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MAJOR ARTICLE

Impact of Intermediate Hyperglycemia and Diabetes on Immune Dysfunction in Tuberculosis

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(See the Editorial Commentary by Wilkinson on pages 79–81.)

Background. People with diabetes have an increased risk of developing active tuberculosis (TB) and are more likely to have poor TB-treatment outcomes, which may impact on control of TB as the prevalence of diabetes is increasing worldwide. Blood transcriptomes are altered in patients with active TB relative to healthy individuals. The effects of diabetes and intermediate hyperglycemia (IH) on this transcriptomic signature were investigated to enhance understanding of immunological susceptibility in diabetes-TB comorbidity.

Methods. Whole blood samples were collected from active TB patients with diabetes (glycated hemoglobin [HbA1c] ≥6.5%) or IH (HbA1c = 5.7% to <6.5%), TB-only patients, and healthy controls in 4 countries: South Africa, Romania, Indonesia, and Peru. Differential blood gene expression was determined by RNA-seq (n = 249).

Results. Diabetes increased the magnitude of gene expression change in the host transcriptome in TB, notably showing an increase in genes associated with innate inflammatory and decrease in adaptive immune responses. Strikingly, patients with IH and TB exhibited blood transcriptomes much more similar to patients with diabetes-TB than to patients with only TB. Both diabetes-TB and IH-TB patients had a decreased type I interferon response relative to TB-only patients.

Conclusions. Comorbidity in individuals with both TB and diabetes is associated with altered transcriptomes, with an expected enhanced inflammation in the presence of both conditions, but also reduced type I interferon responses in comorbid patients, suggesting an unexpected uncoupling of the TB transcriptome phenotype. These immunological dysfunctions are also present in individuals with IH, showing that altered immunity to TB may also be present in this group. The TB disease outcomes in individuals with IH diagnosed with TB should be investigated further.

Keywords. tuberculosis; diabetes; inflammation; hyperglycemia.

Despite much research, precise mechanisms underlying how the host immune response controls *Mycobacterium tuberculosis* are still not fully understood. Studies of comorbidity and coinfection associated with susceptibility to tuberculosis (TB) may provide useful insights. The epidemiological link between diabetes and TB has been known for centuries, yet the biological mechanisms underlying the association are still unclear [1]. People living with diabetes are >3 times more likely to develop active TB once infected [2], and are also more likely to have poor TB-treatment outcomes [3]. Ninety percent of diabetes cases worldwide are type 2 diabetes mellitus (T2DM) [4], characterized by insulin resistance and subsequent insulin insufficiency. Diabetes prevalence has risen from 108 million in 1980 to 415 million in 2014, with an estimated 642 million cases projected by 2040 [4]. The countries with the greatest number of people with diabetes include several countries with concurrent high TB burden, such as China, India, Brazil, Indonesia, and Bangladesh. The estimated population attributable fraction of diabetes for adult TB is estimated at 9%–17% across World Health Organization regions [5], surpassing that for human

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immunodeficiency virus (HIV) in most regions, emphasizing the critical need to target this population for TB control.

T2DM is characterized by a decrease in the ability of insulin to alter metabolism in target cells, leading to overproduction of insulin, pancreatic β-cell exhaustion, and eventual depletion of insulin, causing impaired glucose tolerance. Excessive glucose has toxic effects systemically, on major organs as well as on cells in the immune system. Additionally, T2DM is linked with obesity, with an increased number of adipocytes and with increased secretion of free fatty acids and of proinflammatory cytokines, including tumor necrosis factor–α, interleukin (IL) 1β, and IL-6 [6, 7], from adipocytes. In turn, these abnormalities in glucose control, dyslipidemia, and the chronic inflammatory response lead to an immune dysfunction disorder, affecting the body's defence to *M. tuberculosis*. People with T2DM have impaired immune cell function, with reduced phagocytic ability in response to *M. tuberculosis*, which is correlated with poor glycemic control [8]. Alongside chronic inflammation, including elevated circulating proinflammatory cytokines, people with T2DM-TB comorbidity have enhanced type 1 [9, 10] and regulatory T-cell responses [11] to *M. tuberculosis* antigens (reviewed in [12]).

Spectrums exist in both TB and T2DM, from latent *M. tuberculosis* infection through subclinical incipient TB to active TB disease [13], and from nondiabetes to clinically manifest diabetes via intermediate hyperglycemia (IH). Although not completely sensitive, glycated hemoglobin (HbA1c) measurement can determine an individual's point along this spectrum, with the current threshold for diabetes diagnosis at HbA1c ≥6.5%, and for intermediate hyperglycemia at HbA1c = 5.7% to <6.5% [14]. IH has become an area of interest because it has also been shown to be associated with TB [15, 16].

Blood transcriptomic changes in TB patients have been well described: Active TB patients are distinct from healthy controls (HCs), exhibiting a neutrophil-derived interferon (IFN) [17] and inflammatory response [18] signature. This resolves during successful TB drug treatment [19, 20], and the blood transcriptome in TB is distinct from that in other diseases [21, 22]. Blood-based transcriptomic studies of people living with diabetes are scarce. A recent study [23] found few differences in transcriptomes between TB-only patients and those with T2DM and TB comorbidity, although TB patients with IH were not defined in this study.

