Screening for candidate biomarkers of TB in stimulated blood: another step in the quest for a test?

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It is estimated that each year approximately one-third of all tuberculosis (TB) cases remain either undiagnosed or unreported.[1] Countries are intensifying their efforts to narrow the gaps between TB incidence and notifications through improving surveillance, diagnosis and access to healthcare. A major impediment in this effort is that diagnostic tests are not sufficiently sensitive or specific to accurately identify all TB cases. New tools are urgently needed for the diagnosis of TB to achieve global TB control.

The WHO considers non-sputum biomarker-based tests for rapidly diagnosing TB to be of utmost priority.[1] Human host-based biomarkers have great potential, as they can enhance confirmatory diagnosis particularly in sputum negative and/or culture negative adult, and paediatric cases.[2] In children, the paucibacillary nature of the disease and the more common extrapulmonary presentations are the main reasons why microbiological confirmation is achieved in only a small proportion of children that receive TB treatment.[3]

Host immune biomarkers have been widely used in tests for diagnosing TB infection, such as the interferon-gamma (IFN-γ) release assays (IGRAs), which are based on ex-vivo stimulation of host blood with Mtb-specific antigens. Stimulation of blood or host cells with Mtb antigens gives rise to amplified quantifiable antibody, cytokine or cellular immune responses (reviewed in the study of Yong *et al*[4]).

Samples from the IGRA assay tubes are routinely used to measure IFN-γ, but can also be used for additional analyses, such as measuring gene expression, protein abundance or cellular markers. Mtb antigen-specific cellular responses can assist in the detection of Mtb infection, however they cannot efficiently distinguish active TB from latent tuberculosis infection (LTBI) and other diseases on the background of a LTBI, meaning that they are inadequate for diagnosing active TB. Nevertheless, quantifying additional host response proteins in the IGRA supernatant, with or without the combination of IFN-γ, has been shown to enhance the assay's diagnostic potential.[5]

Several combinations of proteins have been identified as diagnostic signatures in the supernatant of the QuantiFERON-TB Gold In-Tube (QFT-GIT), either using candidate markers measured on Luminex technology to distinguish active TB from LTBI and other diseases[6] or hypothesis-free highly multiplexed SOMAscan aptamer-based proteomics technologies to distinguish active TB from LTBI.[7]

In this edition of Thorax, Yang and colleagues[8] present an eight-protein host signature capable of distinguishing between adult patients with TB disease-both culture confirmed and probable TB-, and those with LTBI, non-TB pneumonia, as well as healthy controls. A total of 630 subjects were recruited in a high-burden TB clinical setting in Shenzen, China. In-house whole blood cultures stimulated with pooled Mtb peptides[9] were quantified in a novel high-throughput antibody-based microarray assay covering 640 human proteins. A two-round screening strategy was followed. Out of the 640 host proteins measured in the Biomarker Screening cohort (n=160), 16 were found to be significantly differentially abundant between TB and non-TB cases, LTBI and healthy controls and were subsequently developed into a smaller custom made multiplexed antibody array. Out of the 16 proteins measured in the model establishment (training) and test cohort (n=368), eight (I-TAC, I-309, MIG, Granulysin, FAP, MEP1B, Furin and LYVE-1) comprised the final signature that was then evaluated in the prospectively recruited prediction cohort (n=102). A series of models for prediction were assessed including random forest algorithm (RF), linear discriminant analysis and support vector machines, with the RF model being superior. The random forest-based eight protein signature had 100% specificity and sensitivity in the training cohort, 83% specificity and 76% sensitivity in the test cohort and 84% specificity and 75% sensitivity in the prediction cohort.

The study provides new insights in the diagnostic potential of a small proteomic signature obtained from stimulated whole blood cultures, which was derived after screening 640 human host proteins. Four out of the eight human host proteins in the final signature had not been previously reported as potential TB biomarkers. To facilitate further mining of the data, the authors have made the assay screening data publicly available to the scientific community. A direct comparison of the in-house culture system with the QFT-GIT assay in a small number of cases showed that the in-house culture system had better sensitivity and specificity than the QFT-GIT assay. The use of the particular Mtb-specific peptide pool for stimulation could have resulted in a more enhanced release of TB-specific host protein response.

Despite the novelties in the biomarker discovery process and the interesting findings, additional work and further validation is needed to fully determine the diagnostic potential of the approach. First, lower performance of the prediction cohort in comparison to the training cohort is noteworthy. It could be attributed to some level of overfitting in the signature discovery process or in the model implementation, but it could also mean that the prospective prediction cohort reflects a more heterogeneous real-world population of patients with symptoms of TB. Second, the fact that heterogeneous groups were employed as a single comparator group (LTBI, non TB pneumonia and healthy controls), may have inflated the performance and may lead to confusion over the intended use of such a test, if developed: screening tool or confirmatory diagnostic test? A combination of different proteins or implementation of different models may show better discrimination of TB from LTBI and TB from non-TB pneumonia, if discovered separately. Finally, the generalisability of the signature needs to be further established as it was derived in a Chinese HIV-uninfected population. Previous host biomarker studies have shown that the performance of signatures can vary across different populations,[10] and may be influenced by immunosuppression, due to HIV for example.

As host proteins are easier to measure than RNA and metabolites, a host protein biomarker test holds promise. However, an assay that requires culture will inevitably be less applicable in low resource point-of-care settings. The benefit of stimulation remains to be shown, as previous studies have highlighted that the effect of stimulation has not always aided in discrimination.[11] Furthermore, the comparative performances of the QFT-GIT and the in-house assay need to be further investigated with larger sample sizes.

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Despite the above limitations, the study from Yang and colleagues[8] is an important advance in the field of host-based proteomic TB biomarker tools. Optimisation of existing screening and diagnostic strategies in conjunction with new research is needed to identify better centralised, near point-of-care and point-of-care tests for TB.

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