

Evaluating the RISK6 signature as a TB diagnostic using a novel laboratory-based Tuberculosis host immune response assay (TB-HIRA)

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Introduction

RISK6, a human-blood transcriptomic signature, has shown promise as a non-sputum-based tool for tuberculosis (TB) screening, triage and treatment monitoring among immunocompetent adults in high TB burden settings [1]. The TB-HIRA is a one-step multiplex qRT-PCR reaction that has been developed to streamline and accelerate the original multi-step singleplex protocol for measuring signature expression.

Aims

- To determine concordance of TB-HIRA with the original RISK6 singleplex assays.
- To determine performance of RISK6 scores using both methods to discriminate between TB disease, QuantiFERON-TB Gold (QFT) positive/latent TB (LTBI) individuals, and controls.

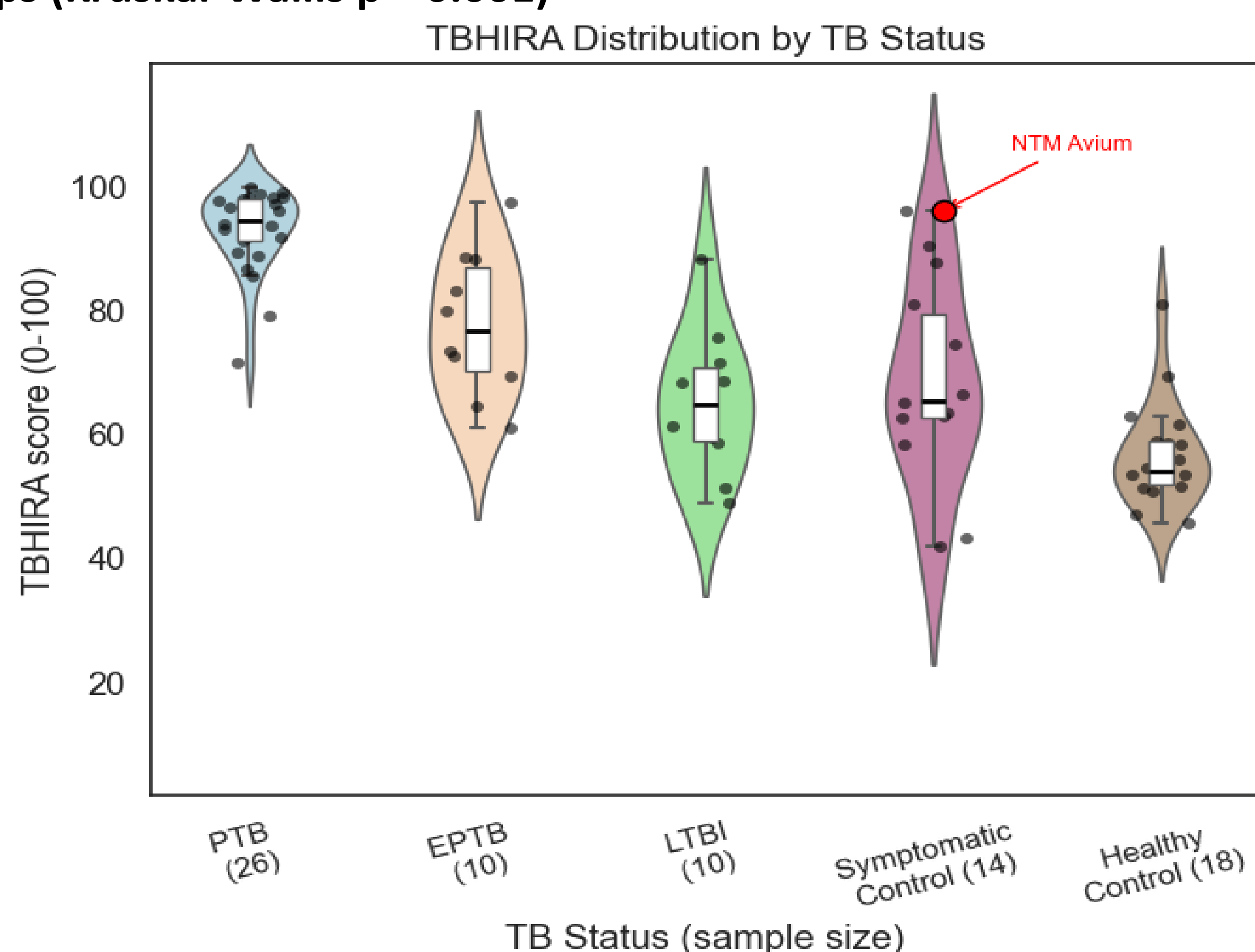
Methods

- Venous blood samples stored in Tempus™ RNA tubes were collected from:
- 26 individuals with drug-sensitive **Pulmonary TB (PTB)** – including a subgroup of 20 patients with longitudinal sampling up to 24 weeks of treatment
 - 10 with drug-sensitive **Extrapulmonary TB (EPTB)**
 - 10 with **Latent TB infection (QFT-positive)**
 - 14 **Symptomatic controls (resp. inpatients)**
 - 18 **Healthy controls (QFT-negative)**

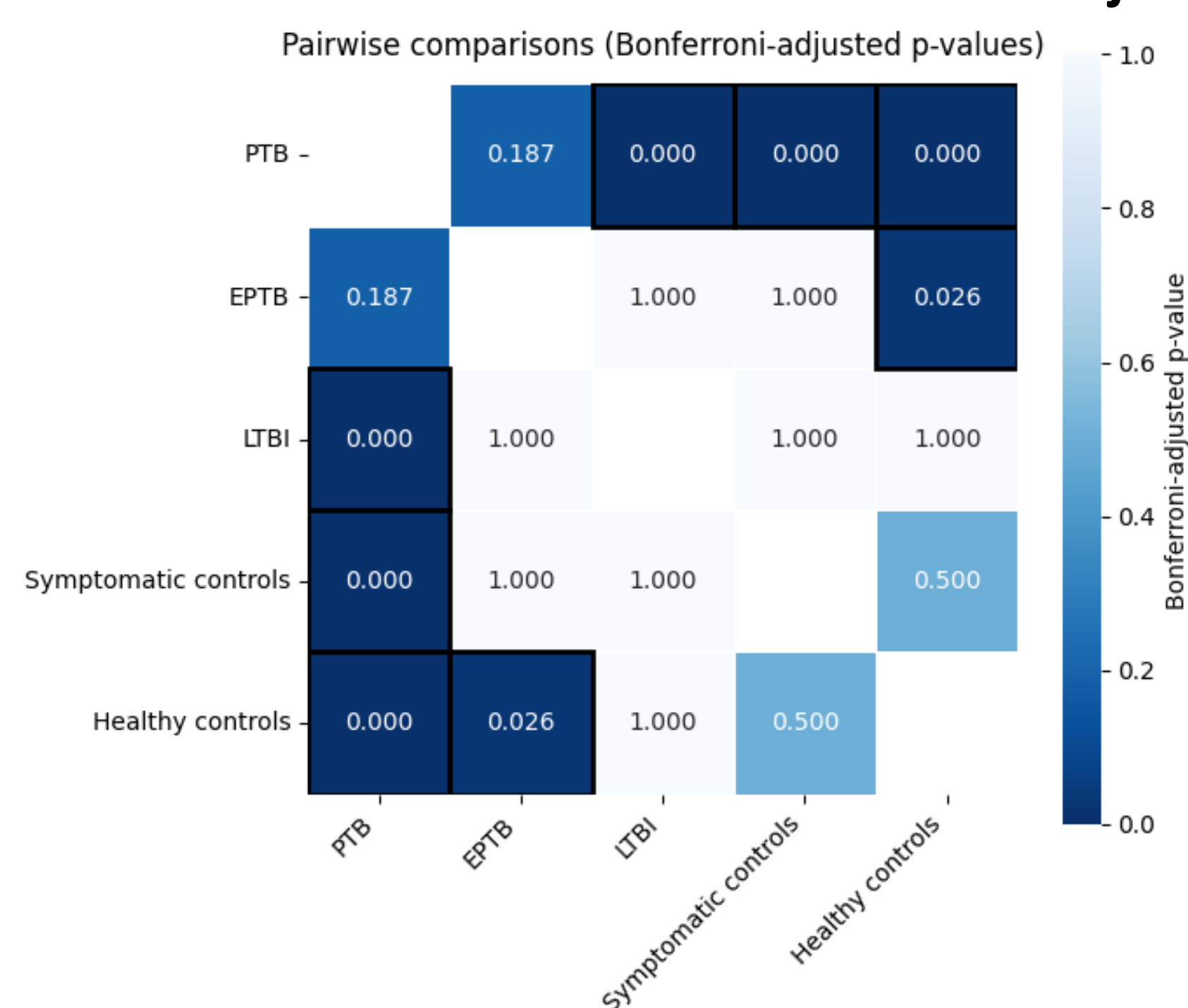
Specimens were blinded and randomised before RNA extraction and throughout analysis. Both TB-HIRA and RISK6 assays were run in RT-PCR/PCR triplicates per sample. RISK6 scores were calculated in R using the risk6.R script [2] and other statistical analyses were performed in Python v3.13.

