REVIEW ARTICLE

The Pathogenesis of Tuberculosis-Diabetes Comorbidity

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Abstract:

Tuberculosis-diabetes co-morbidity (TB-DM) has been a hurdle in the elimination of tuberculosis worldwide. Individuals with Type 2 diabetes mellitus (T2DM) can become vulnerable to bacterial infections, due to compromised cell mediated immunity [1, 2]. Therefore, individuals with diabetes are at an increased risk for developing an active tuberculosis (TB) disease when infected with Mycobacterium tuberculosis (Mtb). It is estimated that these individuals with T2DM are about three times more likely to develop the active TB compared to individuals without diabetes. Approximately 10% of all TB cases are linked to diabetes and the risk of death from TB is roughly double for those with diabetes, especially in middle to low-income areas [3, 4]. Patients with both T2DM and TB have worse outcomes, including slower bacteria conversion, lower rate of cure, higher chances to relapse, increased risk of mortality, and even escalated drug resistance. While it is known that diabetes causes immune dysfunction, there are still many questions as to how diabetes worsens TB outcomes. It is reported that diabetes might change the appropriate mechanisms of immunological factors that maintain host immune defenses towards infectious agents, the production of specific cytokines, increased formation of reactive oxygen species, as well as reduced levels of the antioxidant glutathione (GSH). In this review, we explore recent research that supports possible explanations for tuberculosis-diabetes (TB-DM) comorbidity as well as the potential reasons for the increased risk of mortality and finally promising prophylactic treatments.

1. Introduction

Tuberculosis is the leading cause of infectious death worldwide which is caused by the etiological agent *Mycobacterium tuberculosis (Mtb). Mtb* is transmitted from person to person via aerosol droplets typically by means of coughing. In 2015, there were 10.4 million new cases of TB reported, and 1.8 million deaths worldwide [5]. Furthermore, it is estimated that one third of the world's population is latently infected with *Mtb* [5]. Typical treatment of an active TB disease includes the antibiotics Isoniazid, Rifampin, Ethambutol, and Pyrazinamide which have specific mechanisms of action in combating a *Mtb* infection. However, the treatment of TB disease is becoming more problematic due to the emergence of antibiotic-resistant strains of *Mtb* [5]. Therefore, many new treatment options are being explored as possible alternatives or adjunctive therapies to combat this devastating disease. Despite substantial advancements attended in this field, diabetic patients are still considerably more likely to contract TB than non-diabetic individuals [6-10].

In 2014, Long considered a disease of the affluent "Western" countries of Europe and North America, T2D has now spread to every corner of the world. Indeed, there are now more people with diabetes residing in the "emerging" economies (Asia, Latin America and Africa) than in the industrialized nations (93). which is caused by insulin resistance, and accounts for roughly 90–95% of the total prevalence of diabetes [12]. Glycated hemoglobin (HbA1c) is indicative of the levels of plasma glucose over a period of weeks and is subsequently used as Even when it could be a diagnostic marker, it's more reliable as a follow up test, instead the glucose overload test is used as a more reliable parameter than fasting glycaemia to diagnose type 2 Diabetes Mellitus. While more research is required on the etiology of T2DM (13), current research tenets indicate that genetic factors, obesity, and feasibly a high fat or high sugar diet can be linked to the development of T2DM (13). Additionally, T2DM Additionally, complications arising due to T2DM include health problems such as retinopathy or cardiovascular diseases.Though T2DM affects millions worldwide, the epidemiology and comorbidity studies are largely limited by poor models of the disease that fail to incorporate both the nutritional and polygenic factors involved in the onset of T2DM in humans.

It is commonly accepted that diabetes

decreases the effectiveness of the cell mediated immunity thus making individuals more vulnerable to *Mtb* infection, however, the exact mechanism for *Mtb* susceptibility in individuals with T2DM is not entirely well understood. Some animal studies have suggested that there may be a delay in immune recognition, however, a more recent study on humans advocates that epigenetic reprogramming may be responsible for increasing inflammation thus causing individuals to become more susceptible to the infection [13]. None the less, research has shown that *Mtb* infection can present itself differently amid diabetes co-morbidity.

Differences in Tuberculosis-Diabetes Comorbidity Presentation and the Subsequent Complications:

TB patients with diabetes are more likely to present positive sputum smear when compared to non-diabetic patients [7-9]. Patients with TB-DM are also more likely to present greater fatigability as well as non-pleuritic chest pain in addition to the typical fever and cough presented in a standard active Mtb infection (14, 15). Chest radiographs in TB-DM patients often display a higher prevalence of abnormalities in those with poor glycemic control [16-18]. In particular, TB-DM patients are more likely to have atypical findings such as increased lower lung field cavities, lymphadenopathy, pleural effusion, segmental and lobar consolidation, and the presence of multiple cavities [17-19]. TB-DM patients are also more likely to have lower lobe lesions with greater opacity and parenchymal lesions over the lower fields [18, 21]. Additionally, cavitary lung disease is more than three times more predominant in patients with TB-DM [22]. Likewise, extra pulmonary involvement is also found in patients with TB-DM [23]. Therefore, routine *Mtb* screenings for diabetic patients is usually encouraged so that methods of prevention can be applied in the earliest stages of the disease. Guinea pigs with TB-DM suffered more severe *Mtb* infections than non-diabetic guinea pigs infected with *Mtb* [10]. Furthermore, diabetes has been determined to increase the risk of death among patients with TB co-morbidity [7, 8]. This is because treating Mtb in diabetic patients is profoundly more difficult than in non-diabetic patients due to various types immunosuppression observed as well as other possible ailments that ensue from the comorbidity [19, 24, 25].

