AIDS, Publish Ahead of Print

DOI: 10.1097/QAD.0000000000003262

Isoniazid preventive therapy and TB transcriptional signatures in people with HIV

Ethan D. VALINETZ[1, 10], Daniel MATEMO[2,3], Jill K. GERSH[1], Lara L. JOUDEH[1], Simon C. MENDELSOHN[4], Thomas J. SCRIBA[4], Mark HATHERILL[4], John KINUTHIA[2,6], Anna WALD[1, 7-9], Gerard A. CANGELOSI[5], Ruanne V. BARNABAS[1,6,7], Thomas R. HAWN[1], David J. HORNE[1]

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

[2] Department of Research and Programs, Kenyatta National Hospital, Nairobi, Kenya.

[3] School of Public Health and Community Development Maseno University, Kisumu, Kenya.

[4] South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, and Division of Immunology, Department of Pathology, University of Cape Town, South Africa.

[5] Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, USA

[6] Department of Global Health, University of Washington, Seattle, WA, USA

[7] Department of Epidemiology, University of Washington, Seattle, WA, USA

[8] Department of Lab Medicine & Pathology, University of Washington, Seattle, WA, USA

[9] Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

[10] Division of Infectious Disease, Department of Medicine, Indiana University School of Medicine, Indianapolis, USA

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Corresponding author: David Horne at dhorne@uw.edu

Funding: This research was funded by University of Washington Host Defense Research Training Grant (T32AI007044, EDV), 2015 developmental grant from the University of Washington/Fred Hutch Center for AIDS Research (NIH grant AI027757, DJH), the National Center For Advancing Translational Sciences of the NIH (UL1 TR002319), the Firland Foundation (JKG), and a 2K24AI137310 to TRH. SCM is a recipient of PhD funding from

the Fogarty International Center of the National Institutes of Health (NIH) under Award Number D43 TW010559, the Harry Crossley Clinical Research Fellowship, and the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development under the SAMRC Clinician Researcher Programme. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, the Harry Crossley Foundation, or the SAMRC.

Abstract

Objective(s): To examine the association between isoniazid preventive therapy (IPT) or non-tuberculous mycobacteria (NTM) sputum culture positivity and tuberculosis (TB) transcriptional signatures in people with HIV.

Design: Cross-sectional study.

Methods: We enrolled adults living with HIV who were IPT-naïve or had completed IPT > 6 months prior at HIV care clinics in western Kenya. We calculated TB signatures using gene expression data from qRT-PCR. We used multivariable linear regression to analyze the association between prior receipt of IPT or NTM sputum culture positivity with a transcriptional TB risk score, RISK6 (range 0 – 1). In secondary analyses, we explored the association between IPT or NTM positivity and four other TB transcriptional signatures.

Results: Among 381 participants, 99.7% were receiving antiretroviral therapy and 86.6% had received IPT (completed median of 1.1 years prior). RISK6 scores were lower (mean difference 0.10; 95% CI: 0.06, 0.15; $p < 0.001$) among participants who received IPT than those who did not. In a model that adjusted for age, sex, duration of ART, and plasma HIV RNA, the RISK6 score was 52.8% lower in those with a history of IPT ($p<0.001$). No significant association between year of IPT receipt and RISK6 scores was detected. There was no association between NTM sputum culture positivity and RISK6 scores.

Conclusions: In people with HIV, IPT was associated with significantly lower RISK6 scores compared with persons who did not receive IPT. These data support investigations of its performance as a TB preventive therapy response biomarker.

Key Words: Latent tuberculosis infection, transcriptome, gene expression profiling, HIV infection, antitubercular agents, non-tuberculous mycobacteria

INTRODUCTION

Tuberculosis (TB) remains a significant global health problem, and a leading cause of death among people with HIV [1], highlighting the need for improved diagnostics to identify persons at increased risk of developing active TB. One strategy to end the TB pandemic has been to provide TB preventive therapy to persons at risk for progression to active TB disease. In TB endemic settings, current recommendations are to provide a single course of TB preventive therapy to people with HIV (after excluding active TB) [2]. However, this approach leads to over-treatment (e.g., those who are not infected with *M. tuberculosis* (Mtb)) and undertreatment (e.g., those who are subsequently re-infected with Mtb in high burden settings).

Currently, the identification of infected persons at risk for progression to active TB disease is based on the tuberculin skin test (TST) and interferon gamma release assays (IGRAs), which have several drawbacks. These tests have poor predictive values for progression to active TB as they do not differentiate between persons who have cleared Mtb infection versus those who remain infected with viable organisms [3], and have reduced sensitivity in immunocompromised persons [4, 5], including people with HIV [6]. IGRAs are less specific when used in low-risk populations in non-endemic settings and when applying manufacturer cut-points in high burden settings [4, 7]. Furthermore, the TST is less specific among people who have been vaccinated with BCG[8] or exposed to environmental mycobacteria (non-tuberculous mycobacteria or NTM) [8]. Additionally, TST and IGRA results may not change with TB preventive therapy [9, 10], making it uncertain whether the treatment was successful or whether re-infection with Mtb has occurred. Biomarkers that could accurately identify those at highest risk for progression to active TB disease are urgently needed to efficiently target TB preventive therapy [11]. In addition, biomarkers associated with effective response to TB preventive therapy could inform clinical decisions about whether further treatment is indicated. Transcriptional blood signatures may be scalable as point-of-care tests in TB endemic settings [12].

