The Double Burden

The role of diabetes for tuberculosis risk, manifestations, treatment outcomes and survival

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This review has been accepted as a thesis together with 4 previously published papers by University of Copenhagen 15th of June 2012 and defended on 6th of July 2012

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Dan Med J 2013;60(7):B4673

THE 4 ORIGINAL PAPERS ARE

I. 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

II. Faurholt-Jepsen D, Range N, PrayGod G, et al. The role of diabetes on the clinical manifestations of pulmonary tuberculosis. Trop Med Int Health. 2012 Jul;17(7):877-83. doi: 10.1111/j.1365-3156.2012.03002.x

III. 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

IV. Faurholt-Jepsen D, Range N, PrayGod G, et al. Diabetes is a strong predictor of mortality during tuberculosis treatment: a prospective cohort study among tuberculosis patients from Mwanza, Tanzania. Trop Med Int Health. 2013. doi: 10.1111/tmi.12120

INTRODUCTION AND BACKGROUND

Tuberculosis

The bacterial species Mycobacterium tuberculosis has been a common infectious agent in humans for thousands of years, with the oldest verified case dating back 8000 years [1]. Throughout more recent history, tuberculosis has been an often deadly human companion. Tuberculosis affects primarily the lungs (pulmonary tuberculosis), but can also affect all other extra-pulmonary compartments; e.g. the lymph nodes, bones, skin, and the central nervous system [2].

With the improved living conditions in high income countries alongside with the discovery of antibiotics active against tuberculosis, the tuberculosis incidence fell dramatically, although still comprising a health threat to people with low social status and poor living conditions [3,4]. However, the burden of tuberculosis remained high in low income countries, and, moreover, became one of the largest medical challenges as a severe opportunistic disease of HIV/AIDS [5]. The global challenge of tuberculosis today is the incident nine million new cases each year and the more than one million deaths [6].

One third of the world's population is latently infected with M. tuberculosis, a condition in which the dormant mycobacteria reside in macrophages, thus forever at risk of reactivation and progression to fulminant tuberculosis disease (active tuberculosis) due to co-morbidities compromising the immune defence (e.g. HIV infection, malnutrition, diabetes) [7].

The diabetes epidemic

The on-going urbanization has led to a transition in the living conditions (i.e. poor diet and physical activity patterns) for most inhabitants in high-income countries with a new challenge for public health; the chronic (or non-communicable) diseases [8]. Due to the epidemic-like spreading and severe complications, diabetes (type 2 diabetes) is considered one of the most prominent chronic conditions globally. Succeeding the unhealthy lifestyle transitions in high-income countries, the diabetes epidemic has struck low-income regions, traditionally burdened by the infectious (or communicable) diseases [9].

The prevalence of diabetes has been rapidly increasing, and in 2011 the global prevalence was estimated to 366 million cases [10]. But the trend has not stopped and the projection towards the year 2030 is a prevalence rising to 552 million cases [10]. Although the epidemic was first recognized in high-income countries, the majority of people with diabetes, presently and in the years to come, live in low- and middle income countries, with health systems already burdened by low budgets and other, equally important, diseases.

It is often postulated that diabetes in low-income setting is a disease of the rich, but with the on-going lifestyle transition among all groups as a consequence of the economic development, the chronic diseases also affect the poor [8,11]. Furthermore, diabetes and poverty are interconnected; poor people are more exposed to disease, have less access to the health systems, and, consequently, suffer more often from severe diabetes-related complications [12].

The double burden of tuberculosis and diabetes

The double burden of tuberculosis and diabetes is not a new problem. From ancient time until the beginning of the twentieth century, diabetes was considered a co-morbidity among tuberculosis patients [13–18]. But with the reduced significance of tuberculosis among patients in high-income countries in combination with the low prevalence of diabetes in low-income countries, the

double burden became less significant in the daily clinical work. Before the diabetes epidemic hit sub-Saharan Africa, a study on the association between tuberculosis and diabetes was reported from a hospital in Dar es Salaam, Tanzania [19]. Although the choice of reference population, a small community sample, was not optimal, this study was the first from Africa to report on the association between tuberculosis and diabetes. However, after this study, there were no reports from Sub-Saharan Africa until 20 years later (Paper I), when diabetes was already prevalent in the larger cities throughout the region [20], and tuberculosis control was compromised by, especially, the HIV epidemic [5].

In Omran's classic description of epidemiological transition, the transition was divided in to three stages of disease status; (i) the age of pestilence and famine, (ii) the age of receding pandemics, and (iii) the age of degenerative and man-made diseases [21]. However, the co-occurrence of tuberculosis and diabetes, on both population and individual level, is the result of a diabetes epidemic in regions, where it has not been possible to control tuberculosis [22], and, thus, the double burden is a new phenomenon of the epidemiological transition [23].

A number of studies from the Americas, Europe, Asia and most lately from sub-Saharan Africa have reported strong association between tuberculosis and diabetes [24–28]; on average, the estimated risk of active tuberculosis is thrice as high among people with diabetes [29]. Based on these reports as well as several reviews on the same topic [23,30–32], a collaborative framework on the associations and interactions of tuberculosis and diabetes has been initiated by the WHO and The International Union Against Tuberculosis and Lung Disease to improve joint management of the diseases [33].

It is most frequently stated that diabetes increases the risk of tuberculosis, although the available studies are observational and, due to the limitations of such study designs, do not allow this conclusion of oneway causality. It is evident that the diseases are associated, but the nature of the causality is not fully understood. Furthermore, while the current evidence focuses on the association between diabetes disease and active tuberculosis, few studies consider that even the early stages of the two diseases may interact; e.g., latent tuberculosis gives rise to low-grade inflammation (Vestergaard, in press), which eventually could lead to diabetes [34]. Similarly, not only diabetes, but also prediabetes (impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)), may affect the risk and course of tuberculosis infection and disease, which is also reported in Paper I.

Bi-directional interactions between tuberculosis and diabetes may occur at five different stages of disease and treatment, as suggested in Figure 1; "a" denotes the effects of diabetes on tuberculosis, and "b" denotes the effects of tuberculosis on diabetes. Prediabetes and diabetes may affect the risk of latent, primary and reactivation tuberculosis (Ia), severity of tuberculosis disease (IIa), and tuberculosis treatment outcomes (IIIa), and diabetes treatment may affect the risk of tuberculosis (Va) and tuberculosis treatment outcomes (IVa). Similarly, latent and active tuberculosis may affect risk of prediabetes and diabetes (Ib), severity of diabetes (IIb) and diabetes treatment outcomes (IIIb), and tuberculosis treatment may affect risk of diabetes (Vb) and diabetes treatment outcome (IVb).

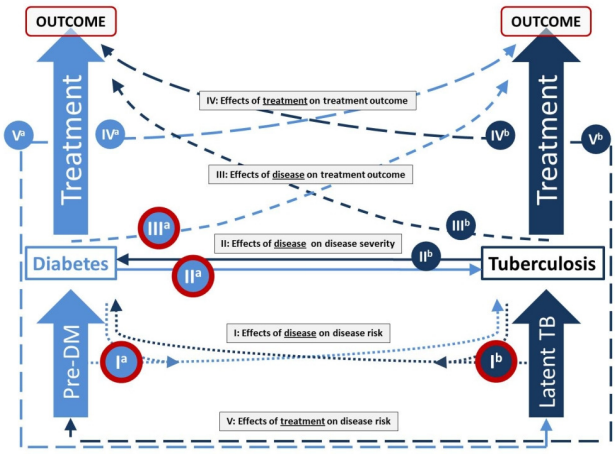

Figure 1
The interactions between tuberculosis and diabetes

In the Tuberculosis and Diabetes Study several of the interactions have been studied:

- Paper I focuses primarily on Ia, the association between diabetes and pulmonary tuberculosis, while dealing with the role of tuberculosis on stress hyperglycaemia; a major threat to the validity of the diabetes diagnosis and further described in the section *Underlying mechanisms of the association*.
- Paper II focuses on the role of diabetes on the clinical manifestations of tuberculosis (e.g. fever) as well as on paraclinical parameters; i.e. haematology, acute phase response, and sputum culture intensity, which all are related to the IIa pathway.
- Finally, Paper IV deals with the role of diabetes as a predictor of mortality during tuberculosis treatment, and Paper III is looking into anthropometric and clinical changes attributable to diabetes over a five months follow-up period. Both studies are related to the IIIa pathway.

