Advancing new diagnostic tests for latent tuberculosis infection due to multidrug-resistant strains of *Mycobacterium tuberculosis* — End of the road?

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A B S T R A C T

An estimated 1.8 billion people worldwide have a latent tuberculosis infection (LTBI), with wide variations in LTBI rates across countries. LTBI can be due to infection with either drug-sensitive or drug-resistant *Mycobacterium tuberculosis* (Mtb) strains. Accurate data on the prevalence of LTBI due to multidrug-resistant (MDR) Mtb strains are unavailable, since the strains cannot be isolated for resistance testing. There are no 'gold standard' tests for accurately diagnosing LTBI. Only three tests are currently available and approved by the World Health Organization (WHO) for the diagnosis of LTBI: the now outdated tuberculin skin test (TST), developed a century year ago, and the two interferon-gamma release assays (IGRAs) developed and rolled out over the past decade, the QuantiFERON (Qiagen, Germany) and T-SPOT.TB (Oxford Immunotec, United Kingdom) tests. These latter tests are not ideal due to issues of sensitivity, specificity, inability to distinguish infection with MDR-Mtb strains, and high costs. Achieving the WHO End TB Strategy target of an 80% reduction in global TB incidence by 2030 will require a major reduction in the number of persons with LTBI progressing to active TB disease. Critical to this will be the development of new diagnostic tests that are better than currently available LTBI tests at predicting who is at risk of progression to active TB disease. The diagnostic product development portfolio for LTBI appears to have reached the end of the road. Every attempt to make optimal use of currently available IGRAs using WHO LTBI guidelines for LTBI testing and treatment must be made to achieve WHO End TB strategy targets.

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Introduction

Latent tuberculosis infection (LTBI) is defined by the World Health Organization (WHO) as "a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens without evidence of clinically manifested active TB" (WHO, 2019). An estimated 1.8 billion people worldwide have LTBI, with wide variations in LTBI rates across countries (Houben and Dodd, 2016; Knight et al., 2019; Huaman and Sterling, 2019; Cohen et al., 2019). LTBI can be due to infection with either drug-sensitive or drug-resistant *M. tuberculosis* (Mtb) strains. Accurate data on the prevalence of LTBI due to MDR-Mtb strains are unavailable, since the strains cannot be isolated for resistance testing. The metric of LTBI burden is currently calculated using a new mathematical model (Knight et al., 2019) that follows cohorts over time, applying the historical annual infection risk to estimate risk trends of new

infections with MDR strains and the prevalence of LTBI. It is currently estimated that 19.1 million people worldwide have LTBI due to MDR-Mtb strains, which equates to one in every 83 persons with LTBI. The primary reason for identifying persons with LTBI is to reduce their risk of progressing to develop active TB in the future, by offering treatment to eradicate LTBI and prevent future disease.

WHO recommendations and available LTBI diagnostic tests

WHO guidelines for the programmatic management of LTBI (WHO, 2020) recommend systematic testing and preventive treatment for three high-risk population groups: persons living with HIV (PLHIV), contacts (household) of microbiologically confirmed pulmonary TB cases, and several clinical risk groups. Identifying persons with LTBI at highest risk for progression of LTBI to active TB remains challenging, despite the availability of online tools for estimating a person's lifetime risk of progression (McGill University, 2019). There are no 'gold standard' tests for accurately diagnosing LTBI. Only three tests are currently available and approved by the WHO for the diagnosis of LTBI: the now outdated tuberculin skin test (TST), developed a century ago, and the two interferon-gamma release assays (IGRAs) developed and rolled out over the past decade, the QuantiFERON (Qiagen, Germany) and T-SPOT.TB (Oxford Immunotec, United Kingdom) tests.

Need for new diagnostic tests for LTBI and for LTBI due to MDR-Mtb strains

There is an urgent need for a point-of-care, easy to use, affordable diagnostic tests for LTBI. IGRAs do not differentiate between LTBI and active disease and they should not be used as diagnostic tests for active TB. The IGRAs and their newer generation variants (Won et al., 2020) have some limitations in terms of interpretation, since they are based on the immune response to Mtb and thus are only indirect tests of LTBI (Haas and Belknap, 2019). IGRAs used on immunocompromised patients appear less sensitive and give false-negative and indeterminate results. Other issues that need to be addressed are how to determine that LTBI treatment has been effective and how to determine when an infection due to a new strain of Mtb has occurred, since treatment responses reported have been variable and checking with a repeat IGRA after LTBI treatment is not recommended. Worryingly, the prevalence of LTBI due to MDR-Mtb strains continues to increase in high MDR-TB countries (Knight et al., 2019), while progress in the development of diagnostic tests to detect these individuals has not been forthcoming, raising concerns about who and when to treat in light of the risk of treating subclinical or early TB disease, and also LTBI due to MDR-Mtb strains (Petersen et al., 2019).

