Impact of Latent Tuberculosis Infection on the Incidence of Type 2 Diabetes Mellitus in HIV-Infected Participants in the Swiss HIV Cohort Study

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Summary: We investigated the link between latent tuberculosis infection (LTBI) and type-2 diabetes (DM) in people living with HIV in the Swiss HIV Cohort Study, and found that LTBI may be associated with a significantly increased risk of developing DM.

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Abstract

While an increased risk of active and latent tuberculosis infection (LTBI) in people with type-2 diabetes (DM) has been demonstrated, it is less well characterized whether LTBI is associated with an increased risk of developing DM. We investigated the link between LTBI and DM in people living with HIV (PHIV) in the Swiss HIV Cohort Study via time-dependent cox proportional hazards models. We found that LTBI significantly increased the risk of developing DM (HR=1.47), which was robust across different adjustment and censoring techniques. Our results thus suggest that LTBI may be associated with an increased risk of developing DM.

Keywords. Latent Tuberculosis, Type-2 Diabetes Mellitus, Heterologous immunity, Inflammatory diseases, Innate immunity, Low-grade inflammation.

Background

The increased risk of acquiring latent tuberculosis infection (LTBI) and developing active tuberculosis (TB) in patients with type-2 diabetes (DM) has been demonstrated by multiple studies [1-3]. However, the effect of LTBI on DM progression is underexplored.

Despite the regular use in clinical practice, it is generally accepted that the binary distinction between LTBI and active TB is an oversimplification: instead, *Mycobacterium tuberculosis* (MTB) infection and disease develop across a spectrum of potential different outcomes [4]. Recently, we have shown in a small animal model that an asymptomatic MTB infection affects the reactivity of the innate immune system by lowering its activation threshold [5]. In humans, we found highly significant differences in matched LTBI(+) and LTBI(-) patients with regard to their ability to control HIV viremia and the frequency of certain opportunistic infections (OIs). In line with an activated innate immune system, evidence on the expansion of NK cell populations in patients with an asymptomatic MTB infection [6] indicates an association between LTBI and changes in the composition of circulating innate immune cells. In sum, we have indirect evidence for changes in the functionality of the innate immune system on a phenotypic level with reduced OIs and decreased HIV viral load associated with LTBI [7], suggesting that a state of equilibrium exists within the spectrum of MTB infection which affects host responses to unrelated infections [8].

However, maintaining an elevated activation state of the innate immune system might come at a cost. It is now widely accepted that chronic inflammation represents a triggering factor in the origin of the metabolic syndrome and plays an important role both in the development of insulin resistance and insulin secretion deficiency [9, 10], eventually leading to diabetes in genetically or metabolically predisposed individuals [10].

Consequently, from an evolutionary perspective, it is plausible to hypothesize that the protective effects of chronic low-grade inflammation against certain infections induced by LTBI come at the cost of increased host vulnerability to metabolic diseases such as DM. In this study, we test this hypothesis by investigating a possible causal link between LTBI and DM in HIV-infected individuals by using the data from the Swiss HIV Cohort Study (SHCS).

Methods

Statistical analysis

Due to the dynamic nature of the MTB infection and other time-varying characteristics of a patient, we used Kaplan-Meier curves and time-dependent cox proportional hazards regression model to compare the risk of developing DM in LTBI and MTB-uninfected patients. In our model, calendar year, TB infection status (LTBI or uninfected), age, body mass index (BMI), antiretroviral treatment (ART) status (on or off treatment), CD4 cell counts, and smoking status (yes or no) are included as time-varying, whereas sex and ethnicity are included as static factors. These factors are included in the analysis to adjust for potential confounding while investigating the effect of the TB infection status on the risk of developing DM. Univariate and multivariate Cox regression analyses were used to model the effect of the TB infection status on the development of DM and to test its robustness given multiple confounders. For the adjusted analysis, we used either multivariable regression or inverse probability weighting (IPW). IPW is a technique for calculating statistics standardized to a pseudo-population different from that in which the data was collected to improve the covariate balance [11].

