Diabetes Mellitus as a Risk Factor for Tuberculosis in HIV-Infected and Uninfected Populations

Rachel W. Kubiak

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2021

Reading Committee:

Paul K. Drain, Chair

Annette L. Fitzpatrick

Mario Kratz

Program Authorized to Offer Degree:

Epidemiology

University of Washington

Abstract

Diabetes Mellitus as a Risk Factor for Tuberculosis in HIV-Infected and Uninfected Populations

Rachel W. Kubiak

Chair of the Supervisory Committee:

Paul K. Drain

Departments of Medicine, Global Health, and Epidemiology

Tuberculosis (TB) is one of the leading infectious causes of morbidity and mortality worldwide. Diabetes and HIV are important risk factors that contribute to the persistently high TB incidence globally. However, the importance of diabetes on TB risk in key sub-populations is not well-established. Additionally, body mass index (BMI) affects both TB and diabetes risk, and may interact with diabetes to change the risk of TB. Finally, sub-clinical TB may also be an important contributor to TB-associated morbidity and mortality but is not well characterized.

We used data from two observational cohorts to: (1) estimate the combined impact of diabetes low and elevated BMI on latent and active TB burdens of disease in southern India; (2) estimate the prevalence of diabetes, associated risk factors, and its impact on 12-month clinical outcomes among adults testing positive for HIV in South Africa; and (3) estimate the prevalence of sub-

clinical TB in this same population of HIV positive adults in South Africa and associated demographic and clinical characteristics.

We found that diabetes was not associated with LTBI in Pondicherry, India. Diabetes-BMI interaction for active TB was statistically significant on both the additive and multiplicative scales. Compared to participants without diabetes, the greatest risk of active TB disease associated with diabetes was among overweight/obese. The burden of TB attributable to diabetes was highest in the low BMI group, suggesting routine screening of low BMI diabetic patients for active TB could be worthwhile in this setting.

Among people living with HIV in Durban, South Africa the prevalence of diabetes was moderate and associated with well-established, traditional risk factors. Limiting diabetes screening to BMI >25 kg/m^2 would capture 85% of participants who screened positive for diabetes, suggesting this could be a useful tool in resource limited settings. Over 12 months of follow-up, screening positive for diabetes was associated with a higher hazard of death but not other HIV-related outcomes. The prevalence of sub-clinical TB was 1.1% and not associated with any of the demographic or clinical characteristics we investigated.

A significant limitation to these findings was incomplete diabetes testing, which is logistically complex and expensive, highlighting an opportunity for diabetes testing strategies that can be more readily implemented worldwide. Additionally, our work provides support for prospective cohort studies of diabetes patients in more diverse settings. Successful clinical management of

incident active TB or HIV in the context of increasingly common non-communicable diseases and limited resources remains an important area of research.

Table of Contents

Acknowledgements

I am deeply grateful to my committee members, Drs. Paul Drain, Annette Fitzpatick, Ruanne Barnabas, Mario Kratz, and Elizabeth Brown for investing their limited time and immense experience in my professional growth. Throughout this long and changing process they modelled professional integrity and personal kindnesses that I aspire to. Special thanks to my committee chair, Dr. Paul Drain, for equipping me to accomplish more than I thought possible, ensuring my timelines were realistic, and showing consideration for me as a whole person. Thank you to Trish and Jay Wenger, my Mom and Dad. Since the day I was born they have supported me far, far more than is reasonable and taught me about love, service, and adventure. Thank you to my partner, Dave Kubiak, who poured immeasurable time and energy into keeping our family going, believes in me when I do not, and reminds me to take time for myself. Family and friends have been the color and light of my days.

Dedication

To Jay and Peter – my little lights.

Chapter 1: Introduction

Tuberculosis (TB) and HIV are leading infectious causes of morbidity and mortality worldwide.[1-3] Meanwhile, the burden of type 2 diabetes mellitus (T2DM) is increasing steadily in low- and middle-income countries (LMICs) as their populations age and rapid urbanization brings changes in diets and shifts to more sedentary lifestyles.[4, 5] Diabetes and HIV increase the risk of TB, but there are important knowledge gaps in how these diseases interact and affect each other. Therefore, it is important to better characterize the populations with multi-morbidity and associated risks in high TB and HIV burden countries. Diabetes and HIV contribute to persistently high rates of TB globally. Earlier detection of active TB in these populations will be critical to achieving the World Health Organization (WHO) End TB Strategy goal of reducing incident cases to <10/100,000 population by 2035.[6]

Diabetes background

Diabetes is a group of non-communicable metabolic diseases wherein blood glucose is elevated but cells are less able to access this energy. Globally, the group of diseases impacts one in 11 adults and remains a major cause of death and disability.[7] Type 2 diabetes mellitus (T2DM) accounts for approximately 90% of diabetes cases with the 10% comprised of type 1 diabetes mellitus (an autoimmune disease), gestational diabetes, and diabetes from other causes.[8] The prevalence of T2DM has increased most rapidly in LMICs, where the burden of HIV and TB may also be high.[9-12]

T2DM is a heterogenous disease but typically occurs when skeletal muscle cells, which are responsible for approximately 80% of postprandial glucose uptake, become resistant to insulin.[13]

β-cells in the pancreas produce more insulin to compensate, but may progressively develop deficiencies in insulin secretion over a period of years.[8] Risk factors for T2DM include age >45 years, history of cardiovascular disease, hypertension, family history of T2DM, physical inactivity, and being overweight or obese as measured by body mass index (BMI).[8, 14] Diabetes can be managed or prevented through a combination of lifestyle and pharmaceutical interventions, including increasing physical activity, nutrition therapy, and psychosocial care.[15] Diabetes can also be treated with prescription medications aimed at decreasing blood glucose levels through several mechanisms including increasing cell sensitivity to insulin (e.g. metformin) and increasing insulin secretion (e.g. sulfonylureas). If not managed well, chronic hyperglycemia, which typically follows chronic low-grade inflammation pursuant to increases in adiposity, can lead to a variety of negative health effects including cardiovascular disease, neuropathy, nephropathy, retinopathy, and skin conditions.

Diabetes risk among people living with HIV

People living with HIV (PLHIV), a virus that causes immune dysregulation, have a unique set of risk factors that increase their risk of T2DM by 2-4 times that of HIV-uninfected adults.[16-21] Markers of chronic inflammation, immunosuppression, lipodystrophy, and some classes of antiretroviral therapies (i.e. protease inhibitors, nucleoside reverse transcriptase inhibitors such as d4T, ddI) have been linked to insulin resistance and the development of T2DM.[19-28] Currently, the Department of Health and Human Services recommends testing for diabetes at the time of HIV diagnosis and, if normal, annually thereafter.[29] Additionally, the effects of traditional risk factors for T2DM appear to differ among PLHIV, who are more likely to develop T2DM at younger ages and lower BMIs.[19]

HIV programs are poised to provide much-needed diabetes care in high HIV burden countries.

In South Africa, diabetes is a leading cause of death and attributed to an increasing share of deaths.[30] Surveys in sub-Saharan Africa found 40-100% of people with diabetes were unaware of their status.[31-35] Diabetic patients in sub-Saharan Africa face challenges with access to diagnostics and medications, which can lead to inadequate glycemic control, hospitalization, higher costs, and increased risk of death.[31, 36-38] HIV programs have established clinical infrastructure for long-term HIV care and treatment. They are uniquely positioned to facilitate diagnosis and treatment of T2DM, which also requires extended follow-up.[39-42] Integrating HIV and diabetes care has the potential to reduce morbidity and mortality through improved patient care.[41]

Diabetes and HIV are major risk factors for active tuberculosis.

TB disease is caused by *Mycobacterium tuberculosis* (Mtb), which is transmitted between humans by inhaled aerosolized droplet nuclei. Approximately 25% of the world's population has latent TB infection (LTBI) wherein a dynamic and persistent immune response is stimulated by Mtb infection without evidence of clinical disease. Of those with LTBI, 5-15% will develop active TB disease over their lifetime, most often in the form of pulmonary TB with accompanying symptoms of cough, fever, night sweats, and/or weight loss. The WHO End TB Strategy recommends targeted screening of LTBI in order to treat those most at risk for active TB disease.[43]

HIV-infection increases the risk of TB by approximately 20 times and is the strongest known risk factor for progression from latent to active disease.[44, 45] Approximately 10% of people with active TB are living with HIV and it is the most common preventable cause of death among PLHIV.[3] The HIV virus weakens the immune system by depleting CD4 T cells, which increases vulnerability to Mtb infection and allows for progression from latent TB infection to active TB disease.[46] Additionally, HIV impairs macrophage function and cytokine production in Mtb-containing granulomas in the lung, disrupting their structure and inhibiting their ability to contain the Mtb infection.[46, 47] TB-HIV co-infection accelerates immune dysfunction, increasing the risk of other opportunistic infections and death.[47, 48]

Among HIV-negative adults, diabetes increases the risk of active TB 3-fold and has been associated with prolonged smear positivity, treatment failure, relapse, and death.[49-53] Low-grade chronic inflammation associated with obesity and T2DM may change the innate and adaptive immune system, possibly contributing to an increase in the risk of progression to active TB disease, although precise biological mechanisms are still being defined.[53-57] Approximately 10-15% of the 10.4 million new adult TB cases worldwide each year are attributable to diabetes.[3, 58] Diabetes prevalence contributes to sustained TB incidence and threatens achieving the WHO End TB Strategy goal.[6, 59] Following the HIV-TB integrated care model, in high TB burden settings the WHO recommends screening diabetes patients for TB symptoms as part of an active case finding strategy, and testing newly diagnosed TB patients for diabetes to link those patients to care.[60, 61]

Existing Knowledge Gaps

Knowledge gap #1: Diabetes and latent TB infection

There remain important knowledge gaps on the impact of diabetes on LTBI and the combined effect of diabetes and nutrition on LTBI. A meta-analysis of 13 studies found a small but statistically significant effect of diabetes on LTBI status (adjusted odds ratio 1.18, 95% CI 1.06-1.30).[62] However, five of the six studies conducted in LMICs, where exposure to TB disease is much higher, were done in populations with significant comorbidities including HIV, chronic kidney disease, or at a long-term care facility. Improved understanding of the relationship between LTBI and diabetes in the general population could inform LTBI screening and treatment guidelines.

We used data from a prospective observational cohort study of TB cases and household contacts in southern India to advance this understanding of LTBI and diabetes. We fit regression models to estimate the impact of diabetes on LTBI prevalence among household contacts of TB cases, and the impact of BMI on this relationship. These results are presented in Chapter 2.

Knowledge gap #2: Body mass and active TB disease

Improved understanding of the nutrition profile of those diabetes patients most at risk for active TB could help direct limited clinical resources. Currently, WHO recommends all diabetes patients living in high TB burden countries to be tested routinely for active TB disease.[61] However, this imposes a high burden on the health care system and is not widely done. Body mass index is easy to measure in an outpatient clinic and could be used to identify individuals at higher risk for TB. A log-linear inverse relationship has been demonstrated between BMI and TB.[63] However, although high BMI may be independently protective of active TB, it also

increases the risk of T2DM, which in turn increases the risk of active TB (**Figure 1**). A mediation analysis showed much of the effect of BMI on TB risk was direct, not mediated through T2DM, although the protective effect of overweight/obesity was considerably reduced among those with diabetes.[64] For people with T2DM, the risk of active TB may be highest at low BMIs,[65] but additional research is needed to identify high risk BMI categories for active TB disease.

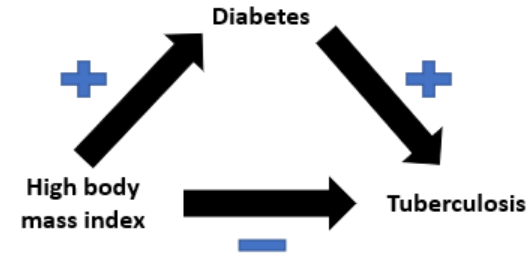


Figure 1. Directed acyclic graph depicting the direct relationship of BMI on TB and the indirect relationship through diabetes

Using data collected as part of a prospective cohort study of TB cases and their household contacts in southern India, we estimated the relative and additive prevalence of diabetes among active TB cases by BMI category. These findings are presented in Chapter 2.

Knowledge gap #3: Diabetes and TB among PLHIV

Although HIV and diabetes by themselves are well-documented risk factors for TB and HIV is a risk factor for diabetes (**Figure 2**), there are limited data on their combined effect.[66, 67] Additionally, long-term exposure to HIV and/or antiretroviral therapy (ART) likely leads to an increased risk of T2DM.[20, 21] Biological mechanisms may involve chronic systemic inflammation caused by the HIV infection and ART-induced metabolic syndrome comprised of insulin resistance, dyslipidemia, and lipodystrophy.[32, 68]

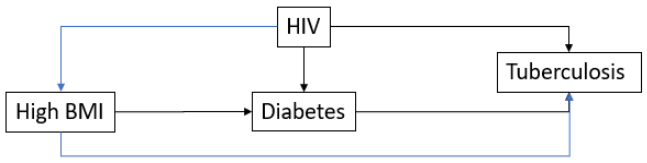


Figure 2. Conceptual diagram depicting the known independent relationships of BMI, HIV, diabetes, and TB with protective effects in blue arrows and risks in black.

In sub-Saharan Africa, two case-control studies and one cross-sectional study suggest HIV-infection may reduce the risk of active TB disease among persons with diabetes. However, the authors noted several study limitations due to measurement error, uncontrolled confounding, and discordant results.[66, 69-71] There are few prospective studies of HIV care outcomes that assess the impact of diabetes co-morbidity on TB. One prospective study found delayed weight gain and hemoglobin recovery at two and five months after TB diagnosis among HIV-infected persons with diabetes.[72] However, follow-up was short and they did not assess important treatment outcomes. Therefore, while current WHO guidelines recommend screening all TB patients for diabetes and all diabetes patients in high-TB settings to be screened for TB,[60, 61] it is unclear how these guidelines should be applied to HIV-infected patients.

We proposed a prospective study using an established cohort to estimate the impact of diabetes on active TB incidence among HIV-infected adults in South Africa. We fit Cox proportional hazard models to assess the impact of diabetes and pre-diabetes on TB and other HIV-related treatment outcomes over a 12-month follow-up period. This work is presented here in Chapter 3.

Knowledge gaps #5: Sub-clinical TB disease prevalence and risk factors among PLHIV

Traditionally, a person infected with TB would be considered to have either latent or active TB disease. However, it is more appropriate to think of TB infection as a spectrum that includes sub-clinical disease between latent infection and active disease (**Figure 3**). Sub-clinical TB disease state is characterized by replicating Mtb in the absence of TB symptoms.[73] It suggests a high likelihood of infectiousness and progression to active disease.[73] There is a paucity of evidence describing risk factors for sub-clinical disease or the prevalence of sub-clinical TB in key populations including PLHIV.[74-76] HIV-infection has been associated with 2-fold higher odds of sub-clinical TB among adults without TB symptoms, but there is limited information available about risk factors and how to target screening in this population.[77]

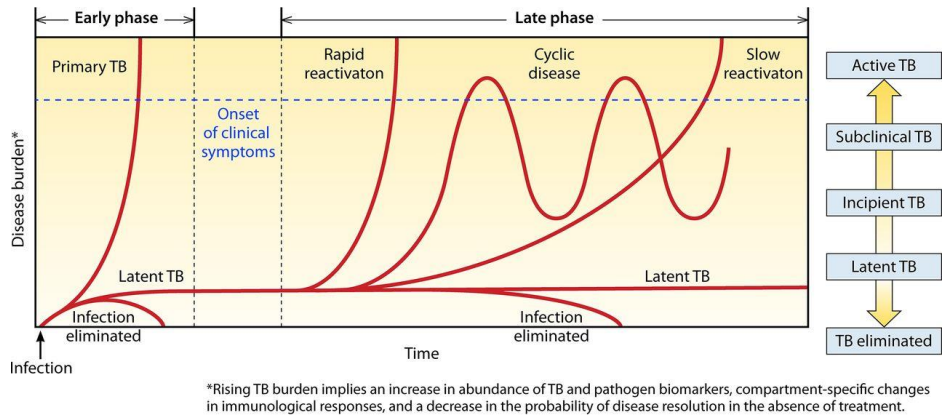


Figure 3. Pathways of tuberculosis disease progression with active TB denoted as primary TB at the top, and latent TB depicted towards the bottom. Source: Drain, et al. 2018.

We proposed estimating the burden of sub-clinical TB and associated risk factors using nearly 500 sputum samples collected irrespective of TB symptoms from adults testing positive for HIV in Durban, South Africa. We estimated the prevalence of sub-clinical TB and used logistic regression to test the predictive power of C-reactive protein and hemoglobin. This work is presented in detail in Chapter 4.

Specific Aims

To address these knowledge gaps at the intersection of HIV, diabetes, and TB, we proposed three specific aims.

1a. Compare the prevalence of LTBI among household contacts with and without diabetes, and active TB disease among participants in southern India with diabetes to those without diabetes.

Our hypothesis was that the prevalence of LTBI and active TB is higher among participants with diabetes compared to those without diabetes.

1b. Test for BMI and DM interaction on the prevalence of LTBI and TB outcomes.

2a. Determine the impact of prevalent diabetes or impaired glucose tolerance (IGT), respectively, on the risk of active TB disease compared to non-diabetic patients in a newly diagnosed HIV-positive cohort within a large urban township in KwaZulu-Natal, South Africa.

Our hypothesis was that the risk of active TB disease does not differ by glycemic control status in an HIV-positive population.

2b. Test if diabetes and IGT exposures increase the risk of incident TB one-year post-HIV diagnosis.

3. Estimate the burden of sub-clinical TB among HIV-positive adults in Durban, South Africa and characteristics associated with this disease state.

Hypothesis: Sub-clinical TB is prevalent in approximately 5% of HIV-positive adults in Durban and is associated with elevated C-reactive protein compared and hemoglobin to asymptomatic, TB-culture negative adults.

Thus, together we aim to better understand the relationship between diabetes and TB in among people living with and without HIV infection, and the continuum of TB disease.