Here, we used an unbiased RNA-seq approach to identify immunological pathways that were altered in T2DM-TB comorbidity compared to TB only, and we also investigated the impact of IH on TB, in order to understand why diabetes increases susceptibility to TB, and whether individuals with IH might also be at increased risk.

METHODS

Patient Recruitment

Adults with newly diagnosed, bacteriologically confirmed pulmonary TB with and without diabetes were recruited between December 2013 and February 2016 at 4 different study sites: Cape Town, South Africa (SUN); Bandung, Indonesia (UNPAD); Lima, Peru (UPCH); and Craiova, Romania (UMFCV). In South Africa and Romania, people with diabetes mellitus (DM) but without TB and healthy individuals were also recruited. Patients were excluded if they were already taking TB treatment, had multidrug-resistant TB, were HIV positive, were pregnant, were taking corticosteroids, or had other serious comorbidity. DM was classified as a laboratory test of HbA1c ≥6.5%, alongside a confirmatory HbA1c ≥6.5% or fasting blood glucose ≥7 mmol/L, as described in this cohort [24]. Patients with IH were characterized with an HbA1c reading of 5.7% to <6.5%. Patients without diabetes, including HCs, had HbA1c values of <5.7%. All patients gave written informed consent, and the study was approved by the London School of Hygiene and Tropical Medicine Observational/Interventions Research Ethics Committee (6449, 11 July 2013) as well as the SUN Health Research Ethics Committee (N13/05/064, 29 July 2013), the UNPAD Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran (number 377/UN6. C2.1.2/ KEPK/ PN/2012), the UMFCV Committee of Ethics and Academic and Scientific Deontology (94, 6 September 2013), and the UPCH Institutional Committee of Ethics in Investigation (61069, 2 September 2013).

To limit confounding, prior to conducting the RNA-seq analysis, patient characteristic variables (age, body mass index [BMI], HbA1c) for each clinical group and for each study setting were initially tested using analysis of variance. Subsequently, variables were tested for normality using the Shapiro-Wilk test: If deemed normally distributed, a pairwise *t* test was performed, if not, a nonparametric Wilcoxon rank-sum test was performed. The participant ethnicities were recorded.

RNA-seq Experiments

Venous blood was collected at TB diagnosis, before initiation of TB treatment, into PAXgene Blood RNA Tubes (PreAnalytiX) and used for RNA-seq analysis, using the polyA tail library preparation method and single-read sequencing (n = 249). Sequencing FASTQ files were aligned to the human genome, and transcript quantification, differential gene expression, biological characterization, and machine learning were performed as described in the Supplementary Methods. RNA-seq quality control data are shown in Supplementary Figure 1 and Supplementary Table 1.

RESULTS

Study Population

A total of 151 TB patients were recruited from 4 locations in South Africa, Romania, Indonesia, and Peru, and classified as having TB only, IH-TB, or DM-TB, depending on laboratory HbA1c results at the time of TB diagnosis (Table 1). The DM-TB group included some patients with

previously diagnosed DM, some of whom were taking insulin (Supplementary Table 2). Additionally, in South Africa and Romania, individuals without disease (HCs) and DM only were recruited. The ages of the participants were not significantly different across the disease categories in any site or when the sites were combined (median age: 46 years for TB only; 46 years for IH-TB; and 49 years for DM-TB; Table 1). As anticipated, the BMI was significantly greater in the DM patients than in all the other patient groups at all sites (Table 1). Overall, participants had 10 different stated ethnic backgrounds, but the participants' ethnicities were not different across disease groups within each site.

Blood TB Transcriptome Signature

First, the effect of DM and IH on gene expression changes in TB patients' blood was investigated in South African samples. As expected, there was substantial up-regulation of gene expression (345 genes) in TB patients compared to HCs (Figure 1A), including up-regulated expression of genes such as complement component C1QA, B, C, and C2; BATF2; SOCS3; Septin 4; ANKRD22; and GBP5, which have been observed previously [20, 25, 26] (Figure 1A). Diabetes alone had a very different impact on the blood transcriptome, with larger numbers of genes differentially expressed, but with low magnitude of change and lower statistical significance (Figure 1B). In contrast, DM had a substantial impact on the blood transcriptome in TB patients, with 1695 genes significantly up-regulated in DM-TB patients compared to HCs, including many ribosomal and transmembrane proteins, as well as proinflammatory cytokines such as IL-1β, IL-15, and IL-18 and the regulatory cytokine IL-10. There were also 1623 genes significantly down-regulated in the DM-TB group compared to HCs, including several zinc finger transcription factors and cytokines such as IL-8, IL-16, and IL-24. Notably, patients with IH-TB exhibited an even greater perturbation of their blood transcriptome relative to HCs, with 2576 genes significantly up-regulated and 2140 genes significantly down-regulated (Figure 1D). These results show that diabetes impacts the peripheral host response to TB and that this effect is already present in individuals with IH. Supplementary Table 3 details all of the differentially expressed genes in the South African cohort. Supplementary Figure 2 shows that sex had no impact on gene expression differences.

These data were validated in a separate patient cohort, recruited in Romania. Similarly large-scale up- and down-regulation of gene expression in both DM-TB and IH-TB were observed relative to HCs, to a much greater extent than that seen in TB only (Supplementary Figure 3). Direct comparison of differentially expressed gene lists in South Africa and Romania showed significant overlap in each case (GeneOverlap $P < .05$; Supplementary Figure 4).