Underlying mechanisms of the association

Mutual risk factors of tuberculosis and diabetes

Mutual risk factors will most likely confound the association between the diseases. The identification of mutual risk factors may be one of the short cuts to prevention of either disease. One example is HIV infection, which may be involved in both diabetes (i.e. anti-retroviral treatment) and tuberculosis (i.e. immune deficiency). Other possible mutual risk factors could be alcohol, smoking, and malnutrition, which all relate to lifestyle and socio-economic status, and have previously proven associated with both tuberculosis and diabetes [7,35–38].

Diabetes increases the risk of infectious diseases

It is evident that diabetes increases the susceptibility of several infectious agents, primarily bacterial and fungal infections [39]. The increased susceptibility to infection is caused by several factors, including altered host-defence and environment for the infecting microorganism. Apparently, the immune system defect is a combination of impaired cellular activity (i.e. decreased phagocytic activity) and altered cytokine release [40–44]. Previous studies have reported that the presence of hyperglycaemia leads to an elevated resting cytokine production in non-stimulated peripheral blood mononuclear cell (PBMCs), whereas reduced concentrations of cytokines have been seen in diabetes patients

after antigen stimulation of PBMCs [44]. It has also been shown that diabetes is associated with lower levels of circulating leucocytes and neutrophils [44–46]. From a recent study comparing diabetes patients (without chronic complications) with non-diabetes controls, it was reported that diabetes patients had a lower percentage of activated macrophages, and that there was a negative correlation between phagocytic activity and increasing levels of fasting blood glucose and glycosylated haemoglobin (HbA1c) [43]. Furthermore, the study showed that optimized glycemic control led to improved phagocytic activity.

During the recent influenza (H1N1) pandemic, diabetes was associated with hospitalization from influenza, although it was not clear whether the association was related to closer monitoring of diabetes patients, due to a generally increased rate of complications among diabetes patients, or whether diabetes led to a true increased risk of infection with influenza [47].

Bacterial infections, such as lower respiratory tract infections, urinary tract infections as well as infections in the skin and mucous membranes, are more common among patients with diabetes [48]. However, the association with these infections may have different causes; glycosuria alters the microenvironment and diabetes improves adherence of the bacteria to uroepithelial cells, both situations causing urinary tract infections [49]. For infections in the skin and mucous membranes, the local immune barrier may be hampered by the diabetic ulcers.

Diabetes increases the risk of tuberculosis

As a consequence of the impaired immune function, it is plausible that diabetes predisposes to pulmonary tuberculosis [29]. The increased risk is most likely caused by altered cellular mechanisms, or, less likely, through altered environment in the lungs. Macrophages are the primary target cells in tuberculosis infection, and with reduced numbers or impaired function of macrophages this will lead to impaired phagocytosis, impaired chemotaxis and reduced release of free radicals. No or poor glycemic control thereby affects the phagocytic function [44].

Tuberculosis increases the risk of diabetes

There is little evidence to suggest that tuberculosis increases the risk of diabetes. However, unpublished data from this project indicate that latent tuberculosis is associated with a higher acute phase response (i.e. low-grade inflammation), and since low-grade inflammation can lead to diabetes [34,50], latent tuberculosis may be a link accelerating the progression to active tuberculosis as well as to clinical diabetes.

It has also been suggested that tuberculosis could lead to chronic hyperglycaemia mediated through persistent insulin deficiency [30], but a more realistic scenario is tuberculosis leading to an acute non-diabetes stress-hyperglycaemia. Stress hyperglycaemia is commonly seen in severely ill patients admitted at intensive care units (i.e. trauma, surgery) [51,52], and the mechanisms behind stress hyperglycaemia are primarily thought to be caused by activation of the hypothalamic-pituitary-adrenal axis, leading to acute insulin resistance and hyperglycaemia [51]. Therefore, severe tuberculosis infection could potentially lead to hyperglycaemia, possibly misinterpreted as diabetes. This is obviously mainly a problem, if the diabetes diagnosis is obtained during tuberculosis infection. This diagnostic challenge is likely to be a player in the Tuberculosis and Diabetes Study due to the timing of the diabetes diagnosis, and this specific problem is dealt with in Paper I.

Tuberculosis disease leads to a weight deficit compared to individuals without tuberculosis, which affects both lean and fat mass [53]. When the body mass recovers during tuberculosis treatment, it is therefore important that the regain also affects lean body mass, since sole build-up of fat may cause insulin resistance and predispose to impaired glucose metabolism [54].

The role of HIV on the association between tuberculosis and diabetes

When assessing the association of tuberculosis and diabetes in a high HIV burdened region, it is essential to mention the role of HIV co-infection, since HIV infection is the most potent risk factor for active tuberculosis [7]. While there is no evidence for a direct association between HIV infection and diabetes, the diseases are interconnected through long-term anti-retroviral treatment (e.g. protease inhibitors) commonly used to keep the HIV infection under control [55,56]. A common adverse effect from, especially, the first generations of anti-retroviral drugs was altered body composition, insulin resistance and impaired glucose tolerance leading to metabolic syndrome and eventually diabetes [55]. Thus, a paradox of this could be that HIV treatment decreases the risk of tuberculosis, while the HIV treatment may ultimately lead to diabetes, which again increases the long-term risk of tuberculosis. However, this paradox is highly speculative, and may not be a major clinical issue.

The Tuberculosis and Diabetes Study

From 2001-2002 members from our research group conducted a nutritional intervention study on tuberculosis patients in Mwanza City, Tanzania. The study investigated the effects of a multi-micronutrient and zinc supplementation (tablets) on treatment outcome and mortality, and found that a micronutrient supplement would speed up weight gain, and possibly reduce the mortality among tuberculosis patients co-infected with HIV [57,58].

Due to the promising findings in the first study, it was decided to conduct two new studies, but with the supplementation based on biscuits instead of tablets. Of the new studies, the first focussed on the effect of a high vs. low dose of multi-micronutrients among HIV uninfected tuberculosis patients as well as HIV co-infected patients with sputum negative tuberculosis [59]. Due to the beneficial effect of multi-micronutrients on mortality found in 2002 among sputum positive tuberculosis patients co-infected with HIV [58], it was decided, for ethical reasons, in the new study that this group should be supplemented with a basis of multi-micronutrients. Therefore a second study among this sub-group was done to assess the effects of high vs. low energy-protein supplementation with all participants receiving multi-micronutrients [60].

The planned recruitment of tuberculosis patients built the fundament for several sub studies [61–64], including the association between tuberculosis and diabetes, the Tuberculosis and Diabetes Study.

Objectives

Overall objective

– to assess the role of diabetes for tuberculosis risk, manifestations, treatment outcomes and survival.

Specific objectives

– to assess the association between diabetes and pulmonary tuberculosis, while assessing the role of HIV as a potential effect modifier (Paper I).