Several blood transcriptional signatures have been described that predict progression to active TB disease in infected people, distinguish between healthy controls and persons with active TB disease, and are associated with TB treatment status [13-19]. Signatures that identify people with incipient TB disease, defined as the period of infection in asymptomatic individuals with increasing Mtb bacillary burden prior to positive sputum conversion and progression to subclinical or active TB disease, may lead to novel intervention strategies [20]. The RISK6 signature, identified by Penn-Nicholson and colleagues [17], was the smallest and best performing set of transcripts that distinguished between adolescents who did and did not progress to active TB disease over a period of 12 months. Although elevated HIV load was associated with higher RISK6 scores, the signature performed similarly regardless of HIV status; however, other transcriptomic signatures have not performed as well among persons with HIV [13, 15]. The CORTIS-HR study [16] examined the performance of a related signature, RISK11, in people with HIV. Antiretroviral therapy (ART), virological suppression, and higher CD4 cell count were found to be associated with lower RISK11 scores, and there was

a trend toward lower scores in participants on isoniazid preventive therapy (IPT). However, the CORTIS-HR study did not collect information about prior use of IPT, or IPT adherence and completion during follow-up. To our knowledge, the association between IPT completion and TB transcriptional signature scores in people with HIV has not been assessed.

Although less commonly diagnosed than TB in endemic settings, NTMs can cause disease in those who are immunocompromised (including people with HIV) or have structural lung disease [21], and disseminated NTM infections in immunocompromised populations have a high mortality rate [22]. When NTMs are isolated from sputum cultures, it is often clinically challenging to distinguish a pathogenic versus colonizing role of the isolated mycobacterial species. Cowman compared transcriptomes in persons with pulmonary NTM infections and persons with other respiratory diseases, finding over 200 transcripts with differential expression between the two groups [23]. To our knowledge, the potential association between NTM sputum culture positivity and TB transcriptional signatures has not been studied.

In the present study, our objective was to examine the association between completion of IPT or presence of culturable NTMs from sputum with RISK6 scores. We also explored the relationship between IPT completion and other TB transcriptional signatures. The overall aim of our study was to examine assays that identify people with HIV who respond to IPT.

METHODS

Study design, setting, and participants

This study was cross-sectional, using clinical information and specimens collected at a single visit from participants of a prospective cohort study [24].

The parent study enrolled participants at two outpatient clinics that provide HIV and primary care for people with HIV in western Kenya where the estimated TB prevalence is 600 per 100,000 persons[25]. Eligible participants had an established diagnosis of HIV infection, were 18-70 years, and were either IPT-naïve or had received IPT more than 6 months prior to study enrollment. Per Kenya Ministry of Health Guidelines, participants were prescribed 6 months of isoniazid (IPT) [26]. Persons were excluded if unable to provide consent in English or Dholuo, if pregnant or incarcerated, or if unwilling to provide a home location. For our analyses, only participants with available IPT history were included.

This study was approved by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (P473/06/2016) and the University of Washington Institutional Review Board (STUDY00001236). All participants signed an informed consent.

Study procedures

At enrollment into the parent study, participants were interviewed to collect demographic information and HIV, TB, and IPT history. Information regarding specific TB treatment regimens was not available and treatment duration was based on self-report. Plasma HIV

RNA measurements and CD4 cell count were confirmed through medical record review; if plasma HIV RNA within 6 months was unavailable from the medical records, this was also tested at enrollment. IPT receipt and duration were confirmed through medical record review; IPT adherence was self-reported. Using 5 tuberculin units (0.1 ml) of purified protein derivative (RT23 solution, Sanofi Pasteur), TSTs were placed in all participants at enrollment and were read within 48-96 hours; a positive result was ≥5 mm induration. Blood was drawn and stored in PAXgene Blood RNA tubes for future use. Participants were instructed on sputum collection and a "spot" sample was collected at the time of enrollment. If this was unsuccessful, the participant was provided with a collection container and instructed to collect an early morning sputum specimen upon awakening 48 – 72 hours after enrollment. Sputum samples were subjected to mycobacterial culture (MGIT Manual Mycobacterial Growth System) and GeneXpert MTB/RIF testing. Isolates were identified as Mtb using the Capilia TB Test Kit; further speciation of NTMs was not performed. Participants diagnosed with sputum-culture positive for Mtb were referred for treatment of TB disease.