Table 1. Characteristics of Study Participants

Characteristic	Country	TB Only	IH-TB	DM-TB	T2DM Only	Healthy Controls	*P* Value (ANOVA)
No. of study participants	South Africa	11	20	15	33	24	
	Romania	10	10	15	19	12	
	Indonesia	14	5	19	…	…	
	Peru	11	9	12	…	…	
	All sites	46	44	61	…	…	
Age, y, median (range)	South Africa	48 (31–56)	44.5 (25–57)	46 (27–57)	49 (29–64)	42 (30–70)	.168
	Romania	43 (30–64)	48.5 (22–63)	47 (22–64)	55 (38–65)	46 (38–61)	.329
	Indonesia	47 (28–62)	51 (37–54)	52 (33–66)	…	…	.430
	Peru	55 (31–69)	52 (31–68)	50.5 (42–58)	…	…	.928
	All sites	46 (28–69)	46 (22–68)	49 (22–66)	…	…	.2645
Sex, % (No. male/female)	South Africa	18 (2/9)	60 (12/8)	47 (7/8)	45 (15/18)	50 (12/12)	
	Romania	60 (6/4)	90 (9/1)	87 (13/2)	73 (14/5)	83 (10/2)	
	Indonesia	50 (7/7)	80 (4/1)	58 (11/8)	…	…	
	Peru	45 (5/6)	55 (5/4)	50 (6/6)	…	…	
	All sites	43 (26/20)	68 (30/14)	60 (37/24)	…	…	
BMI, kg/m^2, median (range)	South Africa	20.1 (14.6–24.1)	18.4 (13.7–27.1)	19.1 (13.9–32.3)	29 (20.52–52.54)	23.7 (17.37–45.20)	1.43x10[-8]
	Romania	20.65 (17.7–25.5)	21 (15.5–22)	21.5 (15.3–36.1)	28.7 (13.1–37.8)	…	6.73x10[-4]
	Indonesia	19.8 (13.76–33.27)	18.67 (13.9–20.09)	19.52 (16.10–31.73)	…	…	.242
	Peru	23.98 (17.35–28.96)	21.94 (18.67–25.56)	22.7 (20.55–33.33)	…	…	.207
	All sites	20.94 (13.76–33.27)	19.44 (13.7–27.1)	21.5 (13.9–36.1)	…	…	1.67x10[-3]
HbA1c, %, median (range)	South Africa	5.3 (4.8–5.7)	6 (5.7–6.3)	10.8 (6.5–14.3)	10.1 (4.7–14.9)	5.3 (4.8–5.6)	2.2x10[-16]
	Romania	5.5 (5.2–5.7)	6 (5.7–6.4)	10.4 (6.6–15)	9 (7.1–14)	5.4 (5.2–5.6)	1.2x10[-13]
	Indonesia	5.55 (5–5.7)	5.9 (5.8–6)	11.9 (7.5–15.9)	…	…	3.2x10[-12]
	Peru	5.3 (5.1–5.7)	5.8 (5.7–6.1)	11.4 (7.1–15.2)	…	…	8.33x10[-9]
	All sites	5.5 (4.8–5.7)	5.95 (5.7–6.4)	11 (6.5–15.9)	…	…	2.2x10[-16]

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; DM-TB, diabetes mellitus–tuberculosis; HbA1c, glycated hemoglobin; IH-TB, intermediate hyperglycemia–tuberculosis; T2DM, type 2 diabetes mellitus; TB, tuberculosis.