- to assess the role of diabetes on the clinical manifestations of tuberculosis, as well as on paraclinical parameters including haematology, acute phase response, and sputum culture intensity (Paper II).
- to assess the role of diabetes as a predictor of tuberculosis culture conversion and mortality among tuberculosis patients during treatment (Paper III).
- to assess the changes in anthropometry as well as some basic clinical parameters over a five months follow-up period to assess the impact of diabetes on the outcome during tuberculosis treatment (Paper IV).

Figure 2
Map of Tanzania showing the location of Mwanza City
Source: www.geology.com/world/tanzania-satellite-image.shtml

METHODOLOGY

Study setting and population

The Tuberculosis and Diabetes Study invited newly diagnosed tuberculosis patients from the districts of Ilemela and Nyamagana in Mwanza City, Tanzania. Mwanza City is located on the shores of Lake Victoria in north-western Tanzania (Figure 2), and has a population (2002) of app. half a million inhabitants (www.tanzania.go.tz). Administratively, the city is divided into 20 wards and 500 streets. Each street is further divided into several informal communal cells with 10-20 households, headed by a ten-cell leader. Most people earn a living through small scale agriculture, fishing, petty trading, business and a few are employed in the public sector.

The city has three major hospitals, three health centres and several dispensaries. Tuberculosis services are provided in four public clinics through the National Tuberculosis and Leprosy Programme (NTLP) of the Tanzanian Ministry of Health and Social Welfare.

The Tuberculosis and Diabetes Study was nested within the framework of two large nutrition intervention studies [59,60], from where all study participants were invited to a blood glucose screening. The patients were recruited from April 2006 to October 2008 with follow up visits at two and five months, and a final survival follow-up after 12 months.

Table 1
Study designs in the Tuberculosis and Diabetes Study

Paper	Design
I	Case-control study 803 tuberculosis patients 350 neighbourhood controls
II	Cross-sectional 1205 tuberculosis patients
III	Prospective cohort study 1205 tuberculosis patients
IV	Prospective cohort study 1239 tuberculosis patients

Study designs

This thesis consists of four papers from the Tuberculosis and Diabetes Study (Table 1). Paper I was a case-control study looking at the role of prediabetes and diabetes on the risk of active tuberculosis, paper II was a cross-sectional study looking into the role of diabetes on the clinical and paraclinical manifestations of tuberculosis, and finally the papers III and IV were prospective cohort studies on the role of diabetes on the tuberculosis treatment outcome. The objectives are described in *Objectives* section.

Criteria for enrolment in the Tuberculosis and Diabetes Study

The recruitment of study participants was carried out in four tuberculosis clinics in Mwanza City; Sekou Toure Regional Hospital, Bugando Medical Centre as well as the Buzuruga and Butimba Health Centres. Newly diagnosed tuberculosis patients (including new cases and relapses) were consecutively invited to participate in the study. Among the microscopically confirmed pulmonary tuberculosis patients (PTB+) enrolled, we asked 400 consecutive patients to participate as cases in a case-control study (Paper I and [53]). Tuberculosis patients consenting to participate in the case-control study were asked to provide detailed contact information about address and their ten-cell leader (leader of an informal communal cell with 10-20 households). The study team requested the ten-cell leader to provide the complete list of individuals in his/her jurisdiction meeting the age- and sex criteria for the recruitment of a non-tuberculosis neighbourhood control. From the list of potential controls one individual was then randomly selected using a lottery method, and invited to participate as neighbourhood control. If the invited control was not eligible or refused, then another candidate was randomly selected.

A full list of inclusion and exclusion criteria for tuberculosis patients and neighbourhood controls are shown in table 2.

A total of 3397 tuberculosis patients were assessed for eligibility, of which 1250 (36.8%) were found eligible and consented to participate in the Tuberculosis and Diabetes Study. Among the 400 non-tuberculosis neighbourhood controls who had accepted to participate, diabetes data were available for 350 (87.5%) and were included in the case-control study (Paper I). A detailed study profile is shown in Figure 3.

Table 2
Criteria for enrolment in the Tuberculosis and Diabetes Study

Tuberculosis patients	
Inclusion criteria	Verified tuberculosis (PTB+ or PTB-)
Exclusion criteria	< 15 years of age Extra-pulmonary tuberculosis Pregnant or lactating women Terminally ill from TB or HIV (judged unlikely to survive > 48 hours) Other severe diseases Non-residents of Mwanza City
Neighbourhood controls*	
Inclusion criteria	Same sex and age (+/- 5 years) as the tuberculosis case Living in the same street as tuberculosis case for at least three months
Exclusion criteria	History of previous active TB or TB treatment History of tuberculosis in the household Evidence of active tuberculosis (i.e. cough, intermittent fevers, excessive night sweating in the past two weeks, unexplained weight loss in the past month) Pregnant or lactating women Terminally ill
** Neighbourhood controls were only included in Paper I*	

Methods

Tuberculosis diagnosis

Patients attending the public clinics and presenting with symptoms suggestive of tuberculosis were asked to bring three sputum samples for microscopic examination. Sputum microscopy was done as part as the routine diagnostic procedure using "spot-morning-spot" samples (method described in [65]) using Ziehl-Nielsen staining technique in combination with culture of M. tuberculosis on Lowenstein Jensen solid media. Sputum smear microscopy was performed in double at (i) the recruiting health facility and (ii) at the Zonal TB Reference Laboratory at Bugando Medical Centre. The smear was graded according to the presence of acid fast bacilli (AFB) as either negative (no AFB) or positive: 1-9 in 100 fields, +1 (10-99 AFB in 100 fields), +2 (1-10 AFB pr. field in >50 fields), or +3 (>10 pr. field in >20 fields). Additionally, early morning sputum samples were collected in a sterile universal bottle for culture of M. tuberculosis on Lowenstein Jensen solid media and graded as either negative (no growth of colonies) or positive: 1-19 colonies, +1 (20-100 colonies), +2 (innumerable discrete colonies), or +3 (confluent growth).

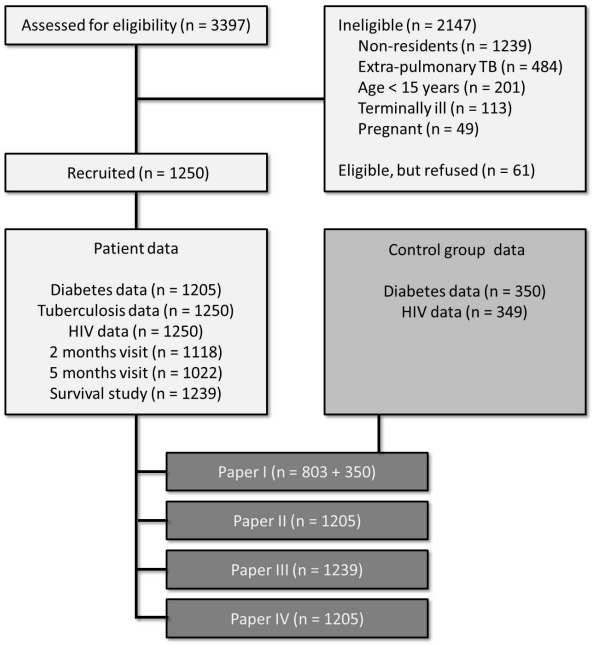

Figure 3
Study profile from the Tuberculosis and Diabetes Study

All enrolled patients full-filled the criteria for active tuberculosis following the national guidelines; a patient was diagnosed with sputum positive pulmonary tuberculosis (PTB+), if two microscopy samples tested positive or one sample tested positive and a chest X-ray was suggestive of tuberculosis. In case of a negative sputum samples, the diagnosis was defined as sputum negative pulmonary tuberculosis (PTB-), in which case the tuberculosis diagnosis was based on clinical suspicion, history of disease, non-response to broad spectrum antibiotics given for 7-14 days as well as a positive x-ray result as suggested by WHO [66].