Results

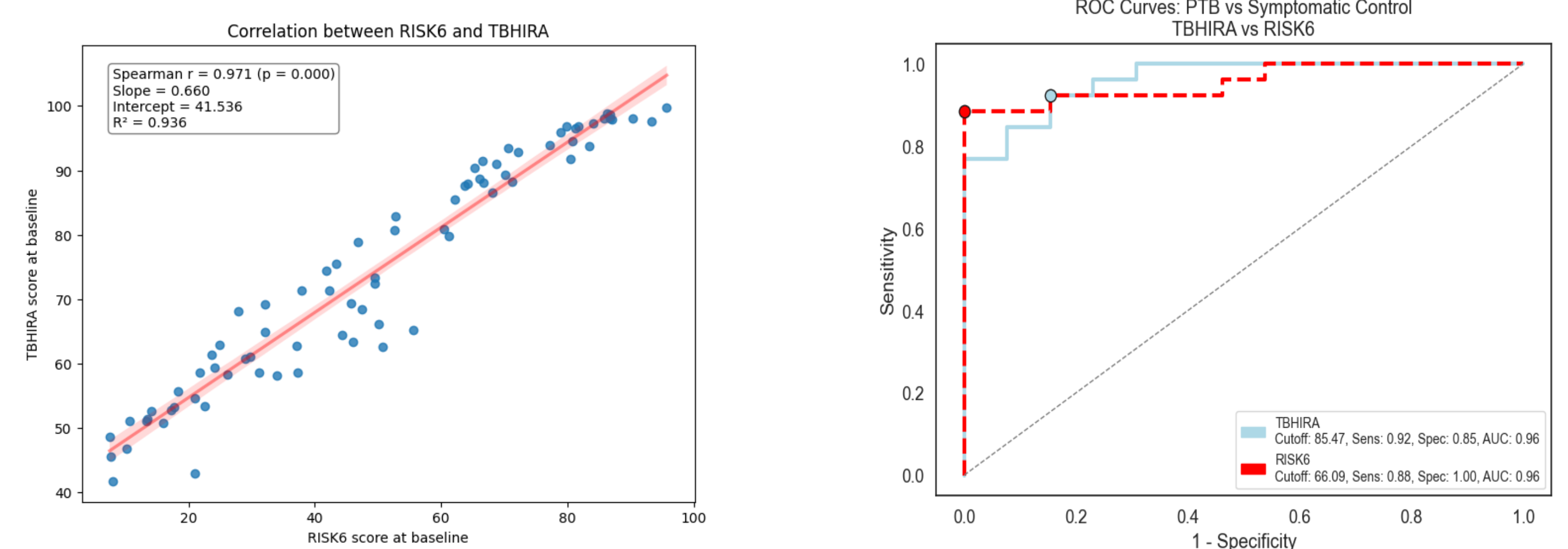
1. The median baseline TB-HIRA score differed significantly across study groups (Kruskal-Wallis $p < 0.001$)