Specifically, diabetic patients may present additional complications such as kidney failure, and hypertension which can influence the effectiveness of treatment [7].

These factors can also cause TB patients with diabetes to have and a higher risk of drug toxicity at lower concentrations of anti-tuberculosis drugs [7]. For example, diabetes has also shown to reduce the effectiveness of rifampicin in TB treatment [7-9, 23, 26].

Diminished Tuberculosis Cure Rate Due to the Comorbidity and Variations in Diabetes Treatment in Response to *Mtb*

Patients with T2DM show a diminished TB cure rate due weakened cell mediated immunity which often results in more drug-resistant *Mtb* and higher patient mortality rates [21, 27-30]. Additionally, post successful antimicrobial treatment, TB-DM patients are at a higher risk of redeveloping an active *Mtb* infection [18]. These patients are also more susceptible to adverse effects after beginning the antimicrobial therapy, resulting in a greater need to transfer into critical care centers during treatment [31, 32]. Strikingly, after two to three months of anti-tuberculosis therapy, TB-DM patients will still have a significantly higher risk of producing a positive sputum culture [27, 31, 32, 33]. The impaired cell mediated immunity and hyperglycemia associated with T2DM can also increase susceptibility of infection to a multidrug resistant *Mtb* strain, thereby further complicating the treatment regimen and success rates [28, 29].

Pre-treatment HBA1C levels and glycemic control are the greatest predictors of treatment outcomes for the TB-DM comorbidity [34, 35]. Poor glycemic control in T2DM patients increases the risk of lung cavitation, positive sputum cultures after 2 months of therapy, and more severe lung disease [18, 36]. Consequently, higher HbA1C levels are correlated with poorer expectations to TB treatment [18]. Moreover, adequate control of blood glucose levels will usually decrease recovery time and improve prognosis. Malnutrition of T2DM patients during *Mtb* infection, however, can lead to increased cortisol levels and inadvertently exacerbate their hyperglycemic state. Therefore, proper nutrition is critical during TB treatment regimens for TB-DM patients [28].

Heightened TB Susceptibility Among Diabetic Patients with Poor Glycemic Control

Diabetic patients with poor glycemic control (HbA_{1C} >8%) exhibit lower interactions between *Mtb* and monocytes resulting in a heightened susceptibility to *Mtb* infection [18, 37-39]. Poor glycemic control and

hyperglycemia correlates to longer hospitalizations, more intense treatment regimens, and an increased risk of mortality, due to weakened immune responses and lower levels of circulating phagocytic immune cells [40, 41]. Furthermore, the prevalence of *Mtb* in adipose tissue found in obese individuals is thought to aid in systemic insulin resistance and ultimately poor glycemic control [42]. It is generally believed that low density lipoprotein cholesterol (LDL-C) levels often decrease acutely during infection and inflammation [43]. However, there is contradictory evidence on lipid levels among TB-DM infected patients. Studies have shown that *Mtb* can be associated with lower levels of cholesterol or LDL-C, and high carbohydrate diets may even help to diminish bacterial burden levels [28-31]. In contrast, other studies have observed TB-DM hosts with hypercholesterolemia, which suggest greater a susceptibility to *Mtb* within this population [44-47]. Therefore, further research is needed to investigate if this incidence is due to TB-DM comorbidity or to hypercholesterolemia alone.

In the study by Alim et al., a diet-induced murine model of T2DM was used to investigate the relationship between mycobacterial infections and T2DM [48]. Alim et al., evaluated immune responses in mice following 30 weeks of an energy dense diet intervention [48]. Compared to the wild-type, the diet-induced T2DM model demonstrated typical clinical symptoms of T2DM including increased body mass, increased HbA_{1c} levels, adipocyte hypertrophy, and mesangial thickening of the glomeruli and basement membrane of the Bowman's capsule. Macrophage functionality was evaluated after both control and diet-induced T2DM mice were challenged with *Mycobacterium fortuitum*, a less virulent alternative of *Mtb.* Their results corroborate clinical findings of increased *Mtb* susceptibility in humans and worse outcomes for T2DM patients with poor glycemic control [18, 34, 38, 49-51]. The diet-induced T2DM mice were unable to mount a significant host immune response against the *M. foruitum* bacterial challenge, exhibiting reduced cytokine secretion, most notably IFN-γ and TNF-α, as well as impaired phagocytic abilities of alveolar and resident peritoneal macrophages [48]. Despite the opposing evidence on the association between cholesterol and *Mtb*, which prompts further investigation, there is a clear link between poor glycemic control and TB-DM disease progression.

