122 (87.1)	139 (96.5)	1194 (95.5)	<0.01
Yes	33 (3.4)	18 (12.9)	5 (3.5)	56 (4.5)	
DST profile					
None to RIF or	683 (90.6)	85 (77.3)	109 (90.1)	877 (89.0)	<0.01
INH	68 (9.0)	24 (21.8)	10 (8.3)	102 (10.4)	
RIF or INH	3 (0.4)	1 (0.9)	2 (1.7)	6 (0.6)	
MDR	266	37	30	333	
Unavailable					
TB Outcomes					

TABLE 4.1 Patient characteristic (at TB treatment start)	**No HIV/DM N=1020 (77.4) N (%)**	**HIV N=147 (11.2) N (%)**	**DM[A] N=151 (11.5) N (%)**	**Total[B] N=1318 N (%)**	**P-value**
Treatment duration (days)[E]					
Mean (STD)	221 (101)	282 (127)	250 (137)	231 (111)	<0.01
Median (IQR)	207 (90)	292 (164)	223 (112)	212 (99)	
Death before treatment initiation					
No	997 (97.8)	139 (94.6)	148 (98.0)	1284 (97.4)	0.07
Yes	23 (2.3)	8 (5.4)	3 (2.0)	34 (2.6)	
Death during TB treatment					
No	945 (94.8)	125 (89.9)	132 (89.2)	1202 (93.6)	<0.01
Yes	52 (5.2)	14 (10.1)	16 (10.8)	82 (6.4)	
Time to death during treatment (days)[F]					
Mean (STD)	81 (82)	77 (82)	82 (90)	81 (83)	
Median (IQR)	57 (82)	46 (139)	64 (112)	57 (87)	0.32
Death before or during TB treatment					
No	945 (92.7)	125 (85.0)	132 (87.4)	1202 (91.2)	<0.01
Yes	75 (7.4)	22 (15.0)	19 (12.6)	116 (8.8)	

Table 1. Abbreviations: DM-diabetes mellitus; STD-standard deviation; IQR-interquartile range; NH-Non-Hispanic; Pulm-pulmonary; EPTB-Extrapulmonary TB; RIF-Rifampin; INH-Isoniazid; AFB-acid fast bacilli; MDR-multi-drug resistant

A. Diabetes mellitus status was self-reported or from abstracted from medical records.
B. Patients with both HIV and DM (n=7) are excluded from the table.
C. Statistically significant, two-sided p-value <0.05
D. Other indicates disabled, not eligible for employment, student, or homemaker
E. Among patients with treatment completion date, excluding deaths during treatment (N=1155)
F. Among patients who died during TB treatment (N=83)

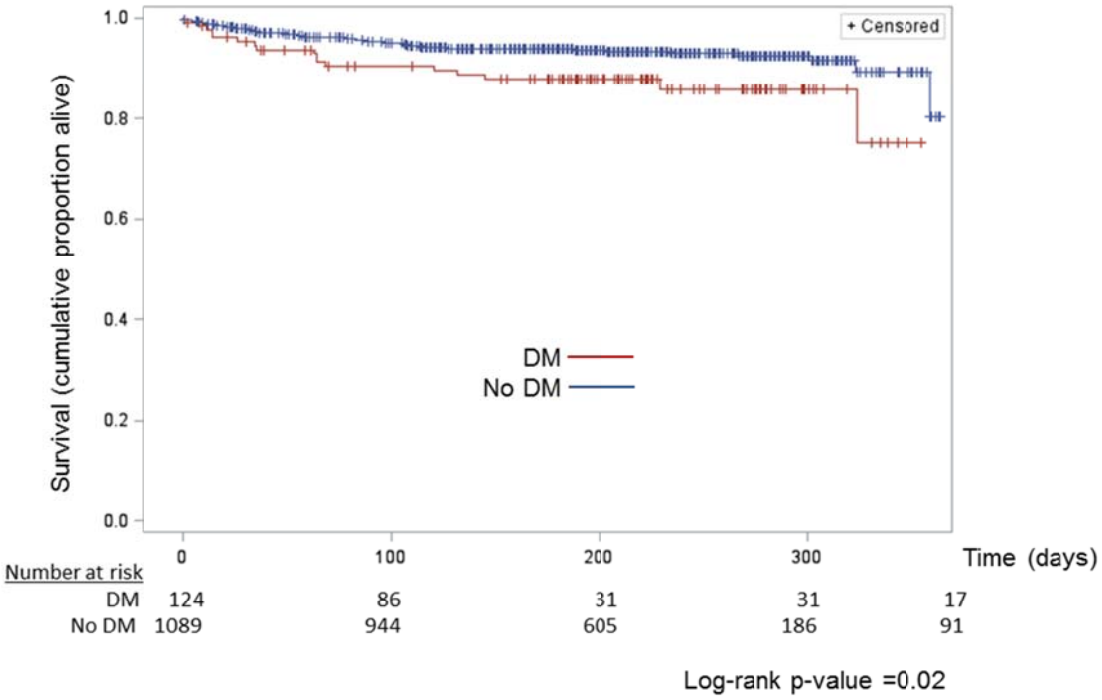
Table 4.2. Bivariate and multivariable hazard rate ratios for baseline patient characteristics associated with death during TB treatment among adult patients in the state of Georgia, 2009-2012

