EDITORIAL

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New tests for detection of *Mycobacterium tuberculosis* infection: sufficient to meet the WHO 2035 targets?

The new WHO End TB Strategy sets ambitious 2035 targets and emphasizes targeted treatment of the *Mycobacterium tuberculosis* infected at risk of developing tuberculosis (TB). New short-course treatment regimens have shown promise for better compliance; however, the available diagnostic tests are insufficient to guide treatment.

This editorial discusses current state of art and future developments in the field of latent TB diagnostics, with emphasis on Quantiferon-TB Gold® Plus (Qiagen), IP-10 release assays, specific skin tests and transcriptomic signatures that suggests a brighter future with more accurate predictive tools.

With 7.3 million annual cases and 1.3 million deaths, TB remains among the most significant infectious killers [1]. Each year, close to half a million new cases of multidrug-resistant cases emerge and HIV-associated TB affects more than a million people [1]. Before diagnosis and treatment, every TB patient will have passed on the bacteria to on average 11 contacts [2], thereby feeding the huge reservoir of *M. tuberculosis* (*Mt*) infected at risk of developing active TB disease. The implementation of direct observed therapy and roll out of PCR-based diagnostics and drug susceptible testing have prevented millions of lives; however, it is clear that radical means are required to curb the epidemic [1].

A central pillar in the new WHO End TB Strategy is a recommendation of target treatment of *Mt* infection among at-risk populations in upper-middle and high-income countries with an incidence <100 per 100,000 population [1,3]. This is an extension of the global recommendations for targeted treatment of children [4] and people living with HIV [5], and is among the core activities expected to facilitate the achievement of the ambitious targets of 90% reduction in TB incidence and 95% reduction in TB deaths by 2035 [1].

### KEYWORDS
- correlate of risk
- IGRA
- IP-10
- prediction of progression
- specific skin test
- transcriptomics

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Morten Ruhwald* & Peter L Andersen

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*Statens Serum Institut, Artillerivej 5, 2300 Copenhagen, Denmark

*Author for correspondence: moru@ssi.dk
The recommendations are supported by models suggesting a significant impact of targeted treatment [6]; however, to meet these ambitious goals there is a need for shorter treatment regimens and better diagnostics to guide the treatment. The introduction of the 3-month weekly isoniazid and rifapentine ensures better compliance and higher treatment success [7–8], however, the currently available toolbox of tests to guide preventive treatment is a bottleneck.

Current diagnostic toolbox to guide targeted treatment

The tuberculin skin test (TST) has been the standard method of determining whether a person is infected with *Mtb* [6] for over a century. The test is performed by intradermal injection of purified protein derivative tuberculin, a precipitate of species-nonspecific antigens [7]. A major limitation to the use of TST, is false-positive reactions occurring in persons infected with nontuberculous mycobacteria and in persons vaccinated with Bacille Calmette–Guérin (BCG) resulting in lower test specificity and compromised potential to guide targeted treatment [9].

The IFN-γ release assays (IGRAs) is an *in vitro* diagnostic alternative to TST [8]. These tests are based on the *Mtb*-specific antigens ESAT-6, CFP10 and TB7.7, and provide an objective readout and solve the problem of false-positive TST results. Two Igra tests are commercially available, the whole blood-based Quantiferon Gold® In-Tube test (QFT, Qiagen, Germany) and the purified peripheral blood mononuclear cells-based test T-Spot.TB® test (Oxford Immunotec, UK). Compared with the TST, IGRAs are more complex and labor intensive, requiring laboratory infrastructure and skilled staff [9]. Although IGRAs have solved the problem of false-positive reactions in BCG vaccinated, both IGRA and TST are unable to differentiate latent infection from active disease [9,10]. Thus, current tests have low-positive predictive value for the development of active TB and require treatment of >30 contacts to prevent one case of TB [11–13].

Imminent future

In 2015, Qiagen launched the fourth generation Quantiferon-TB Gold® Plus (QFT 4G), as a test with improved diagnostic sensitivity for infection in immunosuppressed [14]. QFT 4G builds on the QFT 3G in-tube design and comprises two blood collection tubes, TB1 and TB2, instead of the single TB-Ag tube known from QFT 3G. TB1 contains a cocktail of peptides from ESAT-6 and CFP10 identical to the predecessor [15] but without the TB7.7 antigen [9,14]. TB2 comprise the same ESAT-6 and CFP10 peptides as TB1, and – in addition – an unknown number of shorter peptides intended to activate the CD8-positive T cells. The rationale for the TB2 tube is to boost diagnostic sensitivity for infection, for example, in people living with HIV (PLHIV) with low CD4 T cells. QFT 4G is interpreted positive if either TB1 or TB2 induces an antigen-specific IFN-γ release ≥0.35 IU/ml, a modification of the algorithm that in itself will increase the positivity rate by doubling the chance for samples with low IFN-γ levels (and very high analytical variability [16]) of becoming positive.