Increased Systemic Levels of Circulating Angiogenic Factors in TB-DM

Studies have shown that elevated levels of vascular endothelial growth family members such as VEGF-A, C, D, R1, R2, and R3 regulate angiogenesis and stimulate angiogenic processes [52, 53]. Additional studies have also shown that *Mtb* infection is associated with elevated levels of VEGF-A [54-59] and VEGF-A, C, and R2 can be used as accurate biomarkers of disease severity, bacterial burden, and treatment response in pulmonary TB [60]. This lead Kumar et al. to hypothesize that TB-DM would be coupled with heightened levels of systemic angiogenic factors [4]. The TB-DM group subjects had significantly higher levels of blood glucose, glycated hemoglobin, serum cholesterol, VLDL, LDL, triglycerides, total bilirubin and alkaline phosphatase. It was also observed that the TB-DM group did in fact have significantly higher systemic levels of circulating angiogenic factors VEGF-A, C, D, R1, R2, and R3 than the TB only control group [4]. Kumar et al. then compared the aforementioned circulating angiogenic factors in the TB-DM subjects with having cavitary or non-cavitary disease, unilateral or bilateral disease, and those with or without hemoptysis in order to determine the association of these angiogenic factor levels with disease severity [4]. They found that VEGF-A, C, R2, and R3 are significantly higher in TB-DM subjects with cavitary disease compared to those without it; VEGF-A, C, D, R2, and R3 are higher in those with bilateral disease compared to those with unilateral; and VEGF-A, C, and R2 are higher in those with hemoptysis compared to those without it [4]. This suggests that dysregulated angiogenesis may be associated with an increased risk of hemorrhage from pulmonary TB lesions, and elevated systemic levels of these circulating angiogenic factors are related to disease severity as well as adverse clinical presentation in TB-DM. Kumar et al. then correlated the circulating levels of VEGF-A, C, D, R1, R2, and R3 in TB-DM individuals with smear grades (1+, 2+, and 3+) to determine associations between the systemic levels of circulating angiogenic factors and quantity of bacterial burden in TB-DM [4]. They found that VEGF-A, C, and R2 have a significant positive correlation with smear grades in TB-DM individuals, which suggests a positive association between these factors and total bacterial burden. Additionally, the researchers also found that the systemic levels of VEGF-A, C, D, R1, R2, and R3 displayed a positive relationship with the levels of HbA1c among TB-DM

individuals; further suggesting another connection between TB-DM and poor glycemic control [4]. Therefore, TB-DM comorbidity is associated with elevated levels of circulating angiogenic factors which may reflect dysregulated angiogenesis as well as hyperbolic inflammation.

TB-DM Impaired Immunity and Increased Oxidative Stress

There is long-standing evidence that T2DM can impede the host immune system, and encumber a number of immune responses among T2DM individuals [21]. Interestingly, research has revealed that some diabetics cannot even mount a significant host immune response against *M foruitum*, a less virulent relative of *Mtb*, exhibiting reduced pro-inflammatory cytokine secretion, and impaired phagocytic abilities of alveolar and resident peritoneal macrophages [49, 63-66]. Likewise, the pro-inflammatory cytokines interleukin (IL)-6 and IL- 17 have been reported to be significantly elevated in plasma samples isolated from T2DM patients, which correlates with the enhanced oxidative stress also observed among T2DM individuals [67]. It is postulated that T2DM reduces the production of Th1 producing cytokines IL-12 and interferon (IFN)-γ as well as hinder expression of inducible nitric oxide synthase [68]. This reduction can have drastic downstream effects because, IL-12 normally functions in a *Mycobacterium* infection by activating Natural Killer (NK) cells, as well as differentiating and activating CD4+ T-cells to combat these intracellular microorganisms [69]. Furthermore, the malondialdehyde (MDA), the end-product of lipid peroxidation which is used to indirectly measure reactive oxygen species (ROS) production, levels have been publicized to be significantly elevated in the plasma and monocytes of T2DM patients [67]. Zykova et al. demonstrated that the release of tumor necrosis factor (TNF)-α and IL-1-β from lipopolysaccharide-stimulated macrophages is reduced among diabetic mice when compared to the control mice [70]. Similar to IL-12, IL-1-β plays a role in differentiating CD4+ T-cells but instead into Th17 cells [71]. Th17 cells are critical in clearing pathogens during the host defense mechanism [72]. The level of macrophage inflammatory protein-2, a mediator of lung neutrophil recruitment, has also been shown to be significantly decreased in diabetic mice compared to the control [73]. Additionally, it has been reported that the neutrophils from the diabetic patients also display a diminished

capacity to phagocytose mycobacteria [74]. Neutrophils, while still considered controversial in a *Mtb* infection, are an essential aspect of innate immunity because they phagocytose and eradicate incoming pathogens [75, 76]. These suppressed immune responses are crucial in limiting the spread of *Mtb*, therefore, TB-DM patients are acutely disadvantaged when mounting an effective host immune response.