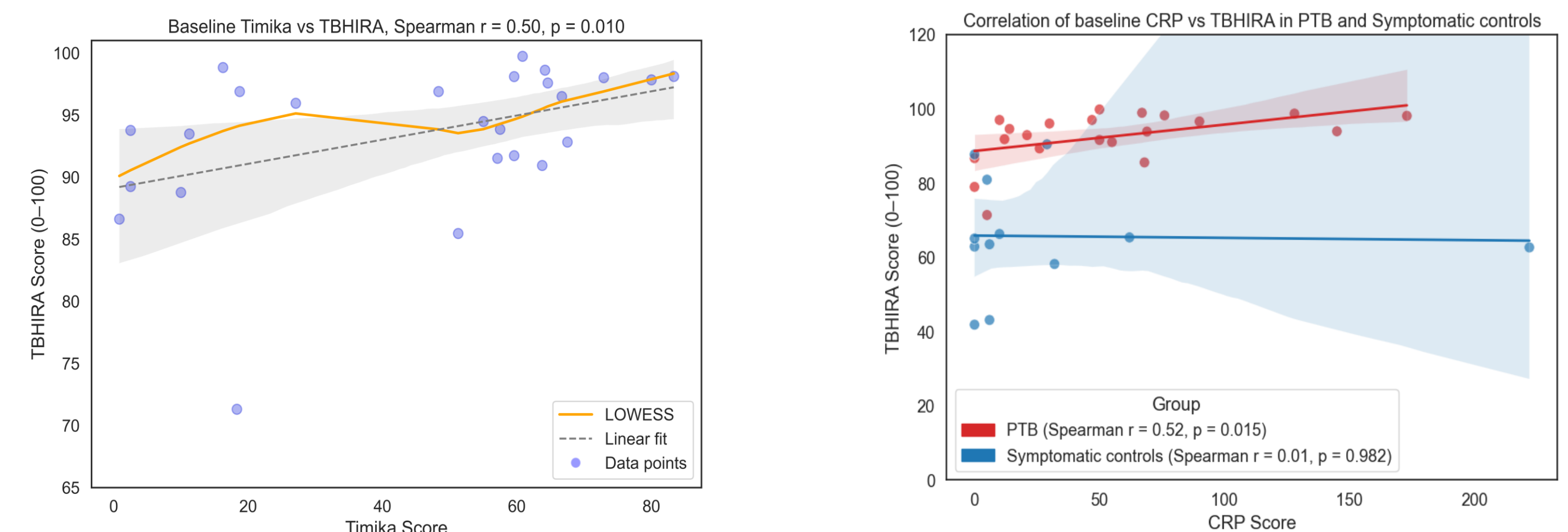
Contingency heatmap of Bonferroni-adjusted p-values for pairwise inter-group comparisons. Darker shades indicate smaller adjusted p-values.



2. Results from RISK6 singleplex and TB-HIRA multiplex assays are well correlated and both have excellent discrimination as a potential respiratory triage test between patients with pulmonary TB and non-TB disease