Laboratory assays

RNA extraction was performed on available PAXgene tubes using the MagMAX RNA Isolation Kit; samples were reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems). qRT-PCR was performed with 44 TaqMan primer-probe sets (Table S1, http://links.lww.com/QAD/C514) using the Fluidigm 96.96 dynamic array platform to determine the expression (cycle threshold, Ct) of transcripts of interest and calculate the RISK6[17], Thompson5[14], Suliman4[18], Maertzdorf4[27], and Sweeney3[13] signature scores. cDNA was first pre-amplified using a pool of the specific TaqMan primer-probe sets for 16 cycles with a 15 second denaturation step at 95°C and 4 minutes at 60°C. The pre-amplified cDNA samples were then loaded on the Fluidigm 96.96 dynamic array platform and amplified for an additional 40 cycles. Quality control procedures were performed by including a positive control on all Fluidigm plates and analyzing the minimum, maximum, and average values for signature scores by plate. The primer-probe sets were included to encompass the components of 5 previously described TB transcriptional signatures from peripheral blood associated with TB disease outcomes. Signature scores were calculated from raw Ct values in RStudio 1.4.1106 as previously described [13, 14, 17, 18, 28].

Data Analysis

The primary aim of the study was to examine the association between IPT (predictor) and RISK6 scores (outcome). Analyses involving the association between IPT status and TB transcriptional signature scores excluded participants with sputum culture-positive for Mtb (5 participants, 3 who received IPT, 2 who did not receive IPT). A sensitivity analysis was performed to analyze the effect of including these participants on the primary analysis. RISK6 scores, ranging from 0 to 1, were log transformed to correct for a non-normal distribution (assessed using Kolmogorov-Smirnov test) and to compare the geometric mean of RISK6 scores by IPT status using the Mann-Whitney U test. We performed multivariable linear regression that included hypothesized confounders of HIV viral load, duration of ART,

age, and sex. HIV viral load has been associated with RISK6 scores [16] [17]. We did not include CD4-cell count due to a high proportion of missing data, but a sensitivity analysis was conducted to assess the effect of including the CD4-cell count in the model. The potential for effect modification by HIV viral load was assessed by adding an interaction term to the model; the HIV viral load term was coded as either detectable or undetectable, with the threshold of 50 copies/mL. Effect modification was also evaluated by stratification according to viral load detection and assessing associations between RISK6 scores and IPT by subgroup using the Mann-Whitney U test. We analyzed the possible association between length of time since IPT receipt, a categorical variable coded as the year IPT was started, and RISK6 scores. For participants with a history of TB treatment, information regarding length of time since TB treatment was not available. The Mann-Whitney U and Kruskal-Wallis tests were used to test for differences in signature score distributions between two groups, or three or more groups, respectively. The Thompson5 and Suliman4 signatures were log transformed for multivariable regression; as the Maertzdorf4 and Sweeney3 signatures include negative values, they were not log transformed. Correlations were tested using Spearman's correlation coefficient (r).

RESULTS

Study Participants

We examined whole blood TB transcriptional signatures in 381 Kenyan adults with HIV who had an available IPT history [24]. The median age was 37 (IQR 31 - 45) years and 58.3% were women (Table 1). Of 381 participants, 330 (86.6%) had received IPT (completed a median of 1.1 years prior to study enrollment) while 51 (13.4%) participants were IPT-naive. Almost all (99.7%) participants were taking ART for HIV. People who had received IPT had been on ART for a median of 6.8 years while those who had not received IPT had been on ART for a median of 0.18 years ($p < 0.001$). The mean CD4 cell count was 434 ± 230 cells/mm^3 and was lower among participants without IPT history (316 ± 181 cells/mm^3) than those with IPT history (443 ± 230 cells/mm^3; $p = 0.005$). Of note, the CD4 cell count was missing for 30 of 51 (58.8%) participants without IPT history compared to 39 of 330 (11.8%) participants with IPT history. As 53 participants did not return to have the TST interpreted, results were available for 328 participants among whom 72 (21.9%) had a TST induration greater than 5mm. Five of 381 participants (1.3%) were sputum culture-positive for Mtb, 43 (11.0%) were positive for NTM, and 333 (87.0%) were culture-negative (Table 1). No participants with sputum culture-positive for Mtb reported TB symptoms, and 4 of 43 participants with sputum cultures positive for NTM reported a cough. Chest radiography was not performed.

Association between IPT status and RISK6 scores

Among the 376 participants without pulmonary TB, the mean RISK6 score among persons with a history of IPT (n = 327) was lower than among persons without (n = 49) a history of IPT (mean difference 0.11, 95% CI: 0.06-0.15; $p < 0.001$; Figure 1A). When adjusted for age,

sex, HIV viral load, and duration of ART, the mean RISK6 score was 52.8% lower (95% CI: 30.0-68.1%; $p < 0.001$) in participants with a history of IPT compared to those without IPT history. Prior IPT history remained significantly associated with RISK6 scores in a multivariable model regardless of whether the HIV viral load was included as a continuous or binary (detectable vs. undetectable) variable (Table 2A).