Although the tuberculosis diagnosis (PTB- or PTB+) obtained from the health facilities was based on microscopy and used for the randomization in the nutritional intervention studies [59,60], it was decided that the final tuberculosis diagnosis in the Tuberculosis and Diabetes Study should rely on culture status; the microscopy results were only used if the culture result was missing.

After the tuberculosis diagnosis was obtained, all patients were started on a standard treatment. During the study period, two different treatment regimens were used [67,68]:

– In the beginning of the study period (until 1st of October 2006), patients followed an eight months course, including initial two months of intensive treatment (intensive phase) and six months of continuation treatment (continuation phase). A four-drug regimen (rifampicin, isoniazid, pyrazinamide, ethambutol) was used during the intensive phase a two-drug regime (ethambutol, isoniazid) during the continuation phase. The administration of drugs followed the DOT-model [5] were drug ingestion was observed daily at the health facility by a health worker during the intensive phase whereas in the continuation phase, patients collected drugs from the health facility on a monthly basis for self-administration at their homes.

- In the last part of the study period (after 1st of October 2006) national guidelines changed to a six months regimen introduced new drug formulations and mode of supervision, and reduced treatment duration to six months. During this period the two months intensive phase was unchanged, however, the continuation phase was reduced to four months and a two-drug regimen with a new combination (rifampicin, isoniazid). In the new guidelines, patients were asked to choose between supervision at home by a treatment supporter or by nurses at the health facility.
- In both time periods, tuberculosis patients who were retreatment cases, received treatment for eight months. Since all retreatment cases in addition to tablets get streptomycin injection for the first two months, their drug intake was observed daily at the health facility during the intensive phase.

Diabetes diagnosis

Fasting blood glucose was determined using point-of-care diagnostic instruments (HemoCue 201+ Glucose System, HemoCue, Sweden) and those with a fasting blood glucose between 5.1-11.0 mmol/L (capillary whole blood levels) completed a standard 2 hour oral glucose tolerance test (OGTT); intake of 75 g of anhydrous glucose dissolved in water and a subsequent two hour relaxation period until a second blood glucose test would be done. The test was performed between 8.00-10.00 AM. All participants were instructed to be fasting from midnight (> 8 hours), and only water was allowed prior to test.

Therefore, final diabetes diagnosis was based on a FBG > 6.0 mmol/L or alternatively a 2h blood glucose >11.0 mmol/L. The diabetes testing was performed as soon as possible (few days) after initiation of TB treatment to eliminate the role of adverse drug effects. Participants already known to have diabetes were only classified as such in the study, if the diagnosis could be reproduced by our methods.

Laboratory work, including HIV diagnosis

Venous blood was drawn in EDTA tubes at local health facilities and transported to the research laboratory, whereupon serum was collected and kept at -80oC until analysed. For HIV diagnosis two rapid tests, Determine HIV 1/2 (Inverness Medical Innovations, Inc., Delaware, USA) and Capillus HIV-1/HIV-2 (Trinity Biotech Plc., Wicklow Ireland) were used. If the tests were discordant, HIV diagnosis was based on a commercial ELISA kit (Organon Uniform II, Organon Teknika, Oss, the Netherlands). Cluster of differentiation 4 (CD4) counts were determined by flow cytometry after CD4 immuno-flourochrome staining of the leucocytes (Partec FACS, Partec GmbH., Germany), and haemoglobin levels (g/dL) and white blood cell (10^9/L) count including differentials were carried out at the research laboratory at the National Institute for Medical Research in Mwanza. Serum concentrations of the acute phase reactants C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were determined at the Department of Clinical Biochemistry, Aalborg University Hospital, Denmark.

Anthropometry and grip strength

Weight and height were measured with the participant barefooted and with minimal clothing (nearest 0.1 kg and 0.1 cm), from which body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Waist circumference was measured between the lower costae and the iliac crest, and an international sex-specific cut-off was used to identify high waist circumference (> 102 cm for men and > 88 cm for women) [69]. Also, triceps skinfold thickness and mid-upper arm circumference were measured allowing for estimation of arm fat area and arm muscle area (method for calculation described in [70]).

Hand grip strength was determined to the nearest 0.1 kg using a digital dynamometer (Takei Scientific Instruments, Japan), and the mean from the two maximum results (one in each hand) was reported.

Questionnaire data

Recall data on known symptoms indicative of tuberculosis or diabetes were collected through interviews using a questionnaire; the patients were asked whether they, during the last month, had experienced fever, polyuria, polydipsia, skin ulcerations, visual impairment, weight loss, cough, haemoptysis, or night sweat. Furthermore, the HIV infected participants were asked about use of anti-retroviral treatment.

Medical records

The medical records of the participants, if available, were checked for preceding events of tuberculosis, to allow a distinction between new or relapsed tuberculosis infections to be made.

Follow-up data

All participants were re-examined after two and five months. Sputum was collected again at two months for those with a positive baseline culture, and similarly a positive culture sample at two months led to a retest at the five months visit. The grading of the culture at two and five months was identical to the baseline procedure. All anthropometric measurements, grip strength and the biological measurements (haemoglobin, white blood cells, CD4) were repeated at the two and five months visit. The diabetes and HIV testing were not repeated, thus the baseline diagnosis was used throughout the study period.

Furthermore, all participants were followed up for their survival status for the minimum of one year after inclusion in the study. Trained research personnel used tracing information provided at recruitment to locate the participants. The survival information was collected using a structured questionnaire on survival status; including information on relapse of tuberculosis within the first year after. Of those confirmed to be dead (all-cause mortality), data on date and place of death was collected. Moreover, mortality information was ascertained using either a death certificate or any other official document. If the survival information could not be obtained, the date of last known contact was used.

Training of staff

Before implementation of the project, tuberculosis clinic staffs were trained on how to take measurements and fill in study questionnaires. During the study the staffs were re-trained on data collection every six month. Moreover, regular supervision visits and study team meetings were done to ensure adherence to the study protocol.

Data analysis

Data were double entered in EpiData (The EpiData Association, Odense, Denmark) and analysed using Stata 11 (StataCorp LP, College Station, USA). In general, normal probability plots were used to assess normality of the continuous variables. Differences in categorical and continuous variables between groups were tested using chi-square test, t-test and oneway analysis (anova). In addition, linear and logistic regression models were used to

adjust for potential confounders and to test for interactions. P-values < 0.05 were considered significant. More specifically:

- Paper I: Logistic regression was used to estimate the role of prediabetes and diabetes as a risk factor for tuberculosis; also stratified by HIV status. The model was adjusted for the acute phase response to reduce the significance of non-diabetes stress-hyperglycaemia
- Paper II: For each of the possible predictors linear (i.e. age, haemoglobin level, white blood cell count, serum CRP, serum AGP, CD4 count, and hand grip strength) or logistic (i.e. sex, tuberculosis status and culture intensity, HIV status, anaemia, reported symptoms, and medical history) regression models were used to test for differences between patients with or without diabetes.
- Paper III: A linear mixed-effects model [71] was used to assess the changes across diabetes status for anthropometry, grip strength and biological measurements at baseline as well as after two and five months of TB treatment (repeated measurements).
- Paper IV: A Kaplan-Meier plot was used to present the time-to-death data, and a Cox proportional hazards analysis was used to assess predictors of mortality in a multivariate analysis including status of diabetes (diabetes vs. non-diabetes), tuberculosis (PTB+ vs. PTB-) and HIV (HIV infected vs. HIV uninfected).

Ethical considerations

Ethical permission was obtained from the Ethics Committee of the National Institute for Medical Research in Tanzania and The Danish National Committee on Biomedical Research Ethics approved the study. The study was registered at ClinicalTrials.gov (NCT00311298).