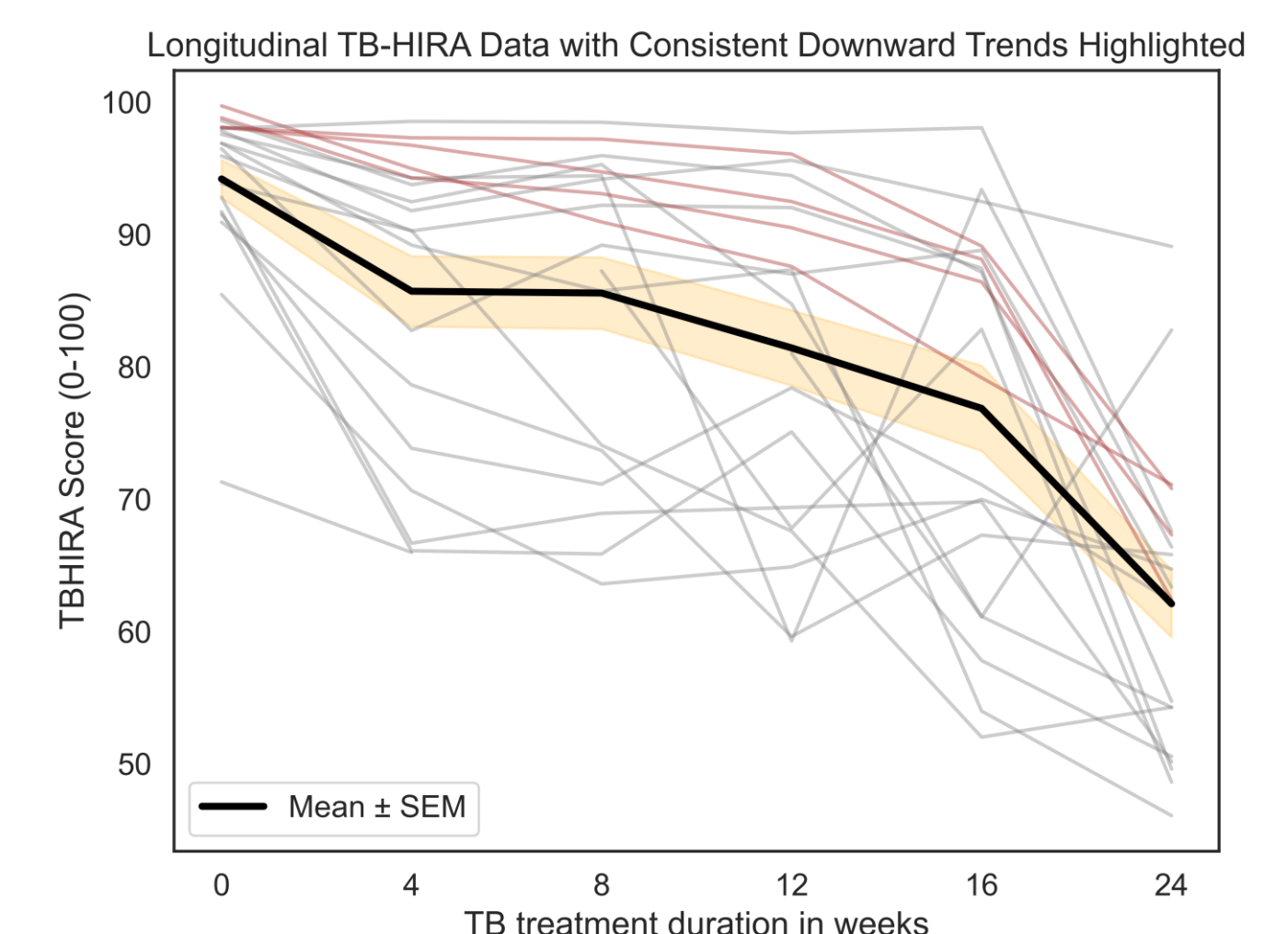


3. TB-HIRA correlates with markers of disease severity (Timika score and CRP) in PTB cases, but is independent of CRP in symptomatic controls



4. TB-HIRA scores showed a variable but overall downward trend over time on treatment in participants with PTB

The median TB-HIRA score was 35% lower at 6 months compared with baseline (62.89 (IQR 53.34 – 67.38) versus 96.69 (IQR 92.55 – 98.03); $p < 0.001$).



Conclusion

- Strong concordance between the multiplex TB-HIRA and original singleplex RISK6 assays, and robust discrimination between patients with PTB and symptomatic controls shows the potential of RISK6 signature (and TB-HIRA) as a non-sputum respiratory triage test.
- TB-HIRA scores in patients with pulmonary TB were correlated with disease severity (expressed by CRP and Timika score).
- Despite variable trajectories, the overall decrease in longitudinal TB-HIRA scores offers potential as a treatment response biomarker.
- TB-HIRA was significantly raised in the single case of NTM Avium, and data from larger well-phenotyped cohorts of TB, NTM and non-TB participants is required.

References:

- Bayaa, R., et al., Multi-country evaluation of RISK6, a 6-gene blood transcriptomic signature, for tuberculosis diagnosis and treatment monitoring. Scientific Reports, 2021. 11(1).
- Penn-Nicholson, A., et al., RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response. Scientific Reports, 2020. 10(1).