We assessed for effect modification by HIV viral load on the association between IPT status and RISK6 scores. RISK6 scores were higher among participants without prior IPT history compared to those with an IPT history in participants with a detectable (mean difference 0.17, 95% CI: 0.09, 0.23; $p < 0.001$) or undetectable (mean difference 0.05, 95% CI: 0.001, 0.09; $p = 0.047$) HIV viral load (Figure 2). However, statistically significant effect modification was not found (interaction term $p = 0.20$).

We performed a number of sensitivity analyses. There was no significant association between duration of ART and RISK6 scores ($p = 0.99$). When duration of ART was removed from the multivariable model, the mean RISK6 score was 54.0% lower (33.0-68.3%; $p < 0.001$) in participants who had received IPT compared to those without IPT history. We found that when CD4 count was also included in the multivariable model, people who had received IPT had a mean RISK6 score 49.5% lower (7.7-72.4%; $p = 0.03$) than those without IPT history. When we included participants who were diagnosed with TB (n=5), among all participants with a history of IPT the mean RISK6 scores were 0.10 lower (unadjusted) and 48% lower (adjusted) than those without prior IPT. Year of IPT receipt was not associated with RISK6 scores when included as a continuous variable ($p = 0.35$) (Figure 1B). However, when categorized by IPT completion from 2007-2015 (n = 86) and 2016-2017 (n = 251), participants who received IPT more recently had an average RISK6 score that was 0.03 lower (0.003-0.05; $p = 0.02$).

Association between positive sputum cultures for NTM and RISK6 scores

The mean RISK6 score in persons with a positive sputum culture for NTM (n=43), a positive sputum culture for Mtb (n=5), and negative sputum cultures (n=333) was not different ($p = 0.35$, Figure 1C). There was no statistically significant difference in RISK6 scores when comparing participants with a positive sputum culture for NTM only to those with a negative sputum culture ($p = 0.62$). In the multivariable regression model adjusting for age, sex, HIV viral load, and duration of ART, the difference between RISK6 scores by sputum culture result remained insignificant, with an estimated geometric mean RISK6 score 1.11 times higher (95% CI: 0.75, 1.64; $p = 0.60$) in participants with a positive sputum culture for NTM compared to those with a negative sputum culture.

Comparison of five TB transcriptional signatures

In exploratory analyses, we evaluated the associations between IPT status and four additional TB transcriptional signatures: Thompson5, Suliman4, Maertzdorf4, and Sweeney3 (Table 2B). The RISK6, Maertzdorf4, and Sweeney3 signatures were most highly correlated with

correlation coefficients between 0.59 and 0.72 (Figure 3). The Thompson5 signature had the weakest association with other signatures (r -0.07 to 0.21). When adjusting for age, sex, and HIV viral load, the Maertzdorf4 (mean difference 0.80, 95% CI: 0.40, 1.21; p = 0.0001) and Sweeney3 (mean difference 0.47, 95% CI: 0.08, 0.86; p = 0.018) signature scores were significantly lower in those who had received IPT compared to participants who had not received IPT. No differences were seen for the Suliman4 or Thompson5 signatures (Table 2B). Of the five transcriptional signatures, only the Maertzdorf4 signature demonstrated a significant association with NTM culture positivity (Supplementary Table 2, http://links.lww.com/QAD/C514). Participants with a positive NTM culture had, on average, a Maertzdorf4 score that was 0.48 higher compared to those with a negative sputum culture (95% CI: 0.08, 0.89; p 0.02).

DISCUSSION

We investigated the association between prior receipt of IPT and RISK6 scores among people with HIV and found that IPT was associated with a lower mean RISK6 score. We hypothesized that RISK6 scores would be lower among people who had received IPT, because IPT has been shown to prevent TB disease, and lower RISK6 scores have been associated with lower risk of incident TB disease. The association remained significant after adjusting for age, sex, HIV viral load, and duration of ART use.

The CORTIS trial, which randomized participants without HIV with positive RISK11 scores to either receive TB preventive treatment or active surveillance only, did not demonstrate a significant reduction in TB incidence in the treatment arm after 15 months of follow-up [29]. One explanation for these results is that the 3-month isoniazid/rifapentine preventive treatment regimen was not sufficient to prevent progression from incipient to active TB disease. Although our data suggest that IPT decreases the RISK6 score, other unmeasured factors critical for TB progression may not be abrogated by treatment. Our findings of lower RISK6 score among participants who had already completed IPT are consistent with the CORTIS-HR trial where people with HIV who were RISK11 negative were more likely to have received IPT at enrollment compared to those who were RISK11 positive [16]. However, our findings differ from a longitudinal analysis of CORTIS[30] which found no difference in RISK11 conversion or reversion frequencies between those on IPT at enrollment, those who started IPT during a 3-month period, and IPT-naïve participants. Potential explanations for this discordance in results could be that RISK11 responses require time after the completion of IPT or differences in TB prevalence between the study settings. We found that the reduction in RISK6 scores due to IPT was independent of HIV viral load, duration of ART, age, or sex. While there was a large difference in time on ART by IPT status, there was no significant association between ART duration and RISK6 scores in unadjusted and adjusted analyses. We hypothesized that a longer period of time since IPT completion would be associated with higher RISK6 scores due to waning of a protective effect of IPT and/or re-infection with Mtb [31] [32]. When participants were grouped by recent (2016-2017) vs remote (2007-2015) IPT completion, we found that remote IPT completion was associated with a higher mean RISK6 score. Together, our data indicate that IPT is associated with lower TB

transcriptional signature scores in people with HIV and are consistent with a model that isoniazid is efficacious for treatment of Mtb infection.