TABLE 4.2 **Patient characteristic** **(at TB treatment start)**	**Died** **N=83/1238 (6.7)** **Died/N (%)**	**cHR (95% CI)**	**aHR[A] (95% CI)**
Diabetes mellitus			
No	66/1089 (6.1)	1.00	1.00
Yes	17/149 (11.4)	**1.88 (1.10, 3.20)**	1.22 (0.70, 2.12)
Age (years)			
16-34	6/402 (1.5)	1.00	1.00
35-44	9/213 (4.2)	**2.78 (0.99, 7.8)**	2.26 (0.79, 6.46)
45-54	19/276 (6.9)	**4.58 (1.83, 11.48)**	**3.01 (1.14, 7.93)**
≥55	49/347 (14.1)	**10.24 (4.39, 23.92)**	**5.49 (2.15, 14.05)**
Sex			
Female	14/414 (3.4)	1.00	1.00
Male	69/824 (8.4)	**2.55 (1.44, 4.53)**	**2.34 (1.31, 4.20)**
Race/ethnicity			
White	20/194 (10.3)	1.00	1.00
NH Black	54/592 (9.1)	0.85 (0.51, 1.42)	1.06 (0.62, 1.82)
NH Asian	4/220 (1.8)	**0.17 (0.06, 0.49)**	0.37 (0.10, 1.41)
Hispanic	5/226 (2.2)	**0.21 (0.08, 0.56)**	0.64 (0.19, 2.17)
Occupation			
Employed	11/492 (2.2)	1.00	1.00
Unemployed	34/479 (7.1)	**3.17 (1.61, 6.26)**	**2.22 (1.09, 4.50)**
Retired	24/124 (19.4)	**9.87 (4.83, 20.16)**	**4.03 (1.83, 8.88)**
Other/unknown	14/143 (9.8)	**4.84 (2.20, 10.66)**	**4.42 (1.98, 9.86)**
Foreign born			
No	69/669 (10.3)	1.00	1.00
Yes	14/568 (2.5)	**0.24 (0.13, 0.42)**	0.84 (0.36, 1.97)
Recent homeless			
No	75/1111 (6.8)	1.00	
Yes	7/124 (5.7)	0.80 (0.37, 1.74)	
Currently in correctional facility	81/1132 (7.2)	1.00	
No	1/103 (1.0)	0.14 (0.02, 1.03)	
Yes			
Heavy alcohol use			
No/unknown	65/1047 (6.2)	1.00	1.00
Yes	18/191 (9.4)	1.51 (0.90, 2.55)	0.84 (0.48, 1.47)
Illicit drug use			
No	73/1113 (6.6)	1.00	
Yes	6/117 (5.1)	0.73 (0.32, 1.68)	
End stage renal disease			
No	75/1206 (6.2)	1.00	
Yes	8/32 (25.0)	**4.21 (2.03, 8.73)**	