Chapter 2: Interaction of nutritional status and diabetes on active and latent tuberculosis: A cross-sectional analysis

Published citation: Kubiak RW, Sarkar S, CR Horsburgh, G Roy, Kratz M, Reshma A, Knudsen S, P Salgame, J Ellner, Drain PK and Hochberg NS. Interaction of nutritional status and diabetes on active and latent tuberculosis: a cross-sectional analysis. *BMC Infect Dis*. 2019;19(1):627. doi: 10.1186/s12879-019-4244-4

Rachel W. Kubiak,[1] Sonali Sarkar,[2] C. Robert Horsburgh, Jr.,[3,4] Gautam Roy,[2] Mario Kratz,[1,5,6] Ayiraveetil Reshma,[2] Selby Knudsen,[3] Padmini Salgame,[7] Jerrold J. Ellner,[3,7,8] Paul K. Drain*[1,9,10] and Natasha S. Hochberg*[3,4,8]

*These authors contributed equally.

[1]Department of Epidemiology, University of Washington, Seattle, USA; [2]Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India; [3]Department of Epidemiology, Boston University School of Public Health, Boston, USA; [4]Section of Infectious Diseases, Department of Medicine, Boston University School of Medicine, Boston, USA; [5]Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine, University of Washington, Seattle, USA; [6]Cancer Prevention Program, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, USA; [7]Center for Emerging Pathogens, Department of Medicine, Rutgers-New Jersey Medical School, Newark, USA; [8]Boston Medical Center, Boston, USA; [9]Department of Global Health, University of Washington, Seattle, USA; and [10]Department of Medicine, University of Washington, Seattle, USA.

ABSTRACT

Background: Malnutrition and diabetes are risk factors for active tuberculosis (TB), possible risk factors for latent TB infection (LTBI), and may interact to alter their effect on these outcomes. Studies to date have not investigated this interaction.

Methods: We enrolled 919 newly diagnosed active TB patients and 1113 household contacts at Primary Health Centres in Puducherry and Tamil Nadu, India from 2014-2018. In cross-sectional analyses, we used generalized estimating equations to measure additive and multiplicative interaction of body mass index (BMI) and diabetes on two outcomes, active TB and LTBI.

Results: Among overweight or obese adults, active TB prevalence was 12-times higher in diabetic compared to non-diabetic participants, 2.5-times higher among normal weight adults, and no different among underweight adults (*P* for interaction <0.0001). Diabetes was associated with 50 additional active TB cases per 100 overweight or obese participants, 56 per 100 normal weight participants, and 17 per 100 underweight participants (*P* for interaction <0.0001). Across BMI categories, screening 2.3-3.8 active TB patients yielded one hyperglycemic patient. LTBI prevalence did not differ by diabetes and BMI*diabetes interaction was not significant.

Conclusions: BMI and diabetes are associated with newly diagnosed active TB, but not LTBI. Diabetes conferred the greatest risk of active TB in overweight and obese adults whereas the burden of active TB associated with diabetes was similar for normal and overweight or obese adults. Hyperglycemia was common among all active TB patients. These findings highlight the importance of bi-directional diabetes-active TB screening in India.

Keywords: body mass index, cross-sectional analysis, diabetes mellitus, latent tuberculosis, prevalence, tuberculosis, underweight, India

BACKGROUND

Active tuberculosis (TB) disease is a major cause of morbidity and the leading infectious cause of mortality globally.[3] Achieving the World Health Organization (WHO) End TB Strategy goal of TB incidence <10/100,000 population by 2035 will require a multi-pronged approach including providing adequate clinical care for comorbidities that are risk factors for active TB disease, and targeted screening and treatment of latent TB infection (LTBI) for those at high risk of progressing to active disease.[78, 79] Diabetes and malnutrition, known risk factors for active TB, have been recognized as two important factors that could prevent achieving the global target of reducing TB incidence by 2035.[80] Diabetes increases the risk of active TB by approximately three-fold.[50, 81] Conversely, for each unit increase in body mass index (BMI), the risk of TB decreases by 13.8% on average.[63] Whereas obesity has a direct protective effect on active TB, it is also a risk factor for diabetes, which may negate protection conferred from high BMI.[64] The rising prevalence of diabetes may be contributing to persistently high TB incidence in high TB burden countries, outweighing the protective effect of high BMI.[59, 82] However, the interaction between BMI and diabetes has not been previously estimated. Improved understanding of the populations most at risk for active TB at national and sub-national levels is essential to implementing the WHO End TB Strategy effectively.[78, 79]

India has high dual burdens of TB and diabetes. Approximately 74 million Indian adults have diabetes (10.4%) and this number is expected to nearly double by 2045.[83] Among 2.7 million incident TB cases in India, 15% may be attributable to diabetes and 32-62% may be attributable to malnutrition.[3, 58, 84] These diseases are associated with personal catastrophic health expenditures in vulnerable populations, which may be partially mitigated through proactive

screening practices and preventative care.[85] Bi-directional diabetes-TB screening is recommended by the WHO and in Indian national guidelines.[61, 86] In India, all TB patients should be screened for random blood glucose (RBG) ≥140 mg/dL.[86] At each diabetes clinic visit, patients should be screened for TB symptoms and referred for TB testing if positive.[86] Given the challenge of providing quality care to this large and growing diabetic patient population, identifying those most likely to have active TB could help to focus limited resources.

The relationship between nutritional status, diabetes, and LTBI is less clear. LTBI is chronic *Mycobacterium tuberculosis* (MTB) infection without evidence of clinical disease affecting one quarter of the world's population, of whom 5-15% will develop active TB over their lifetime.[3] A meta-analysis found no difference in the odds of LTBI among underweight compared to normal weight adults.[87] Diabetes is associated with a higher odds of LTBI by up to 2-fold but studies have had mixed results.[62, 88, 89]

We tested for additive and multiplicative interaction between BMI and diabetes on two separate outcomes, active TB and LTBI, in an observational study of TB patients and their household contacts in south India. Here, we present both the individual and combined effects of BMI and diabetes on active TB disease and LTBI in order to estimate the relative prevalence and burden of these outcomes attributable to diabetes overall and within BMI strata. We also estimated the number of TB patients needed to screen by BMI category in order to identify one instance of hyperglycemia.

METHODS

Study population

We conducted cross-sectional analyses of newly diagnosed active TB patients and their household contacts in southern India as part of the Regional Prospective Observational Research for Tuberculosis (RePORT)-India Consortium.[84] Enrolment began in Pondicherry in May 2014, and in two districts of Tamil Nadu, Cuddalore and Vilupuram, in August 2014 and November 2015, respectively.

Acid-fast bacilli sputum smear positive TB patients were recruited at Revised National TB Control Program District Microscopy Centres and Primary Healthcare Centres. Eligible TB patients for this RePORT-India site were ≥6 years of age; able to provide sputum for a confirmatory culture; enrolled in directly observed therapy, short-course at their local clinic; and willing to be tested for HIV. TB patients with ≥3 doses of anti-TB therapy at enrolment, a history of TB disease or treatment, or a multi-drug resistant TB contact were excluded.

Household contacts were eligible for enrolment if they had lived with the TB patient for at least the previous three months, were ≥6 years old, had no prior TB diagnosis, no known contact with a multi-drug resistant TB patient, and were willing to be tested for LTBI.

Ethics, consent and permissions

All participants enrolled in the study were willing and able to provide written informed consent or assent in conjunction with parental/guardian consent if <18 years. The study protocol was approved by the Jawaharlal Institute of Postgraduate Medical Education and Research Ethics

Committee and Scientific Advisory Committee, and the Institutional Review Boards at Boston University Medical Campus and Rutgers-New Jersey Medical School.

Study procedures

At each participant's enrolment visit, research teams collected demographic and health information and measured participants' height and weight to calculate a BMI. Active TB patients provided a sputum sample at enrolment for Löwenstein-Jensen and liquid mycobacterial growth indicator tube cultures (Becton Dickinson, USA) and received an RBG test by finger stick. The clinics performed HIV testing as part of the standard of care.

At household contact enrolment, which occurred primarily at the household, the study nurse injected 0.5 ml of purified protein derivative into the intradermal layer as a tuberculin skin test (TST) (Span Diagnostics/Arkray Healthcare, India). To determine LTBI status, the study nurse measured induration within five days and the majority within three days. Household contacts with TB symptoms and a positive skin test were asked to provide a sputum sample for AFB smear and culture. Screening for diabetes among household contacts using RBG began in April 2016. Individuals were questioned regarding a history of renal failure. All household contacts were followed for one year; symptom screens (and sputum testing if indicated) were performed to identify incident active TB disease.

Statistical analyses

We used standard BMI categories for the Indian Asian population of underweight (<18.5 kg/m^2), normal weight (18.5-22.9 kg/m^2), and overweight or obese (≥23.0 kg/m^2).[90] A patient was considered to have confirmed diabetes if they reported a prior clinical diagnosis of diabetes.

Active TB was defined as sputum culture-positive for *MTB* either by solid or liquid culture. Household contact LTBI was defined as TST induration ≥5 mm.[91]

We excluded participants enrolled as household contacts who tested culture-positive for MTB following a positive symptom screen, participants enrolled as cases who were not MTB culture-positive, participants <18 years of age, and participants missing both self-reported diabetes status and an RBG measurement.

Among active TB patients, we estimated the number needed to screen (NNS) for elevated RBG as one over the prevalence of RBG ≥140 mg/dL for each BMI category, as per national guidelines for diabetes screening.[86] We estimated the NNS both for those who did not report a prior diagnosis of diabetes and for those who did. To estimate the overall relationship between diabetes and active TB disease, we calculated unadjusted and adjusted prevalence ratios using generalized estimating equations (GEE) with a log link and binomial distribution, or if the model failed to converge, a Poisson distribution with robust standard errors.[92, 93] We accounted for clustering at the family-level in all models using an exchangeable correlation matrix.[92] For the adjusted model, we decided *a priori* to control for age (years), sex, BMI category, smoking (current/not current), and hazardous alcohol use as per the Alcohol Use Disorders Identification Test (AUDIT)-C questionnaire (≥3 for women and ≥4 for men).[94]

To evaluate multiplicative and additive interaction of diabetes and BMI on active TB disease, we calculated BMI-diabetes stratum-specific prevalence ratios and prevalence differences, respectively, adjusting for age and sex.[95] We chose normal BMI, non-diabetic household

contacts who by definition did not have active TB disease as the reference group. We used GEE with a binomial distribution or Poisson distribution with robust standard errors if needed, and a log-link to estimate the prevalence ratios or identity-link to estimate prevalence differences.[92, 93] We employed the same GEE approach to estimate the overall relative unadjusted and adjusted risks of LTBI among household contacts with diabetes compared to those without diabetes. We used non-diabetic household contacts with a normal BMI and no LTBI as the reference group.

We performed two sensitivity analyses of the overall relationship of diabetes with active and latent TB: 1) defining diabetes as RBG ≥200 mg/dL or self-report of a prior clinical diagnosis of diabetes, and 2) excluding participants with a moderately abnormal RBG of 140-199 mg/dL.[86] We used SAS version 9.4 (Cary, NC).

RESULTS

Of the 2032 participants included in analyses, 919 were active TB patients and 1113 were household contacts without active TB disease. Compared to household contacts, TB patients were more often male (79% *versus* 35%), older (mean age 45 *versus* 37 years), and more commonly engaged in hazardous alcohol use (46% *versus* 6%) **(Table 1)**. The majority (61%) of TB patients were underweight compared to only 16% of household contacts.

Overall, 365 (18%) participants reported a prior diabetes diagnosis, and diabetes was more common among active TB patients than their household contacts (32% *versus* 6%). Of the 344 diabetic participants who also received RBG testing, 72% had an RBG ≥200 mg/dL whereas 66/1155 (6%) of those who had not been diagnosed with diabetes had RBG ≥200 mg/dL. The

majority (79%) of participants with known diabetes reported using oral medication to control their diabetes in the past month and 35% were overweight or obese. Of those with RBG ≥200 mg/dL but no prior diabetes diagnosis, 32% were overweight or obese. The proportion of underweight, normal weight, and overweight or obese participants with diabetes was 11% (82/746), 25% (157/653), and 20% (126/633) respectively.

Among TB patients overall, 49% had an RBG ≥140 mg/dL and the NNS ranged from 1.2 to 2.9 **(Table 2)**. Of those who reported a prior diabetes diagnosis, 36% were diagnosed in the year prior to their TB diagnosis, 91% had elevated RBG, and NNS did not vary by BMI category.

The adjusted prevalence of active TB was 2.13-times higher among adults with diabetes compared to those without diabetes (95% confidence interval [CI] 1.95, 2.33) **(Table 3)**. Being underweight was associated with a higher prevalence of TB (adjusted prevalence ratio [aPR] 1.59; 95% CI 1.45, 1.75) whereas being overweight or obese was associated with lower TB prevalence (aPR 0.40; 95% CI 0.32, 0.49). Comparing those with diabetes to those without diabetes, the prevalence of active TB was 1.04-times higher among underweight participants, 2.45-times higher among normal weight participants, and 12-times higher among overweight or obese participants (*P* for interaction <0.0001) **(Table 4)**. Among overweight or obese participants without diabetes, the adjusted risk of active TB was 0.17-times (95% CI 0.11, 0.27) that of normal weight adults without diabetes, but this protective association was not observed among overweight or obese participants with diabetes (aPR 1.73, 95% CI 1.38, 2.17).

On the additive scale, diabetes was associated with an estimated 17 additional TB patients per 100 underweight participants, 56 patients per 100 normal weight participants, and 50 patients per 100 overweight or obese participants (*P* for interaction <0.0001) **(Table 4)**. Among those without diabetes, the prevalence of active TB was higher among underweight participants and lower among overweight or obese participants compared to those of normal weight **(Figure 1)**.

The prevalence of LTBI was no different among household contacts with diabetes (aPR 1.20; 95% CI 0.99, 1.45) or in sensitivity analyses **(Table 2)**. Diabetes was associated with LTBI among underweight participants (aPR 1.97; 95% CI 1.32, 2.93; aPD 0.49; 95% CI 0.27, 0.70) **(Supplemental Tables 1 and 2)**. Interaction with BMI was not significant on the relative or additive scale (*P* for both interactions >0.1).

DISCUSSION

We present evidence of both additive and multiplicative interaction between BMI and diabetes in active TB at the time of diagnosis in this southern Indian cohort. Our findings provide support for current Indian national guidelines recommending bi-directional screening of all diabetes patients for active TB and all TB patients for diabetes irrespective of BMI. Among active TB patients, the NNS to yield one instance of hyperglycemia was low for all BMI categories. The highest relative risk of active TB from diabetes was among overweight and obese adults, whereas the greatest burden of active TB disease due to diabetes was among adults of normal weight. Low BMI was also associated with active TB. LTBI was not associated with prior diabetes diagnosis alone and we found no evidence of BMI-diabetes interaction.

The prevalence of diabetes among TB patients in our study population was similar to previously reported estimates in India.[96, 97] Nearly all TB patients with a prior diabetes diagnosis had elevated RBG suggesting an important opportunity to provide diabetes counselling, referral to diabetes care, and glucose monitoring. Of those who did not report a diabetes diagnosis, 29% still had elevated RBG. Incident TB is associated with hyperglycemia that may resolve over the course of TB treatment independent of diabetes interventions, but may also be a prognostic marker of poor short-term outcomes and longer-term elevated risk of diabetes.[71, 81, 98-100]

Our findings consistently support the inverse association of BMI with active TB risk and increased risk of TB from diabetes.[50, 63] Our observation of highly significant multiplicative and additive BMI-diabetes interaction on active TB adds to the body of literature by identifying for whom and to what extent diabetes and BMI are associated with newly diagnosed active TB in this resource-limited setting. Multiplicative interaction is less heterogeneous than additive interaction, which is more closely tied to the size of the burden of disease in the study population.[101, 102] The additive scale, estimating the difference-in-differences, is less commonly reported but is appropriate for estimating public health impacts and identifying high-risk groups to inform resource allocation.[95, 101] We maximize the programmatic relevance by identifying the high burden of diabetes-associated TB among both normal weight and overweight or obese patients, and the high proportion of TB patients with hyperglycemia at all levels of BMI. Bi-directional screening could lead to earlier diagnosis of both conditions and improved disease management, but additional research is needed to optimize integrated care and identify the most cost-effective screening methods.

Prior studies suggest diabetes may modestly increase the risk of LTBI but the evidence is mixed.[56, 62, 88] A recent meta-analysis found the odds of LTBI was higher among diabetic patients although the effect size was small (1.18, 95% CI 1.06, 1.30).[62] However, LTBI was not associated with diabetes in a prospective cohort study or recent cross-sectional analyses of another Indian cohort.[18, 89] Similarly, in both unadjusted and adjusted models, we found no statistically or clinically significant association of LTBI with prior diabetes diagnosis, or in sensitivity analyses with elevated RBG. These findings add to the evidence that diabetes is not a significant risk factor for LTBI in India and instead suggest diabetes increases the risk of TB activation.

Mechanistic studies provide biological plausibility for increased risk of both MTB acquisition and progression to TB disease in the setting of diabetes and malnutrition. Murine models demonstrate defective innate and adaptive immune responses in the presence of diabetes.[56, 103] In diabetic mice challenged with aerosolized MTB, the priming of the adaptive immune response is delayed resulting in impaired local immune response in the lung and likely increased susceptibility to TB disease.[103] Human studies have found alterations in central memory T cells, effector memory T cells, and T regulatory cells among TB patients with diabetes.[104] Similarly, malnutrition affects a range of immune responses from macrophage phagocytosis and activation to T cell response and IFNγ production that results in increased TB risk.[105] A systematic review of cohort studies identified an inverse log-linear relationship between BMI and active TB,[63] but additional studies of the biological mechanisms involved in the interaction between BMI and diabetes are needed.

Our study has several strengths in addition to estimating interaction on both the multiplicative and additive scale. We present prevalence ratios, which are more intuitive, conservative, and consistent than odds ratios, which do not approximate a risk ratio when the outcome is common.[93, 106] We also had thorough ascertainment of active TB using sputum culture for both household contacts and TB patients. Household contacts with a positive TST and TB symptoms were tested for active TB at enrolment and all household contacts were followed for one year, a high-risk period, to identify incident active TB.

