LTBI-associated Immuno-diagnostic Test Responses (Standardized IGRAs and the TST) as Biomarkers of Incipient TB: Fruitful or Futile?

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Latent TB infection (LTBI) constitutes part of the tuberculosis (TB) disease spectrum (1, 2). The diagnosis and treatment of LTBI is important as global eradication targets will not be attainable without treating LTBI (3).These considerations also apply to drug-resistant TB, which threatens to derail control efforts (4). The WHO has recently recommended that close contacts of index cases of TB, even in TB endemic countries, should be considered for LTBI treatment (even if they are HIV-uninfected or not children) (5). However, the diagnosis of LTBI is challenging. Unlike with active TB, in humans there is no microbiological or histopathological reference standard for LTBI, and one can only infer the potential presence of LTBI using immunodiagnostic tests, which enumerate the magnitude of relatively antigen-specific Th1 effector T-cell responses (6). However, it is epidemiologically well-recognised that only a small proportion of individuals with presumed LTBI (~5 to 10%) will progress to active TB over a lifetime (7).Thus, the more important public health question is whether, and how, we can accurately target treatment by identifying individuals who are most likely to progress to active TB. This state is broadly defined as 'incipient TB' and is characterised by a lack of TB-related symptoms and appropriate chest radiographic abnormalities at the time of testing, and lack of any microbiological evidence of active TB, but a high likelihood of progression to active TB in the short-term, with the potential for perpetuating the transmission cycle (2). The duration from initial exposure to incipient TB or active disease is variable and will depend of several host, mycobacterial, and environmental factors. Epidemiological data suggest that of those infected, ~ 5% will progress to active TB over a five year period with the highest risk being within the first 2 years of exposure (8, 9). Biomarkers to identify incipient TB has remained one of the 'Holy Grails' of TB research.

Given these considerations it has often been asked whether a higher magnitude of the IFN-y release assay (IGRA) response, or larger TST induration diameter, reflecting a higher burden of circulating effector T cells and inferring a higher burden of *Mycobacterium tuberculosis*, predicts a higher likelihood of incipient TB. Indeed, serial IGRA responses increasing in magnitude over time was associated with the development of active disease in several reports suggesting that antigen-driven

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T-cell responses could be a marker of incipient TB (10). However, there are limited and conflicting data regarding this point. Zellweger and Haldar found no association between the magnitude of the IFN- γ response and progression to active TB (11, 12). By contrast, Winje *et al* interrogated a large population-based cohort using QuantiFERON Gold In-Tube (QFT-GIT) and found that a quantitative IGRA readout > 4.0 IU/L was highly associated (> 30 fold risk compared to QFT negativity) with the development of active TB (13). Using a different metric Andrews and co-workers from South Africa found that QFT *conversion* at interferon-γ values higher than 4·00 IU/L (but not below this threshold) was associated with substantially increased risk (42 fold higher risk than non-converters) of developing active TB over a, ~2 year period (14). However, although these data collectively suggested that the magnitude of the T cell response was associated with higher rates of downstream active TB, there remained several unanswered questions. Did this relationship hold true for other immunodiagnostic readouts (like T-SPOT-TB and the TST), what are the implications for clinical practice, and is this relationship meaningful and clinically useful?

The study by Gupta and co-workers (15), here, provides answers to some of these questions. Their findings were based on the results of the prospective UK-based PREDICT study that evaluated 3 immunodiagnostic tests (T-SPOT TB, QuantiFERON TB Gold, and TST) in almost 10 000 participants who were at high risk of LTBI (close contacts of active TB cases or recent migrants) sequentially recruited from 54 centres in the UK (16). They found that, although the magnitude of the IGRA (both QFT-GIT & T-SPOT) and the TST response was a biomarker of incipient TB, the threshold-specific PPV for all 3 immunodiagnostic tests for active TB over a median follow up of ~3 years was poor at < 5%. This is because there were many non-progressors who had a magnitude of response at or above the threshold identifying incipient TB. Moreover, using this higher threshold in clinical practice would result in a substantial drop in test sensitivity to detect active TB cases making the usefulness of such an approach redundant. This is because IGRAs and TSTs are simply poor tests of incipient TB. This is not surprising as only a small proportion of those with LTBI (~5%) will progress to active disease.