Additionally, the immunological evasion processes of *Mtb* exacerbates these mechanisms. For example, mycolic acid, found in the cell wall of *Mtb*, can inhibit the expression of IL-12, monocyte chemotactic protein 1 (MCP-1), and TNF-α in a toll-like receptor 2 (TLR-2) dependent manner in macrophages [77]. *Mtb* is also known to interfere with antigen presentation by major histocompatibility complex (MHC) class II molecules, which is important for priming CD4+ T-cells [78]. While there are many mechanisms by which *Mtb* attempts to alter the host immune system, most individuals who are infected with *Mtb* never develop an active disease due to an effective immune system [79]. However, individuals who have a suppressed immune system, such as HIV and T2DM patients, as outlined here, tend to be clinically affected significantly more than the general population [80, 81]. As discussed previously, T2DM interferes with the CD4+ T-cell response; in contrary, HIV reduces the number of CD4+ T-cells [82]. Hence, T2DM affects the quality of CD4+ T-cell response, while HIV affects the quantity of CD4+ T-cells able to help fight off infections. Nonetheless, due to diminished CD4+ T-cell response both HIV and T2DM induce a susceptibility to *Mtb*.

Metformin's Prophylactic Capabilities

Given that the *Mycobacterial* NDH-I complex is intrinsically comparable to the mitochondrial complex-I (MCI) of human cells and that MCI can be inhibited by metformin, it is thought that metformin also inhibits the NDH-I complex of *Mtb* in a similar manner [83-85]. It has also been presented that metformin treatment may promote the expansion of specific IFN-γ secreting CD8+ T cells in the lungs of mice [86]. Since this insight, studies have been implemented to explore metformin as a potential candidate for combination drug therapy among patients with active TB. One such trial by Marupuru et al. investigated the effects of metformin in TB-DM patients and diabetes patients [87]. The outcome of this study suggests that the patients taking metformin had an enhanced protective effect against *Mtb* [87]. A similar study using *Mtb* infected mice presented that the use of

metformin not only improved TB lung pathology, but reduced chronic inflammation and enhanced specific immune responses as well as improved the efficacy of conventional TB drugs [88]. Singhal et al. revealed that metformin treated T2DM patients with latent TB also expressed increased numbers of *Mtb* specific T cells [88]. They postulated this data suggests that metformin enhances *Mtb*-specific host immunity among TB-DM patients because metformin allows for both protective immunity and pathological immunity to be independently modulated with beneficial effects during *Mtb* infection [88]. Furthermore, their results revealed that metformin not only reduces inflammation, but promotes disease resolution, and generally improves TB treatment outcome [88]. Collectively, this data indicates that metformin is a promising candidate for improving the effectiveness of TB-DM treatment.

Glutathione's Prophylactic Capabilities

It has reported that glutathione (GSH) levels are compromised among individuals with T2DM due to a diminished quantity of GSH synthesis as well as the reduced metabolism of specific enzymes [67, 89]. This is thought to be a leading cause for impairment of macrophages which control *Mtb* infection. The polyol pathway, where glucose is converted to fructose, is also implicated as one of the probable causes for decreased levels of GSH in individuals with T2DM. This is because the enzyme aldose reductase in the polyol pathway competes with glutathione reductase (GSR) for NADPH, it's cofactor. GSH is composed of three amino acids, glutamine, cysteine, and glycine; and prevents cellular damage by detoxifying reactive oxygen species (ROS) [90]. GSH is present in two forms, the reduced form of GSH (rGSH) and oxidized form GSH (GSSG). rGSH possess the antioxidant functionality while GSSG is a byproduct of the oxidation of GSH. In the presence of ROS, two molecules of rGSH are converted to the oxidized form GSSG, and water [90]. GSH can also be synthesized by recycling GSSG back to GSH via GSR.

Lagman et. al investigated not only these underlying mechanisms behind the macrophage impairment in patients with T2DM, but whether their overall diminished immunological ability to control *Mtb* infection is due to reduced levels of GSH by conducting a T2DM clinical trial. The team reported that the levels of GSH in the RBC, plasma, and monocyte samples exhibited significantly lower total GSH as well as

rGSH in individuals with T2DM compared to the healthy individuals [67]. They also observed significantly lower levels of GCLC (catalytic unit of the rate limiting step-enzyme involved in the synthesis of GSH) in the RBCs isolated from individuals with T2DM as well [67]. Additionally, the levels of TGF-β in the plasma samples from individuals with T2DM showed a substantial elevation, which correlates to the decreased expression of GCLC. This is significant because previous studies have revealed that TGF-β can down-regulate the expression of GCLC, ultimately leading to decreased levels of GSH [91, 92]. The group also observed a 50% reduction in the expressions of both GSS and GGT (a notable enzyme that plays an important role in the GSH metabolism) levels in the RBCs isolated from individuals with T2DM, as well as significantly higher levels of GSR in the RBCs isolated from individuals with T2DM [67]. Importantly, treatment with GSH resulted in a significant decrease in the intracellular *in vitro* viability of *Mtb* from both the healthy and T2DM individuals. However, while GSH had a positive impact on the healthy individuals, it was shown to be far more efficacious in controlling *Mtb* infection in individuals with T2DM due to its restorative effects [67]. In conclusion, these findings suggest that the increased susceptibility to *Mtb* infection among individuals with T2DM is at least in part due to having inferior levels of GSH. Therefore, if an individual with TB-DM were able to increase their levels GSH this should improve their prognosis.