Our study also has several limitations. First, our data were cross-sectional, precluding causal inference. Some active TB patients recently diagnosed with diabetes may in fact have transient hyperglycemia caused by TB, biasing results away from the null. Malnutrition is an established risk factor for active TB, but unexpected weight loss is also a common effect.[63] For these analyses estimating the burden of hyperglycemia and diabetes for different BMI categories at the time of TB diagnosis, the order of events is less critical. Second, our results are most relevant to adults because we only included participants ≥18 years in our analyses and type 2 diabetes, which accounts for more than 90% of diabetes, tends to develop in adulthood.[83] Third, only two underweight household contacts had diabetes and both had LTBI, so the reliability of our estimates in this low BMI category is limited. Fourth, bacille Calmette-Guérin vaccination reduces the specificity of TST. However, this effect wanes over 10 years and the vaccination is given in infancy so we expect negligible impact in this adult population.[91, 107] Finally, we compared active TB patients to household contacts, not community-based controls. We believe household contacts are more representative of the lower socio-economic population from which

the cases were drawn. Additionally, we took a conservative statistical approach using prevalence estimates and accounting for family-level similarities with an exchangeable correlation matrix.

Our findings may not be generalizable beyond Indian Asian populations who have a higher likelihood of developing diabetes at every BMI level.[108] We observed a high proportion of diabetes among those of normal or even low BMI, in line with the "*thin-fat phenotype*" observed in India wherein Indians have more body fat and central obesity for each BMI category compared to Caucasians and black Africans.[108]

Conclusions

Our analyses of multiplicative and additive interaction between diabetes and BMI on active TB highlight the high prevalence of diabetes among active TB patients at all levels of BMI in this south Indian cohort. Malnutrition as measured by BMI was also associated with a higher burden of TB, primarily among those without diabetes. BMI and diabetes were not major risk factors for LTBI. Additional research is needed to better understand the biological mechanisms involved, optimize timing of diabetes testing and clinical care for diabetic or hyperglycemic TB patients, assess the cost-effectiveness of bi-directional screening, and estimate the public health impact of diabetes and obesity on active TB in other regions.

Abbreviations

aPD	Adjusted prevalence difference
aPR	Adjusted prevalence ratio
BMI	Body mass index
GEE	Generalized estimating equations
LTBI	Latent tuberculosis infection
MTB	*Mycobacterium tuberculosis*
NNS	Number needed to screen
RBG	Random blood glucose
RePORT	Regional Prospective Observational Research for Tuberculosis
TB	Tuberculosis
TST	Tuberculin skin test

DECLARATIONS

Ethics approval and consent to participate

All participants enrolled in the study were willing and able to provide written informed consent or assent in conjunction with parental/guardian consent if <18 years. The study protocol was approved by the Jawaharlal Institute of Postgraduate Medical Education and Research Ethics Committee and Scientific Advisory Committee, and the Institutional Review Boards at Boston University Medical Campus and Rutgers-New Jersey Medical School.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed are available from the corresponding author on reasonable request.

Competing interests

Paul K. Drain is a member of the editorial board of *BMC Infectious Diseases*.

Funding

This work was supported by Award No. USB1-31150-XX-13 of the U.S. Civilian Research & Development Foundation (CRDF Global) and by the National Science Foundation under Cooperative Agreement No. OISE-9531011 with federal funds from the Government of India's Department of Biotechnology, the Indian Council of Medical Research, the U.S. National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases, Office of AIDS

Research, and distributed in part by CRDF Global. RWK was supported by the National Center for Advancing Translational Sciences of the NIH under TL1 TR002318. NSH is supported in part by a grant from the Warren Alpert Foundation and Boston University School of Medicine.

The content of this article is solely the responsibility of the authors and does not necessarily reflect the views of the Government of India's Department of Biotechnology, the Indian Council of Medical Research, the United States National Institutes of Health, or the United States Civilian Research & Development Foundation.

Authors' contributions

All authors have reviewed and approved this manuscript. All authors gave content of critical intellectual importance and were involved the conception and design, acquisition of data, and/or data analysis and interpretation. Specifically, SS, CRH, GR, PS, JJE, and NSH were responsible for the conception and design of this RePORT-India study. RWK, SS, AR, SK, PS, JJE, and NSH were responsible for data acquisition and management. RWK, CRH, MK, NSH, and PKD, were responsible for the analytical design and played key roles in the interpretation of results. RWK performed the analyses and drafted the manuscript. PKD and NSH contributed equally.

Acknowledgements

We thank the dedicated research staff at JIPMER who worked tirelessly to carry out this study and each of the volunteers for their participation.

Table 1. Baseline characteristics of adult tuberculosis cases and their household contacts in southern India (n=2032).

	Active TB Case (n=919) n (%) or mean ± std	**Household Contact** (n=1113) n (%) or mean ± std
Sociodemographics		
Male (n=1969)	729 (79.3)	392 (35.2)
Age (years)	44.9 ± 14.0	36.8 ± 14.4
Years of schooling	6.9 ± 4.7	8.0 ± 5.1
Married/Living together	673 (73.2)	702 (63.1)
Household income ≤5,000 rupees (n=1817)[a]	465 (50.6)	395 (44.0)
Hazardous alcohol use	426 (46.4)	70 (6.3)
Current smoker	215 (23.4)	107 (9.6)
Body Mass Index (kg/m^2)		
Underweight (<18.5)	564 (61.4)	182 (16.4)
Normal (18.5-22.9)	266 (28.9)	387 (34.8)
Overweight/obese (≥23.0)	89 (9.7)	461 (48.9)
Diabetes measurements		
Prior diabetes diagnosis	296 (32.2)	69 (6.2)
Random blood glucose (mg/dL) (n=1635)	180.8 ± 103.4	132.0 ± 63.8
Random blood glucose ≥ 200 mg/dL (n=1635)	263 (28.6)	58 (5.2)
Prior diabetes diagnosis or random blood sugar ≥200 mg/dL	343 (37.3)	94 (8.5)
Tuberculosis testing		
Tuberculin skin test positive	NA	605 (54.4)
Tuberculin skin test induration (mm)[b]	NA	7 (2-11)
Days to MGIT positivity (n=916)	8.5 ± 4.1	NA
Other Comorbidities		
Self-reported history of renal failure	1 (0.1)	3 (0.3)
HIV positive	3 (0.3)	NA

MGIT, mycobacterial growth indicator tubes; NA, Not Available; Std, standard deviation.

[a] Equivalent to ~75 USD.

[b] Median (interquartile range)

Table 2. Number of active tuberculosis patients screened to identify one instance of elevated random blood glucose (n=915).

		Body mass index (kg/m^2)		
		<18.5	18.5-22.9	≥23.0
Overall	Total (n)	562	264	89
	RGB ≥140 (%)	34.9	67.8	82
	Number needed to screen	2.9	1.5	1.2
No prior diabetes	Total (n)	484	121	18
	RGB ≥140 (%)	26.2	39.7	44.4
	Number needed to screen	3.8	2.5	2.3
Diabetes diagnosis	Total (n)	78	143	71
	RGB ≥140 (%)	88.5	91.6	91.6
	Number needed to screen	1.1	1.1	1.1

RGB, random blood glucose (mg/dL)

Table 3. Prevalence of latent tuberculosis infection and active tuberculosis disease among diabetic compared to non-diabetic adults.[a]

	LTBI (n=1113)			TB (n=2032)		
	n (%)	PR (95% CI)	aPR (95% CI)[b]	n (%)	PR (95% CI)	aPR (95% CI)[b]
No history of diabetes	561/1044 (53.7)	1.00 (Referent)	1.00 (Referent)	623/1667 (37.4)	1.00 (Referent)	1.00 (Referent)
Prior diabetes diagnosis	44/69 (63.8)	1.24 (1.04, 1.48)	1.20 (0.99, 1.45)	296/365 (81.1)	3.83 (2.01, 7.30)	2.13 (1.95, 2.33)
Sensitivity analyses						
RBG < 200	553/1019 (54.2)	1.00 (Referent)	1.00 (Referent)	576/1595 (36.1)	1.00 (Referent)	1.00 (Referent)
RBG ≥ 200 or prior diabetes diagnosis	52/94 (55.3)	1.04 (0.86, 1.25)	1.00 (0.82, 1.21)	343/437 (78.5)	2.17 (1.90, 2.48)	2.06 (1.90, 2.23)
RBG < 140	517/928 (55.7)	1.00 (Referent)	1.00 (Referent)	440/1368 (32.2)	1.00 (Referent)	1.00 (Referent)
RBG ≥ 200 or prior diabetes diagnosis	52/94 (55.3)	1.01 (0.84, 1.22)	0.97 (0.80, 1.18)	343/437 (78.5)	2.47 (2.25, 2.71)	2.19 (2.00, 2.39)

aPR, adjusted prevalence ratio; CI, confidence interval; LTBI, latent tuberculosis infection; PR, prevalence ratio; RBG, random blood glucose (mg/dL); TB, tuberculosis.

[a] All models account for clustering at the family-level with an exchangeable correlation matrix. For LTBI, the referent group is LTBI-negative household contacts. For TB, the reference group is all household contacts.

[b] Adjusted for age, sex, body mass index category, smoking, and hazardous alcohol use.

Table 4. Relative and additive effect modification of diabetes on active tuberculosis prevalence by body mass index.[a]

	BMI Category (kg/m^2)											
	<18.5				18.5-22.9				≥23.0			
	TB cases/ total	%	aPR (95% CI)	aPD (95% CI)	TB cases/ total	%	aPR (95% CI)	aPD (95% CI)	TB cases/ total	%	aPR (95% CI)	aPD (95% CI)
No history of diabetes	484/ 664	72.9	2.44 (2.09, 2.85)	0.40 (0.34, 0.45)	121/ 496	24.4	**1.00 (Referent)**	**0.00 (Referent)**	18/ 507	3.6	0.17 (0.11, 0.27)	-0.16 (-0.20, -0.13)
Prior diabetes diagnosis	80/ 82	97.6	2.38 (1.98, 2.86)	0.61 (0.53, 0.68)	145/ 157	92.4	2.45 (2.05, 2.92)	0.56 (0.49, 0.63)	71/ 126	56.4	1.73 (1.38, 2.17)	0.24 (0.15, 0.34)
Effect of diabetes within BMI strata			1.04 (0.93, 1.16)	0.17 (0.12, 0.23)			2.45 (2.05, 2.92)	0.56 (0.49, 0.63)			12.01 (6.98, 20.71)	0.50 (0.41, 0.59)

aPD, adjusted prevalence difference; aPR, adjusted prevalence ratio; BMI, body mass index; CI, confidence interval; TB, tuberculosis.

P for interaction additive and relative scales <0.0001.

[a] Adjusted for age and sex, and accounting for clustering at the family-level with an exchangeable correlation matrix.

Supplemental Table 1. Relative scale for effect modification of diabetes on latent tuberculosis infection prevalence by body mass index category in south India[a]

	BMI Category											
	<18.5				18.5-22.9				≥23			
	LTBI/Total	%	aPR	95% CI	LTBI/Total	%	aPR	95% CI	LTBI/Total	%	aPR	95% CI
No history of diabetes	103/180	57.2	1.18	0.99, 1.40	180/375	48.0	**1.00**	**Referent**	278/489	56.9	1.12	0.99, 1.27
Prior diabetes diagnosis	2/2	100	1.55	1.13, 2.12	7/12	58.3	1.04	0.62, 1.76	35/55	63.6	1.24	0.97, 1.58
Effect of diabetes within strata of BMI			1.97	1.32, 2.93			1.04	0.62, 1.76			1.14	0.90, 1.43

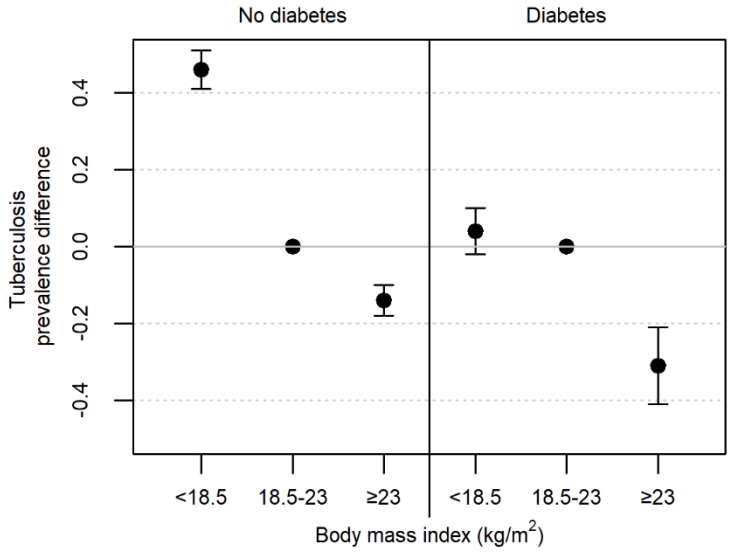
aPR, adjusted prevalence ratio; BMI, body mass index; CI, confidence interval; LTBI, latent tuberculosis infection.
P for interaction 0.109
[a] Adjusted for age and sex, and accounting for clustering at the family-level with an exchangeable correlation matrix.

Supplemental Table 2. Additive scale for effect modification of diabetes on latent tuberculosis infection prevalence by body mass index category in south India[a]

	BMI Category											
	<18.5				18.5-22.9				≥23			
	LTBI/Total	%	aPD	95% CI	LTBI/Total	%	aPD	95% CI	LTBI/Total	%	aPD	95% CI
No history of diabetes	103/180	57.2	0.09	0.00, 0.19	180/375	48.0	**0.00**	**Referent**	278/489	56.9	0.05	-0.01, 0.11
Prior diabetes diagnosis	2/2	100	0.36	0.19, 0.53	7/12	58.3	0.03	-0.26, 0.32	35/55	63.6	0.12	-0.03, 0.27
Effect of diabetes within strata of BMI			0.49	0.27, 0.70			0.03	-0.26, 0.32			0.10	-0.04, 0.24

aPD, adjusted prevalence difference; BMI, body mass index; CI, confidence interval; LTBI, latent tuberculosis infection.
P for interaction 0.119
[a] Adjusted for age and sex, and accounting for clustering at the family-level with an exchangeable correlation matrix.

Figure 1. Adjusted difference in prevalence of active tuberculosis between body mass index categories, by diabetes status.



Legend: Points represent the adjusted prevalence difference and vertical bars represent 95% confidence intervals. Adjusted for age and sex, and accounting for clustering at the family-level with an exchangeable correlation matrix.

Chapter 3: Clinic-based diabetes screening at the time of HIV testing and associations with poor clinical outcomes in South Africa: A cohort study

Rachel W. KUBIAK,[1] Mario KRATZ,[1,2,3] Ayesha A. MOTALA,[4] Sean GALAGAN,[5] Sabina GOVERE,[6] Hilary THULARE,[6] Elizabeth R. BROWN,[7,8] Mahomed-Yunus S. MOOSA,[9] Paul K. DRAIN.[1,5,10]

[1]Department of Epidemiology, University of Washington, Seattle, Washington; [2]Cancer Prevention Program, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington; [3]Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine, University of Washington, Seattle, Washington; [4]Department of Diabetes and Endocrinology, University of KwaZulu-Natal, Durban, South Africa; [5]Department of Global Health, University of Washington, Seattle, Washington; [6]AIDS Healthcare Foundation, Durban, South Africa; [7]Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, Washington; [8]Department of Biostatistics, University of Washington, Seattle, Washington; [9]Department of Infectious Diseases, University of KwaZulu-Natal, Durban, South Africa; [10]Department of Medicine, University of Washington, Seattle, Washington.

ABSTRACT

Background: HIV clinical care programs in high burden settings are uniquely positioned to facilitate diabetes diagnosis, which is a major challenge. However, in sub-Saharan Africa, data on the burden of diabetes among people living with HIV (PLHIV) and its impact on HIV outcomes is sparse.

Methods: We enrolled adults presenting for HIV testing at an outpatient clinic in Durban. Those who tested positive for HIV-infection were screened for diabetes using a point-of-care hemoglobin A_{1c} (HbA_{1c}) test. We assessed baseline risk factors for diabetes (HbA_{1c} ≥6.5%) and the outcomes of HIV viral suppression (<50 copies/ml) 4-8 months after antiretroviral therapy initiation and hospitalization, tuberculosis, and death over 12 months. We used log-binomial, Poisson, and Cox proportional hazard models adjusting for confounders.

Results: Among 1369 PLHIV, 0.5% (n=7) reported a prior diabetes diagnosis, 20.6% (95% CI 18.5-22.8%, n=282) screened positive for pre-diabetes (HbA_{1c} 5.7-6.4%) and 3.5% (95% CI 2.7-4.6%, n=48) for diabetes. In univariable and multivariable analyses, older age, higher BMI, and mean arterial pressure were associated with diabetes. The number needed to screen to identify one new PLHIV with diabetes was 28.6 persons overall and 20.4 restricting to those with BMI ≥25 kg/m^2. Compared to PLHIV without diabetes, those with diabetes had 3.10-times (95% CI 1.05-9.19) adjusted hazard of death. Other outcomes were non-significant.

Conclusions: Diabetes and pre-diabetes were common among adults testing positive for HIV and associated with death. Clinic-based diabetes screening could be targeted to higher risk groups and may improve HIV treatment outcomes.

Key words: Diabetes mellitus, HIV, hyperglycemia, pre-diabetes, mortality, tuberculosis

INTRODUCTION

Diabetes is a chronic non-communicable disease that increases the risks of infection, hospitalization, and death and remains a major cause of global morbidity and mortality.[83, 109] In sub-Saharan Africa, the age-adjusted burden of diabetes is estimated to be 15.5 million people (4.4%) and is projected to reach 40.7 million people by the year 2045.[83] As antiretroviral therapies (ART) have increased life expectancy for people living with HIV (PLHIV), diabetes may play a larger role in chronic care and management. Among people living with HIV (PLHIV), diabetes is common and associated with age and long-term HIV or ART exposure.[20, 21]

In sub-Saharan Africa, where the burden of diabetes and HIV are high, there is limited data on the prevalence of diabetes among PLHIV and the impact of diabetes on clinical outcomes. Available data suggest PLHIV with diabetes may be at increased risk of active tuberculosis (TB) and death compared to PLHIV without diabetes.[66, 70, 71, 75]

A major challenge for diabetes care is early diagnosis.[31, 83, 110] In South Africa, country-wide diabetes prevalence is 5.4% and more than twice that in urban settings.[111, 112] However, prevalence surveys show 31-85% of people with diabetes are unaware of their disease status.[110, 112, 113] Clinics have established infrastructure for HIV screening and are uniquely positioned to facilitate diabetes diagnosis and treatment, but diabetes screening rates are thought to be low.[114, 115]

We examined the burden of diabetes, pre-diabetes, and associated risk factors among newly diagnosed HIV-positive South African adults. We estimated the number needed to screen (NNS) overall and using age and/or body mass index (BMI) to guide testing of patients at high risk for diabetes, and assessed relationships between diabetes and key clinical outcomes.