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The authors must be commended on undertaking such a challenging study both in terms of recruitment and analysis. The findings are helpful to clinicians and public health physicians who are using immunodiagnostics tests in screening programmes. It suggests that alternative biomarkers of incipient TB are urgently needed. A weakness of the study though, despite the drawbacks of the IGRAs, was the lack of serial testing (discussed below). Such an approach would have only been feasible if the TST was not performed at baseline (as tuberculin contains RD-1 antigen and can boost downstream IGRA responses (17).To try and circumvent the poor predictive value and specificity, alternative immunodiagnostic readouts have been investigated including: (i) different cytokine readouts e.g. combination of IL-2/ IFN-γ, (ii) T cell responses to alternative antigens e.g. HBHA and Ag85a (18-20), (iii) cell activation markers e.g. CD4+HLA-DR+ T cells (21), and (iv) readouts from alternative compartments including RD-1-based skin tests that are being commercialised (22).

Other investigators have uncovered biosignatures of incipient TB. Several studies have identified blood-based transcriptional signatures associated with progression to active TB (23-26) with a positive predictive value ~10 fold higher than the IGRAs. These genomic biosignatures, consisting of 3 to 16 gene transcripts, were able to predict TB progression in participants with LTBI with reasonable accuracy, though performance was variable when validated against other cohorts. Suliman *et al* (27) derived a 4-gene signature, which correlated with TB disease progression and performed well when validated against other transcriptomic signatures. However, using a RT-PCR based readouts may not be user-friendly or cost-effective for TB-endemic settings. Very recently a 3 to 5 protein biosignature of incipient TB was derived and validated (28), and a novel ultrasensitive phage-based amplification assay for incipient TB was described (29). These data suggest that a point-of-care assay may be a realistic goal once better biomarkers are developed and validated.

Another broader issue raised by this study is the ambiguous and confusing interpretation of IGRA readouts. On the one hand, positive IGRA responses are often interpreted as a marker of LTBI and hence 'protection' given that ~95% never progress to active disease, and serial IGRA responses often

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decrease during the course of successful TB treatment (30). By contrast, the work of Gupta and others suggest that IGRAs are a biomarker of incipient TB and hence TB risk. Which is it, protection or risk? This answer is both depending on the clinical context. Thus the conundrum can be resolved by recognising that TB is a spectrum of infection, which is a dynamic interplay between host and pathogen at the level of the granuloma, and this may change over time reflecting timepoint-specific host immunity and mycobacterial disease burden (and hence changing levels of TB-specific effector T-cells in blood). A temporal compartment-specific effect may also influence interpretation due to translocation of antigen-specific T cells from the blood to the disease site, e.g the lung (31). Thus, serial measurements may often be required to determine whether IGRA responses are stable, increase in magnitude over time (conversion), or reduce in magnitude over time (reversion), possibly suggesting clearance of infection (32). This concept has been well outlined in a recent review (6). Thus, IGRA readouts can be a marker of protection or susceptibility depending on the context. This will explain why patients with stable persistently positive IGRA responses remain asymptomatic and do not progress to active TB over many years, whilst those with increasing counts/ responses progress to active TB, whilst others may revert to presumably clear their infection. Thus, selecting vaccine candidates simply on their ability to induce or drive antigen-specific IFN-y responses is counterintuitive; rather, selection based on preventing sustained conversion seems more logical and is an approach that has recently been used (33).

For now, the findings of Gupta and colleagues is clinically useful and points us in the right direction. The bottom-line is that better biomarkers of incipient TB are required, and nascent biomarker signatures require urgent prospective clinical validation. It is hoped that these resource-intensive and challenging prospective validation studies [like the CORTIS study (34)] will be fruitful rather than futile as TB remains the foremost infectious diseases killer globally.

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