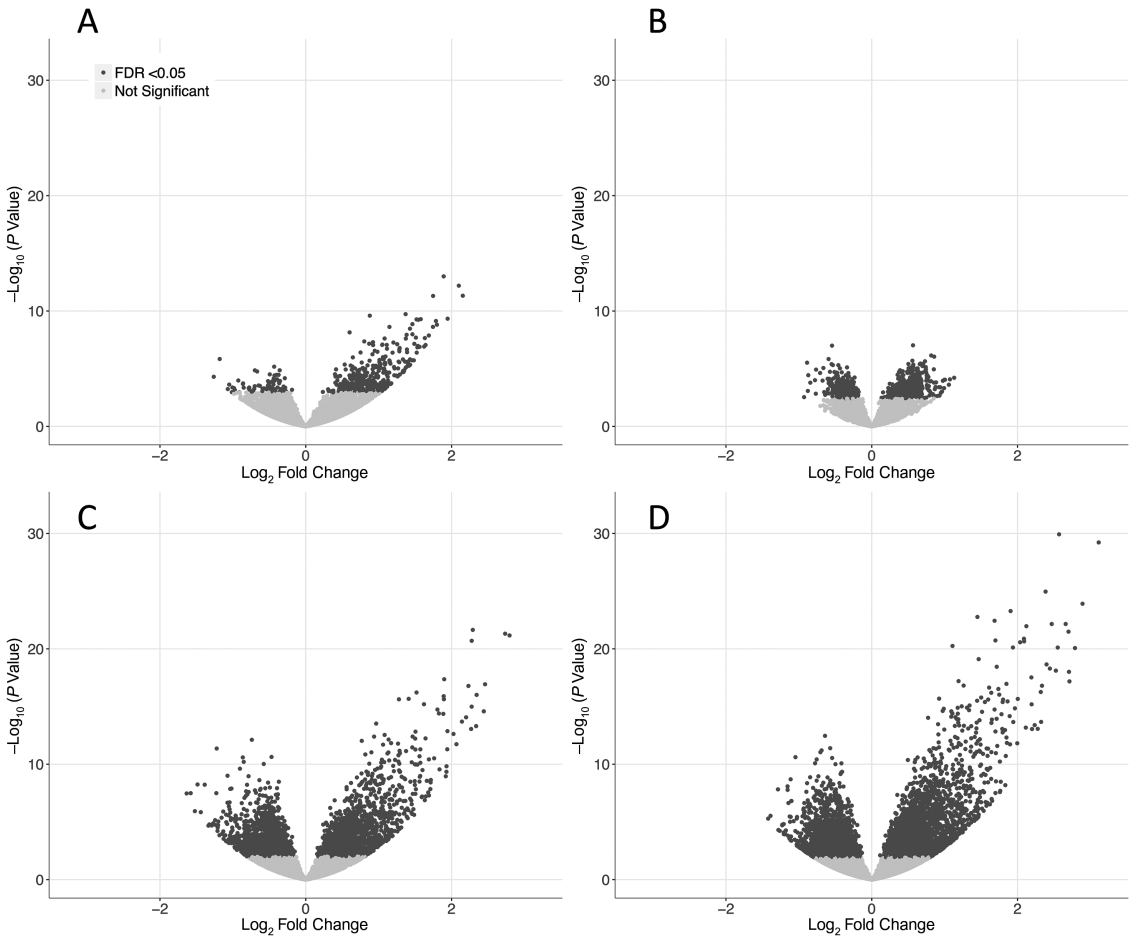

Figure 1. Differential expression analysis of all the disease phenotypes in South Africa compared to healthy controls before the initiation of tuberculosis (TB) treatment. Gene expression profiles of TB only (n = 11, A), diabetes mellitus (DM) only (n = 33, B), DM-TB (n = 15, C), and intermediate hyperglycemia (IH)–TB (n = 20, D), each relative to healthy controls (n = 24). Genes that were deemed statistically significantly differentially expressed had an adjusted $P < .05$ after multiple testing correction (Benjamini-Hochberg). Black corresponds to the genes whose expression was significantly changed, and gray shows genes without significant expression change. Abbreviation: FDR, false discovery rate.

Log fold change (FC)–FC plots demonstrate concordant and discordant differential expression of genes when samples in different disease categories are compared to controls; discordant genes show significant differences in both comparisons, yet in opposite directions, whereas concordant genes show significant differences in both comparisons in the same direction. Here, all 4 disease groups with HCs in the South African cohort were compared (Figure 2). The main differences, showing the most discordance, were seen between the DM-only group vs HC comparison against all 3 comparisons of patients with TB (TB only vs HCs; DM-TB vs HCs; and IH-TB vs HCs), showing that the TB transcriptome is dominant. The greatest concordance was between DM-TB vs HCs and IH-TB vs HCs, showing that patients in these categories exhibited similar changes in blood gene expression. The comparison between TB only vs HCs and IH-TB vs HCs showed quantitative differences, with concordance between the genes but to a larger degree in the IH-TB group, indicating extra perturbation when hyperglycemia is present (Figure 2).

To determine whether differential gene expression could be used to discriminate disease phenotype, principal component analysis (PCA) was performed on all study participants from South Africa. The resulting PCA plot, based on the differentially expressed genes from the initial analysis, showed 2 main clusters: HCs and people with DM only clustered together, while the second main cluster comprised people with IH-TB and DM-TB comorbidity (Figure 3).

Modular Analysis in DM and TB Comorbidity

Modular analysis, which tests for enrichment sets of co-regulated genes sharing related biological functions [27, 28], was performed. As observed previously [17, 20], in the South African cohort, TB-only patients exhibited up-regulation of specific modules, including the type I IFN