METHODS

Study population

Beginning in September 2013, we enrolled adults presenting for outpatient HIV screening at the iThembalabantu People's Hope Clinic, a large public clinic in the Umlazi township of Durban, South Africa. Eligible participants were ≥18 years, ART-naïve, English or Zulu speaking, had not received anti-fungal therapy within three months, and were not pregnant. For this analysis, we included only HIV-positive participants who had point-of-care hemoglobin A_{1c} (HbA_{1c}) testing for diabetes, which was initially performed at the clinician's discretion and then for all HIV-positive participants from January 2017 to February 2019.

Ethics approval and consent to participate

All study participants provided written informed consent in English or Zulu. The study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BF052/13) and the University of Washington Institutional Review Board (49563).

Study procedures

Prior to HIV testing, a research assistant collected demographic (age, sex, ethnicity, education, measures of socioeconomic status) and health information (cigarette and alcohol use; history of diabetes, kidney disease, liver disease) using a standardized in-person questionnaire. Research assistants also measured the participant's blood pressure, height, and weight. Serial HIV rapid testing and referral for ART was performed at the clinic by routine clinic staff as per local standard of care.[116]

Among PLHIV, a research nurse obtained blood samples, screened participants for TB, and performed a fingerpick HbA_{1c} test (A1cNow®$^{+}$, PTS Diagnostics, Indianapolis, IN). A1cNow®+ is an immunoassay that has been certified by the National Glycohemoglobin Standardization Program (NGSP), which works to harmonize HbA_{1c} tests.[117] Participants were referred to a clinician for additional testing and care according to national guidelines.[118]

At three, six, and 12 months after enrollment, a research assistant reviewed pharmacy and medical records and attempted to telephone the participant at least three times. At each time point they documented available HIV outcomes including HIV viral load, hospitalizations, and death. Hospitalized participants had additional chart review to determine the cause. Opportunistic infections were documented including herpes simplex lesions, oral/esophageal candida, pneumonias, cytomegalovirus, cryptosporidiosis, bacterial meningitis, toxoplasmosis, and herpes zoster. TB diagnoses were confirmed in

the South African TB Registry. Participants who could not be contacted, had no recent medical records, and were not confirmed to be attending another clinic were searched for in the South African National Death Registry.

Study definitions

We defined diabetes as HbA_{1c} ≥6.5%, which is consistent with World Health Organization (WHO) and American Diabetes Association (ADA) guidelines for lab-based high-performance liquid chromatographic HbA_{1c} testing.[8, 119] We considered pre-diabetes to be HbA_{1c} of 5.7-6.4%, diabetes as HbA_{1c} ≥6.5%, and hyperglycemia to be inclusive of both diabetes and pre-diabetes using HbA_{1c} ≥5.7%.[8] Measured cofactors for diabetes included body mass index (BMI) (overweight 25-29.9 kg/m^2; obese ≥30 kg/m^2), mean arterial pressure (MAP, [(2*diastolic blood pressure) + systolic blood pressure]/3), and anemia (hemoglobin <12 g/dL in women or <13 g/dL in men).[120] We used the nine-question validated Household Food Insecurity Access Scale (HFIAS) to ascertain food insecurity and defined it as the presence or absence of any food insecurity.[121] HIV viral load suppression was defined as ≤50 copies/mL 4-8 months after ART initiation. Tuberculosis was defined as acid-fast bacilli smear positive, GeneXpert positive, culture positive, or empiric treatment initiation.

Statistical analysis

We performed statistical analyses using SAS version 9.4 (Cary, NC). We used the Agresti-Coull method to calculate 95% confidence intervals (CI) for the prevalence of diabetes and

pre-diabetes.[122] We tested for correlates of diabetes at baseline and the intermediate study outcomes of ART initiation, missing ≥1 clinic visit, and missing ≥1 ART pharmacy refill using the χ-square or Fisher's exact test for categorical variables and ANOVA for continuous variables. To estimate the relative risk (RR) of diabetes at baseline, we used log-binomial regression models or Poisson regression with robust standard errors if the model failed to converge.[93] To identify potential predictors of screening positive for diabetes in this cohort, we performed multivariable backwards regression including characteristics statistically significantly associated with diabetes ($p<0.2$) in univariate analyses.

We estimated the number needed to screen (NNS) to yield one case of diabetes or hyperglycemia, the proportion identified, and proportion missed overall and for a range of age and BMI cut points. We generated receiver operating characteristic (ROC) curves using logistic regression to estimate the area under the curve (AUC) and identified the value at which sensitivity and specificity were maximized using Youden index.[123]

We estimated the risk of key HIV clinical outcomes by diabetes status in univariate analysis and after adjusting for age, sex, BMI, ART initiation, opportunistic infections, and anemia. We used log-binomial regression or Poisson regression with robust standard errors to estimate the RR of 1) not achieving viral suppression and 2) ART default or lost to follow-up. We used Cox proportional hazard models to estimate the relative difference in time to 1) first hospitalization, 2) tuberculosis diagnosis, or 3) death. We performed

sensitivity analyses excluding participants enrolled prior to 2017 when systematic HbA_{1c} screening was implemented.

RESULTS

Description of study population

Of the 7,877 adults who presented to iThembalabantu Clinic for HIV screening and were provisionally enrolled into the study, 3,104 tested positive for HIV. Hemoglobin A_{1c} testing following a positive HIV test was performed for 1,369 participants, all of whom were included in this analysis. The majority (57%) of participants were diagnosed with HIV at the time of enrollment. Those with an earlier diagnosis prior to study enrollment reported their diagnosis occurred a median of 10 months prior (IQR 0-44.5 months). Anemia was common among both men (30%) and women (54%) (**Table 1**). The majority of participants were overweight (24%) or obese (38%). Only 0.5% (n=7) reported a prior diagnosis of diabetes. Overall, 41% of participants were men, mean age was 33.4 ± 9.5 years, and median CD4 count was 364 cells/mm^3 (IQR 214-551 cells/mm^3). A viral load measurement was available for 871 participants collected 4-8 months after ART initiation (mean 6.1 months, standard deviation 0.9) at which point 83% (n=723) were virally suppressed. At 12-months, 51% of participants were reached by phone call.

Correlates of diabetes and pre-diabetes at time of HIV testing

Overall, 3.5% (95% CI 2.7-4.6%, n=48) of participants screened positive for diabetes and 20.6% (95% CI 18.5-22.8%, n=282) for pre-diabetes (**Table 1**). The mean age of

participants who screened positive for diabetes was higher than those with pre-diabetes or normal HbA_{1c} (p <0.001). Of those with pre-diabetes or diabetes, 74% were overweight or obese compared to 58% of those with normal HbA_{1c} (p <0.001). Participants with diabetes also had higher MAP (mean 103.6 mmHg) compared to those with pre-diabetes (95.9 mmHg) or normal HbA_{1c} (92.9 mmHg) (p-value <0.001).

In univariate regression analyses, several variables, including age, sex, completion of high school, income category, food insecurity, smoking status, alcohol use, Karnofsky score, BMI category, MAP, anemia, and hemoglobin were associated with risk of diabetes. In a multivariable model, each additional year of age was associated with a 4% increase in the risk of screening positive for diabetes (95% CI 1.01-1.08, p=0.010) after controlling for BMI category, current alcohol use, Karnofsky score, and MAP (**Table 2**). Obesity was associated with 2.57-times (95% CI 1.02-6.28, p=0.041) the risk of screening positive for diabetes after controlling for confounding factors compared to those who had a BMI <25 kg/m^2.

Diabetes and hyperglycemia screening at HIV testing

Overall, the NNS to identify one newly diagnosed PLHIV at this urban clinic with diabetes was 29 people and the NNS for hyperglycemia was four people (**Table 3**). The NNS for one new case of hyperglycemia was three people among those ≥45 years, though only 21% of cases could potentially be identified relying solely on this metric. Using a BMI ≥25 kg/m^2, the NNS to identify one instance of hyperglycemia is four people and 74% of cases

could be identified. Applying South African and international guidelines of BMI plus one additional risk factor, 14 people would need to be screened to identify one case of diabetes and four for one case of hyperglycemia.

Age and BMI were predictive of diabetes with AUCs of 0.72 and 0.69, respectively (**Figure 1A**). Age most accurately predicted diabetes using a cut point of 38.6 years. At this point sensitivity (i.e. the proportion of those with diabetes who would be eligible for screening) was maximized at 62.5% and specificity (i.e. the proportion of the without diabetes who would not be eligible for screening) was maximized at 77.1%. Taking age and BMI together the AUC was 0.77. At 30 years and 25 kg/m^2 (specificity: 62.7%, sensitivity: 70.8%), the NNS is 24 people (**Figure 1B**) and 95.8% of diabetes cases would be captured (**Figure 1C**).

Using age alone to identify hyperglycemia, combined sensitivity and specificity were maximized at 30.3 years with a specificity of 45.8% and sensitivity of 64.9% (AUC 0.60). The predictive accuracy of BMI alone for hyperglycemia was maximized at 25.2 kg/m^2 with a specificity of 43.8% and sensitivity of 73.9% (AUC 0.64).

Diabetes and clinical outcomes

The majority of participants initiated ART; 93.0% of those with normal HbA_{1c} initiated ART, 86.9% with moderately elevated HbA_{1c}, and 87.5% with high HbA_{1c} (p=0.029).

Among those who initiated ART, there was no difference in the proportion who defaulted, missed ≥1 pharmacy refill, or missed ≥1 clinic visit by HbA_{1c} level.

In adjusted analyses, PLHIV with diabetes were not less likely to achieve viral suppression (adjusted RR 0.75, 95% CI 0.20-2.86) or at higher risk of TB diagnosis (adjusted hazard ratio 1.73, 95% CI 0.69-4.34) (**Table 4**). Cumulative survival was poorer among participants with diabetes than those without diabetes (**Figure 1D**). The adjusted mortality rate was 3-times higher among those with diabetes than those without (95% CI 1.05-9.19).

Among those with hyperglycemia, there was no difference in the risk of lack of viral suppression, TB, hospitalization, death, or loss to follow-up compared to those without hyperglycemia.

Sensitivity Analyses

Prior to systematically screening all participants using the point-of-care HbA1c test, 151 were tested, among which 12 had an HbA_{1c} ≥6.5. Excluding these participants, the prevalence of diabetes was 2.2%, yielding a NNS of 46.4 people. Elevated HbA_{1c} was statistically significantly associated with traditional risk factors including higher age and MAP but not higher BMI (**Supplemental Table 1**) or death (**Supplemental Table 2**).

DISCUSSION

In this cohort of newly diagnosed HIV-positive adults in Durban, South Africa, the prevalence of pre-diabetes was high and diabetes was low with few people reporting a prior diabetes diagnosis. Consistent risk factors for diabetes included older age, higher BMI, and higher MAP. Diabetes was associated with mortality over the 12 months following HIV diagnosis. Our data support screening all PLHIV for diabetes or targeting those with a BMI $\geq 25 kg/m^2$ if universal screening is not practical.

The prevalence of diabetes among PLHIV in this cohort was 3.5%, or 2.2% in sensitivity analyses, which is similar to observations from other studies of PLHIV in urban South Africa and other sub-Saharan African countries.[21, 124-128] The prevalence of diabetes in the general population in South Africa is estimated to be slightly higher with recent estimates ranging from 5.4% to 10.1%.[83, 110, 129] Risk factors for diabetes in this population included those that are well-established among HIV-uninfected adults and PLHIV in sub-Saharan Africa, such as older age and hypertension.[8, 130]

Missed opportunities for diagnosing diabetes remain a major concern in this population. In our cohort, the proportion of people aware of their diabetes was much lower than in national prevalence surveys;[112, 113] only one of the 48 participants with $HbA_{1c} \geq 6.5\%$ had received a prior diabetes diagnosis. The Society for Endocrinology, Metabolism, and Diabetes of South Africa recommends diabetes screening for PLHIV at the time of ART initiation or regimen change while South Africa's national HIV guidelines do not specify

recommendations for routine diabetes screening.[118] Inclusion of diabetes screening recommendations in HIV guidelines may help improve uptake.

As in the general population, age and BMI could be used to further increase the yield of diabetes screening by restricting screening to those more likely to have hyperglycemia among PLHIV. We were unable to directly assess if PLHIV were likely to demonstrate elevated HbA_{1c} at relatively lower ages and BMIs compared to HIV-negative adults because of a lack of comparison group. However, data from this cohort suggests screening PLHIV with a BMI $\geq 25 kg/m^2$ could reduce the NNS while capturing nearly all diabetes cases. The AUC suggests the optimal age threshold is lower than guideline of ≥45 years for HIV-negative adults, which is supported by research showing PLHIV are more likely to develop diabetes at younger ages.[23, 131]

We decided *a priori* to test the validity of age and BMI because they are known to be important predictors of diabetes, are quickly and easily ascertainable in an outpatient clinic, and are used to inform screening eligibility for HIV-negative adults.[118] Age and BMI were independently associated with elevated HbA_{1c} in this cohort using stepwise logistic regression, further supporting the potential use of these measurements to inform diabetes screening practices. BMI was not significant in the sensitivity analysis, which could be due to lack of statistical power or indicative of PLHIV being susceptible to diabetes at lower BMIs as reported previously.[19]

Our study contributes to the literature suggesting the risk of active TB among PLHIV with diabetes compared to those without diabetes is low, in contrast to the 3-fold elevated risk of TB among people without HIV infection.[50] Of three studies examining the association of diabetes with active TB among HIV-infected adults, two found an association with higher odds of TB (n=232, aOR 4.7, 95% CI 1.1-20.8; n=521, aOR 2.4, 95% CI 1.0-5.9), and one found no effect (n=382, aOR 0.14, 95% CI 0.01-1.81).[66, 70, 71] In this cohort, ascertainment of TB cases over the 12 month study period was thorough and statistical significance was not achieved.

The presence of multiple morbidities complicates patient care, especially when the diseases differ in pathogenesis and management. Regular clinic visits for ART maintenance are an opportunity for non-communicable disease care. In South Africa, PLHIV on maintenance therapy can have better managed diabetes than the general population, but evidence is mixed.[115, 128, 132-134] In this cohort, there was no significant difference in ART initiation or maintenance, but time to death was faster among those with diabetes overall but not in the sensitivity analyses likely due to lack of statistical power. In Brazil and the United States, risk of death among PLHIV was 2-3-times higher for diabetic patients.[135, 136] As testing and diagnosis of diabetes and other non-communicable diseases increase for PLHIV, it will be important to consider how to optimize care for patients and clinics and to study the cost-effectiveness of different approaches to integrated care.

Our study had several strengths and limitations. Our study cohort was large and representative of the township population at risk for HIV. We comprehensively ascertained HIV outcomes by calling the participant and/or family member, and reviewing hospital records, pharmacy records, and national registries. We estimated RRs, which are more consistent, conservative, and interpretable than odds ratios, and used time-to-event analyses where relevant to not introduce bias. However, we relied on a single point-of-care HbA_{1c} test to screen for diabetes, whereas guidelines recommend two lab-based HbA_{1c} tests several weeks apart.[8, 137-141] Point-of-care HbA_{1c} tests are not standardized, require regular calibration, and therefore are not recommended as diagnostic tests by the WHO or ADA. In prior validation diagnostic studies, A1cNow®+ had excellent correlation (>90%) with gold standard high performance liquid chromatography HbA_{1c}, 82% specificity, 100% sensitivity, but moderately overestimated HbA_{1c} levels (mean difference 0.2-0.3% units).[142, 143] The coefficients of variation (2.7-2.9%) were reasonable for a point-of-care test, although not below the recommended level of <2%.[144] Additionally, HbA_{1c} may moderately overestimate diabetes among PLHIV especially with concurrent iron deficiency and therefore our estimate of the prevalence of diabetes may be high, although we adjusted for anemia in outcome analyses.[8, 137-141] Diabetes was selectively screened for in prior to 2017 and 22 of the 48 diabetes cases detected were among the 163 participants tested for HbA1c at that time, and therefore our estimate of the prevalence of diabetes may be high. Lastly, all major risk factors for diabetes were not ascertained in this cohort; therefore, we could not test for associations with all potential risk factors.

Conclusions

In summary, in urban South Africa hyperglycemia was common among adults testing positive for HIV in an outpatient setting and associated with a higher risk of death. Diabetes prevalence was lower than the general population, but the majority reported no prior diabetes diagnosis indicating an opportunity for disease detection. The NNS to identify one new case of hyperglycemia was low overall and could be further reduced using age or BMI. Further research is needed to refine diabetes screening guidelines among PLHIV using other methods of diabetes testing, assess the impact of diabetes rapid diagnosis and treatment on HIV outcomes, and optimize integrated chronic disease care.

LIST OF ABBREVIATIONS

ART – Antiretroviral therapy

AUC – Area under the curve

BMI – Body mass index

HbA_{1c} – Hemoglobin A_{1c}

MAP – Mean arterial pressure

NNS – Number needed to screen

OR – Odds ratio

PLHIV – People living with HIV

ROC – Receiver operating characteristic

RR – Risk Ratio

TB – Tuberculosis

DECLARATIONS

Competing interests

The authors declare no conflicts of interest.

Funding

This work was supported by the National Institute for Allergy and Infectious Diseases (K23 AI108293; R03 AI143351; F31 AI138633) of the National Institutes of Health.

Authors' Contributions

R.W.K., P.K.D. designed the study. S.G., S.G., H.T., M.S.M, and P.K.D. were responsible for participant recruitment, data acquisition and management. R.W.K. performed analyses with input from E.R.B and A.A.M. R.W.K., M.K., and P.K.D. drafted the manuscript and A.A.M., S.G., S.G., H.T., E.R.B. and M.S.M. provided critical review.