TABLE 4.2 **Patient characteristic** **(at TB treatment start)**	**Died** **N=83/1238 (6.7)** **Died/N (%)**	**cHR (95% CI)**	**aHR[A] (95% CI)**
HIV status			
Negative/unknown	68/1098 (6.2)	1.00	1.00
Positive	15/140 (10.7)	1.59 (0.90, 3.54)	**1.99 (1.05, 3.75)**
AFB smear status			
Negative	33/655 (5.0)	1.00	
Positive	35/498 (7.0)	1.40 (0.87, 2.25)	
Unknown	15/85 (17.7)	**3.74 (2.03, 6.89)**	
Baseline culture			
Negative/unknown	12/298 (4.0)	1.00	1.00
Positive	71/940 (7.6)	**1.86 (1.01, 3.43)**	1.66 (0.89, 3.08)
TB site of disease			
Pulmonary only	56/913 (6.1)	1.00	
Pulmonary and extra-pulmonary	11/105 (10.5)	1.67 (0.87, 3.18)	
Extra-pulmonary only	16/220 (7.3)	1.09 (0.63, 1.91)	
Previous TB treatment			
No	78/1167 (6.7)	1.00	
Yes	4/70 (5.7)	0.86 (0.31, 2.34)	
Any lung cavity			
No	54/780 (6.9)	1.00	
Yes	28/427 (6.6)	0.95 (0.60, 1.51)	
Milliary TB			
No	78/1187 (6.6)	1.00	
Yes	5/51 (9.8)	1.51 (0.61, 3.72)	
Drug resistance profile			
None to RIF or INH	59/827 (7.1)	1.00	
RIF or INH	7/96 (7.3)	0.97 (0.44, 2.13)	
MDR	1/6 (16.7)	1.86 (0.26, 13.57)	
Unavailable	16/309 (5.2)	0.73 (0.42, 1.27)	

Table 2. Abbreviations: cHR-crude hazard ratio; aHR-adjusted hazard ratio; CI-confidence interval; STD-standard deviation; IQR-interquartile range; NH-Non-Hispanic; RIF-Rifampin; INH-Isoniazid; AFB-acid fast bacilli; MDR-multi-drug resistant
A. Adjusted HR model included all covariates with listed estimates in the last column. Covariates were chosen based on statistically significant associations with diabetes mellitus and death during TB treatment.

Figure 4.1. Unadjusted cumulative all-cause mortality among tuberculosis patients with and without diabetes mellitus one year from initiation of TB treatment



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CHAPTER 5: SUMMARY AND CONCLUSIONS

Although diabetes mellitus (DM) is an established risk factor for the development of active tuberculosis (TB) disease,[1, 2] the effects of DM on clinical TB disease remain difficult to describe definitively.[3, 4] Epidemiologic studies, including three presented in this dissertation, report heterogeneous results with respect to the relation between DM and 1) clinical TB severity at the time of presentation and 2) TB treatment outcomes. The three studies have contributed additional information to help clarify the association between DM and TB disease.

The first study reported findings consistent with many previously published results that explored the relation between DM and clinical TB symptoms at the time of TB treatment initiation. Like previous studies, our results suggested that compared to TB patients without DM, those patients with TB and DM (TB-DM) have more TB symptoms, including symptoms characteristic of more severe disease, at the time of TB diagnosis. Specifically, patients with TB-DM were more likely to present with symptoms of cough, cough with blood, and cavitary lung disease.

The second study was one of the first to explore the association between DM and time to sputum culture conversion in a cohort of patients with multi drug-resistant (MDR) TB. Previous studies suggest that TB patients with DM take longer to convert sputum cultures from positive to negative. Our findings, which showed little difference in time to sputum culture conversion among MDR TB patients with DM, challenged previous results.[4] Unlike previous studies, our analyses were conducted among a cohort of MDR TB patients. In addition, our study adjusted for important confounders including alcohol use, smoking status, and cavitary lung disease.

Third, we reported new data on the relation between DM and time to death during TB treatment in Chapter 4. The majority of prior studies that examined the association between DM and mortality have reported an increased risk of death among TB-DM patients.[4-7] However, the majority of previous studies of mortality among TB-DM patients did not adjust for important confounders such as age, HIV or other co-morbidities, smoking, or other factors associated with both DM status and risk of death. We also found a statistically significant unadjusted relation between DM and time to all-cause death during TB treatment, but after adjusting for important confounders the association between TB and DM moved toward the null.