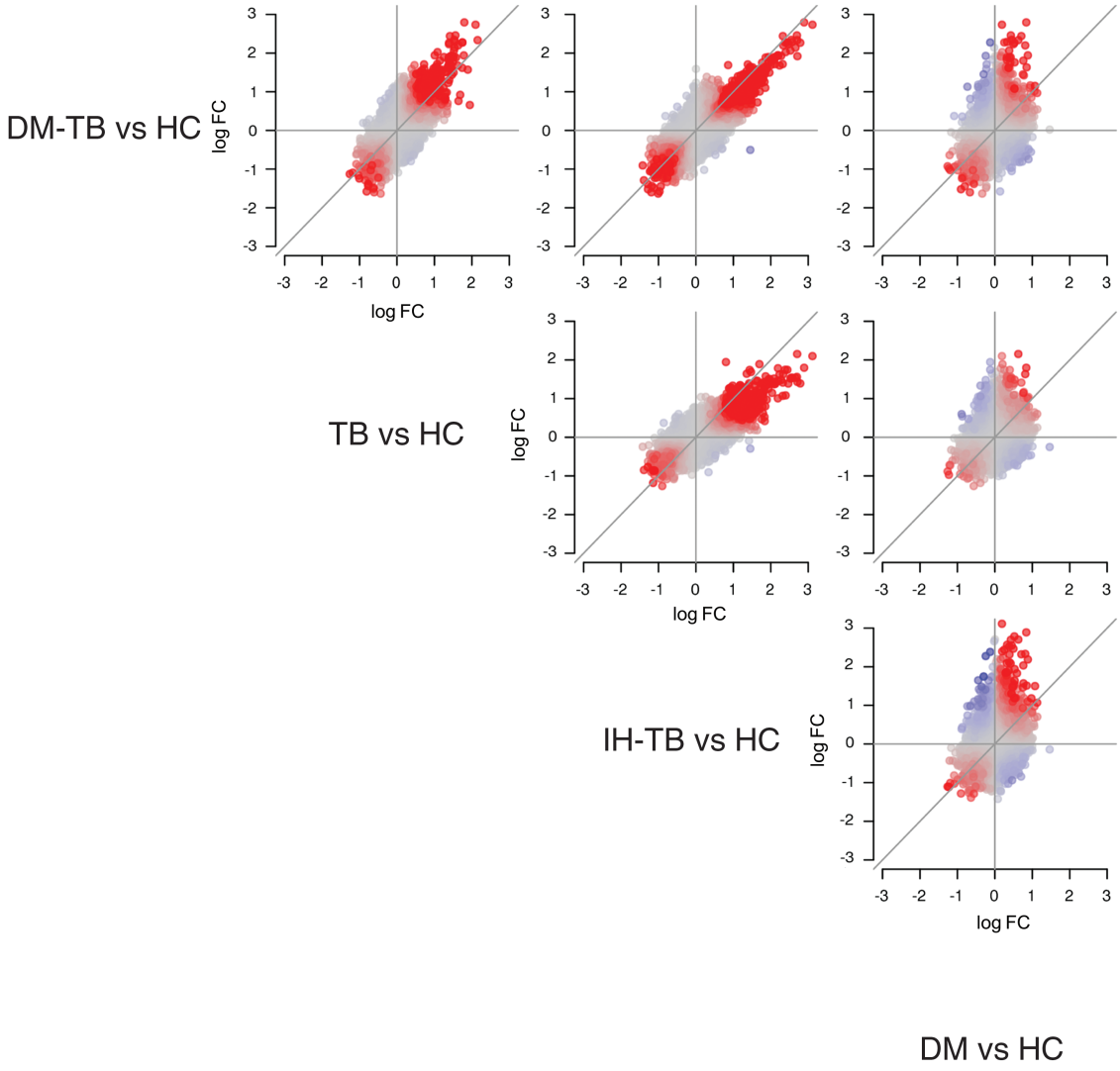

Figure 2. Concordance and discordance of gene expression between the comparisons of each disease group and healthy controls in South Africa. Log fold change (FC) and *P* value between groups was calculated with R-package DESeq2. A disco.score was calculated for each pair of corresponding genes. The axes show $\log_2$ FC between the conditions indicated by the labels. For example, on the top left plot the x-axis corresponds to the comparison between tuberculosis (TB) and healthy controls (HC), and the y-axis shows the $\log_2$ FC between diabetes mellitus (DM)-TB and HC. Red dots show genes that are significantly different from the controls in the same direction (concordant genes), and blue dots show genes that are significantly different in both comparisons, but in opposite directions. Intensity of color indicates the strength of concordance/discordance as measured by the disco.score.

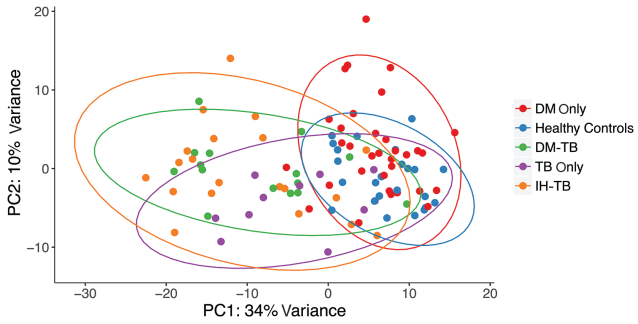

Figure 3. Principal component analysis (PCA) of South African participants. The list of all genes that were significantly differentially expressed in any patient group comparison with healthy controls was used in a PCA of all the samples obtained from participants recruited in South Africa. Abbreviations: DM, diabetes mellitus; IH, intermediate hyperglycemia; PC, principal component; TB, tuberculosis.

response, complement, and activated dendritic cells (Figure 4 and Supplementary Figure 5). In contrast, in diabetes patients, mainly respiration and protein synthesis modules were significantly up-regulated compared to HCs; these changes were not present in the comorbidity groups. Notably, module enrichments in DM-TB and IH-TB patients were similar to each other, which included up-regulation of modules differentially expressed in the TB-only group with a greater magnitude, especially for modules related to the inflammatory response and to myeloid cell function. There was stronger down-regulation of modules in IH-TB and DM-TB groups compared to TB only: these included natural killer (NK) cell modules and adaptive immune response modules such as T-cell activation, differentiation, and B cells (Figure 4).

Figure 4. Transcriptional modules that were significantly differentially expressed in tuberculosis (TB)-only, diabetes mellitus (DM)-TB, intermediate hyperglycemia (IH)-TB, and DM only compared to healthy controls in South Africa before initiation of TB treatment. Transcripts were evaluated using a preexisting modular framework. Significantly up-regulated (red) and down-regulated (blue) modules are shown: the length of each bar corresponds to the effect size (magnitude of change) of that module, and the color saturation represents the adjusted *P* value (<.0001). The amount of color represents the proportion of genes within that module that were differentially expressed.