Conclusion

It is well documented that patients with diabetes are at a higher risk for developing an active TB infection than non-diabetic patients. Recently, the incidence of diabetes is rising among underdeveloped countries where *Mtb* is also in high occurrence. Analysis of the presented data suggests that while we are closer to understanding the reasons behind the increased TB-DM comorbidity there is still much to be done in the way of preventing and eradicating these diseases. The relationship between TB and diabetes, and the increasing incidence of TB-DM around the globe thus requires further research and proactive detection methods to optimize the treatment of the two diseases and the subsequent comorbidity that ensues.

References

1 Gan YH (2013) Host susceptibility factors to bacterial infections in type 2 diabetes. PLoS Pathog 9: e1003794.

2 Tan KS, Lee KO, Low KC, Gamage AM, Liu Y, et al. (2012) Glutathione deficiency in type 2 diabetes im- pairs cytokine responses and control of intracellular bacteria. J Clin Invest 122: 2289–2300.

3 Narasimhan P, Wood J, MacIntyre C.R, Mathai D, Risk factors for tuberculosis, Pulm. Med. 2013 (2013)

4 Benoit, S.R., et al., *Association of Diabetes and Tuberculosis Disease among US-Bound Adult Refugees, 2009-2014*. Emerg Infect Dis, 2017. 23(3): p. 543-545.

5 Tuberculosis (TB). *World Health Organization*. World Health Organization, n.d. Web. 4 November 2016.

6 Lienhardt C, Fielding K, Sillah JS, Bah B, Gustafson P, et al. Investigation of the risk factors for tuberculosis: A case-control study in three countries in West Africa. Int J Epidemiol. 2005;34:914–923.

7 Ponce-De-Leon A, Garcia-Garcia Md Mde L, Garcia-Sancho MC, Gomez-Perez FJ, Valdespino-Gomez JL, et al. Tuberculosis and diabetes in southern Mexico. Diabetes Care. 2004;27:1584–1590.

8 Pablos-Mendez A, Blustein J, Knirsch CA. The role of diabetes mellitus in the higher prevalence of tuberculosis among Hispanics. Am J Public Health. 1997;87:574–579.

9 Alisjahbana B, van Crevel R, Sahiratmadja E, den Heijer M, Maya A, et al. Diabetes mellitus is strongly associated with tuberculosis in Indonesia. Int J Tuberc Lung Dis. 2006;10:696–700.

10 Perez A, Brown HS, 3rd, Restrepo BI. Association between tuberculosis and diabetes in the Mexican border and non-border regions of Texas. Am J Trop Med Hyg. 2006;74:604–611.

11 Diabetes. *World Health Organization*. World Health Organization, n.d. Web. 7 October 2016.

12 Center for Disease Control (2014) National Diabetes Statistics Report

13 Prada-Medina, Cesar A., et al. “Systems Immunology of Diabetes-Tuberculosis Comorbidity Reveals Signatures of Disease Complications.” *Scientific Reports*, vol. 7, no. 1, 2017.

14 Abdul Hashim K., and Muhammed Anas Ayoob. “Impact of diabetes mellitus on clinical presentation and treatment

response of smear positive pulmonary tuberculosis patients." *International Journal of Research in Medical Sciences*, www.msjonline.org/index.php/ijrms/article/view/2918/2690. Accessed 26 Aug. 2017.

15 Patel, Anandk, et al. "Radiological presentation of patients of pulmonary tuberculosis with diabetes mellitus." *Lung India*, vol. 28, no. 1, 2011, p. 70.

16 Chiang CY, Lee JJ, Chien ST, Enarson DA, Chang YC, et al. Glycemic control and radiographic manifestations of tuberculosis in diabetic patients. PLoS One. 2014;9(4):e93397.

17 Huang LK, Wang HH, Lai YC, Chang SC. The impact of glycemic status on radiological manifestations of pulmonary tuberculosis in diabetic patients. PLoS One. 2017;12(6):e0179750.

18 Mahishale V, Avuthu S, Patil B, Lolly M, Eti A, et al. Effect of Poor Glycemic Control in Newly Diagnosed Patients with Smear-Positive Pulmonary Tuberculosis and Type-2 Diabetes Mellitus. Iran J Med Sci. 2017 Mar;42(2):144-151.

19 Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. Lancet Infect Dis. 2009 Dec;9(12):737-46.

20 Lee KS, Song KS, Lim TH, Kim PN, Kim IY, et al. Adult-onset pulmonary tuberculosis: findings on chest radiographs and CT scans. AJR Am J Roentgenol. 1993 Apr;160(4):753-8.

21 Saiki O, Negoro S, Tsuyuguchi I, Yamamura Y. Depressed immunological defence mechanisms in mice with experimentally induced diabetes. Infect Immun. 1980 Apr;28(1):127-31.

22 Magee MJ, Kempker RR, Kipiani M, Gandhi NR, Darchia L, et al. Diabetes mellitus is associated with cavities, smear grade, and multidrug-resistant tuberculosis in Georgia. Int J Tuberc Lung Dis. 2015 Jun;19(6):685-92.