Although SHCS provides data in high temporal resolution, it is not possible to have information on each time-dependent factor with the same frequency, which is a requirement for a time-dependent cox proportional hazards regression model. To account for the missing data regarding BMI and CD4 cell counts, we applied interpolation, smoothing, and last observation carried forward (LOCF) techniques (Example provided in Figure S1). TB infection status, ART status, and smoking status are binary and change depending on the information provided between consecutive tests or follow-up visits. CD4 cell counts are used as a continuous variable (divided by 200). Age and BMI are included as time-updated categorical variables.

Observation time started with the first available MTB test (either tuberculin skin test (TST) or Interferon-gamma Release Assay (IGRA)) and ended at the time of first clinical diagnosis of either active TB (extrapulmonary or pulmonary TB) or DM, or at the time of the last follow-up visit. Any negative MTB test result following a positive one is omitted, and the patient is assumed to have LTBI until the time of active TB diagnosis, DM diagnosis or end of observation time. Patients with an initial negative MTB test result are assumed to be MTB-uninfected, and their infection status changes at the time of their first positive test result if there is any. By construct, this design defines *i)* LTBI period as having a positive MTB test and no diagnosis of active TB, *ii)* no infection period as having a negative MTB test until the first positive test or active TB diagnosis during follow-up.

Regular screening for DM was introduced to SHCS in April 2000, therefore patients are assumed to be at risk of DM from April 2000 onwards. Observation time of a patient with an MTB test pre-dating April 2000 is assumed to start at April 2000 with their infection status at that time. Definitions used in the SHCS for diabetes mellitus, pulmonary tuberculosis, and extrapulmonary tuberculosis diagnoses are provided in Table S4.

Selection of the study population

Using a time-dependent model restricted our study population in various ways. To interpolate and smooth the CD4 cell count and BMI measurements, we only included patients with at least two CD4 and BMI measurements separated at least 20 days apart over the course of the observation time, which was a technical requirement for the smoothing algorithm. Patients without an MTB test and patients with MTB tests only after a clinical diagnosis (either active TB or DM) are excluded from the analysis. Baseline characteristics of the patients included in our analysis are provided in Table S1.

Sensitivity analysis

We performed three different sensitivity analyses in total, regarding *i)* adjustment for different combinations of confounders, *ii)* different censoring techniques, *iii)* considering Hepatitis C infection (HCV) and MTB infection treatment with Isoniazid (INH).

Although receiving ART, smoking, and low CD4 cell counts during HIV infection are independently associated with a higher risk of developing DM, causal mechanisms of these associations and how they modify the effect of LTBI are not well characterized. To test the robustness of our results, we repeated our analysis by adjusting for these three factors in combinations of two and in isolation in addition to the rest of the risk factors.

Censoring at the time of active TB diagnosis allows us to quantify the causal effect of LTBI in isolation. One may argue that this approach violates the consistency requirement for causal inference [11] since progression to active TB is not a process to be manipulated in a target trial where the treatment group is hypothetically exposed to *Mycobacterium tuberculosis.* Therefore, we repeated our analysis where observation time ended at the time of the first clinical diagnosis of DM or at the time of the last follow-up visit, ignoring the time and the existence of any active TB diagnosis.

Since HCV and alcohol consumption can be considered as potential confounders when investigating the effects of LTBI, and INH treatment can alter the definition of our exposure variable of interest (LTBI), we repeated our analysis including the average daily alcohol consumption, HCV serology where patients were segregated into two as never being infected with HCV or not, and by excluding all patients who ever received INH treatment. Contrary to our main and the other two sensitivity analyses mentioned above, this analysis was a point analysis, i.e., static (time-independent) versions of the variables were used.

Results

We observed 60 DM events for ~9844 person-years (974 patients) of follow-up in patients with LTBI, and 433 DM events for ~105426 person-years (9867 patients) in patients without an MTB infection. Kaplan-Meier curves representing the two groups (Figure 1) show an increased risk of DM in patients with LTBI ($p_{log\text{-}rank}$=0.006). They show that after 3600 days (~10 years), ~5.5% of the patients with LTBI, and ~3.8% of the patients without an MTB infection (NTBI) developed DM.

In the Cox-Proportional Hazard model, LTBI was associated with a higher hazard of DM as compared to MTB-uninfected (NTBI) patients (Figure 2). The hazard ratio was 1.49 (1.14, 1.95) without adjustment, 1.44 (1.09, 1.90) in the multivariable model, and 1.47 (1.06, 2.03) after adjustment by IPW, with at least a 5% level of significance for all results (Table S2). Note that although numerically the hazard ratios are close to each other, the most reliable hazard ratio corresponding to the average *causal* effect of LTBI on the development of DM is given by the results where IPW adjustment is used [11].