Acknowledgements

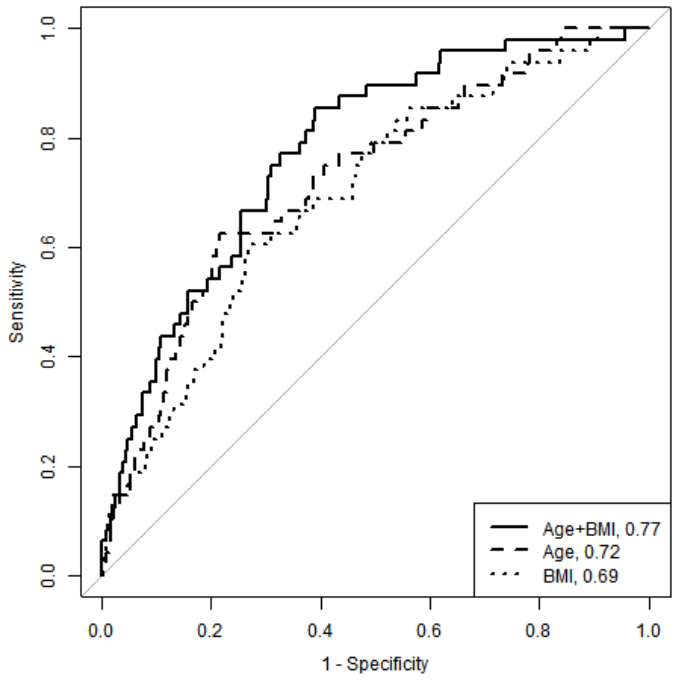
We thank all participants in this study, the clinical sites that shared their space, and the research staff and nurses who performed the study.

Availability of data and materials

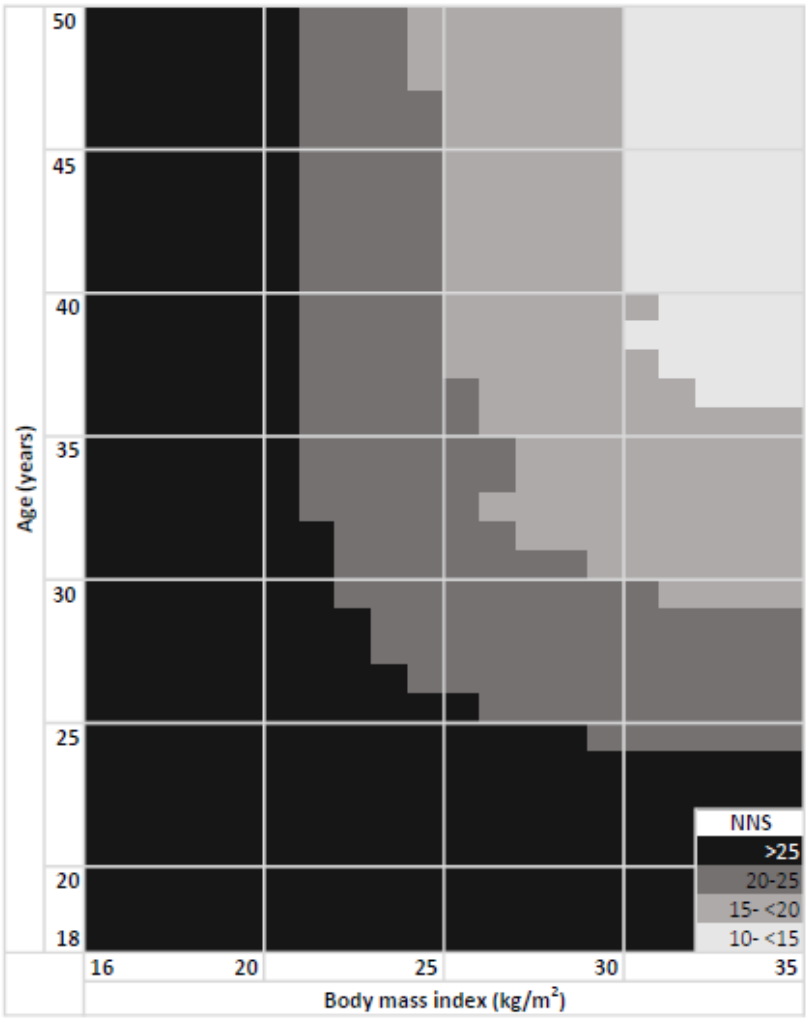
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Figure 1. Accuracy (A), number needed to screen (B), and percent of cases captured (C) using age (years) and body mass index as discrete screening criteria for hemoglobin A_{1c} ≥6.5%. Kaplan-Meier survival curves by hemoglobin A_{1c} level (D).

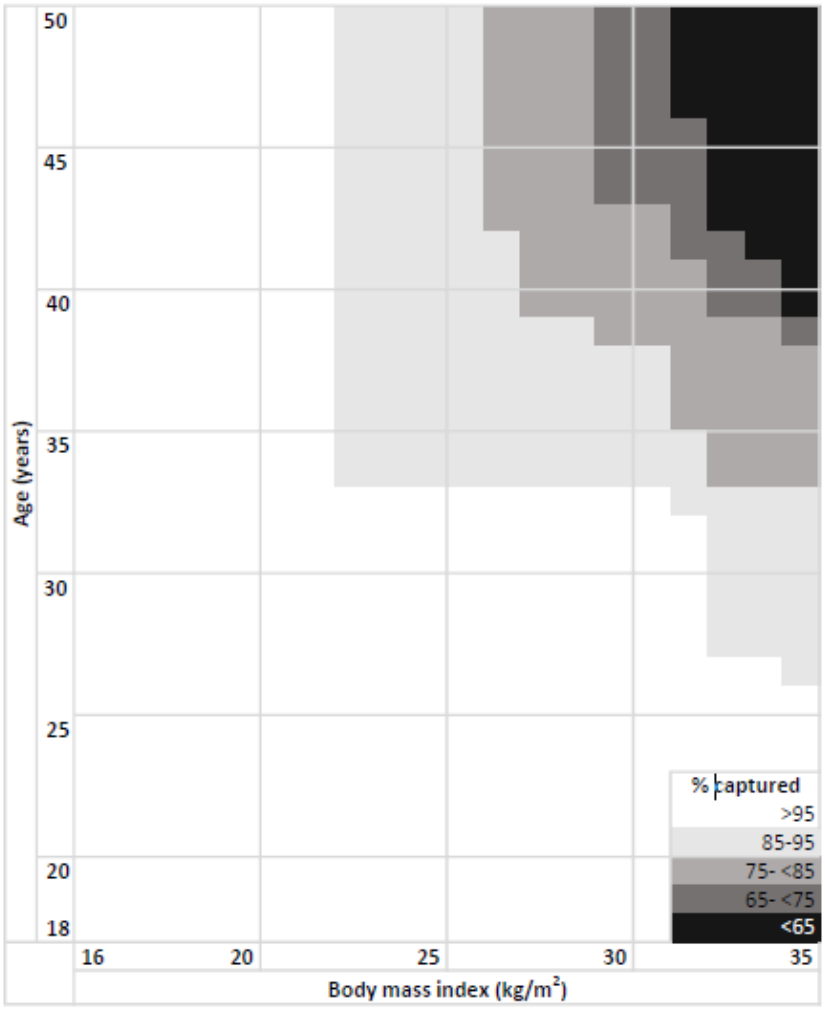
A)



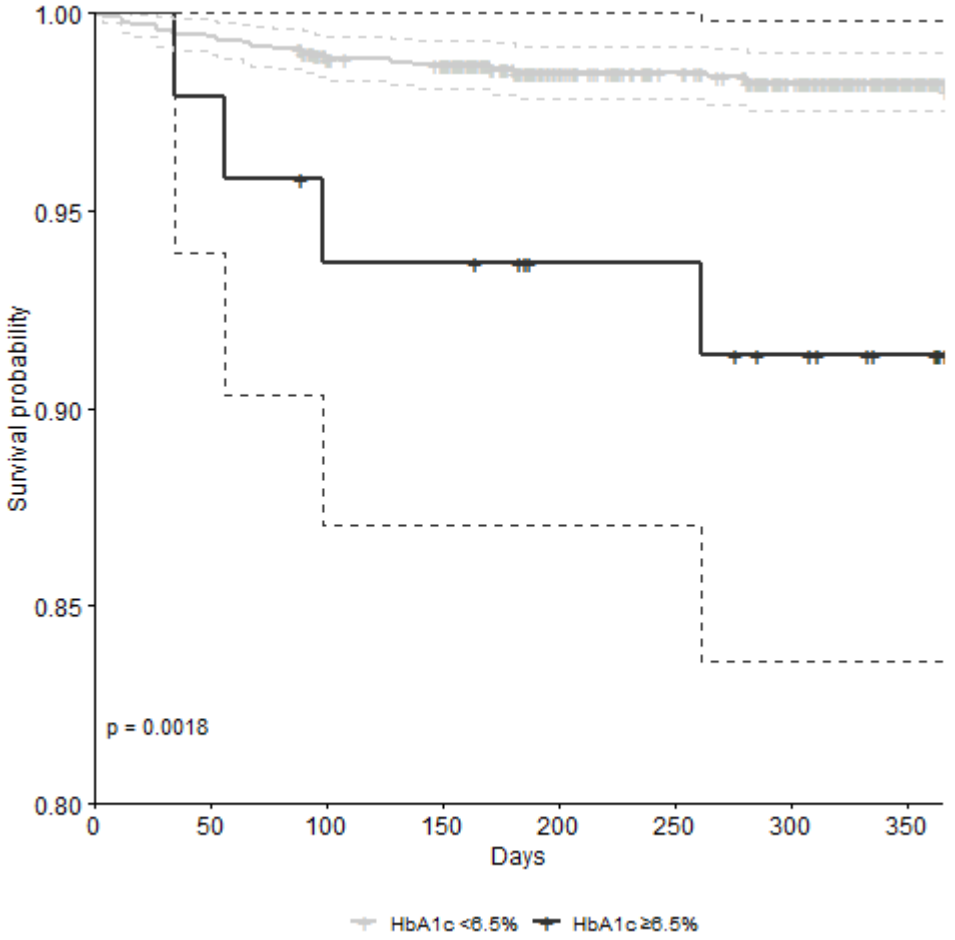
B)


50
45
40
35
30
25
20
18
Age (years)
16
20
25
30
35
Body mass index (kg/m²)
NNS
>25
20-25
15- <20
10- <15

C)


50
45
40
35
30
25
20
18
Age (years)
% captured
>95
85-95
75- <85
65- <75
<65
16
20
25
30
35
Body mass index (kg/m²)

D)



Legend: A) Distance from the receiver operating characteristic curve to the no discrimination line is maximized for HbA_{1c} ≥6.5% at age alone at 38.6 years (specificity: 77.1%, sensitivity: 62.5%, positive likelihood ratio: 2.72), BMI alone at 31.6 kg/m^2 (specificity: 70.8%, sensitivity: 60.4%, positive likelihood ratio: 2.07).
B) Number need to screen to identify one instance of HbA_{1c} ≥6.5%. The lower leftmost black square represents screening the entire population (NNS=28.5) and the upper rightmost light grey square is the most restrictive screening algorithm represented (NNS=12.7).
C) Proportion of participants with HbA_{1c} ≥6.5% captured. The lower leftmost white square represents screening the entire population (100% captured) and upper rightmost black square is the most restrictive screening algorithm represented (43.8% captured).
D) Kaplan-Meier curves of days to death from time of enrollment by HbA_{1c} ≥6.5% (black) and HbA_{1c} <6.5% (grey).

Table 1. Baseline characteristics of people living with HIV screened for diabetes in South Africa.

	Overall (n=1369) N (%) or mean ±SD	**HbA1c ≤5.6%** (n=1039) N (%) or mean ±SD	**HbA1c 5.7-6.4%** (n=282) N (%) or mean ±SD	**HbA1c ≥6.5%** (n=48) N (%) or mean ±SD	**p-value**
Sociodemographics					
Male	554 (40.5)	436 (42.0)	103 (36.5)	15 (31.3)	0.107
Age (years)	33.4 ± 9.5	32.5 ± 8.9	35.2 ± 10.2	41.2 + 10.6	**<0.001**
Zulu or Xhosa (n=1211)	1300 (98.4)	1003 (98.6)	256 (97.7)	41 (97.6)	0.526
Married	79 (5.8)	54 (5.2)	20 (7.0)	5 (10.4)	0.179
Completed high school or higher degree	808 (59.0)	618 (59.5)	168 (59.6)	22 (45.8)	0.167
Employed ≥20 hours/week	268 (19.6)	210 (20.2)	51 (18.1)	7 (14.6)	0.491
Income <2000 ZAR/month (n=1365)	857 (62.8)	644 (62.2)	176 (62.4)	37 (77.1)	0.113
Food insecure (mild, moderate, severe)	32 (2.3)	18 (1.7)	10 (3.6)	4 (8.3)	**0.004**
<5km from clinic	939 (68.7)	694 (66.9)	209 (74.4)	36 (75.0)	**0.035**
Current smoker	265 (19.4)	216 (20.8)	44 (15.7)	5 (10.4)	**0.042**
Current alcohol use	469 (34.3)	376 (36.3)	90 (31.9)	3 (6.3)	**<0.001**
Prior diabetes diagnosis (n=538)	7 (1.3)	6 (1.3)	0 (0)	1 (16.7)	0.082
Uses insulin	2 (28.6)	1 (20)	0 (0)	1 (100)	0.286
Self-reported liver disease	5 (0.4)	3 (0.3)	2 (0.7)	0 (0)	0.407
Self-reported kidney disease	27 (2.0)	16 (1.5)	10 (3.6)	1 (2.1)	0.099
Clinical characteristics and laboratory testing					
Karnofsky score (n=1368)	88.0 ± 6.3	88.2 ± 5.9	87.7 ± 6.7	84.6 ± 9.7	**<0.001**
Body mass index (kg/m^2) (n=1368)	28.3 ± 6.9	27.5 ± 6.6	30.3 ± 6.8	33.6 ± 8.9	
<25	524 (38.0)	438 (42.2)	79 (28.0)	7 (14.6)	**<0.001**
25-29.9	322 (23.5)	275 (26.5)	38 (13.5)	9 (18.8)	
≥30	522 (38.2)	325 (31.3)	165 (58.5)	32 (66.7)	
Hypertension	276 (20.2)	214 (20.6)	47 (16.7)	15 (31.3)	0.052
Mean arterial pressure (mmHg)	93.9 ± 15.9	92.9 ± 15.3	95.9 ± 17.0	103.6 ± 18.2	**<0.001**
Hepatitis B (n=911)	52 (5.7)	37 (5.7)	13 (6.0)	2 (4.7)	0.944
Anemia (n=1240)	541 (43.6)	403 (42.1)	115 (47.3)	23 (59.0)	**0.049**

Hemoglobin (g/dL) (n=1240)	12.4 ± 2.1	12.5 ± 2.1	12.2 ± 1.9	11.8 ± 2.2	**0.049**
Women	11.4 ± 1.8	11.6 ± 1.7	11.7 ± 1.6	10.9 ± 1.9	0.097
Men	13.2 ± 2.1	13.6 ± 2.1	13.0 ± 2.1	13.3 ± 1.8	**0.044**
CD4 count (cells/mm^3) (n=1361)*	364 [214-551]	367 [222-555]	343 [203-518]	406 [141-606]	
<200	312 (22.8)	232 (22.4)	67 (24.1)	13 (27.1)	0.429
200-350	342 (25.0)	257 (24.8)	77 (27.7)	8 (16.7)	
>350	707 (51.6)	546 (52.8)	134 (48.2)	27 (56.3)	

N (%) or mean ± standard deviation.

P-values obtained using Chi-square or Fisher's exact test for categorical variables and ANOVA for continuous variables

* Median [interquartile range]

Table 2. Univariate and multivariate analysis of the risk of diabetes (hemoglobin A1c ≥6.5%) by baseline characteristics (n=1369).

	RR (95% CI) n=1369	**p-value**	**aRR* (95% CI)** n=1254	**p-value**
Male	1.50 (0.82-2.73)	0.189		
Age per 1-year increase	1.07 (1.04-1.09)	**<0.001**	1.04 (1.01-1.08)	**0.010**
Married	1.90 (0.77-4.66)	0.162		
Did not complete high school	1.70 (0.97-2.97)	0.062		
Employed ≥20 hours/week	1.43 (0.65-3.14)	0.379		
Income <2000 ZAR/month (n=1365)	1.99 (1.03-3.87)	**0.007**		
Food insecure (mild, moderate, severe)	3.80 (1.45-9.94)	**0.006**		
Lives <5 kilometers from clinic	1.37 (0.72-2.60)	0.340		
Current smoker	0.48 (0.19-1.21)	0.120		
Current alcohol use	0.13 (0.03-0.41)	**<0.001**	0.19 (0.06-0.64)	**0.008**
Karnofsky score per 10-unit increase (n=1368)	0.60 (0.46-0.77)	**<0.001**	0.54 (0.37-0.80)	**0.002**
Body mass index (kg/m^2) (n=1368)				
<25	*Ref.*		*Ref.*	
25-29.9	2.09 (0.79-5.56)	0.139	1.39 (0.47-4.07)	0.550
≥30	4.59 (2.04-10.30)	**<0.001**	2.57 (1.04-6.37)	**0.041**
Mean arterial pressure (mmHg) (n=1368)	1.03 (1.01-1.04)	**<0.001**	1.02 (1.00-1.04)	**0.026**
Hepatitis B (n=911)	0.81 (0.20-3.24)	0.761		
Anemia (n=1240)	1.86 (0.99-3.48)	0.053		
Hemoglobin (g/dL) (n=1240)	0.88 (0.77-1.01)	0.072		
CD4 count (cells/mm^3) (n=1361)				
<200	1.09 (0.57-2.09)	0.792		
200-350	0.61 (0.28-1.33)	0.217		
>350	*Ref.*			

*Variables tested for inclusion in adjusted model if p<0.2 in unadjusted model and retained in adjusted model through backwards regression

Table 3. Prevalence of elevated hemoglobin A1c and the number needed to screen at the time of HIV testing according to national screening guidelines.