Interferon and Inflammatory Responses

To determine the reproducibility of results across geographical regions and different patient ethnicities, RNA-seq data from DM-TB, IH-TB, and TB-only patients from all 4 study sites were combined. There was no systematic bias due to sample origin site (Supplementary Figure 6). In the combined dataset, DM-TB and IH-TB patients showed similar altered gene expression patterns in blood compared to patients with TB only (Figure 5). DM-TB patients had 292 up-regulated and 130 down-regulated genes, whereas IH-TB patients had 432 up-regulated and 126 down-regulated genes (Supplementary Table 4).

In modular analysis, both DM-TB and IH-TB showed a general trend of up-regulation, especially of modules involved in inflammation. Interestingly, however, patients with DM-TB and IH-TB also exhibited down-regulation of several IFN modules compared to TB only (Supplementary Figure 7). The most significantly differentially expressed are shown in Figure 6. The genes within these modules were differentially expressed in DM-TB and IH-TB, but of a greater magnitude in DM-TB, indicating an association with the extent of hyperglycemia.

Transcriptomic Signatures

We applied known Kaforou et al [29] and Sweeney et al [30] TB biomarker signatures that could distinguish TB from latent TB infection, to test validity in DM patients, using the random forest algorithm. As expected, the Kaforou signature validated well in our TB-only dataset (area under the curve [AUC] = 0.96; Figure 7, blue line). However, in the DM-TB comorbidity cohort, the performance was significantly reduced for both Kaforou (AUC = 0.87; Figure 7, orange line, test for difference between receiver operating characteristic [ROC] curves, P = .018) and Sweeney (AUC = 0.84; Figure 7, green line) signatures. This implies that a TB biomarker signature for use in the general population needs to be derived from patient cohorts including those with DM. ROC curves showing sensitivities and specificities for these comparisons are shown in Supplementary Table 5.

DISCUSSION

The principal finding of this study was that DM comorbidity influences blood transcriptomes in patients with TB, weakening the performance of published TB biosignatures. Of potentially greater public health importance, the modulation seen with diagnosed DM was also evident in those with intermediate hyperglycemia, below the current HbA1c cutoff for DM diagnosis. There was greater up-regulation of genes involved in the inflammatory response in diabetes and TB comorbidity and also a reduced up-regulation of the IFN response, principally of genes in the type I IFN pathway.

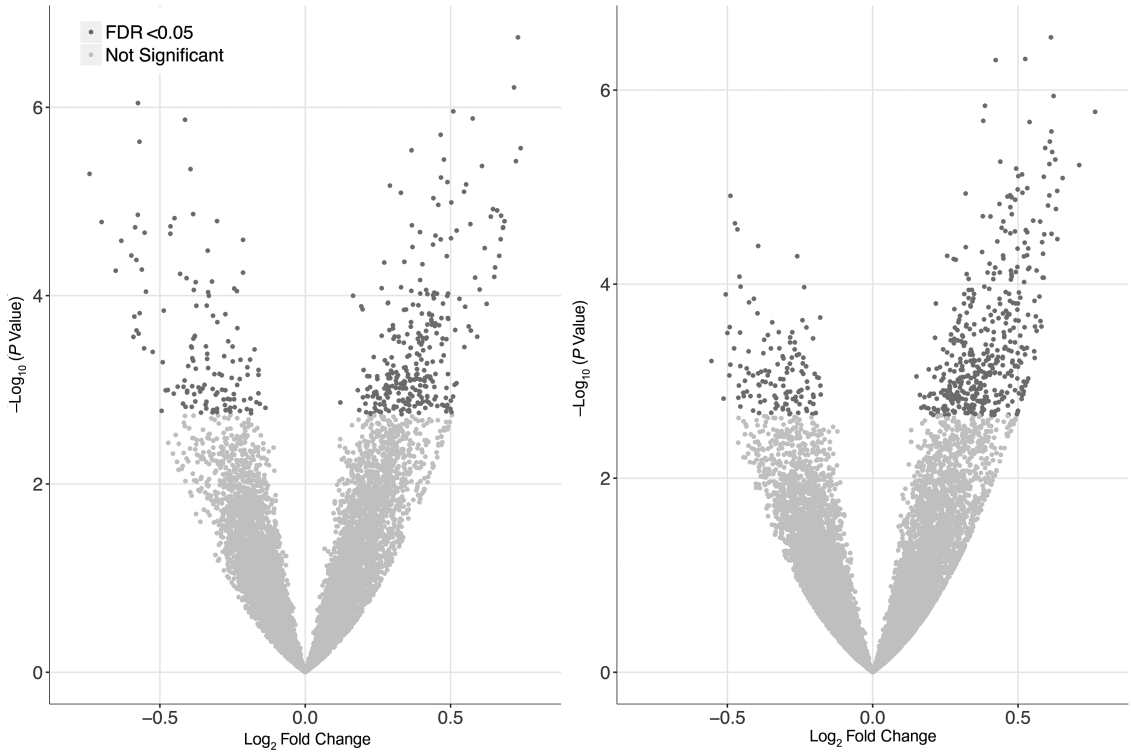

Figure 5. Differential gene expression analysis of diabetes mellitus (DM)–tuberculosis (TB) and intermediate hyperglycemia (IH)–TB patients relative to TB-only patients, in the combined dataset from all 4 field sites. Samples collected in South Africa, Romania, Peru, and Indonesia from DM-TB (*A*) patients and IH-TB patients (*B*) were compared with patients with TB only in a combined analysis. Genes significantly differentially expressed after multiple testing correction are shown in black (P < .05). Genes in gray are not statistically significantly altered compared to patients with untreated TB only. Abbreviation: FDR, false discovery rate.