We found an association between the Maertzdorf4 signature and NTM sputum culture positivity, but the other signatures had no significant association with NTM sputum culture positivity. One possible explanation is that the transcripts included in these TB signatures are not differentially expressed in active NTM infection, which may exhibit a different transcriptional profile. A previous study demonstrated differential expression of 25 genes in people with pulmonary NTM infection versus other respiratory diseases [23]; none of these genes are included in the five TB signatures we measured. Another possibility is that some of the participants with a positive NTM sputum culture were colonized or that laboratory contamination occurred. The diagnosis of pulmonary NTM disease requires clinical findings or chest imaging compatible with NTM infection; most participants in our study did not have symptoms compatible with NTM or TB disease, and none had chest imaging available. Additionally, one positive sputum culture alone is not sufficient to make the diagnosis of NTM lung disease [33]. Interestingly, the Maertzdorf4 signature did not discriminate well between TB and sarcoidosis [27], suggesting that this signature may be associated with disorders involving granulomatous inflammation, including NTM disease. Further identification of the NTM isolated from positive sputum cultures was not available, so some of the participants may have had sputum cultures positive for NTM species that rarely cause disease. Additional studies including persons with confirmed NTM disease are needed to further describe the association between NTM and TB transcriptional signatures.

A study of RISK6 performance that included people with HIV found an AUC of 92.6% (95% CI, 86.8-98.5) for discriminating between persons with TB and asymptomatic controls [34], all of whom were HIV-infected. The participants with TB were recruited in South Africa from healthcare clinics and were newly diagnosed with TB based on positive sputum GeneXpert MTB/RIF of liquid culture. Study results strongly suggested that disease severity in patients with TB impacts the performance of transcriptomic signatures, with higher disease burdens improving RISK6 performance[34]. In our study, the five participants diagnosed with pulmonary TB were symptom free, had sputum-smear negative disease, 4 of the 5 were GeneXpert MTB/RIF negative, and the one GeneXpert positive result was low on semiquantitative grading. Although the poor performance of RISK6 in our study for discriminating persons with TB is not clear, it may relate in part to a presumed lower bacillary burden compared to the South Africa cohort.

Our study had several limitations. Only one sputum sample was collected from each participant which may have led to underdiagnosis of TB as multiple samples increase detection. Adherence to IPT was based on self-report, which may be susceptible to recall or social desirability bias. This could have significantly impacted our results because receipt of IPT was our main predictor of interest. However, since participants who were less adherent to their prescribed IPT would be expected to have higher RISK6 scores, this would likely have attenuated the association we observed. Another potential source of bias is that persons who are more likely to initiate and maintain IPT may be more likely to also be adherent to

HIV treatment. We attempted to mitigate against this by adjusting for HIV viral load in our model, but this may not have been sufficient to fully address this source of bias. An additional limitation is that, given our study design, we cannot conclude that there is a causal relationship between IPT receipt and lower RISK6 scores. We also do not know whether the difference in mean RISK6 scores by IPT status would have been associated with different clinical outcomes, such as TB incidence, because of the cross-sectional study design. Respiratory viral and bacterial organisms were associated with a higher RISK11 score in persons with and without HIV [30]. The frequency of chronic viral infections (e.g. hepatitis B and C) may differ by a person's engagement with healthcare (e.g. IPT receipt). We did not evaluate for the presence of chronic or acute viral infections in the study participants.

In conclusion, we demonstrated that prior receipt of isoniazid preventive therapy was associated with a lower mean RISK6 score in a cohort of Kenyan people with HIV. Prospective studies measuring RISK6 and other TB transcriptional signatures before and after TB preventive therapy are needed to describe the nature of their relationship more definitively. Inclusion of a follow up period monitoring for incident TB might also allow for an assessment of whether changes in TB transcriptional signatures scores could be used to monitor successful response to IPT.

Acknowledgements: We would like to thank the participation of the individual study subjects and their families. We thank Lamar Fleming at the Fred Hutchinson Cancer Research Center for assistance with Fluidigm assays.