Outcome	$N_{outcome}$ /N_{total}	%	NNS	% hyperglycemic cases identified	% hyperglycemic cases missed
Overall					
Diabetes (HbA1c ≥6.5%)	48/1369	3.5	28.6	100	0
Hyperglycemia (HbA1c ≥5.7%)	330/1369	24.1	4.1	100	0
Age ≥45 years					
Diabetes (HbA1c ≥6.5%)	16/169	9.5	10.5	33.3	66.7
Hyperglycemia (HbA1c ≥5.7%)	65/169	38.5	2.6	19.7	80.3
BMI ≥25 kg/m^2					
Diabetes (HbA1c ≥6.5%)	41/844	4.9	20.4	85.4	14.6
Hyperglycemia (HbA1c ≥5.7%)	244/844	28.9	3.5	73.9	26.1
Age ≥45 years and/or BMI ≥25 kg/m^2					
Diabetes (HbA1c ≥6.5%)	42/888	4.7	21.3	87.5	12.5
Hyperglycemia (HbA1c ≥5.7%)	254/888	28.6	3.5	77.0	23.0

BMI, body mass index; HbA1c, hemoglobin A1c; NNS, number needed to screen

Table 4. Associations of diabetes with HIV outcomes 12 months after HIV diagnosis

	Event/Total$_{Unexposed}$ N (%)	**Event/Total$_{Exposed}$** N (%)	**HR or RR (95% CI)**	**p-value**	**aHR or aRR* (95% CI)**	**p-value**
HbA1c ≥6.5%						
HIV viral load ≥50 copies/mL	145/849 (17.1)	3/22 (13.6)	0.80 (0.28-2.31)	0.678	0.75 (0.20-2.86)	0.672
Hospitalized**	80/1321 (6.1)	4/48 (8.3)	1.36 (0.50-3.72)	0.547	1.38 (0.49-3.87)	0.544
Tuberculosis**	96/1321 (7.3)	5/48 (10.4)	1.48 (0.60-3.647)	0.390	1.73 (0.69-4.34)	0.240
Died**	38/1321 (2.9)	4/48 (8.3)	2.84 (1.02-7.97)	**0.047**	3.10 (1.05-9.19)	**0.041**
Default or lost to follow-up	230/1321 (17.4)	8/48 (16.7)	0.96 (0.50-1.82)	0.894	0.94 (0.35-2.50)	0.904
HbA1c ≥5.7%						
HIV viral load >50 copies/mL	109/668 (16.3)	39/203 (19.2)	1.18 (0.85-1.64)	0.046	1.23 (0.87-1.73)	0.238
Hospitalized**	65/1039 (6.3)	19/330 (5.8)	0.91 (0.55-1.52)	0.717	0.93 (0.55-1.57)	0.929
Tuberculosis**	77/1039 (7.4)	24/330 (7.3)	1.01 (0.64-1.61)	0.955	1.07 (0.66-1.74)	0.775
Died**	30/1039 (2.9)	12/330 (3.6)	1.23 (0.63-2.41)	0.542	1.14 (0.56-2.32)	0.727
Default or lost to follow-up	184/1039 (17.1)	54/330 (16.4)	0.92 (0.70-1.22)	0.576	0.82 (0.61-1.10)	0.186

aHR, adjusted hazard ratio; aRR, adjusted risk ratio; CI, confidence interval; HR, hazard ratio; HbA1c, hemoglobin A1c; RR, risk ratio.

*Adjusting for age, sex, body mass index, antiretroviral therapy initiation, anemia, and opportunistic infections excluding tuberculosis when it is the primary exposure of interest and otherwise inclusive of tuberculosis diagnosis

** Hazard ratio

Supplemental Table 1. Sensitivity analysis including only participants enrolled when routine diabetes screening was performed - Univariate and multivariate analysis of the risk of diabetes (hemoglobin A_{1c} ≥6.5%) by baseline characteristics (n=1206).

	RR (95% CI) n=1206	**p-value**	**aRR* (95% CI)** n=1200	**p-value**
Male	0.93 (0.43-2.00)	0.859		
Age per 1-year increase	1.08 (1.05-1.11)	**<0.001**	1.05 (1.02-1.09)	**0.004**
Married	1.51 (0.37-6.26)	0.568		
Did not complete high school	1.81 (0.84-3.87)	0.128		
Employed ≥20 hours/week	1.10 (0.42-2.88)	0.849		
Income <2000 ZAR/month	1.48 (0.65-3.37)	0.356		
Food insecure (mild, moderate, severe)	NA			
Current smoker	0.69 (0.24-1.98)	0.486		
Current alcohol use	0.23 (0.07-0.76)	**0.016**	0.26 (0.07-0.88)	**0.030**
Karnofsky score per 10-unit increase	0.57 (0.39-0.82)	**0.003**	0.62 (0.39-0.98)	**0.042**
Body mass index (kg/m^2)				
<25	*Ref.*			
25-29.9	2.08 (0.78-5.54)	0.141		
≥30	2.06 (0.79-5.36)	0.139		
Mean arterial pressure (mmHg)	1.03 (1.01-1.05)	**<0.001**	1.03 (1.01-1.04)	**0.015**
Hepatitis B	1.32 (0.32-5.49)	0.704		
Anemia	1.66 (0.76-3.62)	0.205		
Hemoglobin (g/dL)	0.91 (0.77-1.08)	0.299		
CD4 count ($cells/mm^3$)				
<200	1.44 (0.60-3.48)	0.418		
200-350	0.96 (0.36-2.53)	0.935		
>350	*Ref.*			

*Variables tested for inclusion in adjusted model if p<0.2 in unadjusted model and retained in adjusted model through backwards regression

Table 4. Sensitivity Analysis including only participants enrolled when routine diabetes screening was performed – Associations of diabetes with HIV outcomes 12 months after HIV diagnosis

	Event/Total$_{Unexposed}$ N (%)	**Event/Total$_{Exposed}$** N (%)	**HR or RR (95% CI)**	**p-value**	**aHR or aRR* (95% CI)**	**p-value**
HbA1c ≥6.5%						
HIV viral load ≥50 copies/mL	136/792 (17.2)	2/13 (15.4)	0.90 (0.25-3.24)	0.867	0.66 (0.10-4.36)	0.664
Hospitalized**	73/1180 (6.2)	2/26 (8.3)	1.30 (0.32-5.28)	0.718	1.20 (0.29-5.02)	0.800
Tuberculosis**	93/1180 (7.9)	3/26 (11.5)	1.51 (0.48-4.76)	0.484	1.28 (0.40-4.11)	0.681
Died**	37/1180 (3.1)	2/26 (7.7)	2.58 (0.62-10.69)	0.192	1.79 (0.41-7.86)	0.443
Default or lost to follow-up	194/1180 (16.4)	3/26 (11.5)	0.70 (0.24-2.05)	0.517	1.01 (0.32-3.20)	0.993
HbA1c ≥5.7%						
HIV viral load >50 copies/mL	106/639 (16.6)	32/166 (19.3)	1.16 (0.81-1.66)	0.409	1.17 (0.81-1.70)	0.398
Hospitalized**	63/968 (6.5)	12/238 (5.0)	0.78 (0.42-1.44)	0.717	0.73 (0.39-1.36)	0.316
Tuberculosis**	75/968 (7.8)	21/238 (8.8)	1.19 (0.73-1.93)	0.491	1.08 (0.65-1.79)	0.773
Died**	29/968 (3.0)	10/238 (4.2)	1.39 (0.68-2.84)	0.375	1.02 (0.47-2.21)	0.956
Default or lost to follow-up	166/968 (17.2)	31/238 (13.0)	0.76 (0.53-1.08)	0.130	0.82 (0.59-1.15)	0.248

aHR, adjusted hazard ratio; aRR, adjusted risk ratio; CI, confidence interval; HR, hazard ratio; HbA1c, hemoglobin A1c; RR, risk ratio.

*Adjusting for age, sex, body mass index, antiretroviral therapy initiation, anemia, and opportunistic infections excluding tuberculosis when it is the primary exposure of interest and otherwise inclusive of tuberculosis diagnosis

** Hazard ratio

Chapter 4: Sub-clinical tuberculosis prevalence and characteristics among HIV-positive adults in Durban, South Africa

Rachel W. Kubiak[1], Sabina Govere[2], Sean R. Galagan[3], Hilary Thulare[2], Mahomed-Yunus Moosa[4], Paul K. Drain[1,3,5]

[1] Department of Epidemiology, University of Washington, Seattle, USA

[2] AIDS Healthcare Foundation, Durban, South Africa

[3] Department of Global Health, University of Washington, Seattle, Washington

[4] Department of Infectious Diseases, University of KwaZulu-Natal, Durban, South Africa

[5] Department of Medicine, University of Washington, Seattle, Washington.

ABSTRACT

Setting: An outpatient clinic in Durban, South Africa

Objective: Assess the burden of and characteristics associated with sub-clinical TB among HIV-positive adults in Durban, South Africa

Design: We administered a standardized four symptom TB screening questionnaire to adults presenting for HIV testing at an outpatient clinic, and collected sputum for culture regardless of TB symptoms. We defined sub-clinical TB as *Mycobacterium TB* culture-positive with no TB symptoms and active TB as culture-positive with at least one TB symptom. We compared culture-negative participants to those with sub-clinical TB, and used logistic regression to test the predictive power of C-reactive protein (CRP).

Results: Among 437 participants, five (1.1%) had sub-clinical TB and 38 (8.7%) had active TB. There was no significant difference between sub-clinical TB and culture-negative participants by mean body mass index (27.6 ±5.6 versus 26.1 ±6.8 kg/m^2) or median CD4 count (389 [106-408] versus 335 [187-484] cells/mm^3). A ten-unit increase in CRP was associated with higher odds of culture positivity (OR=1.13, 95% CI 1.01-1.19) overall, but not for sub-clinical TB compared to TB-negative participants (OR=0.85, 95% CI 0.48-1.50).

Conclusion: Routinely collected information at an outpatient clinic were not strong predictors of sub-clinical TB among adults living with HIV.

INTRODUCTION

Early detection of active tuberculosis (TB) leads to earlier anti-TB treatment initiation, reduced morbidity and transmission, and is a key component of the World Health Organization's End TB Strategy.[78] TB has traditionally been characterized as either latent or active, but more recent evidence describes a spectrum of disease and includes a sub-clinical disease state between these two. Sub-clinical TB is characterized by replicating *Mycobacterium tuberculosis* (MTB) in the absence of TB symptoms indicating a high likelihood of infectiousness and progression to active disease.[73] This disease state is an opportunity to identify and treat people before they progress to active disease. However, identifying people who should be tested for sub-clinical disease is challenging, since they will be missed by the recommended 4-symptoms screening questions.

Given the challenges with testing and identification of key populations, there is limited data on the prevalence of sub-clinical TB with estimates ranging from 1-6%.[74-76] Additionally, there is a need for more data on who is at risk for sub-clinical disease and would benefit from targeted screening. HIV-infection has been associated with 2-fold higher odds of sub-clinical TB among adults without TB symptoms, but there is limited information available about other risk factors and how to target screening in this population.[77]

We assessed burden of and characteristics associated with sub-clinical TB among HIV-positive adults in Durban, South Africa.

METHODS

Study Design

We enrolled adults at the time they received a positive HIV test result at the iThembalabantu People's Hope Clinic, a large outpatient clinic in the Umlazi township of Durban, South Africa. Eligible participants were ≥18 years, did not report prior use of anti-retroviral therapy (ART), were English or Zulu speaking, had not received anti-fungal therapy within three months, and were not pregnant. These cross-sectional analyses include all participants enrolled from January 2014 to December 2015 who were asked to provide sputum for culture irrespective of TB symptoms.

All study participants provided written informed consent in English or Zulu. The study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BF052/13) and the University of Washington Institutional Review Board (49563).

Study Procedures

A research assistant collected sociodemographic information and medical history using a standardized in-person questionnaire. They directly measured the participant's blood pressure, height, and weight. HIV testing and referral for ART was performed at the clinic by routine clinic staff as per local standard of care.[116]

All participants who tested positive for HIV were asked to provide sputum regardless of symptoms for mycobacterium culture on Middlebrook 7H11 agar. If the participant was unable to expectorate sputum, sputum was induced by hypertonic saline nebulization. Two cultures were performed for each viable sputum sample. GeneXpert testing was performed according to local guidelines for those who were symptomatic or had clinical suspicion for TB.

Statistical Analyses

We defined sub-clinical TB as sputum culture-positive for MTB with no chronic cough, fever, night sweats, or weight loss. We defined active TB as sputum culture-positive with at least one TB symptom. We did not include GeneXpert positivity in our case definition because it was only performed on those who met symptom or clinical eligibility criteria.

We estimated the prevalence of sub-clinical TB and generated 95% confidence intervals (CI) using the Agresti-Coull method.[122] We tested demographic and clinical characteristics for associations with sub-clinical TB compared to MTB-negative participants using Fisher's exact test for categorical variables and Student's t-test for unequal variance for continuous variables. Finally, we used logistic regression to test the predictive power of C-reactive protein (CRP) and hemoglobin and estimated the area under the curve (AUC). Data were analyzed using SAS version 9.4 (Cary, NC).

RESULTS

Among 442 participants enrolled in the study, cultures were contaminated for two and missing for three who were excluded from further analysis. Among the 437 with at least one culture result, 60.6% (n=256) were female, mean age was 33.5 years (standard deviation 9.3 years), and median CD4 count was 301 cells/mm^3 (interquartile range [IQR] 171-467 cells/mm^3) (**Table 1**). More than half (58%, 130/223) of participants required sputum induction. GeneXpert testing was positive for 26 (23.2%) of the 112 participants for whom testing was indicated.

Overall, among the 437 participants, sub-clinical TB was diagnosed in 1.1% (95% CI 0.4-2.7%, n=5) and active TB in 8.7% (95% CI 6.4-11.7%, n=38). Among the 43 participants with culture-confirmed TB, 9.4% (n=5) of cases were sub-clinical. The prevalence of sub-clinical TB among participants without TB symptoms was 3.0% (95% CI 1.1-7.0%, n=5/167). One participant with sub-clinical TB also had a GeneXpert test done, which was positive. The prevalence of active TB among those with TB symptoms was 14.0% (95% CI 10.4-18.7%, n=38/270).

There were no statistically significant differences between sub-clinical TB and culture-negative participants by sex (60% versus 40% female, p=0.394), mean age (34.3 ±8.6 versus 33.1 ±9.3 years), mean body mass index (27.6 ±5.6 versus 26.1 ±6.8 kg/m^2), or median CD4 count (389 [106-408] versus 335 [187-484] cells/mm^3) (**Table 2**).

Mean CRP was higher among culture-negative participants (18.7 ± 42.5 mg/L) compared to those with sub-clinical TB (7.2 ±8.7 mg/L, p=0.037) with wide standard deviations. Conversely, median CRP was lower in culture-negative participants 3.5 mg/L [1.2-11.8 mg/L] than sub-clinical TB cases 5.2 mg/L [2.9-5.7 mg/L]. Among those without TB symptoms, CRP was not significantly associated with sub-clinical TB (odds ratio [OR]=0.85, 95% CI 0.48-1.50). A ten-unit increase in CRP was associated with 1.13-fold higher odds of culture positivity (95% CI 1.01-1.19) (**Table 3**).

The odds of having sub-clinical TB versus a culture-negative finding was not significantly associated with hemoglobin level (OR=1.06, 95% CI 0.61-1.84). A one-unit increase in hemoglobin was associated with 0.79-fold lower odds of culture positivity (95% CI 0.67-0.93).

DISCUSSION

The prevalence of sub-clinical TB was low in this cross-sectional analysis of adults testing positive for HIV in Durban, South Africa. Demographic and clinical indicators were similar among those without TB symptoms, irrespective of the presence of culture-confirmed TB. Hemoglobin and CRP were not independent predictors of sub-clinical TB and therefore have limited utility as screening tools for identifying sub-clinical disease.

The prevalence of sub-clinical TB in this group of PLHIV was 1.1%, similar to the 1.6% prevalence reported among diabetes patients in South Africa and more broadly in a South African TB prevalence survey, but below the 4% reported in another cohort of newly diagnosed HIV-positive adults in Durban or 6% among household contacts of an index TB case.[74-77] In the South African TB prevalence survey, the proportion of TB cases that were asymptomatic was 45%, compared to 9% in this HIV-positive population and 23% previously reported among PLHIV.[74, 77] The relatively high proportion of symptomatic TB cases among PLHIV may mean the proportion of culture-positive cases with sub-clinical disease is smaller.

CRP is a non-specific marker of inflammation. In our cohort, we believe it is likely that many asymptomatic culture-negative participants had other types of infections that contributed to elevated CRP such that we were unable to distinguish between those diseases and sub-clinical TB disease. We note that among those who did not have TB symptoms, 34% had a cough that had developed within the prior two weeks and 3% had hemoptysis.

This study has several strengths including collecting sputum from each sequential participant, inducing sputum if necessary, and using gold standard testing for TB. Our analyses were underpowered due to the low number of participants with sub-clinical TB and we were unable to assess the long term natural progression of sub-clinical TB.

In conclusion, sub-clinical TB was present in PLHIV at the time they sought an HIV test but we were unable to distinguish between PLHIV with and without sub-clinical TB using routinely collected clinical characteristics or chemistries. CRP was moderately elevated among culture-positive compared to culture-negative adults overall, but not when restricted to adults without TB symptoms limiting its utility as a sub-clinical TB screening tool.

ACKNOWLEDGEMENTS

We thank the participants for their time and study staff for their dedication to this research.

This work was supported by the National Institute for Allergy and Infectious Diseases (K23 AI108293, R03 AI143351) of the National Institutes of Health.

Table 1. Demographic and clinical characteristics (n=437)

	N (%) or mean ±standard deviation
Demographics	
Female	256 (41)
Age (years)	33.5 ±9.3
Income <2,000 ZAR/month	335 (77)
Obtained a least high school degree	157 (36)
Food insecure	24 (5)
Current smoker	108 (25)
Medical History	
Ever tested for HIV	310 (72)
Ever tested positive for HIV	25 (8)
History of tuberculosis treatment	20 (5)
Clinical characteristics	
Current cough	165 (38)
Hemoptysis	13 (3)
Loss of appetite	122 (28)
Fatigue	231 (53)
Body mass index (kg/m^2)	25.8 ±6.8
<18.5	30 (7)
18.5-24.9	213 (49)
≥25.0	194 (44)
CD4 count ($cells/mm^3$) (n=426)	301 [171-467]
C-reactive protein (mg/L) (n=423)	4.1 [1.3-17.3]
Hemoglobin (g/dL) (n=323)	11.7 ±2.1
Anemia (n=323)	209 (65)

Table 2. Demographic and clinical characteristics of culture-negative participants and sub-clinical tuberculosis cases (n=399).