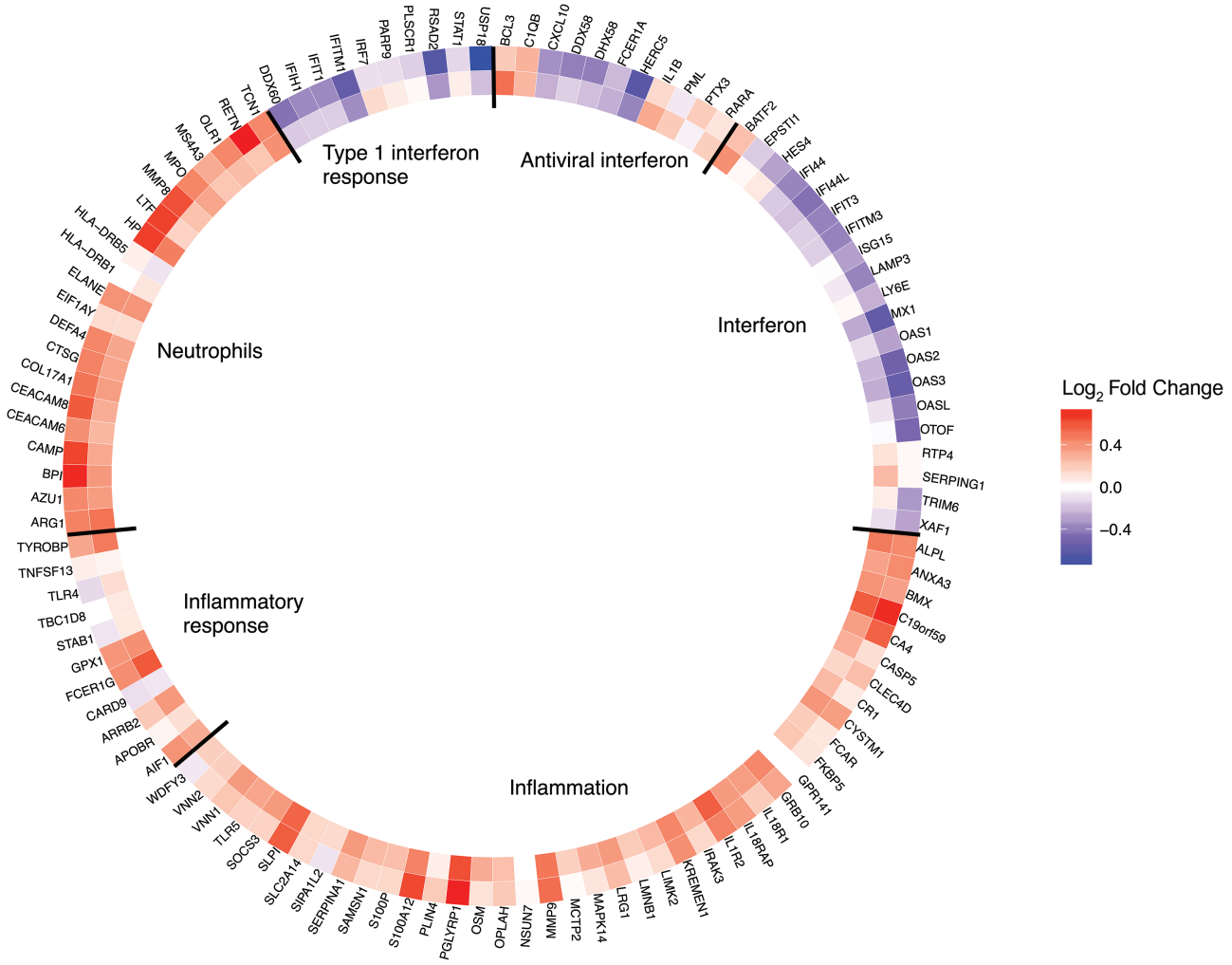

Figure 6. Summary of modular analysis in all 4 field sites. The fold changes of the genes within the top significantly differentially expressed modules are shown (adjusted $P < .05$). Inside: intermediate hyperglycemia–tuberculosis (TB) compared to TB only. Outside: diabetes mellitus–TB compared to TB only. Up-regulated genes are shown in red, and down-regulated genes are in blue. The saturation of color represents the magnitude of differential expression.

IH-TB patients were more similar to DM-TB patients than to people with TB only, even though their hyperglycemia was below the diabetes diagnostic threshold. Separation of the TB patients with HbA1c <6.5% facilitated the discovery of differences between the groups, which was not clearly evident in a previous transcriptomics study [23]. Our results demonstrate that patients with intermediate levels of hyperglycemia exhibit immune dysfunction, which could lead to an increased susceptibility to active TB disease. It was previously proposed that adverse effects and disease susceptibility would occur at higher HbA1c values, later in diabetes progression [31]. Infectious diseases, including TB, can themselves induce transient hyperglycemia [32] and poor outcome; further studies are warranted to establish the interplay between glycemic control, TB, and immune dysfunction. Targeted control of diabetes in TB could lead to better TB outcomes, and analysis of the direct impact of glycemic control in immune dysfunction is merited.