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Figure Legends:

Figure 1: RISK6 scores by IPT status, year of IPT receipt, and sputum culture result. RISK6 scores were calculated and stratified by history of IPT use (A), year of IPT receipt (B), or NTM sputum culture positivity (C). IPT, isoniazid preventive therapy; Mtb, *Mycobacterium tuberculosis*; NTM, non-tuberculous mycobacteria

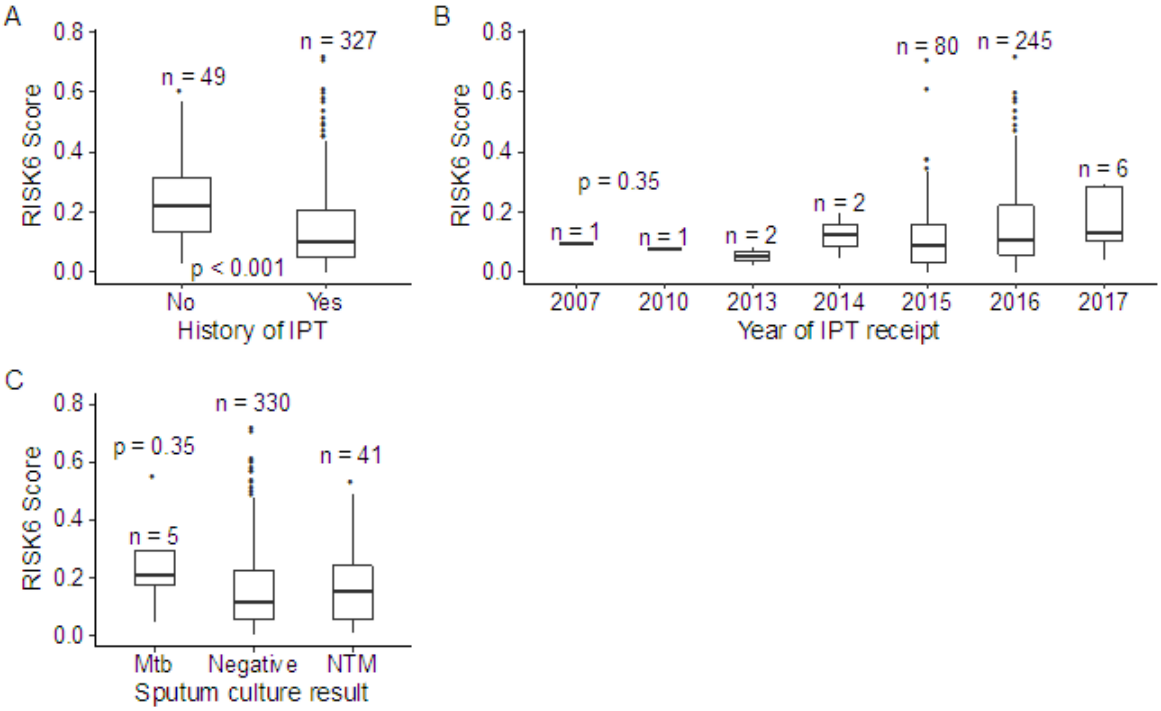

Figure 2: RISK6 scores versus IPT status by HIV viral load. RISK6 scores stratified by history of IPT use and presence of a detectable HIV viral load. Left panel, HIV viral load detectable (>50 copies/mL); right panel, HIV viral load undetectable (<50 copies/mL)

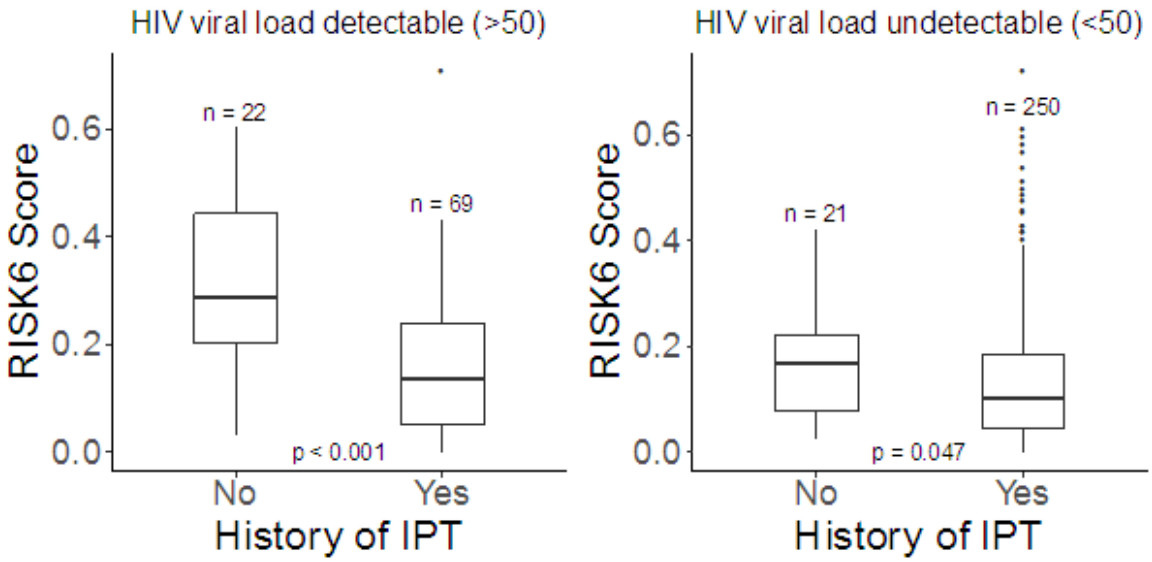

Figure 3: Correlation between TB transcriptional signatures. Pearson's correlation coefficients (r) between each of the five TB transcriptional signatures are depicted numerically as well as with a colored heat map ranging from -1 (blue) to +1 (red).