	M. tuberculosis **culture-negative n=394**	**Sub-clinical tuberculosis n=5**	**p-value**
Demographics			
Female	237 (60)	2 (40)	0.394
Age (years)	33.1 ±9.3	34.3 ±8.6	0.770
Income <2,000 ZAR/month	300 (77)	5 (100)	0.594
Obtained a least high school degree	147 (37)	1 (20)	0.655
Food insecure	21 (5)	0 (0)	1.00
Current smoker	97 (25)	2 (40)	0.602
Medical history			
Ever tested for HIV	288 (73)	4 (80)	1.00
Ever tested positive for HIV	23 (8)	0 (0)	1.00
History of tuberculosis treatment	18 (5)	0 (0)	1.00
Clinical characteristics			
Current cough	134 (34)	1 (20)	0.666
Hemoptysis	12 (3)	0 (0)	1.00
Loss of appetite	96 (24)	0 (0)	0.343
Fatigue	197 (50)	1 (20)	0.372
Body mass index (kg/m^2)	26.1 ±6.8	27.6 ±5.6	0.577
<18.5	21 (5)	0 (0)	1.00
18.5-24.9	191 (49)	3 (60)	
≥25.0	182 (46)	2 (40)	
CD4 count ($cells/mm^3$) (n=389)	335 [187-484]	389 [106-408]	0.452
C-reactive protein (n=385)	3.5 [1.2-11.8]	5.2 [2.9-5.7]	**0.037**
Hemoglobin (n=290)	11.8 ±2.1	12.1 ±0.9	0.687
Anemia (n=290)	177 (62)	2 (67)	1.00

Table 3. Odds of tuberculosis outcome compared to culture-negative participants

	Sub-clinical tuberculosis (n=399)			**Sub-clinical or active tuberculosis** (n=437)		
	OR (95% CI)	p-value	AUC	OR (95% CI)	p-value	AUC
C-reactive protein (per 10-unit)	0.85 (0.48-1.50)	0.574	0.493	1.13 (1.08-1.19)	**<0.001**	0.792
Hemoglobin (per 1-unit)	1.06 (0.61-1.84)	0.835	0.542	0.78 (0.67-0.93)	**0.004**	0.665

AUC, Area under the curce; OR, Odds ratio.

Conclusions

This doctoral dissertation describes the prevalence and associated risk factors for latent, sub-clinical, or active TB in India and South Africa, two high-burden TB countries with very different at-risk populations for TB. Diabetes was a key exposure of interest because it is known to increase risk for active TB generally, but the impact is not clear not in key subpopulations including PLHIV or across BMI categories.

In Chapter 2, we showed that diabetes was not associated with LTBI in Pondicherry, India, where TB-incidence and the prevalence of diabetes are high. Diabetes-BMI interaction for active TB was statistically significant on both the additive and multiplicative scales. The greatest risk of active TB disease from diabetes was among overweight/obese participants (12-fold risk compared to those without diabetes), while diabetes had no significant effect on TB risk within the undernourished group. Our findings support screening all diabetic patients for TB regardless of BMI. Based on additive interaction and the prevalence of diabetes in the different BMI groups using normal BMI and no diabetes as the reference group, we found that the burden of TB attributable to diabetes was highest in the low BMI group, suggesting routine screening of low BMI diabetic patients for active TB could be worthwhile in this setting. Additionally, hyperglycaemia was common among TB patients in all BMI categories indicating routine clinical testing of new TB patients would yield a high proportion of patients who may benefit from more intensive clinical care.

In Chapter 3, we showed that among PLHIV in Durban, South Africa diabetes was associated with well-established, traditional risk factors such as older age, higher BMI, and high blood

pressure measurements. Pre-diabetes was common and the proportion of people who screened positive for diabetes was moderate with few participants reporting any prior diagnosis. Although two thirds of PLHIV who screened positive for diabetes were <45 years old, nearly all had a BMI >25 kg/m^2, which could be a useful cut point for screening in resource limited settings. Screening positive for diabetes was not associated with incident active TB over 12 months of follow-up, which could have been due to lack of statistical power and the substantial effect of HIV on active TB risk. Diabetes was associated with mortality over the 12 months following HIV diagnosis.

In Chapter 4, we observed the prevalence of sub-clinical TB was 1.1% among adults presenting for HIV testing in Durban, South Africa, while the prevalence of active TB was 8.7%. There were no statistically significant differences in demographic or clinical characteristics between culture-negative and sub-clinical TB study participants. Our findings indicate that sub-clinical TB is present among adults newly diagnosed with HIV infection, but we do not yet have a way of easily identifying the adults without TB symptoms who would benefit from TB testing.

Collectively, this body of work is a reminder of heterogeneity in disease etiology and populations at risk in different geographic locations. In Aim 1, we found diabetes was an important contributor to the burden of active TB disease in India. By contrast, in Aim 2 we found that among PLHIV in urban South Africa, diabetes was relatively less common and not associated with active TB. This highlights the importance of defining the source population from which study subjects are drawn and thinking critically about the generalizability to the target

population. Therefore, our finding in Aim 3 of a low prevalence of sub-clinical TB among PLHIV in South Africa is most relevant to other settings with high co-burdens of HIV and TB.

Throughout, our findings would have been strengthened by more accurate ascertainment of the diabetes exposure or outcome. Diagnosing diabetes is somewhat logistically complex for the patient, involving multiple measurements over weeks or months. It may also require overnight fasting or a more expensive laboratory-based HbA_{1c} test. Coordinating clinic visits to obtain fasting blood glucose measurements or funding lab-based testing was not feasible within these research studies. This body of research highlights a need for diabetes testing strategies that can be more readily implemented worldwide. Additionally, our work provides support for prospective cohort-based studies of diabetes patients in more diverse settings inclusive of sub-Saharan Africa, the Indian subcontinent, and South America. Successful clinical management of incident active TB or HIV in the context of increasingly common non-communicable diseases and limited resources remains an important area of research.

References

1. Bor J, Herbst AJ, Newell ML, Bärnighausen T. Increases in adult life expectancy in rural South Africa: valuing the scale-up of HIV treatment. *Science*. 2013;339(6122):961-5. doi:10.1126/science.1230413

2. Reniers G, Blom S, Calvert C, et al. Trends in the burden of HIV mortality after roll-out of antiretroviral therapy in KwaZulu-Natal, South Africa: an observational community cohort study. *Lancet HIV*. 2017;4(3):e113-e121. doi:10.1016/S2352-3018(16)30225-9

3. *Global Tuberculosis Report 2018*. Geneva, Switzerland: World Health Organization, 2018. http://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf?ua=1

4. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2095-128. doi:10.1016/S0140-6736(12)61728-0

5. Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2197-223. doi:10.1016/S0140-6736(12)61689-4

6. Lonnroth K, Castro KG, Chakaya JM, et al. Tuberculosis control and elimination 2010-50: cure, care, and social development. *Lancet*. 22 2010;375(9728):1814-29. doi:10.1016/s0140-6736(10)60483-7

7. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 2018;14(2):88-98. doi:10.1038/nrendo.2017.151

8. American Diabete Association. 2. Classification and Diagnosis of Diabetes. *Diabetes care*. 2018;41(Suppl 1):S13-S27. doi:10.2337/dc18-S002

9. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27(5):1047-53.

10. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract*. 2014;103(2):137-49. doi:10.1016/j.diabres.2013.11.002

11. *Global Report on Diabetes*. Geneva, Switzerland: World Health Organization, 2016. http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf?ua=1

12. NCD Risk Factor Collaboration – Africa Working Group. Trends in obesity and diabetes across Africa from 1980 to 2014: an analysis of pooled population-based studies. *Int J Epidemiol*. 2017;46(5):1421-1432. doi:10.1093/ije/dyx078

13. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care*. 2009;32 Suppl 2:S157-63. doi:10.2337/dc09-S302

14. Scott RA, Langenberg C, Sharp SJ, et al. The link between family history and risk of type 2 diabetes is not explained by anthropometric, lifestyle or genetic risk factors: the EPIC-InterAct study. *Diabetologia*. 2013;56(1):60-9. doi:10.1007/s00125-012-2715-x

15. American Diabetes Association. 5. Facilitating Behavior Change and Well-being to Improve Health Outcomes. *Diabetes Care*. 2020;43(Suppl 1):S48-S65. doi:10.2337/dc20-S005

16. Tien PC, Schneider MF, Cole SR, et al. Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study. *AIDS*. 2007;21(13):1739-45. doi:10.1097/QAD.0b013e32827038d0

17. Rasmussen LD, Mathiesen ER, Kronborg G, Pedersen C, Gerstoft J, Obel N. Risk of diabetes mellitus in persons with and without HIV: a Danish nationwide population-based cohort study. *PLoS One*. 2012;7(9):e44575. doi:10.1371/journal.pone.0044575

18. Brown TT, Cole SR, Li X, et al. Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Arch Intern Med*. 2005;165(10):1179-84. doi:10.1001/archinte.165.10.1179

19. Hernandez-Romieu AC, Garg S, Rosenberg ES, Thompson-Paul AM, Skarbinski J. Is diabetes prevalence higher among HIV-infected individuals compared with the general population? Evidence from MMP and NHANES 2009-2010. *BMJ Open Diabetes Res Care*. 2017;5(1):e000304. doi:10.1136/bmjdrc-2016-000304

20. Nduka CU, Stranges S, Kimani PK, Sarki AM, Uthman OA. Is There Sufficient Evidence for a Causal Association between Antiretroviral Therapy and Diabetes in HIV-infected Patients? A Meta-analysis. *Diabetes Metab Res Rev*. 2017;doi:10.1002/dmrr.2902

21. Prioreschi A, Munthali RJ, Soepnel L, et al. Incidence and prevalence of type 2 diabetes mellitus with HIV infection in Africa: a systematic review and meta-analysis. *BMJ Open*. 2017;7(3):e013953. doi:10.1136/bmjopen-2016-013953

22. De Wit S, Sabin CA, Weber R, et al. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Diabetes Care*. 2008;31(6):1224-9. doi:10.2337/dc07-2013

23. Butt AA, McGinnis K, Rodriguez-Barradas MC, et al. HIV infection and the risk of diabetes mellitus. *AIDS*. 2009;23(10):1227-34. doi:10.1097/QAD.0b013e32832bd7af

24. Hadigan C, Kattakuzhy S. Diabetes mellitus type 2 and abnormal glucose metabolism in the setting of human immunodeficiency virus. *Endocrinol Metab Clin North Am*. 2014;43(3):685-96. doi:10.1016/j.ecl.2014.05.003

25. Ledergerber B, Furrer H, Rickenbach M, et al. Factors associated with the incidence of type 2 diabetes mellitus in HIV-infected participants in the Swiss HIV Cohort Study. *Clin Infect Dis*. 2007;45(1):111-9. doi:10.1086/518619

26. Coelho AR, Moreira FA, Santos AC, et al. Diabetes mellitus in HIV-infected patients: fasting glucose, A1c, or oral glucose tolerance test - which method to choose for the diagnosis? *BMC Infect Dis*. 2018;18(1):309. doi:10.1186/s12879-018-3221-7

27. Capeau J, Bouteloup V, Katlama C, et al. Ten-year diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral treatment. *AIDS*. 2012;26(3):303-14. doi:10.1097/QAD.0b013e32834e8776

28. Tripathi A, Liese AD, Jerrell JM, et al. Incidence of diabetes mellitus in a population-based cohort of HIV-infected and non-HIV-infected persons: the impact of clinical and therapeutic factors over time. *Diabet Med*. 2014;31(10):1185-93. doi:10.1111/dme.12455

29. *Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV*. http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf.

30. Collaborators GDaI. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396(10258):1204-1222. doi:10.1016/S0140-6736(20)30925-9

31. Hall V, Thomsen RW, Henriksen O, Lohse N. Diabetes in Sub Saharan Africa 1999-2011: epidemiology and public health implications. A systematic review. *BMC Public Health*. 2011;11:564. doi:10.1186/1471-2458-11-564

32. Young F, Critchley JA, Johnstone LK, Unwin NC. A review of co-morbidity between infectious and chronic disease in Sub Saharan Africa: TB and diabetes mellitus, HIV and

metabolic syndrome, and the impact of globalization. *Global Health*. 2009;5:9. doi:10.1186/1744-8603-5-9

33. Isa SE, Oche AO, Kang'ombe AR, et al. Human Immunodeficiency Virus and Risk of Type 2 Diabetes in a Large Adult Cohort in Jos, Nigeria. *Clin Infect Dis*. 2016;63(6):830-5. doi:10.1093/cid/ciw381

34. Jerene D, Hiruy N, Jemal I, et al. The yield and feasibility of integrated screening for TB, diabetes and HIV in four public hospitals in Ethiopia. *Int Health*. 2017;9(2):100-104. doi:10.1093/inthealth/ihx002

35. Ekeke N, Ukwaja KN, Chukwu JN, et al. Screening for diabetes mellitus among tuberculosis patients in Southern Nigeria: a multi-centre implementation study under programme settings. *Sci Rep*. 2017;7:44205. doi:10.1038/srep44205

36. Beran D, McCabe A, Yudkin JS. Access to medicines versus access to treatment: the case of type 1 diabetes. *Bull World Health Organ*. 2008;86(8):648-9.

37. Mbanya JC, Mbanya D. Diabetes cost in sub-Saharan Africa. *J Cardiovasc Risk*. 2003;10(3):191-3. doi:10.1097/01.hjr.0000078379.16042.f6

38. Brown JB, Ramaiya K, Besançon S, et al. Use of medical services and medicines attributable to diabetes in Sub-Saharan Africa. *PLoS One*. 2014;9(9):e106716. doi:10.1371/journal.pone.0106716

39. Nigatu T. Integration of HIV and noncommunicable diseases in health care delivery in low- and middle-income countries. *Prev Chronic Dis*. 2012;9:E93.

40. Duffy M, Ojikutu B, Andrian S, Sohng E, Minior T, Hirschhorn LR. Non-communicable diseases and HIV care and treatment: models of integrated service delivery. *Trop Med Int Health*. 2017; 22(8):926-937. doi:10.1111/tmi.12901

41. Background Paper: Integration of services for HIV and Noncommunicable Diseases in Low and Middle Income Countries. Geneva, Switzerland: World Health Organization, 2017.

42. Green A. NIH project focuses on integration of HIV and NCD care. *Lancet*. 2016;388(10054):1869. doi:10.1016/S0140-6736(16)31846-3

43. *2017 Global Tuberculosis Report*. Geneva, Switzerland: World Health Organization, 2017. http://www.who.int/tb/publications/global_report/en/

44. Dolin PJ, Raviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990-2000. *Bull World Health Organ*. 1994;72(2):213-20.

45. Narasimhan P, Wood J, Macintyre CR, Mathai D. Risk factors for tuberculosis. *Pulm Med*. 2013;2013:828939. doi:10.1155/2013/828939

46. Diedrich CR, Flynn JL. HIV-1/mycobacterium tuberculosis coinfection immunology: how does HIV-1 exacerbate tuberculosis? *Infect Immun*. 2011;79(4):1407-17. doi:10.1128/IAI.01126-10

47. Pawlowski A, Jansson M, Sköld M, Rottenberg ME, Källenius G. Tuberculosis and HIV co-infection. *PLoS Pathog*. 2012;8(2):e1002464. doi:10.1371/journal.ppat.1002464

48. Whalen C, Horsburgh CR, Hom D, Lahart C, Simberkoff M, Ellner J. Accelerated course of human immunodeficiency virus infection after tuberculosis. *Am J Respir Crit Care Med*. 1995;151(1):129-35. doi:10.1164/ajrccm.151.1.7812542

49. Baker MA, Harries AD, Jeon CY, et al. The impact of diabetes on tuberculosis treatment outcomes: a systematic review. *BMC Med*. 2011;9:81. doi:10.1186/1741-7015-9-81

50. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. *PLoS Med*. 2008;5(7):e152. doi:10.1371/journal.pmed.0050152

51. Harries AD, Kumar AM, Satyanarayana S, et al. Addressing diabetes mellitus as part of the strategy for ending TB. *Trans R Soc Trop Med Hyg*. 2016;110(3):173-9. doi:10.1093/trstmh/trv111

52. Stevenson CR, Critchley JA, Forouhi NG, et al. Diabetes and the risk of tuberculosis: a neglected threat to public health? *Chronic Illn*. 2007;3(3):228-45. doi:10.1177/1742395307081502

53. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. *Lancet Infect Dis*. 2009;9(12):737-46. doi:10.1016/S1473-3099(09)70282-8

54. Hodgson K, Morris J, Bridson T, Govan B, Rush C, Ketheesan N. Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections. *Immunology*. 2015;144(2):171-85. doi:10.1111/imm.12394

55. Restrepo BI, Schlesinger LS. Host-pathogen interactions in tuberculosis patients with type 2 diabetes mellitus. *Tuberculosis*. 2013;93 Suppl:S10-4. doi:10.1016/S1472-9792(13)70004-0

56. Restrepo BI, Schlesinger LS. Impact of diabetes on the natural history of tuberculosis. *Diabetes Res Clin Pract*. 2014;106(2):191-9. doi:10.1016/j.diabres.2014.06.011

57. Ronacher K, Joosten SA, van Crevel R, Dockrell HM, Walzl G, Ottenhoff TH. Acquired immunodeficiencies and tuberculosis: focus on HIV/AIDS and diabetes mellitus. *Immunol Rev*. 2015;264(1):121-37. doi:10.1111/imr.12257

58. Lönnroth K, Roglic G, Harries AD. Improving tuberculosis prevention and care through addressing the global diabetes epidemic: from evidence to policy and practice. *Lancet Diabetes Endocrinol*. 2014;2(9):730-9. doi:10.1016/S2213-8587(14)70109-3

59. Pan SC, Ku CC, Kao D, Ezzati M, Fang CT, Lin HH. Effect of diabetes on tuberculosis control in 13 countries with high tuberculosis: a modelling study. *Lancet Diabetes Endocrinol*. 2015;3(5):323-30. doi:10.1016/S2213-8587(15)00042-X

60. Harries AD, Kumar AM, Satyanarayana S, et al. Diabetes mellitus and tuberculosis: programmatic management issues. *Int J Tuberc Lung Dis*. 2015;19(8):879-86. doi:10.5588/ijtld.15.0069

61. *Collaborative framework for care and control of tuberculosis and diabetes*. Geneva, Switzerland: World Health Organization, 2011.