Independently of TB infection status, older age, higher BMI, being male, belonging to an ethnic group other than white, and being on ART were associated with higher hazard ratios, which are accepted risk factors for DM. Our sensitivity analysis showed that the higher hazard of LTBI relative to NTBI patients was robust across different approaches for censoring and for different combinations of confounders (Table S3), where its mean values varied between 1.28 to 1.46. Similarly, we did not observe a significant change in the hazard of LTBI relative to NTBI when HCV and alcohol consumption was included as confounders and patients receiving INH treatment were excluded from the analysis (Figure S3).

Discussion

In this study, we assessed the association of LTBI with the development of DM at the population level in a large prospective, nationwide clinical cohort. Compared to MTB-uninfected patients, LTBI was associated with a significant increase in the risk of developing DM, and its effect was robust across different censoring techniques and different combinations of confounders. While the increased risk of active TB and LTBI for DM patients has been well established, the effect of LTBI on progression to DM is less well characterized [12, 13]. The main finding of our study supports the hypothesis that the heterologous immunity promoted by the continuous interaction of *Mycobacterium tuberculosis* with the immune system [5, 7] comes at the cost of making the host more vulnerable to inflammatory diseases, providing an evolutionary perspective on the trade-off of maintaining an elevated immune response. Chronic inflammation being a triggering factor in the origin of the metabolic syndrome and the development of insulin resistance speaks for a causal association and permits us to build our hypothesis in the context of LTBI-DM relationship, but in the opposite direction compared to the conventional analyses in the literature.

The lack of a gold standard in tests for defining LTBI is a potential limitation to all studies in the field [7]. Especially for HIV-infected individuals, LTBI tests - which rely on the T cell memory response - tend to have a high false negative rate due to immunosuppression [7]. Therefore, we adjusted for the CD4 T cell counts as a time-dependent confounder in the multivariate analysis. LTBI positivity itself might indicate different stages within the spectrum of Mycobacterium tuberculosis infection [4], which is a possibility we do not consider in this study and assume it is a binary state.

Besides established risk factors such as sex, ethnicity, and age; two other prominent risk factors potentially associated with DM are antiretroviral therapy (ART) and smoking [14, 15]. Therefore, considering ART as a potential confounder is crucial for our analysis. Similarly, epidemiological studies demonstrate a clear association between cigarette smoking and a decreased risk of DM [16].

Another difficulty in defining LTBI is the dynamic nature of the course of MTB infection over a lifetime [7]. Therefore, most of the studies chose to define LTBI as a static condition. One of the novel aspects of our study is to include all time-dependent factors in a longitudinal fashion, which permits us to select our study population in a way that the events of interest (such as testing positive for MTB infection, being diagnosed with DM, or being diagnosed with active TB) are properly aligned in time to assess the causal effect of LTBI on DM progression. By including IPW as an adjustment technique, we were able to assess the hazard ratio corresponding to the average *causal* effect of LTBI on the development of DM**,** whereas conditional adjustment might provide less reliable results due to the unknown mediating effects of each confounder in LTBI patients. As in any observational study, it is impossible to exclude residual confounders. However, our results were robust to adjustments for a broad range of measured confounders in a large number of sensitivity analyses. Our study population is limited to HIV-positive people only, thus we cannot verify whether the effect of LTBI on developing DM holds for HIV-negative people. Therefore, more studies investigating the effects of LTBI on progression to different inflammatory diseases are necessary to better support the hypothesis proposed in this work.

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Figure 1: Survival curves of the two groups estimated using a Cox proportional hazards model. Survival curves of NTBI (no tuberculosis infection) and LTBI (latent tuberculosis infection) estimated using a Cox proportional hazards model and adjusted using inverse probability weighting (IPW). Censoring is applied at the time of first clinical diagnosis either for DM or active tuberculosis (TB) if there is any, otherwise at the time of last visit.