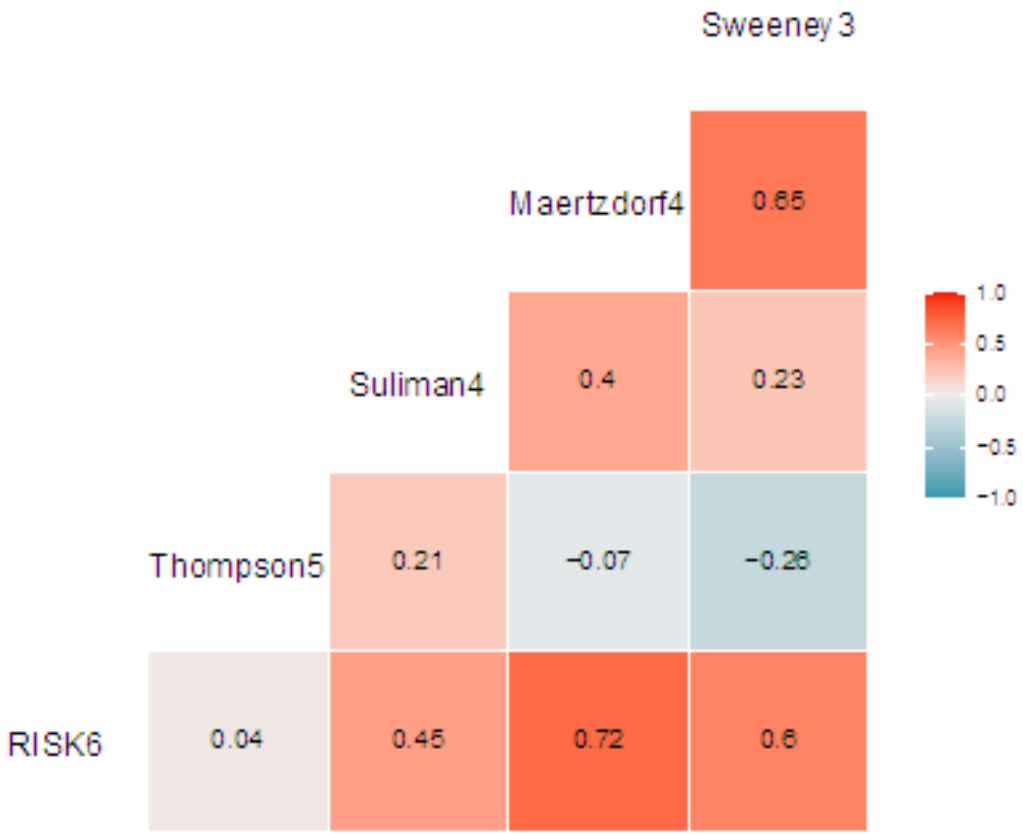

Table 1. Characteristics of participants by IPT status

Characteristic	Overall, N = 381[1]	No History of IPT, N = 51[1]	Prior History of IPT, N = 330[1]	p-value[2]
Age, in years (median (IQR))	37 (31 - 45)	31 (27 – 40.5)	38 (31.2 - 46)	0.011
Sex				0.5
Female	222 (58.3%)	32 (62.7%)	190 (57.6%)	
Male	159 (41.7%)	19 (37.3%)	140 (42.4%)	
BMI	23.9 (20.9)	23.0 (5.4)	24.0 (22.4)	0.5
Tobacco status				0.5
Never	345 (90.6%)	47 (92.2%)	298 (90.3%)	
Yes, currently	10 (2.6%)	0 (0%)	10 (3.0%)	
Yes, in past but not now	26 (6.8%)	4 (7.8%)	22 (6.7%)	
Drinks alcohol (n = 380)				0.5
No	332 (87.4%)	43 (84.3%)	289 (87.8%) (n = 329)	
Yes	48 (12.6%)	8 (15.7%)	40 (12.2%)	
Diabetes (n = 368)				0.14
No	367 (99.7%)	49 (98.0%) (n = 50)	318 (100%) (n = 318)	
Yes	1 (0.3%)	1 (2.0%)	0 (0%)	
Taking ART (n = 378)				0.13
No	1 (0.3%)	1 (2.0%) (n = 50)	0 (0%) (n = 328)	
Yes	377 (99.7%)	49 (98.0%)	328 (100%)	
Duration of ART use, in years (median (IQR)) (n = 376)	5.8 (2.4 – 9.2)	0.1 (0.04 – 1.8) (n = 47)	6.7 (3.4 – 9.6) (n = 329)	<0.001
Most recent CD4 count (n = 312)	434.6 (229.6)	315.9 (181.2) (n = 21)	443.2 (230.6) (n = 291)	0.005
Detectable HIV viral load?[16]				<0.001
No	278 (75.1%)	24 (52.2%)[17]	254 (78.4%)[18]	
Yes	92 (24.9%)	22 (47.8%)	70 (21.6%)	
HIV viral load (n = 370)	14,745.8 (181,552.8)	98,012.2 (507,422.3) (n = 46)	2,924.0 (25,623.7) (n = 324)	0.2
Current ART regimen (n = 375)				<0.001
AZT + 3TC + EFV	4 (1.1%)	1 (2.0%) (n = 49)	3 (0.9%) (n = 326)	