23 Siddiqui AN, Khayyam KU, Sharma M. Effect of Diabetes Mellitus on Tuberculosis Treatment Outcome and Adverse Reactions in Patients Receiving Directly Observed Treatment Strategy in India: A Prospective Study. Biomed Res Int. 2016;2016:7273935.

24 Riza, Anca Lelia, et al. "Clinical management of concurrent diabetes and tuberculosis and the implications for patient services." *The Lancet Diabetes & Endocrinology*, vol. 2, no. 9, 2014, pp. 740–753.

25 Degner, Nicholas R, et al. *The Effect of Diabetes and Comorbidities on*

Tuberculosis Treatment Outcomes. 2017, www.atsjournals.org/doi/pdf/10.1164/ajrccm-conference.2017.195.1_MeetingAbstracts.A7587. Accessed 26 Aug. 2017.

26 Kang YA, Kim SY, Jo KW, Kim HJ, Park SK, et al. Impact of diabetes on treatment outcomes and long-term survival in multidrug-resistant tuberculosis. Respiration. 2013;86(6):472-8.

27 Baker MA, Harries AD, Jeon CY, Hart JE, Kapur A, et al. The impact of diabetes on tuberculosis treatment outcomes: a systematic review. BMC Med. 2011 Jul 1;9:81.

28 Fisher-Hoch SP, Whitney E, McCormick JB, Crespo G, Smith B, et al. Type 2 diabetes and multidrug-resistant tuberculosis. Scand J Infect Dis. 2008;40(11-12):888-93. PubMed PMID: 18728934; NIHMSID: NIHMS205077; PubMed Central PMCID

29 Gómez-Gómez A, Magaña-Aquino M, López-Meza S, Aranda-Álvarez M, Díaz-Ornelas DE, et al. Diabetes and Other Risk Factors for Multi-drug Resistant Tuberculosis in a Mexican Population with Pulmonary Tuberculosis: Case Control Study. Arch Med Res. 2015 Feb;46(2):142-8.

30 Workneh MH, Bjune GA, Yimer SA. Diabetes mellitus is associated with increased mortality during tuberculosis treatment: a prospective cohort study among tuberculosis patients in South-Eastern Amahra Region, Ethiopia. Infect Dis Poverty. 2016 Mar 21;5:22.

31 Alisjahbana B, Sahiratmadja E, Nelwan EJ, Purwa AM, Ahmad Y, et al. The effect of type 2 diabetes mellitus on the presentation and treatment response of pulmonary tuberculosis. Clin Infect Dis. 2007 Aug 15;45(4):428-35.

32 Gil-Santana L, Almeida-Junior JL, Oliveira CA, Hickson LS, Daltro C, et al. Diabetes Is Associated with Worse Clinical Presentation in Tuberculosis Patients from Brazil: A Retrospective Cohort Study. PLoS One. 2016;11(1):e0146876.

33 Restrepo BI, Fisher-Hoch SP, Crespo JG, Whitney E, Perez A, et al. Type 2 diabetes and tuberculosis in a dynamic bi-national border population. Epidemiol Infect. 2007 Apr;135(3):483-91.

34 Chiang CY, Bai KJ, Lin HH, Chien ST, Lee JJ, et al. The influence of diabetes, glycemic control, and diabetes-related comorbidities on pulmonary tuberculosis. PLoS One. 2015;10(3):e0121698.

35 Leung CC, Lam TH, Chan WM, Yew WW, Ho KS, et al. Diabetic control and

risk of tuberculosis: a cohort study. Am J Epidemiol. 2008 Jun 15;167(12):1486-94.

36 Park SW, Shin JW, Kim JY, Park IW, Choi BW, et al. The effect of diabetic control status on the clinical features of pulmonary tuberculosis. Eur J Clin Microbiol Infect Dis. 2012 Jul;31(7):1305-10.

37 Gomez, D.I., et al., *Reduced Mycobacterium tuberculosis association with monocytes from diabetes patients that have poor glucose control.* Tuberculosis (Edinb), 2013. 93(2): p. 192-7.

38 Lee, P.H., et al., *Glycemic Control and the Risk of Tuberculosis: A Cohort Study.* PLoS Med, 2016. 13(8): p. e1002072.

39 Komura, T., et al., *CD14+ monocytes are vulnerable and functionally impaired under endoplasmic reticulum stress in patients with type 2 diabetes.* Diabetes, 2010. 59(3): p. 634-43.

40 Kornfeld, H., et al., *High Prevalence and Heterogeneity of Diabetes in Patients With TB in South India: A Report from the Effects of Diabetes on Tuberculosis Severity (EDOTS) Study*. Chest, 2016. 149(6): p. 1501-8.

41 Workneh, M.H., G.A. Bjune, and S.A. Yimer, *Prevalence and Associated Factors of Diabetes Mellitus among Tuberculosis Patients in South-Eastern Amhara Region, Ethiopia: A Cross Sectional Study*. PLoS One, 2016. 11(1): p. e0147621.

42 Erol A. Visceral adipose tissue specific persistence of Mycobacterium tuberculosis may be reason for the metabolic syndrome. *Medical hypotheses.* 2008;71(2):222-228.

43 Cabana VG, Siegel JN, Sabesin SM. Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. *Journal of lipid research.* 1989;30(1):39-49.