Advancing LTBI diagnostic tests

The pace of development of more accurate tests for LTBI has been slow. IGRA tests were approved for sale in 2004 and no other IGRA tests for LTBI have been approved by the WHO. Several other new LTBI tests have been evaluated. The IGRA test LIOFeron TB/LTBI was introduced in 2019 by Lionex GmbH (Braunschweig, Germany) and contains the alanine dehydrogenase (Ala-DH) of Mtb. This test differs from the QuantiFERON-TB Gold Plus test in that the first antigen tube (TB-A) contains full-length ESAT-6, CFP-10, and TB7.7, and the highly purified recombinant Ala-DH is included in the second antigen tube (TB-B). A recent study (Della Bella et al., 2020) reported that the LIOFeron TB/LTBI assay may have higher sensitivity than the QuantiFERON-TB Gold Plus test and that further evaluation is required in controlled studies in different geographical areas. Chemiluminescence immunoassays (CLIA) have also been studied in comparison to the QuantiFERON-TB Gold test (Brantestig et al., 2020).

The C-Tb test (Statens Serum Institute, Copenhagen, Denmark) is a skin test that uses ESAT-6 and CFP-10 instead of purified protein derivative (PPD). The test aims to combine the operational advantages of the TST with the performance characteristics of IGRAs. This test performed better than the TST in bacillus Calmette–Guérin (BCG)-vaccinated people, had a high concordance with the QuantiFERON-TB Gold In-Tube test, and positivity was correlated with the exposure risk (Ruhwald et al., 2017). Further evaluations are awaited. C-Tb was found to be safe in people living with HIV and children less than 5 years of age, giving a positivity rate similar to the QuantiFERON test (Ruhwald et al. 2017).

Whole blood biomarkers that can better predict the risk of TB progression are being studied using RNA sequencing of blood from cohorts; these studies are identifying gene signatures for the risk of progression to active TB (Zak et al., 2016; Wang et al., 2019; Suliman et al., 2018; Warsinske et al., 2018; Deng et al., 2019; Gupta et al., 2020). Mtb-specific CD4+ T-cell activation markers in blood may discriminate pulmonary and extrapulmonary TB from LTBI (Silveira-Mattos et al., 2019). Recently, Mtb has been demonstrated in mesenchymal and hematopoietic stem cells (Mayito et al., 2019); thus whole genome sequencing of bone marrow specimens from LTBI patients may show the presence of Mtb with *rpo* gene mutations in stem cell CD34 populations.

Despite these advances, no diagnostic tests are currently available that can accurately detect LTBI, distinguish subclinical or early clinical disease from LTBI, and identify LTBI due to drug-resistant strains of Mtb. These will be essential in order to provide patient-centered quality LTBI services (WHO, 2020; Alsdurf and Menzies, 2020).

The future

Worryingly, the prevalence of LTBI due to MDR-Mtb strains will continue to increase in high MDR-TB countries (Knight et al., 2019). The WHO-recommended treatment regimens for LTBI contain TB drugs to which MDR-Mtb strains are resistant. While the new American Thoracic Society/US Centers for Disease Control and Prevention/European Respiratory Society/Infectious Diseases Society of America (ATS/CDC/ERS/IDSA) Clinical Practice Guidelines have now reached a consensus to provide fluoroquinolone-based preventive treatment to contacts of infectious MDR-Mtb patients (Migliori et al., 2020), accurately defining the drug sensitivity of the infecting Mtb strain remains impossible using currently available LTBI diagnostic tests. Achieving the WHO End TB Strategy target of an 80% reduction in global TB incidence by 2030 will require a major reduction in the number of persons with LTBI progressing to active TB disease. Critical to this will be the development of new diagnostic tests that are better than currently available LTBI tests at predicting who is at risk of progression to active TB disease. New diagnostic tests could be aligned with clinical prediction tools to quantify individual TB risks for contacts (Li et al., 2020).

Conclusions

The current WHO-recommended tests for LTBI are not ideal due to issues of sensitivity, specificity, inability to distinguish infection with MDR-Mtb strains, and the high costs. While it appears that the diagnostic product development portfolio for LTBI has reached the end of the road after 27 years of investment, since the WHO declared TB a global emergency, every attempt must be made to ensure optimal use of currently available IGRAs using WHO LTBI

guidelines for LTBI testing and treatment. This will be essential to achieve the End TB Strategy goals.

Ethical approval

Not required.

Conflict of interest

All authors have a specialist interest in TB. All authors declare no conflicts of interest.

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