AZT + 3TC + LPV/r	7 (1.9%)	2 (4.1%)	5 (1.5%)	
AZT + 3TC + NVP	34 (9.1%)	1 (2.0%)	33 (10.1%)	
TDF + 3TC + EFV	182 (48.5%)	41 (83.7%)	141 (43.3%)	
TDF + 3TC + LPV/r	7 (1.9%)	0 (0%)	7 (2.1%)	
TDF + 3TC + NVP	122 (32.5%)	2 (4.1%)	120 (36.8%)	
Other	19 (5.1%)	2 (4.1%)	17 (5.2%)	
WHO stage of HIV infection				0.005
Stage 1	112 (29.4%)	24 (47.1%)	88 (26.7%)	
Stage 2	164 (43.0%)	15 (29.4%)	149 (45.2%)	
Stage 3	96 (25.2%)	9 (17.6%)	87 (26.4%)	
Stage 4	9 (2.4%)	3 (5.9%)	6 (1.8%)	
History of TB (n = 378)				0.7
No	320 (84.7%)	44 (88.0%) (n = 50)	276 (84.1%) (n = 328)	
Yes	58 (15.3%)	6 (12.0%)	52 (15.9%)	
Presence of TB symptoms (n = 50)				0.11
No	333 (87.6%)	41 (80.4%)	292 (88.8%) (n = 329)	
Yes	47 (12.4%)	10 (19.6%)	37 (11.2%)	
TST induration (n = 328)				0.6
<5 mm	256 (78.0%)	37 (82.2%) (n = 45)	219 (77.4%) (n = 283)	
>5 mm	72 (22.0%)	8 (17.8%)	64 (22.6%)	
Sputum culture result				0.081
Mycobacterium tuberculosis	5 (1.3%)	2 (3.9%)	3 (0.9%)	
Negative	333 (87.4%)	41 (80.4%)	292 (88.5%)	
Non-tuberculous mycobacteria (NTM)	43 (11.3%)	8 (15.7%)	35 (10.6%)	

[1] Mean (SD); n / N (%)

[2] Welch Two Sample t-test; Fisher's exact test

BMI, body mass index; ART, antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; TST, tuberculin skin test

Table 2A. Bivariate, multivariate, and sensitivity analyses of RISK6 scores and covariates

	Bivariate analysis		**Multivariate analysis (VL continuous)**		**Multivariate analysis (VL binary*)**	
Variable	RISK6 coefficient	p-value	RISK6 coefficient	p-value	RISK6 coefficient	p-value
IPT receipt	0.44	<0.001	0.46	<0.001	0.48	<0.001
Age	0.99	0.37	1.00	0.62	1.00	0.62
Male Sex	0.79	0.06	0.80	0.08	0.77	0.04
HIV viral load (continuous)	1.00	0.07	1.00	0.30	N/A	N/A
HIV viral load (binary)	1.39	0.02	N/A	N/A	1.29	0.08
Duration of ART	0.99	0.99	1.01	0.23	0.99	0.39

*VL binary means HIV viral load was characterized as either >50 copies/mL or <50 copies/mL.

Table 2B. Summary of bivariate and multivariate models of each signature by IPT status

Signature	**Unadjusted mean difference**	**Adjusted coefficient***	**Adjusted coefficient****
RISK6 (95% CI) p	0.10 (0.06, 0.15) < 0.001	0.46 (0.32, 0.67) < 0.001	0.52 (0.36, 0.75) < 0.001
Thompson5 (95% CI) p	-0.02 (-0.050 0.01) 0.17	1.04 (0.98, 1.15) 0.17	1.06 (0.98, 1.15) 0.17
Suliman4 (95% CI) p	0.01 (-0.05, 0.08) 0.76	0.91 (0.73, 1.13) 0.41	0.91 (0.73, 1.13) 0.41
Maertzdorf4 (95% CI) p	0.76 (0.37, 1.16) < 0.001	-0.81 (-1.21, -0.04) < 0.001	-0.84 (-1.25, -0.44) < 0.001
Sweeney3 (95% CI) p	0.59 (0.27, 0.91) < 0.001	-0.47 (-0.86, -0.08) 0.02	-0.52 (-0.92, -0.13) 0.01

*Adjusted model included age, sex, and HIV viral load (continuous) as covariates

**Adjusted model included age, sex, and HIV viral load (binary) as covariates