44 Deniz O, Gumus S, Yaman H, et al. Serum total cholesterol, HDL-C and LDL-C concentrations significantly correlate with the radiological extent of disease and the degree of smear positivity in patients with pulmonary tuberculosis. *Clinical biochemistry*. 2007;40(3-4):162-166.

45 Kozarevic D, McGee D, Vojvodic N, et al. Serum cholesterol and mortality: the Yugoslavia Cardiovascular Disease Study. *American journal of epidemiology.* 1981;114(1):21-28.

46 Perez-Guzman C, Vargas MH, Quinonez F, Bazavilvazo N, Aguilar A. A cholesterol-rich diet accelerates bacteriologic sterilization in pulmonary tuberculosis. *Chest*. 2005;127(2):643-651.

47 Taylor GO, Bamgboye AE. Serum cholesterol and diseases in Nigerians. *The American journal of clinical nutrition.* 1979;32(12):2540-2545.

48 Alim, M.A., et al., *Anti-mycobacterial function of macrophages is impaired in a diet induced model of type 2 diabetes.* Tuberculosis (Edinb), 2017. 102: p. 47-54.

49 Kumar, N.P., et al., *Diminished systemic and antigen-specific type 1, type 17, and other proinflammatory cytokines in diabetic and prediabetic individuals with latent Mycobacterium tuberculosis infection.* J Infect Dis, 2014. 210(10): p. 1670-8.

50 Lachmandas, E., et al., *The effect of hyperglycaemia on in vitro cytokine production and macrophage infection with Mycobacterium tuberculosis.* PLoS One, 2015. 10(2): p. e0117941.

51 Lachmandas, E., et al., *Diabetes Mellitus and Increased Tuberculosis Susceptibility: The Role of Short-Chain Fatty Acids.* J Diabetes Res, 2016. 2016: p. 6014631.

52 Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol 2009;21:154e65.

53 Jeltsch M, Leppanen VM, Saharinen P, Alitalo K. Receptor tyrosine kinase-mediated angiogenesis. Cold Spring Harb Perspect Biol 2013;5.

54 Matsuyama W, Hashiguchi T, Matsumuro K, Iwami F, Hirotsu Y, Kawabata M, et al. Increased serum level of vascular endothelial growth factor in pulmonary tuberculosis. Am J Respir Crit Care Med 2000;162:1120e2.

55 Abe Y, Nakamura M, Oshika Y, Hatanaka H, Tokunaga T, Okhubo Y, et al. Serum levels of vascular endothelial growth factor and cavity formation in active pulmonary tuberculosis. Respiration 2001;68:496e500.

56 Alatas F, Alatas O, Metintas M, Ozarslan A, Erginel S, Yildirim H. Vascular endothelial growth factor levels in active pulmonary tuberculosis. Chest 2004;125:2156e9.

57 Mihret A, Bekele Y, Bobosha K, Kidd M, Aseffa A, Howe R, et al. Plasma cytokines and chemokines differentiate between active disease and non-active tuberculosis infection. J Infect 2013;66:357e65.

58 Ota MO, Mendy JF, Donkor S, Togun T, Daramy M, Gomez MP, et al. Rapid diagnosis of tuberculosis using ex vivo host biomarkers in sputum. Eur Respir J 2014;44:254e7.

59 Riou C, Perez Peixoto B, Roberts L, Ronacher K, Walzl G,Manca C, et al. Effect of standard tuberculosis treatment on plasma cytokine levels in patients with active pulmonary tuberculosis. PLoS One 2012;7:e36886.

60 Kumar NP, Banurekha VV, Nair D, Babu S. Circulating angiogenic factors as biomarkers of disease severity and bacterial burden in pulmonary tuberculosis. PLoS One 2016;11:e0146318

61 Geerlings, S.E. and A.I. Hoepelman, *Immune dysfunction in patients with diabetes mellitus (DM).* FEMS Immunol Med Microbiol, 1999. 26(3-4): p. 259-65.

62 Restrepo, B.I., et al., *Tuberculosis in poorly controlled type 2 diabetes: altered cytokine expression in peripheral white blood cells.* Clin Infect Dis, 2008. 47(5): p. 634-41.

63 Stew, S.S., et al., *Differential expression of monocyte surface markers among TB patients with diabetes co-morbidity.* Tuberculosis (Edinb), 2013. 93 Suppl: p. S78-82.

64 Lecube, A., et al., *Phagocytic activity is impaired in type 2 diabetes mellitus and increases after metabolic improvement.* PLoS One, 2011. 6(8): p. e23366.

65 Richard, C., et al., *Individuals with obesity and type 2 diabetes have additional immune dysfunction compared with obese individuals who are metabolically healthy.* BMJ Open Diabetes Res Care, 2017. 5(1): p. e000379.

66 Delamaire, M., et al., *Impaired leucocyte functions in diabetic patients.* Diabet Med, 1997. 14(1): p. 29-34.

67 Lagman M, Ly J, Saing T, Kaur Singh M, Vera Tudela E, Morris D, Chi PT, Ochoa C, Sathananthan A, Venketaraman V. Investigating the causes for decreased levels of glutathione in individuals with type II diabetes. PLoS One. 2015 Mar 19;10(3):e0118436.

