Tuberculosis immunodiagnosis: delving below the surface

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It is one hundred and thirty years since Franz Ziehl and Friedrich Neelsen developed the rapid stain for acid-fast bacilli; accurate point-of-care diagnosis of active tuberculosis (TB) remains a major unmet clinical need. With the sensitivity of the Ziehl–Neelsen stain in sputum less than 50% and more than 20% of TB cases negative on both acid-fast stain and culture for Mycobacterium tuberculosis, there has long been a yawning gap in the diagnostic toolkit for TB. New rapid molecular methods have recently improved detection of M tuberculosis nucleic acids in sputum providing diagnostic sensitivity that is much higher than sputum-smear microscopy but lower than conventional serological tests. Although serological tests for TB are point-of-care, they lack diagnostic accuracy and are devoid of clinical utility. After thorough review of the evidence, and on account of their continued widespread misuse in many high-burden countries, WHO recently tried to bury current commercial serological test kits with a negative endorsement warning against their use.

Research into cellular immunodiagnosis has been more fruitful, delivering a tangible advance in clinical practice in the form of interferon-gamma release-assays (IGRA). IGRA detect M tuberculosis infection, providing a new standard-of-care for diagnosis of latent TB infection (LTBI), but they cannot distinguish active TB from LTBI. Hence, their potential role in evaluation of vaccine-induced responses. This method, known as Antibodies in Lymphocyte Supernatant (ALS), was previously used with BCG vaccine as antigen to detect BCG-specific antibodies in TB patients. It showed promising results as a biomarker for active TB in both adults and children. In this issue of Thorax, Ashenafi et al evaluated this assay in parallel with the tuberculin skin test (TST) and an IGRA (Quantiferon TB-Gold-in-tube) in adult TB patients (with and without HIV to coinfection) and asymptomatic controls with and without LTBI in Ethiopia.

By comparing these tests with a definitive clinical diagnosis of TB, which was carefully defined by microbiological, cytological and clinical characteristics, they were able to construct receiver-operating characteristic curves and an optimal cut-off for ALS positivity. Using this, they showed that the ALS assay was sensitive (91% of all cases of active TB), and could discriminate active TB from LTBI where the test was only positive in 16% of cases; its specificity was 94% in asymptomatic controls without LTBI. As expected, and in contrast with ALS, neither the TST nor the IGRA discriminated active TB from LTBI. Additionally, in a subset of patients, they were able to show that the number of IgG+ plasmablasts in peripheral blood mononuclear cells (PBMCs), as determined by flow cytometry, correlated with the titres of BCG-specific IgG antibodies secreted by the cultured PBMCs in the ALS assay.

In the context of the existing literature with ALS in TB, the main advance provided by this study is that the diagnostic sensitivity of this assay is well maintained in HIV coinfection, including advanced immunosuppression. Given the difficulty of reliably diagnosing TB in HIV coinfection, advances in this patient population are urgently needed. Intriguingly, there was a significant inverse correlation between CD3 T cell numbers and BCG-specific IgG titres among TB patients. This paradoxical correlation of increasing diagnostic sensitivity with advancing HIV-induced immunosuppression is analogous to the detection of the M tuberculosis cell wall component lipoarabinomannan (LAM), in urine, where mycobacterial dissemination in immunosuppression results in increased diagnostic sensitivity. An interesting pattern is emerging where certain new diagnostic tests for TB may find clinical application in specific subgroups of patients. By contrast with BCG-specific IgG titres, and as expected and previously documented, M tuberculosis-specific interferon-gamma production measured by Quantiferon TB-Gold-in-tube was significantly reduced in HIV coinfection and decreased with declining CD4 T cell numbers.

Are there immune responses that differ sufficiently between active TB and LTBI to enable development of an immunodiagnostic test that is specific for active TB?

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LTBI, this result is probably an overestimate of the real specificity in clinical practice. The actual target population for this assay is patients with suspected active TB and a high proportion of such patients who turn out not to have active TB will have LTBI. With 16% of the LTBI group scoring positive in this assay, specificity in a population of real-life TB suspects will, accordingly, be lower than 94%. Although 12 of the 96 patients with suspected TB in this study turned out not to have TB, the number of false-positive results in this group is not reported. Moreover, non-TB illnesses might also cause false-positive results and these 12 patients were the only group in whom this might have been assessed.

The case-control study design precludes reliable estimates of positive (PPV) and negative predictive values (NPV) as these parameters require the appropriate real-life population in which the test would be deployed, that is, prospectively presenting TB suspects. These values also depend crucially on the prevalence of the target condition (active TB) and confounders that may cause false-positive results (such as LTBI and non-TB illnesses) in the study population.

Hence, the next step rigorously to validate this new immunodiagnostic assay is a large prospective study in routine clinical practice of patients with suspected TB in whom the diagnosis is unknown at the point of recruitment, as has been done for IGRA. Those patients who transpire to not have TB, but who instead have non-TB illnesses and/or LTBI, will be the appropriate real-life ‘control’ group, enabling reliable generalisable estimates of diagnostic test performance, PPV and NPV.

If future studies confirm that the diagnostic performance of ALS confers clinical utility in routine practice, there are logistical issues that will need to be addressed. Although the ALS measures antibodies, it is not yet a point-of-care test, requiring 72 h incubation in sterile tissue culture conditions at 37°C with 5% ambient CO2.

Despite its limitations, this proof-of-principle paper by Ashenafi et al shows what can be achieved by dissecting out the components of the immune response that lie below the surface that is conventionally measured. This is analogous to recent advances in T cell-based diagnosis, where dissecting out the T cell subsets that contribute to the interferon-gamma measured by current IGRAs, and indeed dissecting out the T cell subsets contributing to the M tuberculosis-specific T cell response as a whole, enables differentiation of untreated active TB, treated TB and LTBI.

After decades in the doldrums, research-based development of new TB diagnostics has entered an exciting phase. And using recent advances in immunology to delve beneath the surface of conventional immunological readouts is accelerating to accelerate the development of new, more informative immunodiagnostic paradigms.

Competing interests AL is inventor for patents underpinning T cell-based diagnosis. The IFN-γ ELISpot IGRA was commercialised by an Oxford University spin-out company (T-SPOT.TB, Oxford Immunotec) in which Oxford University and AL have minority shares of equity and royalty entitlements.

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