Strengths and limitations

The three studies had several strengths. An important strength was that the first study, which examined the association between DM and TB clinical severity, enrolled only new TB patients. Because previous TB treatment history is commonly a key confounder in studies examining TB clinical presentation characteristics or TB treatment outcomes, by excluding patients with previous treatment history we excluded the possibility of confounding by previous treatment history. The first study also used a novel point-of-care screening test to measure blood glucose levels with glycated hemoglobin (HbA1c). Previous studies of TB-DM patients have typically relied on self-reported DM status or less accurate measures of blood glucose control (i.e., random blood glucose). By using HbA1c, we measured patients' 90-day average blood glucose levels and therefore the primary exposure in our study (DM status) was not influenced by daily fluctuations in blood glucose from regular variation in diet or time of day of measurement. The ability to categorize study participants as TB patients with normal glucose, pre-DM, or DM was another strength of

using HbA1c to measure DM status in the first study. To our knowledge, previous studies of TB-DM have not estimated the association of pre-DM with TB clinical characteristics.

The second study also had notable strengths. First, few previous studies of the effect of DM on MDR TB treatment outcomes exist. To our knowledge, the second study was the first to examine the effect of DM on 1) time to sputum culture conversion adjusted for potential confounders and 2) default from second-line treatment among MDR TB patients. In addition, all adult MDR TB patients from the country of Georgia were included during 2009 to 2012 so the results of our study were representative of all MDR TB patients in the country. Finally, Cox proportional hazards models used in the second study were adjusted for several important covariates, thereby we controlled for potential confounding factors (e.g., by including patient characteristics associated with both DM and culture conversion in multivariable models).

Key strengths of the third study included its novel objectives and the ability to control for confounding covariates. To our knowledge, only one previous study examined the association between DM and time to death during TB treatment in the US. In addition, we believe no previous studies have 1) compared the clinical presentation of TB-DM patients to clinical presentation characteristics of TB-HIV patients. Unlike previously published studies estimating the association between DM and mortality during TB treatment, we were able to estimate the hazard of all cause mortality using multivariable models with good statistical power adjusting for potentially important confounders.

The three studies also had noteworthy limitations. The first study was limited by small sample size. As a result, the generalizability of study findings from the first study to all new TB patients in the country of Georgia may be limited. Similarly, the statistical power to

detect differences between TB patients with and without DM in the study's secondary outcomes (two-month sputum acid fast bacilli [AFB] smear and culture conversion) was low.

The second study was limited by the classification of DM status. To determine if MDR TB patients had DM, the study relied on self-reported status and review of hospital records. However, systematic screening for blood glucose levels was not performed. Therefore, some MDR TB patients were likely classified as not having DM despite truly having high blood glucose consistent with DM. Another critical limitation from the second study was loss to follow-up of MDR TB patients. A large proportion of patients did not have medical or laboratory records available after the baseline enrollment, which could have biased the estimated relation between DM status and time to sputum culture conversion.

The primary limitation of the third study was potential misclassification of DM status, the primary exposure variable of interest. The second study used self-report or medical records review to determine DM status of TB patients. Although many TB patients received a fasting blood glucose measurement and were consequently diagnosed with DM, not all patients were systematically screened for DM with a measure of blood glucose.

All three studies shared two important limitations. First, all three studies had only one cross-sectional measurement of DM status. Because DM status was only measured at one time point at the beginning of TB treatment in all three studies, we could not differentiate between TB patients with DM (or pre-DM) and those who had temporary hyperglycemia induced by TB disease or any another temporary factor. Some of the TB patients categorized with DM (or pre-DM) may have returned blood glucose levels to normal during the course of TB treatment. Among TB patients who were accurately diagnosed with DM before becoming infected with TB, multiple measures of patient DM status would not be necessary. Nonetheless, in all three studies multiple measures of blood

glucose would have improved the ability to determine if effect modification existed between DM, blood glucose control, and TB treatment outcomes.

Second, all three studies were limited by the extent of TB drug regimen adherence during treatment. Patient adherence to TB drug regimens is an important factor that is strongly associated with successful response to treatment. For example, patients with poor adherence to TB regimens are more likely to remain smear and culture positive for longer time, more likely to default from treatment, and more likely to die during TB treatment. In addition, because TB treatment requires long duration (typically more than 6 months for drug susceptible TB and more than 24 months for MDR TB) of therapy, a high proportion of patients are likely to be non-adherent. The extent to which regimen adherence was associated with DM status was unknown in the three studies. Consequently, we were unable to measure potential confounding by this characteristic.