68 Yamashiro S, Kawakami K, Uezu K, et al. Lower expression of Th1-related cytokines and inducible nitric oxide synthase in mice with streptozotocin-induced diabetes mellitus infected with Mycobacterium tuberculosis. *Clinical and experimental immunology.* 2005;139(1):57-64.

69 Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood.* 2008;112(5):1557-1569.

70 Zykova SN, Jenssen TG, Berdal M, Olsen R, Myklebust R, Seljelid R. Altered cytokine and nitric oxide secretion in vitro

by macrophages from diabetic type II-like db/db mice. *Diabetes.* 2000;49(9):1451-1458.

71 Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. *Nature.* 2008;453(7198):1051-1057.

72 Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annual review of immunology.* 2009;27:485-517.

73 Amano H, Yamamoto H, Senba M, et al. Impairment of endotoxin-induced macrophage inflammatory protein 2 gene expression in alveolar macrophages in streptozotocin-induced diabetes in mice. *Infection and immunity.* 2000;68(5):2925-2929.

74 Raposo-García S, Guerra-Laso JM, García-García S, Juan-García J, López-Fidalgo E, Diez-Tascón C, Nebreda-Mayoral T, López-Medrano R, Rivero-Lezcano OM. Immunological response to Mycobacterium tuberculosis infection in blood from type 2 diabetes patients. Immunol Lett. 2017 Apr 1;186:41-45.

75 Warren E, Teskey G, Venketaraman V. Effector Mechanisms of Neutrophils within the Innate Immune System in Response to Mycobacterium tuberculosis Infection. J Clin Med. 2017 Feb 7;6(2).

76 Appelberg R. Neutrophils and intracellular pathogens: beyond phagocytosis and killing. *Trends in microbiology.* 2007;15(2):87-92.

77 Sequeira PC, Senaratne RH, Riley LW. Inhibition of toll-like receptor 2 (TLR-2)-mediated response in human alveolar epithelial cells by mycolic acids and Mycobacterium tuberculosis mce1 operon mutant. *Pathogens and disease.* 2014;70(2):132-140.

78 Pancholi P, Mirza A, Schauf V, Steinman RM, Bhardwaj N. Presentation of mycobacterial antigens by human dendritic cells: lack of transfer from infected macrophages. *Infection and immunity.* 1993;61(12):5326-5332.

79 Comstock GW. Epidemiology of tuberculosis. *The American review of respiratory disease.* 1982;125(3 Pt 2):8-15.

80 Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. *PLoS medicine.* 2008;5(7):e152.

81 Vasilyeva IA, Belilovsky EM, Borisov SE, Sterlikov SA. WHO GLOBAL TUBERCULOSIS REPORTS: COMPILATION AND INTERPRETATION. *Tuberkulez i*

Bolezni Lëgkih, Vol 95, Iss 5, Pp 7-16 (2017). 2017(5):7.

82 Douek DC, Brenchley JM, Betts MR, et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature*. 2002;417(6884):95-98.

83 Yagi T, Yano T, Di Bernardo S, Matsuno-Yagi A. Procaryotic complex I (NDH-1), an overview. (BBA) - Bioenergetics. 1998;1364:125–33.

84 Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature. 1998;393:537–44.

85 Wheaton WW, Weinberg SE, Hamanaka RB, Soberanes S, Sullivan LB, Anso E, et al. Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. eLife. 2014;13(3):e02242.

86 Pearce E. L, Walsh M. C, Cejas P. J, Harms G. M, Shen H., Wang L. S, Jones R. G, Choi Y, Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460, 103–107 (2009).

87 Marupuru S, Senapati P, Pathadka S, Miraj SS, Unnikrishnan MK, Manu MK. Protective effect of metformin against tuberculosis infections in diabetic patients: an observational study of south Indian tertiary healthcare facility. Braz J Infect Dis. 2017 May - Jun;21(3):312-316.

88 Singhal A, Jie L, Pavanish K, et al. (2014). Metformin as adjunct antituberculosis therapy. Science translational medicine. 6. 10.1126/scitranslmed.3009885.

89 Martina V, Bruno GA, Zumpano E, Origlia C, Quaranta L, Pescarmona GP. Administration of glutathione in patients with type 2 diabetes mellitus increases the platelet constitutive nitric oxide synthase activity and reduces PAI-1. J Endocrinol Invest. 2001 Jan;24(1):37-41.

90 Griffith OW Biologic and pharmacologic regulation of mammalian glutathione synthesis. Free Radic Biol Med (1999) 27: 922–935.

91 Franklin CC, Rosenfeld-Franklin ME, White C, Kavanagh TJ, Fausto N (2003) TGFbeta1-induced sup- pression of glutathione antioxidant defenses in hepatocytes: caspase-dependent post-translational and caspase-independent transcriptional regulatory mechanisms. FASEB J 17: 1535–1537.

92 Bakin AV, Stourman NV, Sekhar KR, Rinehart C, Yan X, et al. (2005) Smad3-ATF3 signaling mediates TGF-beta suppression of genes encoding Phase II

detoxifying proteins. Free Radic Biol Med
38: 375–387.