Written and oral information was presented to all eligible participants before written consent was obtained. Written consent was obtained from parents/legal guardians of any participants between 15-18 years of age. Pre-HIV test counselling was mandatory, and post-HIV-test counselling was offered to all HIV-positive participants. Patients with diagnosed HIV or diabetes were referred for further management.

ABSTRACTS OF 4 ORIGINAL PAPERS

Paper I: Diabetes is a risk factor for pulmonary tuberculosis: a case-control study from Mwanza, Tanzania

BACKGROUND

Diabetes and TB are associated, and diabetes is increasingly common in low-income countries where tuberculosis (TB) is highly endemic. However, the role of diabetes for TB has not been assessed in populations where HIV is prevalent.

METHODS

A case-control study was conducted in an urban population in Tanzania among culture-confirmed pulmonary TB patients and non-TB neighbourhood controls. Participants were tested for diabetes according to WHO guidelines and serum concentrations of acute phase reactants were measured. The association between diabetes and TB, and the role of HIV as an effect modifier, were examined using logistic regression. Since blood glucose levels increase during the acute phase response, we adjusted for elevated serum acute phase reactants.

RESULTS

Among 803 cases and 350 controls the mean (SD) age was 34.8 (11.9) and 33.8 (12.0) years, and the prevalence of diabetes was 16.7% (95% CI: 14.2; 19.4) and 9.4% (6.6; 13.0), respectively. Diabetes was associated with TB (OR 2.2, 95% CI: 1.5; 3.4, p<0.001). However, the association depended on HIV status (interaction, p = 0.01) due to a stronger association among HIV uninfected (OR 4.2, 95% CI: 1.5; 11.6, p = 0.01) compared to HIV infected (OR 0.1, 95% CI: 0.01; 1.8, p = 0.13) after adjusting for age, sex, demographic factors and elevated serum acute phase reactants.

CONCLUSION

Diabetes is a risk factor for TB in HIV uninfected, whereas the association in HIV infected patients needs further study. The increasing diabetes prevalence may be a threat to TB control.

Paper II: The role of diabetes on the clinical manifestations of pulmonary tuberculosis

OBJECTIVE

Diabetes is associated with pulmonary tuberculosis (TB), possibly due to impaired immunity, and diabetes may exacerbate the clinical manifestations of TB. Our aim was to assess the role of diabetes in the clinical manifestations of TB.

METHODS

We studied 1250 patients with pulmonary TB in an urban population in a cross-sectional study in Tanzania. All participants were tested for diabetes and HIV co-infection, and TB culture intensity was assessed. Levels of white blood cells, haemoglobin, acute phase reactants, CD4 count and HIV viral load were measured, and a qualitative morbidity questionnaire was used to identify the prevalence of disease-related symptoms.

RESULTS

Tuberculosis patients with diabetes had a higher neutrophil count (B 0.5×10^9 cells/l, 95% CI 0.2; 0.9, P = 0.001) than non-diabetic TB patients. Serum C-reactive protein (B 18.8 mg/l, CI 95% 8.2; 29.4, P = 0.001) and alpha-1-acid glycoprotein (B 0.2 g/l, CI 95% 0.03; 0.3, P = 0.02) were similarly higher in patients with diabetes. Diabetes did not affect culture intensity or HIV status, but self-reported fever was three times higher among participants with diabetes than in those without diabetes (OR 2.9, CI 95% 1.5; 5.7, P = 0.002).

CONCLUSION

Diabetes is associated with small changes in the manifestations of TB, but may have little clinical significance.

Paper III: The role of diabetes co-morbidity for tuberculosis treatment outcomes: a prospective cohort study from Mwanza, Tanzania

BACKGROUND

Due to the association between diabetes and pulmonary tuberculosis (TB), diabetes may threaten the control of TB. In a prospective cohort study nested in a nutrition trial, we investigated the role of diabetes on changes in anthropometry, grip strength, and clinical parameters over a five months follow-up period.

METHODS
Among pulmonary TB patients with known diabetes status, we assessed anthropometry and clinical parameters (e.g. haemoglobin) at baseline and after two and five months of TB treatment. A linear mixed-effects model (repeated measurements) was used to investigate the role of diabetes during recovery.

RESULTS
Of 1205 TB patients, the mean (standard deviation) age was 36.6 (13.0) years, 40.9% were females, 48.9% were HIV co-infected, and 16.3% had diabetes. TB patients with diabetes co-morbidity experienced a lower weight gain at two (1.3 kg, CI95% 0.5; 2.0, p = 0.001) and five months (1.0 kg, CI95% 0.3; 1.7, p = 0.007). Similarly, the increase in the level of haemoglobin was lower among TB patients with diabetes co-morbidity after two (Δ 0.6 g/dL, CI95% 0.3; 0.9 p < 0.001) and five months (Δ 0.5 g/dL, CI95% 0.2; 0.9 p = 0.004) of TB treatment, respectively.

CONCLUSION
TB patients initiating TB treatment with diabetes co-morbidity experience delayed recovery of body mass and haemoglobin, which are important for the functional recovery from disease.

Paper IV: Diabetes is a strong predictor of mortality during tuberculosis treatment: a prospective cohort study among tuberculosis patients from Mwanza, Tanzania.

OBJECTIVE
Strong evidence suggests diabetes may be associated with tuberculosis (TB) and could influence TB treatment outcomes. We assessed the role of diabetes on sputum culture conversion and mortality among patients undergoing TB treatment.

METHODS
A total of 1250 Tanzanian TB patients were followed prospectively during TB treatment with sputum culture after 2 and 5 months. Survival status was assessed at least 1 year after initiation of treatment. At baseline, all participants underwent testing for diabetes and HIV, and the serum concentration of the acute phase reactant alpha-1 glycoprotein (AGP) was determined.

RESULTS
There were no differences between participants with and without diabetes regarding the proportion of positive cultures at 2 (3.8% vs. 5.8%) and 5 (1.3% vs. 0.9%) months (P > 0.46). However, among patients with a positive TB culture, relatively more patients with diabetes died before the 5-month follow-up. Within the initial 100 days of TB treatment, diabetes was associated with a fivefold increased risk of mortality (RR 5.09, 95% CI 2.36; 11.02, P < 0.001) among HIV uninfected, and a twofold increase among HIV co-infected patient (RR 2.33 95% CI 1.20; 4.53, P = 0.012), while diabetes was not associated with long-term mortality. Further adjustment with AGP did not change the estimates.

CONCLUSION
Diabetes considerably increases risk of early mortality during TB treatment. The effect may not be explained by increased severity of TB, but could be due to impaired TB treatment response. Research is needed to clarify the mechanism and to assess whether glycaemic control improves survival.

GENERAL DISCUSSION AND PERSPECTIVES

Introduction

This study is the first assessing the association between tuberculosis and diabetes in a population where tuberculosis patients are heavily burdened by HIV co-infection. We found that prediabetes and diabetes were highly associated with pulmonary tuberculosis, and that the association was strongest among HIV uninfected. While diabetes only marginally affected the baseline clinical status of tuberculosis, diabetes had a huge effect on the treatment outcome with delayed weight gain, delayed normalization of haemoglobin levels as well as a substantial effect on tuberculosis mortality.

In this thesis I have focussed on type 2 diabetes, and all patients with a diabetes diagnosis have been considered as such. However, we cannot rule out the existence of other variants of diabetes, e.g. type 1 diabetes or previously accepted definitions such as malnutrition-related diabetes which consists of a group of patients with hyperglycaemia and nutritional deficiencies, but without the classical symptoms, signs and metabolic characteristics known for type 1 and 2 diabetes [72,73]. The existence and consequences of diabetes in Africa is poorly investigated and should be prioritized. Although this thesis primarily deals with the association between tuberculosis and diabetes, we also found unexpected high diabetes prevalence among neighbours to tuberculosis patients. This is of major concern, since those diagnosed with diabetes in general were relatively young and lean. It is also unknown whether diabetes among non-obese, non-tuberculous Africans has the same long-term implications for the individual (i.e. risk for co-morbidities, impaired quality of life, and risk of premature death) as has been reported from high-income countries.