Developing TB control strategies targeting people with diabetes is critical, due to the rapidly increasing global T2DM burden, especially in countries with high TB incidence [33]. People living with diabetes have an increased risk of developing active TB following infection [34], and therefore this patient group may contribute disproportionately to onward TB transmission. DM-TB comorbidity is more likely to result in poor TB treatment outcomes [3], including failure and relapse, adding an extra burden on healthcare systems. Globally, population level diabetes interventions might provide effective TB control strategies [35].

In a parallel study conducted within the TANDEM Consortium analysis involving patients from the same 4 countries, TB patients with IH as well as with DM were more likely to be sputum smear positive compared to normoglycemic TB controls [24]. The impact of IH on TB disease susceptibility has been less well investigated, but in 2 studies conducted in India [15, 36] and 1 in Kenya [37], both IH and diabetes prevalences

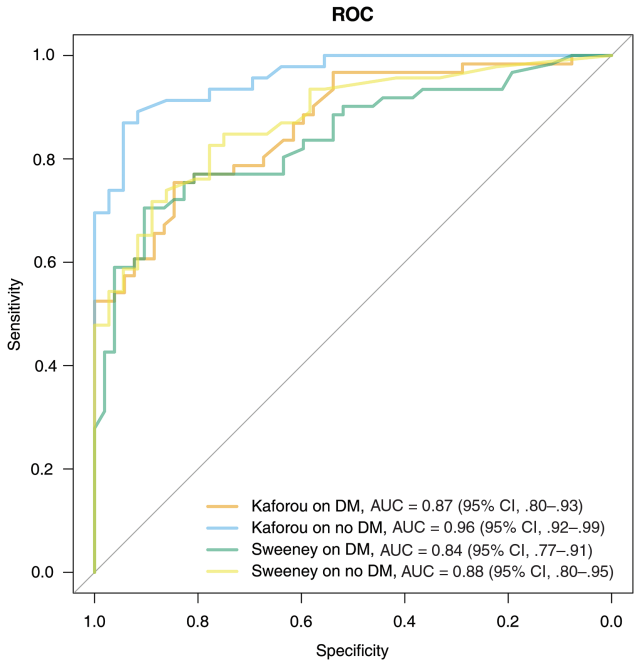

Figure 7. Predictive model of known signature in predictive model of known signature in TANDEM data. Receiver operating characteristic curves are based on a machine learning model generated from 2 different external datasets of transcriptome profiles of patients with tuberculosis and healthy controls (Kaforou et al [29] and Sweeney et al [30] training set). The random forest model was applied to the TANDEM cohort (test set), separately to individuals with and without diabetes mellitus. Abbreviations: AUC, area under the curve; CI, confidence interval; DM, diabetes mellitus; ROC, receiver operating characteristic.

in patients with pulmonary TB were substantially higher than in the general population, indicating that IH is also associated with the development of active TB. Our data strongly indicate that immune abnormalities occurring in DM-TB are already present in IH-TB, and it is possible that existing altered immune function in hyperglycemia underlies the epidemiological link.

This study shows that TB patients with either IH or diabetes have an altered immune phenotype, characterized by an excessive inflammatory response and a reduced type I IFN response. Both TB [18] and DM are proinflammatory conditions, and a degree of synergy between the 2 diseases is likely responsible for driving lung pathology and clinical symptoms. In active TB only, type I IFN response genes are up-regulated compared to healthy infected and uninfected individuals [17, 20, 22, 38]. Although surmised to be a deleterious excessive response in TB-only patients, the reduced IFN response seen in IH-TB and DM-TB patients might indicate an insufficient response, permitting continued growth of mycobacteria. Several IFN-related modules were present in the analysis performed, containing overlapping gene sets including genes encoding proteins involved in both type I and type II IFN pathways, all of which were all less up-regulated in the IH-TB and DM-TB groups compared to TB alone. The balance between inflammatory and type I IFN responses is critical for control of *M. tuberculosis* [39], and our finding reveals a potential mechanism of TB susceptibility in IH as well as in diabetes patients. Further work is required to confirm whether the type II IFN response is also affected, although we noted greater down-regulation of T-cell and NK-cell modules in IH-TB and DM-TB compared to TB alone. A recent study performed in Pakistan found raised serum concentrations of IFN-γ and IL-13 in both IH-TB and DM-TB, although TB patients with up to 1 month of anti-TB therapy were included [40]. Further work could also include an analysis of regulatory microRNAs and long noncoding RNAs, to understand better the mechanisms underpinning altered transcriptomes and immune responses in DM-TB.

Immunological differences in DM-TB compared to TB only include enhanced proinflammatory responses [12], and increased circulating cytokines have also been described in IH-TB [41]. Immune abnormalities are detectable in individuals latently infected with *M. tuberculosis* with IH [42], and it is possible that concurrent intermediate hyperglycemia and latent TB infection drives the development of both diseases, in a similar manner to the synergism between TB and HIV disease. This may indicate a relationship between moderate HbA1c values and TB susceptibility that warrants careful clinical considerations, and investigation of clinical disease outcome.

To conclude, these data have uncovered an enhanced inflammatory profile together with decreased IFN responses, associated with DM-TB comorbidity, which may be responsible for the increased susceptibility to TB in people with diabetes. Furthermore, immune dysfunction exists even at intermediate levels of hyperglycemia, potentially causing TB susceptibility with implications for TB control. HbA1c values need to be considered in TB studies, as abnormal glycemia clearly affects the immune response.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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