Figure 2: Time-to-event analysis of the incidence of Type-2 Diabetes in patients with LTBI compared to MTB-uninfected patients. Hazard ratios of various factors obtained from a Cox proportional hazards model for the incidence of Type 2 Diabetes (DM); the lines indicate the 95% CIs obtained in the regression model; the squares indicate the regression coefficients (see Table S1 for the underlying numerical values). Censoring is applied at the time of first clinical diagnosis either for DM or active tuberculosis (TB) if there is any, otherwise at the time of last visit. CI, confidence interval; IPW, inverse probability weighting; HR, hazard ratio; NTBI, no tuberculosis infection; LTBI, latent tuberculosis infection; BMI, body mass index; CD4, CD4 cell count per µl multiplied by (1/200); ART, antiretroviral therapy.

Figure 1

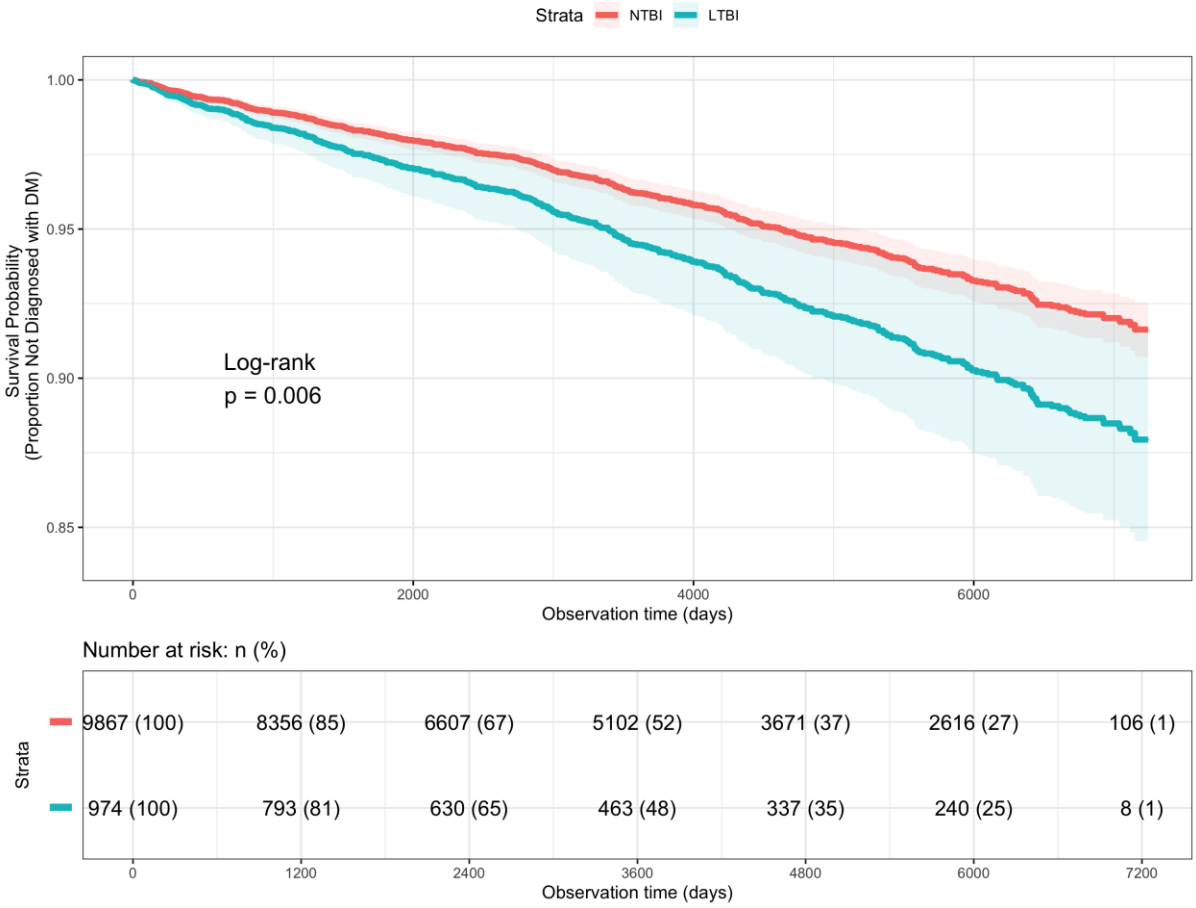

Figure 2