Registration studies and the first independent evaluations found that the QFT 4G provides a marginal and nonsignificant increase in the detection rates in TB patients and contacts [14,17–18]. The million-dollar question yet to be answered experimentally is whether the extra detected individuals are found among those that progress to TB – hereby truly improving the test; or whether it picks up more in the group who never progress to TB and, therefore, will be a detriment to the predictive value. Nevertheless, the fourth tube adds cost and complexity to an already laborious test, wherefore it is difficult to appreciate QFT 4G as the panacea enabling the End TB Strategy targets.

Further ahead

Many activities are underway aiming to improve the IGRA. Several groups have explored the use of alternative readout markers expressed in higher levels compared with IFN-γ. It is now established that cell-mediated immune responses can be detected with other cytokines and chemokines, among which IP-10 is the leading alternative [19]. IP-10 is a chemokine expressed in 100-fold higher levels than IFN-γ, allowing for simpler detection technology, such as the lateral flow quick test format or even extraction from dried blood spots [19,20]. IP-10-based IGRAs are highly concordant with IFN-γ-based IGRA, but the high magnitude of response allows for improved sensitivity in children and PLHIV with confirmed *Mtb* infection [19]. Of particular relevance for the implementation of the End TB strategy, the lateral flow-based IP-10 IGRA would enable implementation of a rapid
and simple IGRA like diagnostic with minimal equipment and training.

Another approach to improving the predictive potential of the IGRA is alternative antigens associated with controlled infection. One example is heparin-binding hemagglutinin, a protein expressed on the surface of several mycobacterial species. Heparin-binding hemagglutinin-specific cellular immune responses are associated with Mtb contamination suggesting a not yet fully explored predictive potential when combined with specific antigens, such as ESAT-6 and CFP10 [21], in a two-tube test vis-a-vis QFT 4G.

The era of specific skin tests?

Specific skin tests are another promising and field friendly alternative to IGRA. C-Tb developed by Statens Serum Institut (Copenhagen, Denmark) is a skin test based on a recombinant double ESAT-6 protein and single CFP10 monomer expressed in Lactococcus lactis. Phase II data suggest that C-Tb drives induration sizes on level with TST and delivers positivity rates in TB cases and unexposed controls on par with QFT [22,23]. As C-Tb is unaffected by previous BCG vaccination, the responses seen in infected are more clearly separated from uninfected, and the cutoff can be lower and universal (at ≥5 mm) irrespective of HIV infection or age [18,19]. Phase III trial data recently shown at conferences suggest that C-Tb is safe and delivers positivity rates highly concordant with QFT 3G in recent contacts, but also that C-Tb appears more robust than QFT in PL HIV with active TB and low number of circulating CD4 T cells [24] [Ruhwald, Aggerbaek, Pers. Comm.]. Diaskintest developed by Pharmstandard (Moscow, Russia) using recombinant ESAT-6 and CFP10 dimer expressed in Escherichia coli is another specific skin test. Despite having been on the market in several former soviet republics since 2005, few results of this test has emerged in the international scientific literature. Diaskintest seems to deliver IGRA like diagnostic performance in BCG vaccinated, but reports suggest a relatively high number of adverse events associated with this test [25].

Prediction beyond the IGRA

Outside the IGRA concept, a recent landmark paper from Zak et al. suggested that whole-blood transcriptomic mRNA expression signatures accurately predict risk of progression in latently infected individuals [26]. A correlate of risk was identified by mining RNA-sequencing data from large prospective cohorts of Mtb-infected adolescents from South Africa, and a 63 mRNA transcript from 16 genes was migrated to a chip-based PCR assay for high-throughput analysis. Validation in independent prospective cohorts from South Africa and Gambia demonstrated sensitivity of 54% and specificity of 83% for progression to active disease within a year [26]. The mRNA signature was not in itself specific for infection, but indicates a future two-step approach with a doorstep IGRA followed by a PCR-based risk prediction in IGRA-positive cases.

In conclusion, the field of immunodiagnostics is in transition. Innovative new specific skin tests and field-friendly IGRA like test formats will enable specific and likely cheaper diagnostics. Transcriptomic signatures could be a game changer, guiding rational use of targeted treatment. Encouraging, the predictive potential of transcriptomic signatures could also provide a revolution in TB vaccine development, enabling much smaller and cost-effective efficacy trials in high-risk populations to triage vaccine candidates [27]. Advances on all fronts are required to meet the ambitious 2035 targets of the End TB strategy, but without a new and efficacious TB vaccine and drastic improvements in living conditions and global access to healthcare, the targets still seem a high bar to meet.

Financial & competing interests disclosure

M Ruhwald and PL Andersen are employed by SSI, a governmental not-for-profit research organization that holds intellectual property rights on several antigens used for immunodiagnostic tests and vaccines for tuberculosis. M Ruhwald is registered as inventor on patents disclosing the use of IP-10 as immunodiagnostic marker for Mycobacterium tuberculosis infection. All rights have been assigned to SSI. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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