With the data from The Tuberculosis and Diabetes Study, we have data on the association of tuberculosis and diabetes from Africa as well as Asia, the Americas and Europe [24–27]. Nevertheless, there is still a research gap when it comes to underlying mechanisms, but also related to screening tools for (i) tuberculosis among diabetes patients and (ii) diabetes among tuberculosis patients. With the recent "Collaborative framework for care and control of tuberculosis and diabetes" [33], which is a joint report by the WHO and the International Union Against TB Lung Disease, the double burden was put on the international health agenda. Although our data were not available for this large report, the suggestions from the report on bi-directional screening and joined management and care of the diseases will be highly relevant for African populations, where the tuberculosis incidence remains high [6].

Strengths and limitations

The study population

The study was nested within the framework of two large intervention studies [59,60], comprising a well-defined and well-examined tuberculosis population. The patients were recruited over three consecutive years, almost all eligible patients consented to participate, and, except for fatalities, very few dropped out. During the recruitment period the national treatment guidelines changed [67,68], which caused some challenges related to the nutritional intervention, while the diabetes component remained unchanged.

The neighbourhood control group was selected for comparison with the PTB+ patients. As such, the control group was not representative for the background population in Tanzania, but suitable for analysing the association between tuberculosis

and diabetes, since the controls had similar age, sex, and socio-economic background. This said, being a selected control group for tuberculosis patients may introduce selection bias with respect to the estimate of diabetes prevalence. We cannot generalize the diabetes prevalence found in this group onto population level, since many factors in the neighbourhood of a tuberculosis patient (e.g. social status, living conditions) may not represent the population as a whole. Also, since diabetes in low-income countries is becoming common among individuals from all socio-economic groups [74], it is difficult to know whether we over- or underestimate the national prevalence.

The study designs

Due to the setup of the study, we were able to combine multiple designs; case-control (Paper I), cross-sectional (Paper II) and prospective cohort designs (Papers III and IV).

Paper I established the association, which was assessed from the prevalence of diabetes within non-tuberculous controls and tuberculosis patients. We randomly matched neighbourhood controls to a group of PTB+ patients on the parameters age, sex and neighbourhood; thus, we attempted to minimize the role of confounders. We used the case-control design to help determine if the exposure (diabetes) was associated with the outcome (tuberculosis). Despite the usefulness of the design to describe the association it does not assess the causation. Furthermore, the case-control design is sensitive to selection bias, since we identify the participants on the outcome and trace back to investigate the exposure.

Paper II was a cross-sectional study, where we assessed the association between diabetes and several clinical and paraclinical outcomes, when the tuberculosis patients were about to initiate treatment. The advantage of the design was the capability of, at low cost, investigating several associations, which later on could be more rigorously studied in new studies using designs with a higher level of evidence, such as a prospective cohort study. The cross-sectional design has some limitations, and, as was also the case for the case-control design, it does not allow differentiating cause and effect from simple associations.

In the papers III and IV we used a prospective cohort design, which ranges higher in the hierarchy of evidence compared to the case-control and cross-sectional designs. By following the tuberculosis patients over time, we were able to demonstrate chronological order between diabetes and the treatment outcome, thus allowing the establishment of causation. However, while the design per se is considered to be strong on causality, our methods did introduce some limitations related to this; if hyperglycaemia to some extent was caused by tuberculosis severity, the causality would be the opposite of what was hypothesized. Finally, a potential danger of cohort studies is losses to follow-up, but fortunately we experienced low drop-out rates (except losses due to death).

The validity of the diabetes diagnosis and the potential role of stress hyperglycaemia

The diabetes diagnosis was considered an epidemiological diagnosis based on the 1999 WHO definitions [75]. However, the definitions were based on patients without co-morbidities, and, therefore have limitations in a population burdened by infectious diseases. The choice of method was decided to be based on a combination of capillary FBG levels and a standard 2h OGTT. However, since the population consisted of tuberculosis patients, who had lost significant body mass and, additionally, were at risk of stress hyperglycaemia and fluctuations in glucose levels, it was decided that the range of the FBG for offering the OGTT should be expanded from the commonly used 5.6-6.0 mmol/L; those with a FBG between 5.1-11.0 mmol/L had to undergo the OGTT, whereas those with FBG <5.1 or >11.0 mmol/L did not and were instantly diagnosed with normoglycemia or diabetes, respectively.

As mentioned, the major concern was stress hyperglycaemia [51], which potentially would lead to false-positive diagnosis of diabetes. The problems related to stress hyperglycaemia had briefly been touched in one review [30], but there were no data on the frequency and magnitude of stress hyperglycaemia in the available studies. Only two studies had reported fluctuations in the diabetes prevalence during tuberculosis treatment and found divergent result. In a 20 year old study from Nigeria the prevalence of glucose intolerance among 54 patients declined during tuberculosis treatment [76], but in a more recent study from Indonesia, the proportion of patients with diabetes co-morbidity did not change during the treatment period [24]. In the current study we chose to adjust for the acute phase response in the statistical models. The argument for performing this adjustment was that if tuberculosis patients with more severe disease had hyperglycaemia, it would expectedly be accompanied by an acute phase response.

To improve the validity of the diagnostic test for diabetes we could have used other tests such as HbA1c, which is considered more stable on a day-to day basis, unaffected by fasting state and can be performed at any time of the day [77]. Since HbA1c reflects the average blood glucose level over eight to 12 weeks [77], it may be less affected by acute stress hyperglycaemia. However, HbA1c is more expensive compared to OGTT, and was not yet internationally recommended when we conducted the study. Furthermore, HbA1c is also sensitive to long periods (weeks) of stress hyperglycaemia [78] and may just as well provide transient false-positive tests in tuberculosis patients.

In epidemiological studies relying on direct measurement of blood glucose, it is a problem to ensure that the patient is fasting prior to the diabetes testing [75,79]. In the other studies published on the association between tuberculosis and diabetes, the diabetes diagnosis was based on FBG levels, random non-FBG or register data [24,80,81], but none of the studies using FBG mentioned how they ensured that the patients were fasting at the time of the test. We instructed the participants to arrive fasting in the morning, we repeatedly asked about intake of food and drinks prior to the test, and we asked again among those participants with blood glucose levels suggestive of diabetes. If the participant was not fasting, we postponed the testing to another day. We tried hard to ensure that the patients were reliably fasting, and it could only have been better controlled if we had kept the participants overnight in the clinic. To overcome the fasting dilemma, we could have chosen to discard the fasting levels and rely only on the blood glucose level after completion of the OGTT, but this would have made the study less comparable with the other studies available. Also, we did not perform OGTT on all, thus a final diagnosis would always be a combination of the two tests. However, in preliminary analyses, we diagnosed diabetes based on the results from the OGTT and found the same associations between diabetes and tuberculosis as well as interaction between diabetes and HIV.

Ideally, in a tuberculosis population with unknown diabetes status diabetes testing should be done both during and after tuberculosis treatment to eliminate the role of stress hyperglycaemia. Although not mentioned previously in this thesis, we did

retest a sub group of patients for diabetes (n = 271) and found that a large proportion of those who tested positive for diabetes at baseline no longer fulfilled the criteria for diabetes after two months (Faurholt-Jepsen, unpublished data). Despite this shift in diagnosis for some of the patients, the diabetes prevalence remained high (15%), since some of the patients previously tested negative now had blood glucose levels suggestive of diabetes. This finding may just reflect the common clinical experience that people experience large fluctuations in the day-to-day blood glucose levels, but also that stress hyperglycaemia, and even false low glucose levels compared to habitual state, may be prevalent. Identifying hyperglycaemia, regardless of the underlying mechanism, seems to be essential for the individual tuberculosis patient, since the state clearly is associated with adverse (and fatal) treatment outcomes (Paper III and IV).