Remaining gaps in knowledge and future research recommendations

Additional research is greatly needed to better understand the association between TB patients' blood glucose levels and TB treatment outcomes. Few studies have described the trajectory of blood glucose levels among patients receiving anti-TB medications. Because infection with TB may cause immune-related acute inflammation, TB patients may have hyperglycemia at the time of TB diagnosis that results from the innate or adaptive immune response and not due to chronic metabolic complications typically associated with DM. The proportion of TB patients with hyperglycemia at the time of anti-TB therapy initiation that will return their blood glucose levels to normal after or during successful TB treatment is uknown. Adequately powered studies are needed to measure blood glucose levels at multiple times during and after the course of TB treatment. For example, we suggest measuring blood

glucose levels at the time of TB diagnosis, monthly during TB treatment, and six months after the completion of TB treatment. Such a study, linked with appropriate clinical TB outcome measures (TB severity at baseline, time to culture conversion, and TB final outcome), would help distinguish whether increased risk of poor TB outcomes is associated with a failure to reduce an initially high blood glucose during TB treatment. If consistent hyperglycemia during TB therapy is associated with slower culture conversion and/or higher risk of poor TB treatment outcomes (i.e., failure or death) a study of blood glucose trajectories during TB treatment would also help establish guidelines for targeted blood glucose levels that are appropriate for clinical interventions aimed at improving DM control during TB treatment.

Additional longitudinal cohort studies are needed to clarify the association between DM and key TB treatment outcomes including *Mycobacterium tuberculosis* complex culture conversion time and treatment result after completed therapy. After adjusting for confounders, our studies did not find a statistically significant association between DM and culture conversion, treatment success, or mortality. However, additional studies with improved measurements of DM and more frequent measurement of longitudinal TB culture status are needed. For example, a cohort of new TB patients screened could first be screened for DM at the time of TB diagnosis with both HbA1c and fasting blood glucose. Patients with TB who were newly diagnosed with DM should have the DM status confirmed with an oral glucose tolerance test. During the course of TB treatment all patients in the study should receive bi-weekly sputum culture tests to determine a precise date of culture conversion. In an ideal observational study, patients should also continue to receive bi-weekly culture tests even after initially converting to negative in order to determine if re-current positive cultures occur.

Experimental studies are also needed to better assess the effect of DM on TB treatment outcomes. While it is not feasible to randomly assign patients to the exposure of interest (DM) and determine TB treatment outcomes, alternative studies can be implemented that will help answer questions related to whether glucose control improves TB outcomes. For example, a cluster-randomized trial among TB clinics could randomize an intervention intended to improve DM care and glucose control among TB-DM patients. In the intervention arm TB clinics could implement regular glucose monitoring among TB-DM patients with initially high blood glucose levels. Pharmacotherapy with Metformin or other glucose control interventions intended to reduce hyperglycemia could be provided to TB-DM to determine if the level of blood glucose control is associated with *M. tuberculosis* sputum culture conversion time or final TB treatment outcome.

Prior studies suggest persons with DM are at increased risk of developing active TB disease.[1, 2] Whether the increased risk of active TB among persons with DM results from a greater likelihood of acquiring a new exogenous infection (i.e., being exposed to a person with infectious TB) or is due to reactivation of a previous latent infection (i.e., failing to maintain TB bacteria in a latent state within macrophages) remains understudied. Additional cohort studies of patients at risk of developing active TB are needed to explain why patients with DM have increased incidence of TB disease. For example, an ideal study would include a large cohort screened for DM and latent TB in a setting with high background prevalence of TB. Among patients identified to have DM, one could obtain follow-up information to determine if the incidence of active TB disease was different in those DM patients who had initial latent TB infection compared to those DM patients with no latent TB infection at baseline. The incidence rates of active TB in these two groups of patients with DM could also be compared to patients without DM who did and did not have latent TB infection at

the study's start. Cohort studies of DM patients screened for latent TB and followed for incident TB disease would help clarify whether DM patients with latent TB infection should be targeted for preventative TB prophylactic therapy.

