T CELL BIOMARKERS FOR DIAGNOSIS OF TUBERCULOSIS: CANDIDATE VALIDATION AND DESIGN OF A NOVEL SIMPLIFIED INTRA-CELLULAR CYTOKINE STAINING ASSAY FOR CLINICAL TRANSLATION

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Background
Current diagnosis of tuberculosis (TB) disease is dependent on detection of *Mycobacterium tuberculosis* (Mtb) in sputum. Distinction between people with TB disease and those with asymptomatic Mtb infection is essential to define treatment strategy, and remains challenging whenever Mtb is not detectable. Several studies have independently identified diagnostic biomarkers based on Mtb-specific T cell phenotype or functional profiles, upon stimulation of peripheral blood mononuclear cells. The performance of these biomarkers has not been directly compared in the same cohort, nor have these biomarkers been validated using a whole blood assay, which would be easier to implement for diagnostic purposes. The objectives of our study were to compare published candidate T cell-based diagnostic biomarkers for TB, to identify novel marker combinations that improve diagnostic performance, and to design a simplified assay for clinical translation.

Methods
We recruited 25 adults with microbiologically-confirmed TB disease (GeneXpert+) and 25 healthy adults with asymptomatic Mtb infection (QuantiFERON-TB Gold test+). Whole blood was stimulated with no antigen, whole cell mycobacteria (BCG), a selection of mycobacterial immunodominant epitopes or Mtb-specific peptides (QuantiFERON TB antigens) for 12 hours. Samples were fixed, cryopreserved and stored until batched flow cytometry analysis. Cells were stained with the following antibodies: CD3, CD4, CD8, IFNγ, TNFα, IL2, CD27, HLA-DR and analysed on a LSRII flow cytometer, including reference beads for absolute counts.

Results
Overall, among previously published T-cell biomarkers, expression of HLA-DR on Mtb-specific IFN-γ+ CD4 T cells gave improved discrimination of active TB from latent infection as compared to CD27 expression or cytokine patterns alone, with Area Under the Curve (AUC) of 0.98, 91% sensitivity and 90% specificity. In comparison, novel combinations of cytokines and expression of phenotypic markers on Mtb-specific T cells yielded an equivalent AUC, with >95% sensitivity and specificity. Candidate biomarkers based on whole blood stimulation with mycobacterial antigens and minimal sets of 4 flow cytometric markers were identified, that were interpretable in >80% participants and yielded >0.95 AUC for TB diagnosis, with >95% sensitivity and specificity. The choice of antigen stimulant had minimal effects on assay performance and mostly affected the rates of interpretable results, based on responsiveness.

Conclusions
We identified novel biomarker combinations that can be measured with a field-friendly whole blood assay using a minimal 4-antibody flow cytometry panel, which yielded equivalent diagnostic performance for TB as validated biomarkers that require more complex panels. Active TB patients could be distinguished from persons with latent infection with >95% accuracy. Our data support further development of a simple whole blood diagnostic test for TB for clinical translation.