The role of HIV and anti-retroviral treatment

Most of the HIV co-infected participants did not know their status prior to the study, but one out of eight HIV infected patients was already receiving anti-retroviral treatment when the study commenced. Due to the adverse effects (e.g. metabolic dysfunction) from the anti-retroviral treatment [55], it was relevant to explore if these patient had a higher proportion of diabetes and if it had other consequences for the association between tuberculosis and diabetes, but no differences were identified. However, HIV disease did interact with diabetes, with the highest diabetes-associated risk of tuberculosis among HIV uninfected (paper I).

Perspectives and future studies

In the African region, the double burden of tuberculosis and diabetes is becoming a major health problem. Although the tuberculosis incidence may have stabilized [6], the incidence of diabetes will continue to increase [82] and possibly interfere with tuberculosis control [32]. As mentioned, the WHO initiative "Collaborative framework for care and control of tuberculosis and diabetes" [33] has recently been launched, and this clearly shows that the large health organizations are concerned about the increasing burden of tuberculosis and diabetes. The report was made since no international guidelines of joint management existed, thus the intention of the framework was "to guide national programmes, clinicians and others engaged in care of patients and prevention and control of diabetes and TB on how to establish a coordinated response to both diseases, at organizational and clinical levels". More specifically, the framework has made recommendations on bi-directional detection and management, so patients with either disease are screened and, if applicable, treated for the other.

Bi-directional screening

Based on current evidence, research of bi-directional screening methods and improved care of patients with both tuberculosis and diabetes seem feasible. However, if one of the diseases is rare, the positive predictive value of bi-directional screening would be too low [83].

The concept of bi-directional screening is known from the HIV and tuberculosis programmes where patients attending either the tuberculosis or the HIV ward are examined for the other disease [84]. Thus, a similar approach could prove useful to identify diabetes or tuberculosis among patients already diseased by the other condition [31]. Since it may be difficult to trace diabetes or tuberculosis among individuals without clinical symptoms, it seems sensible to explore approaches with bi-directional screening, which make the most of existing patient contacts. Patients attending diabetes clinics are already routinely examined for diabetes-related complications, but these patients could possibly benefit from a tuberculosis screening tool based on symptoms (e.g. cough, weight loss, unexplained fever etc.), and saving the diagnostic tests for tuberculosis suspects only. Similarly, tuberculosis patients should routinely receive diabetes screening (e.g. FBG, HbA1c) to identify the patients with a potential underlying diabetes diagnosis.

Separation of patients with and without tuberculosis

Since patients with diabetes are at increased risk of tuberculosis, it is important to prevent that they are exposed. Thus, diabetes patients without established tuberculosis co-morbidity should be tested for tuberculosis in a non-tuberculosis area to avoid exposure, and, furthermore, such patients should be treated to improve glycaemic control, which may improve their immune response to tuberculosis infection [43]. Likewise, diabetes patients with verified tuberculosis should be treated isolated from those without. Separating the patients is not necessarily an expensive approach and could possibly be implemented in most settings. As a result of this, tuberculous diabetes patients would also receive better care, since they would be treated by health professionals with special expertise in both diseases; such approach was already implemented in England in 1954 [85], where Luntz and colleagues arranged specialized wards for tuberculous diabetes patients. In addition to improved management in health facilities, diabetes clinicians should inform their patients about the risk of community acquired tuberculosis infection, about preventing tuberculosis exposure in the household by screening or testing family members for tuberculosis, and about how to reduce the impact from potential mutual risk factors (e.g. smoking, alcohol, malnutrition).

Research priorities

To further explore the causative links between tuberculosis and diabetes as well as the role of the interaction on morbidity, treatment outcomes and mortality of both diseases, there is a need of better designed epidemiological and clinical studies. As shown in figure 1 (section *The double burden of tuberculosis and diabetes*), there are several possible causal interactions between the diseases and even between the pre-disease conditions, latent tuberculosis and prediabetes (i.e. IFG, IFT). While several studies report an overall association, little is known about the causal pathways. Furthermore, what is not mentioned in the figure is the nature of the risk factors leading to pre-disease and whether such risk factors interconnect. To become infected with tuberculosis exposure is essential, and impaired immunity will increase the susceptibility. Several risk factors are associated with impaired immunity increasing the susceptibility of primary infection, and, interestingly, a number of these, such as tobacco, alcohol, malnutrition as well as poor social status and living conditions, could be considered mutual risk factors, since they are also associated with diabetes [3,4,72]. Mutual risk factors for tuberculosis and diabetes should be prevented, in order to prevent tuberculosis and diabetes and their synergistic interactions with respect to serious outcomes.

Diagnosis and treatment of diabetes among tuberculosis patients

The recommended method for diagnosing diabetes, a combination of FBG and OGTT, has limitations in populations with concurrent diseases [75]. Since diabetes status is almost always un-

known among tuberculosis patients in low-income countries, primary diabetes testing needs to be performed under conditions with tuberculosis co-morbidity. However, the validity of these tests has never been tested in a tuberculosis population. This could be done among tuberculosis patients who survive throughout the entire treatment period which allows a retest after cure and cessation of tuberculosis treatment. To introduce alternative and less labour demanding procedures, the validity of other tests such as HbA1c should be assessed in parallel.

For all tuberculosis patients presenting with hyperglycaemia, the first priority must be better management and care, and maybe even medical intervention to stabilize the blood glucose, to help them survive the initial phase of the treatment. Optimally, medical intervention should be done in a randomized trial to investigate whether medical treatment has any effect on survival and treatment outcome. Also, such study would make it possible to validate various blood glucose testing methods before, during and after successful tuberculosis treatment. In the study from Indonesia, all participants with diabetes initiated oral anti-diabetes medicine within 2-4 weeks of tuberculosis treatment [86], but since the Indonesian study only reported two deaths out of 634 enrolled patients, mortality may not be a problem in the population. It is questionable to treat everybody with anti-diabetes medicine if their diabetes diagnosis is obtained at a time with severe co-morbidity. We do not know the frequency of stress-hyperglycaemia in a tuberculosis population, and attempts to medically control hyperglycaemia in severe illness have been both favourable and harmful [87]. This problem opts for a randomized place-controlled study to assess the usefulness of controlling hyperglycaemia in a tuberculosis population with high mortality rates. Otherwise, the risk of mortality during tuberculosis treatment among individuals with or without diabetes could be assessed in a prospective cohort study, but such study would need a large number of participants and many resources, and, thus, may not be feasible.

Another approach, to assess the role of diabetes or hyperglycaemia on mortality, would be to include other screening tools such as performance scores (e.g. TBscore, Karnofsky score), which predict mortality among tuberculosis patients [88,89]. Based on such scores, is has been reported that tuberculosis patients with diabetes has reduced performance status [86], however, it needs to be explored whether such scores can predict mortality among tuberculosis patients with diabetes.

Epidemiological and clinical studies

In addition to the available cross-sectional and retrospective cohort studies, new long-term prospective cohort studies should be implemented. Studies within population with latent tuberculosis or diabetes/prediabetes could explore the long-term risk of the opposite disease, e.g. the role of prediabetes or diabetes on the development of latent and active tuberculosis. Furthermore, studies within populations without disease could identify risk factors related to one or both diseases (e.g. malnutrition, changes related to lifestyle transition, birth record data etc.); especially risk factors for prediabetes and diabetes in low-income countries are poorly examined.