Public health and clinical implications

The findings from the three studies indicate that considerable more epidemiologic data is needed to accurately characterize the burden conferred by DM on global TB control. Several recently published systematic reviews[1, 4, 8] and commentaries[3, 9, 10] suggest that important barriers to TB control, including increased TB incidence and greater risk of poor TB treatment outcomes, are attributable to DM. While the three studies do not address the impact of DM on TB incidence, we have explored the public health and clinical implications of DM on TB presentation and TB treatment outcomes.

The first study reported results consistent with previous publications that TB-DM patients have altered clinical presentation characteristics when compared to TB patients without DM. For example, most previous studies that examined sputum smear results among TB patients with and without DM reported a greater proportion AFB-positive[7, 11-15] and higher smear grade[14] among patients with DM. Also similar to the findings of our first paper, previously published studies also reported more cough[11, 12] and hemoptysis[11-13] among TB patients with DM. Finally, our first study findings were also consistent with earlier studies that reported more frequent lung cavitation[12-15] among TB patients with DM compared to TB patients without DM. If TB-DM patients have differences in clinical presentation of TB disease, this suggests they may also have different initial response to TB infection.[10] Moreover, because initial evidence suggests that TB-DM patients may have higher AFB smear grades and more severe cough, they also may be more likely to transmit TB infection to contacts. In settings that do not systematically conduct contact investigations

on all active smear-positive TB cases, prioritization of investigations could be justified for smear-positive TB-DM patients who may be more likely to infect contacts. In such cases of prioritizing contact investigations, many other transmission risk factors and disease characteristics of index TB patients must also be considered.

The primary results from the second paper are inconsistent with the findings of previously published studies that compared time to sputum culture conversion in TB patients with and without DM. Four previous studies among TB patients receiving first-line anti-TB therapy found that compared to patients without DM, TB patients with DM required more time to convert sputum cultures from positive to negative.[7, 16-18] However, most previous studies of TB-DM and culture conversion were among drug sensitive patients. The second study results were consistent with the estimated hazard of culture conversion in a study of MDR TB patients with and without DM from Peru, Latvia, Estonia, Russia, and Manila. Both studies showed that MDR TB patients were modestly but not significantly at risk of slower time to sputum culture conversion. Consequently, initial observational epidemiologic data suggest that the clinical impact of DM on TB culture conversion time may be important among drug susceptible TB patients but not among MDR TB patients. Thus preliminary studies imply that TB-DM patients on first-line anti-TB therapy may need additional culture monitoring or altered treatment regimens to ensure timely conversions. But very early evidence suggests that patients with MDR TB and DM may not be at increased risk of remaining sputum culture positive for longer periods of time and therefore altered second-line TB regimens may not be needed in this subgroup.

The third study results were inconsistent with the three previous studies that used survival analysis to estimate the association between DM and time to death in TB patients.[5, 6, 19] Previous studies have reported an increased hazard of death during TB treatment among

patients with TB-DM compared to TB patients without DM. Although our unadjusted findings also suggested a significantly increased hazard of death during TB treatment, after adjusting for age, HIV, and other important confounders, our estimated hazard for the effect of DM on time to death during TB treatment was no longer significantly different than TB patients without DM. While our third study did not measure DM care or DM medication use, anecdotal information indicated that most TB-DM patients received DM care. Previously published studies (mentioned above) also did not measure DM care characteristics. However, the discrepancy in risk of death among our study and previous studies is likely due (in part) to differences in DM care during TB treatment. Additional epidemiologic data is needed to estimate the effect of DM care on TB treatment outcomes of patients with TB and DM. If patients with good DM care (i.e., well-controlled blood glucose) are at decreased risk of death during TB therapy, guidelines for the treatment of DM among TB patients should include recommended strategies that lead to improved care for both TB and DM clinical outcomes.

Overall, we reported that TB patients with DM had more severe clinical TB symptoms at the time of TB diagnosis. All three studies found that compared to TB patients without DM, those patients with TB and DM were more likely to present with AFB smear positive TB or higher grade of AFB smear. Despite more severe clinical disease at the time of TB treatment initiation among TB-DM patients, we did not detect a clinically meaningful difference in response to anti-TB therapy in this group.

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