62. Lee MR, Huang YP, Kuo YT, et al. Diabetes Mellitus and Latent Tuberculosis Infection: A Systemic Review and Metaanalysis. *Clin Infect Dis*. 2017;64(6):719-727. doi:10.1093/cid/ciw836

63. Lönnroth K, Williams BG, Cegielski P, Dye C. A consistent log-linear relationship between tuberculosis incidence and body mass index. *Int J Epidemiol*. 2010;39(1):149-55. doi:10.1093/ije/dyp308

64. Lin HH, Wu CY, Wang CH, et al. Association of Obesity, Diabetes, and Risk of Tuberculosis: Two Population-Based Cohorts. *Clin Infect Dis*. Feb 2018;66(5):699-705. doi:10.1093/cid/cix852

65. Li Y, Guo J, Xia T, et al. Incidence of pulmonary tuberculosis in Chinese adults with type 2 diabetes: a retrospective cohort study in Shanghai. *Sci Rep*. 2020;10(1):8578. doi:10.1038/s41598-020-65603-y

66. Bailey SL, Ayles H. Association between diabetes mellitus and active tuberculosis in Africa and the effect of HIV. *Trop Med Int Health*. 2017;22(3):261-268. doi:10.1111/tmi.12822

67. Corbett EL, Watt CJ, Walker N, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med*. 2003;163(9):1009-21. doi:10.1001/archinte.163.9.1009

68. Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. *Top HIV Med*. 2009;17(4):118-23.

69. Kibirige D, Ssekitoleko R, Mutebi E, Worodria W. Overt diabetes mellitus among newly diagnosed Ugandan tuberculosis patients: a cross sectional study. *BMC Infect Dis*. 2013;13:122. doi:10.1186/1471-2334-13-122

70. Faurholt-Jepsen D, Range N, Praygod G, et al. Diabetes is a risk factor for pulmonary tuberculosis: a case-control study from Mwanza, Tanzania. *PLoS One*. 2011;6(8):e24215. doi:10.1371/journal.pone.0024215

71. Boillat-Blanco N, Ramaiya KL, Mganga M, et al. Transient Hyperglycemia in Patients With Tuberculosis in Tanzania: Implications for Diabetes Screening Algorithms. *J Infect Dis*. Apr 2016;213(7):1163-72. doi:10.1093/infdis/jiv568

72. Faurholt-Jepsen D, Range N, Praygod G, et al. The role of diabetes co-morbidity for tuberculosis treatment outcomes: a prospective cohort study from Mwanza, Tanzania. *BMC Infect Dis*. 2012;12:165. doi:10.1186/1471-2334-12-165

73. Drain PK, Bajema KL, Dowdy D, et al. Incipient and Subclinical Tuberculosis: a Clinical Review of Early Stages and Progression of Infection. *Clin Microbiol Rev*. 2018;31(4)doi:10.1128/CMR.00021-18

74. Bajema KL, Bassett IV, Coleman SM, et al. Subclinical tuberculosis among adults with HIV: clinical features and outcomes in a South African cohort. *BMC Infect Dis*. 2019;19(1):14. doi:10.1186/s12879-018-3614-7

75. Berkowitz N, Okorie A, Goliath R, Levitt N, Wilkinson RJ, Oni T. The prevalence and determinants of active tuberculosis among diabetes patients in Cape Town, South Africa, a high HIV/TB burden setting. *Diabetes Res Clin Pract*. 2018;138:16-25. doi:10.1016/j.diabres.2018.01.018

76. Bekken GK, Ritz C, Selvam S, et al. Identification of subclinical tuberculosis in household contacts using exposure scores and contact investigations. *BMC Infect Dis*. 2020;20(1):96. doi:10.1186/s12879-020-4800-y

77. Gunasekera K, Cohen T, Gao W, Ayles H, Godfrey-Faussett P, Claassens M. Smoking and HIV associated with subclinical tuberculosis: analysis of a population-based prevalence survey. *Int J Tuberc Lung Dis*. 2020;24(3):340-346. doi:10.5588/ijtld.19.0387

78. *The End TB Strategy*. Geneva, Switzerland: World Health Organization, 2014.

79. Implementing the End TB Strategy: The Essentials. Geneva, Switzerland: World Health Organization, 2015.

80. Odone A, Houben RM, White RG, Lönnroth K. The effect of diabetes and undernutrition trends on reaching 2035 global tuberculosis targets. *Lancet Diabetes Endocrinol*. Sep 2014;2(9):754-64. doi:10.1016/S2213-8587(14)70164-0

81. Young F, Wotton CJ, Critchley JA, Unwin NC, Goldacre MJ. Increased risk of tuberculosis disease in people with diabetes mellitus: record-linkage study in a UK population. *J Epidemiol Community Health*. 2012;66(6):519-23. doi:10.1136/jech.2010.114595

82. Dye C, Bourdin Trunz B, Lönnroth K, Roglic G, Williams BG. Nutrition, diabetes and tuberculosis in the epidemiological transition. *PLoS One*. 2011;6(6):e21161. doi:10.1371/journal.pone.0021161

83. *IDF Diabetes Atlas*. 7th edn. Brussels, Belgium: International Diabetes Federation, 2017.

84. Hochberg NS, Sarkar S, Horsburgh CR, et al. Comorbidities in pulmonary tuberculosis cases in Puducherry and Tamil Nadu, India: Opportunities for intervention. *PLoS One*. 2017;12(8):e0183195. doi:10.1371/journal.pone.0183195

85. Brinda EM, Kowal P, Attermann J, Enemark U. Health service use, out-of-pocket payments and catastrophic health expenditure among older people in India: the WHO Study on global AGEing and adult health (SAGE). *J Epidemiol Community Health*. 2015;69(5):489-94. doi:10.1136/jech-2014-204960

86. *National framework for joint TB-diabetes collaborative activities*. Geneva, Switzerland: World Health Organization, 2017.

87. Saag LA, LaValley MP, Hochberg NS, et al. Low body mass index and latent tuberculous infection: a systematic review and meta-analysis. *Int J Tuberc Lung Dis*. 2018;22(4):358-365. doi:10.5588/ijtld.17.0558

88. Barron MM, Shaw KM, Bullard KM, Ali MK, Magee MJ. Diabetes is associated with increased prevalence of latent tuberculosis infection: Findings from the National Health and Nutrition Examination Survey, 2011-2012. *Diabetes Res Clin Pract*. 2018;139:366-379. doi:10.1016/j.diabres.2018.03.022

89. Shivakumar S, Chandrasekaran P, Kumar AMV, et al. Diabetes and pre-diabetes among household contacts of tuberculosis patients in India: is it time to screen them all? *Int J Tuberc Lung Dis*. 2018;22(6):686-694. doi:10.5588/ijtld.17.0598

90. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 2004;363(9403):157-63. doi:10.1016/S0140-6736(03)15268-3

91. Centers for Disease Control and Prevention. Guidelines for the investigation of contacts of persons with infectious tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. *MMWR Recomm Rep*. 2005;54(RR-15):1-47.

92. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. *Am J Epidemiol*. 2005;162(3):2. doi:10.1093/aje/kwi188

93. Barros AJ, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Med Res Methodol*. 2003;3:21. doi:10.1186/1471-2288-3-21

94. Frank D, DeBenedetti AF, Volk RJ, Williams EC, Kivlahan DR, Bradley KA. Effectiveness of the AUDIT-C as a screening test for alcohol misuse in three race/ethnic groups. *J Gen Intern Med*. 2008;23(6):781-7. doi:10.1007/s11606-008-0594-0

95. Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. *Int J Epidemiol*. 2012;41(2):514-20. doi:10.1093/ije/dyr218

96. Balakrishnan S, Vijayan S, Nair S, et al. High diabetes prevalence among tuberculosis cases in Kerala, India. *PLoS One*. 2012;7(10):e46502. doi:10.1371/journal.pone.0046502

97. Raghuraman S, Vasudevan KP, Govindarajan S, Chinnakali P, Panigrahi KC. Prevalence of Diabetes Mellitus among Tuberculosis Patients in Urban Puducherry. *N Am J Med Sci*. 2014;6(1):30-4. doi:10.4103/1947-2714.125863

98. Gupte AN, Mave V, Meshram S, et al. Trends in HbA1c levels and implications for diabetes screening in tuberculosis cases undergoing treatment in India. *Int J Tuberc Lung Dis*. 2018;22(7):800-806. doi:10.5588/ijtld.18.0026

99. Marik PE, Bellomo R. Stress hyperglycemia: an essential survival response! *Crit Care Med*. 2013;41(6):e93-4. doi:10.1097/CCM.0b013e318283d124

100. Magee MJ, Salindri AD, Kyaw NTT, Auld SC, Haw JS, Umpierrez GE. Stress Hyperglycemia in Patients with Tuberculosis Disease: Epidemiology and Clinical Implications. *Curr Diab Rep*. 2018;18(9):71. doi:10.1007/s11892-018-1036-y

101. VanderWeele TJ, Knol MJ. A tutorial on interaction. *Epidemiol Methods*. 2014;3(1):39. doi:10.1515/em-2013-0005

102. Rothman KJ, Greenland S, Walker AM. Concepts of interaction. *Am J Epidemiol*. 1980;112(4):467-70.

103. Vallerskog T, Martens GW, Kornfeld H. Diabetic mice display a delayed adaptive immune response to Mycobacterium tuberculosis. *J Immunol*. 2010;184(11):6275-82. doi:10.4049/jimmunol.1000304

104. Kumar NP, Moideen K, Viswanathan V, Kornfeld H, Babu S. Effect of standard tuberculosis treatment on naive, memory and regulatory T-cell homeostasis in tuberculosis-diabetes co-morbidity. *Immunology*. 2016;149(1):87-97. doi:10.1111/imm.12632

105. Sinha P, Davis J, Saag L, et al. Undernutrition and Tuberculosis: Public Health Implications. *J Infect Dis*. 2018;doi:10.1093/infdis/jiy675

106. Zocchetti C, Consonni D, Bertazzi PA. Relationship between prevalence rate ratios and odds ratios in cross-sectional studies. *Int J Epidemiol*. 1997;26(1):220-3.

107. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis*. 2006;10(11):1192-204.

108. Yajnik CS. Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. *J Nutr*. 2004;134(1):205-10. doi:10.1093/jn/134.1.205

109. Collaborators GCoD. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet.* 2017;390(10100):1151-1210. doi:10.1016/S0140-6736(17)32152-9

110. Stokes A, Berry KM, Mchiza Z, et al. Prevalence and unmet need for diabetes care across the care continuum in a national sample of South African adults: Evidence from the SANHANES-1, 2011-2012. *PLoS One*. 2017;12(10):e0184264. doi:10.1371/journal.pone.0184264

111. *Mortality and causes of death in South Africa, 2016: Findings from death notification*. Pretoria, South Africa: Statistics South Africa, 2018.

112. Hird TR, Pirie FJ, Esterhuizen TM, et al. Burden of Diabetes and First Evidence for the Utility of HbA1c for Diagnosis and Detection of Diabetes in Urban Black South Africans: The Durban Diabetes Study. *PLoS One*. 2016;11(8):e0161966. doi:10.1371/journal.pone.0161966

113. Beagley J, Guariguata L, Weil C, Motala AA. Global estimates of undiagnosed diabetes in adults. *Diabetes Res Clin Pract*. 2014;103(2):150-60. doi:10.1016/j.diabres.2013.11.001

114. Njuguna B, Vorkoper S, Patel P, et al. Models of integration of HIV and noncommunicable disease care in sub-Saharan Africa: lessons learned and evidence gaps. *AIDS*. Jul 2018;32 Suppl 1:S33-S42. doi:10.1097/QAD.0000000000001887

115. *Chronic care of HIV and noncommunicable diseases: How to leverage the HIV experience*. UNAIDS, 2011. http://www.unaids.org/sites/default/files/media_asset/20110526_JC2145_Chronic_care_of_HIV_0.pdf

116. *The South African antiretroviral treatment guidelines.* 2017.

117. Knaebel J, Irvin BR, Xie CZ. Accuracy and clinical utility of a point-of-care HbA1c testing device. *Postgrad Med*. May 2013;125(3):91-8. doi:10.3810/pgm.2013.05.2664

118. The Society for Endocrinology, Metabolism and Diabetes of South Africa Type 2 Diabetes Guidelines Expert Committee. The 2017 SEMDSA Guideline for the Management of Type 2 Diabetes. *JEMDSA*. 2017;22(1):S1-S196.

119. *Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus*. Geneva, Switzerland: World Health Organization, 2011.

120. *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. Geneva, Switzerland: World Health Organization, 2011.

121. Coates J, Swindale A, Biliinsky P. *Household Food Insecurity Access Scale (HFIAS) for Measurement of Food Access: Indicator Guide (v. 3)*. Washington, D.C.: FHI 360/FANTA, 2007.

122. Brown LD, Cai TT, Dasgupta A. Interval Estimation for a Binomial Proportion. *Statistical Science*. 2001;16(2):101-133.

123. YOUDEN WJ. Index for rating diagnostic tests. *Cancer*. 1950;3(1):32-5.

124. Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr*. 2011;57(4):284-9. doi:10.1097/QAI.0b013e318221863f

125. Oni T, Youngblood E, Boulle A, McGrath N, Wilkinson RJ, Levitt NS. Patterns of HIV, TB, and non-communicable disease multi-morbidity in peri-urban South Africa- a cross sectional study. *BMC Infect Dis*. 2015;15:20. doi:10.1186/s12879-015-0750-1

126. Magodoro IM, Esterhuizen TM, Chivese T. A cross-sectional, facility based study of comorbid non-communicable diseases among adults living with HIV infection in Zimbabwe. *BMC Res Notes*. 2016;9:379. doi:10.1186/s13104-016-2187-z

127. Mathabire Rücker SC, Tayea A, Bitilinyu-Bangoh J, et al. High rates of hypertension, diabetes, elevated low-density lipoprotein cholesterol, and cardiovascular disease risk factors in HIV-infected patients in Malawi. *AIDS*. 2018;32(2):253-260. doi:10.1097/QAD.0000000000001700

128. Manne-Goehler J, Montana L, Gómez-Olivé FX, et al. The ART Advantage: Health Care Utilization for Diabetes and Hypertension in Rural South Africa. *J Acquir Immune Defic Syndr*. 2017;75(5):561-567. doi:10.1097/QAI.0000000000001445

129. Bailey SL, Ayles H, Beyers N, et al. Diabetes mellitus in Zambia and the Western Cape province of South Africa: Prevalence, risk factors, diagnosis and management. *Diabetes Res Clin Pract*. 2016;118:1-11. doi:10.1016/j.diabres.2016.05.001

130. Njuguna B, Kiplagat J, Bloomfield GS, Pastakia SD, Vedanthan R, Koethe JR. Prevalence, Risk Factors, and Pathophysiology of Dysglycemia among People Living with HIV in Sub-Saharan Africa. *J Diabetes Res*. 2018;2018:6916497. doi:10.1155/2018/6916497

131. Guaraldi G, Orlando G, Zona S, et al. Premature age-related comorbidities among HIV-infected persons compared with the general population. *Clin Infect Dis*. 2011;53(11):1120-6. doi:10.1093/cid/cir627

132. Chang AY, Gómez-Olivé FX, Manne-Goehler J, et al. Multimorbidity and care for hypertension, diabetes and HIV among older adults in rural South Africa. *Bull World Health Organ*. 2019;97(1):10-23. doi:10.2471/BLT.18.217000

133. Nugent R, Barnabas RV, Golovaty I, et al. Costs and cost-effectiveness of HIV/noncommunicable disease integration in Africa: from theory to practice. *AIDS*. 2018;32 Suppl 1:S83-S92. doi:10.1097/QAD.0000000000001884

134. Manne-Goehler J, Siedner MJ, Montana L, et al. Hypertension and diabetes control along the HIV care cascade in rural South Africa. *J Int AIDS Soc*. 2019;22(3):e25213. doi:10.1002/jia2.25213

135. Moreira RC, Pacheco AG, Paula A, et al. Diabetes Mellitus is Associated with Increased Death Rates Among HIV-Infected Patients in Rio de Janeiro, Brazil. *AIDS Res Hum Retroviruses*. 2016;32(12):1210-1218. doi:10.1089/AID.2015.0265

136. Park J, Zuñiga JA, García AA. Diabetes negatively impacts the ten-year survival rates of people living with HIV. *Int J STD AIDS*. 2019;30(10):991-998. doi:10.1177/0956462419857005

137. Kim PS, Woods C, Georgoff P, et al. A1C underestimates glycemia in HIV infection. *Diabetes care*. 2009;32(9):1591-3. doi:10.2337/dc09-0177

138. Eckhardt BJ, Holzman RS, Kwan CK, Baghdadi J, Aberg JA. Glycated Hemoglobin A(1c) as screening for diabetes mellitus in HIV-infected individuals. *AIDS Patient Care STDS*. 2012;26(4):197-201. doi:10.1089/apc.2011.0379

139. Glesby MJ, Hoover DR, Shi Q, et al. Glycated haemoglobin in diabetic women with and without HIV infection: data from the Women's Interagency HIV Study. *Antivir Ther*. 2010;15(4):571-7. doi:10.3851/IMP1557

140. Slama L, Palella FJ, Abraham AG, et al. Inaccuracy of haemoglobin A1c among HIV-infected men: effects of CD4 cell count, antiretroviral therapies and haematological parameters. *J Antimicrob Chemother*. 2014;69(12):3360-7. doi:10.1093/jac/dku295

141. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. *Acta Haematol*. 2004;112(3):126-8. doi:10.1159/000079722

142. Strauss SM, Rosedale M, Pesce MA, et al. Point-of-Care HbA1c Testing with the A1cNow Test Kit in General Practice Dental Clinics: A Pilot Study Involving Its Accuracy and Practical Issues in Its Use. *Point Care*. 2014;13(4):142-147. doi:10.1097/POC.0000000000000039

143. Ginde AA, Cagliero E, Nathan DM, Camargo CA. Point-of-care glucose and hemoglobin A1c in emergency department patients without known diabetes: implications for opportunistic screening. *Acad Emerg Med*. Dec 2008;15(12):1241-7. doi:10.1111/j.1553-2712.2008.00240.x

144. Hirst JA, McLellan JH, Price CP, et al. Performance of point-of-care HbA1c test devices: implications for use in clinical practice - a systematic review and meta-analysis. *Clin Chem Lab Med*. Feb 2017;55(2):167-180. doi:10.1515/cclm-2016-0303