Although most focus has been on the possible of effects of diabetes on active tuberculosis, it is also possible that latent or active tuberculosis lead to the onset of prediabetes and diabetes. In the current population we have seen that latent tuberculosis in household contacts and neighbourhood controls may give rise to low-grade inflammation (Vestergaard, unpublished), and there is evidence to suggest that low-grade inflammation can lead to diabetes [90,91].

Mechanistic studies on immunity, pharmaco-kinetics and drug interactions

The release of cytokine interferon gamma (IFN-γ) seems to be affected by diabetes [92,93]. We have, yet unpublished, data showing that diabetes is associated with a marked reduction in the levels of IFN-γ among both PTB+ patients and non- tuberculosis controls. This finding indicates a diabetes-associated defect in the Th1 cell line [93]. The implication of this and other immune defects associated with diabetes (or hyperglycaemia) should be further explored to identify the specific consequences related to tuberculosis. Such studies should also try to distinguish between primary infection and reactivation of latent infection.

It has previously been reported that diabetes disease may impair the bioavailability [94,95] of tuberculosis drugs as well as increase the risk for multidrug resistant tuberculosis [96,97]. From an Indonesian study it was reported that tuberculosis patients with diabetes had lower plasma levels of rifampicin [94]. Since the effect was seen during the continuation phase of treatment, it could possibly be mediated through increased body weight and alterations in hepatic induction [94]. Furthermore, diabetes may cause gastrointestinal malabsorption, which in itself could lead to altered bioavailability [95], and explain the adverse treatment outcome we and others have reported [26,86,98,99].

There may also be direct interactions between tuberculosis drugs and oral anti-diabetic drugs. Rifampicin and isoniazid (tuberculosis drugs) act as inducer and inhibitor, respectively, of the enzymatic drug metabolism in the liver, which is also the location of the elimination of several oral anti-diabetics [32], more specifically, rifampicin may in fact increase the glucose lowering effects of metformin [100,101]. However, these interactions (drug-disease and drug-drug) have only been poorly investigated and should be repeated in other populations.

It is obvious to focus on interactions directly related to tuberculosis and diabetes, but the role of other common co-morbidities and treatments should be considered. In our study population, half of the tuberculosis patients and one tenth of the controls were HIV co-infected. Most of the HIV co-infected participants were treatment naïve, since the diagnosis was obtained solely due to the participation in the study. While it is a fact that altered glucose metabolism is an adverse effect from older generations of anti-retroviral treatment (e.g. protease inhibitors) [55,56], little is known about the new generations of anti-retroviral treatment. We are currently exploring this in a study among 400 Ethiopian HIV patients, where we look into the changes in body composition and glucose metabolism within the first year of treatment, and this study will hopefully cast some light on the current role of antiretroviral treatment on the risk of diabetes.

Health services research

In addition to the epidemiological, clinical and mechanistic studies suggested, there is a need for integrated health services research. The double burden is mainly a problem in high burdened tuberculosis countries, but the countries are spread across the globe with different genetic background, stages of nutrition transition, and HIV burden. This diversity is also reflected in the availability of health care, which vary between countries, but also between urban and rural areas. Future research on tuberculosis

and diabetes interactions will benefit from additional local health services research to ease the flow of patients between health facilities, and to identify and improve current guidelines and protocols. Such research will also help to identify existing gaps in health systems to allow appropriate treatment of tuberculosis and diabetes co-morbidity.

SUMMARY

One third of the world's population is latently infected with Mycobacterium tuberculosis, and with the lifestyle changes succeeding the on-going urbanization, populations already burdened by tuberculosis are experiencing a dramatic increase in chronic diseases, with diabetes being a serious challenge.

Tuberculosis and diabetes are not only becoming co-existing diseases. In fact, the diseases interact, and there is evidence to suggest that especially diabetes disease increases the susceptibility for developing active tuberculosis disease. Furthermore, it is plausible that tuberculosis leads to, either transient or permanent, impairment of the glucose metabolism, which ultimately will turn into diabetes. A number of studies from the Americas, Europe, Asia, and, most lately, from sub-Saharan Africa have reported strong association between tuberculosis and diabetes; on average, the estimated risk of active tuberculosis is thrice as high among people with diabetes. The study from sub-Saharan Africa was conducted in Tanzania and is the basis of this thesis.

Based on available evidence on the association between tuberculosis and diabetes, the primary aim of the study was to assess the role of diabetes for tuberculosis risk, manifestations, treatment outcomes and survival in a Tanzanian population of tuberculosis patients and non-tuberculosis neighbourhood controls. The study was conducted in Mwanza City in northern Tanzania, with a population exceeding half a million inhabitants, with tuberculosis and HIV being common infections in the region, but with little knowledge about the prevalence of diabetes. We recruited newly diagnosed pulmonary tuberculosis patients from spring 2006 and continuously till the fall 2009, with all participating in a nutritional intervention running in parallel with the medical tuberculosis treatment.

All participants underwent diabetes and HIV testing as well as a series of measurements such as anthropometric, clinical and paraclinical parameters. The population was followed up during treatment (2 and 5 months) to assess treatment outcome as well as after one year to assess their survival status.

Based on data from 1250 tuberculosis patients and 350 neighbourhood controls, we found that 38 and 21%, respectively, had impaired glycaemia, and that the prevalence of diabetes was 17 and 9% among tuberculosis patients and controls, respectively. This difference in prevalence between patients and controls was equivalent to an adjusted odds ratio of more than four, indicating a strong association between tuberculosis and diabetes. Furthermore, we found that diabetes was associated with tuberculosis among both participants with or without HIV co-infection.

Despite the strong association, diabetes had only moderate clinical implications when the tuberculosis patients initiated the tuberculosis treatment; the patients with diabetes co-morbidity had a minor elevation in the immune response and more frequently reported to have fever. Furthermore, diabetes did not seem to delay time to sputum conversion during treatment. Nevertheless, diabetes co-morbidity led to impaired treatment outcome with slower recovery of weight and haemoglobin and a more than four times higher mortality rate within the initial phase of tuberculosis treatment.

In conclusion, in the African region, the double burden of tuberculosis and diabetes is becoming a major health problem. Although the tuberculosis incidence has stabilized during the last decade, the increasing incidence of diabetes will possibly interfere with tuberculosis control and may, consequently, make the tuberculosis incidence increase again. Future research strategies should focus on enhanced diagnostic tools to identify tuberculosis patients with diabetes co-morbidity, and on the role of disease-disease, drug-disease and drug-drug interactions between tuberculosis and diabetes diseases and treatments.

LIST OF ABBREVIATIONS

AFA	Arm fat area
AFB	Acid fast bacilli
AGP	Alpha-1-acid glycoprotein
AIDS	Acquired immune deficiency syndrome
AMA	Arm muscle area
ART	Antiretroviral therapy
BMI	Body mass index
CD4	Cluster of differentiation 4
CI	Confidence interval
CRP	C-reactive protein
DOT	Daily observed therapy
EDTA	Ethylenediamminetetraacetate
ELISA	Enzyme-linked immunosorbent assay
FBG	Fasting blood glucose
HbA1c	Glycosylated haemoglobin A1c
HIV	Human immunodeficiency virus
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
INF-γ	Interferon gamma
MUAC	Mid upper arm circumference
NIMR	National institute for medical research
NTLP	National Tuberculosis and Leprosy Programme
OGTT	Oral glucose tolerance test
OR	Odds ratio
PBMC	Peripheral blood mononuclear cell
PTB+	Smear-positive pulmonary tuberculosis
PTB-	Smear-negative pulmonary tuberculosis
RR	Relative risk
SD	Standard deviation
TB	Tuberculosis
TSF	Triceps skinfold thickness
WHO	World Health Organization

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