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MINIREVIEW

From Latent Tuberculosis Infection to Tuberculosis. News in Diagnostics (QuantiFERON-Plus)

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Abstract

It is estimated that one third of the world's population have latent tuberculosis infection and that this is a significant reservoir for future tuberculosis cases. Most cases occur within two years following initial infection. The identification of individuals with latent tuberculosis infection is difficult due to the lack of an ideal diagnostic assay and incomplete understanding of latent infection. Currently, there are three tests: the oldest tuberculin skin test, T-SPOT.TB and the latest QuantiFERON-Plus for the detection of *Mycobacterium tuberculosis* infection. The interpretation of the test results must be used in the conjunction with a patient's epidemiological history, risk assessment, current clinical status, radiography and microbiological methods to ensure accurate diagnosis.

Key words: *Mycobacterium tuberculosis*, interferon-gamma release assays, latent tuberculosis infection, tuberculin skin test, tuberculosis

Introduction

The World Health Organization estimated that one third of the world's population has latent tuberculosis infection (LTBI) and the risk of progressing to TB is very heterogeneous (WHO, 2008). LTBI provides a potential reservoir for the reactivation and future development of active TB (Rangaka *et al*., 2012; Turetz and Ma, 2016). The active disease develops in 5–10% of those with LTBI over the course of their lifetimes (Turetz and Ma, 2016; Salgame *et al*., 2015). The highest risk of the progression from LTBI declines exponentially. Most TB cases occur within the first two years after a person has been infected (Mack *et al*., 2009; Salgame *et al*., 2015). In countries with intermediate (for example Poland) and high incidence of TB it is not practical to provide mass treatment for LTBI (Salgame *et al*., 2015; Korzeniewska-Koseła, 2016). It is well established that only a minority of patients with LTBI will develop TB (Mack *et al*., 2009). However, among children, immunocompromised individuals and patients receiving biological treatment, the risk of the progression to TB is significantly higher (Salgame *et al*., 2015). A major component of TB control is the identification of patients with LTBI in risk groups and the provision of chemoprophylaxis to prevent the development of active TB in those infected (Turetz and Ma, 2016; Uplekar *et al*., 2016). In Poland, prior to biological treatment, children and older patients are treated prophylactically. LTBI is a state of persistent T-cell responses to *Mycobacterium tuberculosis* antigens without clinical symptoms or signs of active TB, such as cough, hemoptysis, fever, night sweats, weight loss and opacity in chest radiographs (Mack *et al*., 2009; Lim, 2016; Getahun *et al*., 2015). There is no diagnostic gold standard for LTBI and direct identification of LTBI is not possible. Diagnostic tests are designed to identify the immune response against *M. tuberculosis*. Currently, there are two accepted methods for LTBI identification: the *in vivo* tuberculin skin test (TST) and *ex-vivo* interferon-gamma release assays (IGRAs). Two IGRAs are commercially available: QuantiFERON-TB Gold Plus (Qiagen, Germany) and T-SPOT.TB (Oxford Immunotec, UK). Both IGRAs are approved by the U.S. Food and Drug Administration and Conformité Européenne (Rangaka *et al*., 2012; Turetz and Ma, 2016; Salgame *et al*., 2015, Pai *et al*., 2014). However, none of the assays mentioned above, can distinguish between LTBI and active TB and none can identify which patients with LTBI will develop active TB (Rangaka *et al*., 2012; Turetz and Ma, 2016; Lim, 2016).

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Tuberculin Skin Test

The TST has been continuously in use for 100 years in clinical medicine and has been used to identify people with latent *M. tuberculosis* infection (Mack *et al.*, 2009). A TST is performed by intradermal injection of a purified protein derivative (PPD) of tuberculin, on the palmar surface of the forearm, at a volume of 2 units. The induration at the injection site is measured after 48–72 hours, by measuring the diameter of the area of induration, transversely to the long axis of the forearm (Borkowska *et al.*, 2011; Kruczak *et al.*, 2009; Kang *et al.*, 2005). In the case of patients who are latently infected with TB, tuberculin will stimulate a delayed type hypersensitivity (DTH) response *via* T lymphocytes. Tuberculin induces DTH where T cells and macrophages produce lymphokines that cause oedema, fibrin deposition, and inflow of other inflammatory cells (Turetz and Ma, 2016; Borkowska *et al.*, 2011; Kruczak *et al.*, 2009; Lalvani, 2007). In Poland, a RT23 type (renset tuberculin, 23 series) of PPD tuberculin has been used since 1966, produced at the Institute of Serum and Vaccine in Copenhagen (Borkowska *et al.*, 2011). TST is interpreted on the basis of the diameter and the clinical characteristics of a patient (Turetz and Ma, 2016). The TST has limitations, however. False positive and negative results can occur. There are 2 causes of false positive results: Bacillus-Calmette-Guerin (BCG) vaccination and nontuberculous mycobacterial infections (Rangaka *et al.*, 2012; Turetz and Ma, 2016; Pai *et al.*, 2014). In populations vaccinated with BCG, the skin reaction may be positive in some individuals even after 15 years following vaccination (Borkowska *et al.*, 2011). The specificity of the test is low because tuberculin contains more than 200 different antigens from microorganisms other than *M. tuberculosis*. In addition, false negative responses can occur if the patient is too young or too old, in immunocompromised patients (HIV infection), as well as in cases of those taking immunosuppressive medications or in those with active TB (Turetz and Ma, 2016; Pai *et al.*, 2014; Borkowska *et al.*, 2011). In Poland, where the whole population is vaccinated with BCG, it is important to establish whether the positive result of the TST is connected with a previous vaccination or with ongoing *M. tuberculosis* infection (Borkowska *et al.*, 2011).

Interferon Gamma Release Assays

The IGRAs are an alternative to the TST for the diagnosis of LTBI. These assays identify cellular immune responses to *M. tuberculosis* by measuring interferon-gamma (IFN-γ) after stimulation of T cells with *M. tuberculosis*-specific antigens (Turetz and Ma, 2016; Diel *et al.*, 2011). Two tests are available: T-SPOT.TB is based on the Elispot-enzyme-linked immunospot and QuantiFeron TB Gold Plus on the enzyme-linked immunosorbent assays (ELISA) technique. In the case of the T-SPOT.TB, whole blood is used and the test is based on measurement of the number of peripheral mononuclear cells that produce IFN-γ after stimulation with two antigens: early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP 10). Both antigens are encoded in the region of difference 1 (RD1) (Trajman *et al.*, 2016; van Ingen *et al.*, 2009). The second test, QFT-Plus, uses specialized whole blood collection tubes. The antigens used in this test are a peptide cocktail simulating the ESAT-6 and CFP 10. QFT-Plus comprises two distinct TB antigen tubes and both tubes contain ESAT-6 and CFP 10. TB1 tube is designed to elicit cell mediated immunity (CMI) responses from CD4+ T-helper lymphocytes and the TB2 tube contains an additional set of peptides targeted to the induction of CMI responses from CD8+cytotoxic T lymphocytes (2015b). The QFT-Plus measures the level of IFN-γ in the peripheral blood by the ELISA technique (Trajman *et al.*, 2016). The antigens used in the IGRAs do not cross-react with the vaccination strain *Mycobacterium bovis* BCG and with most species of nontuberculous mycobacteria (NTM). However, the RD1-coding region of antigens ESAT-6 and CFP 10, similar to that of *M. tuberculosis*, is present in *Mycobacterium kansasii*, *Mycobacterium szulgai*, *Mycobacterium marinum*, and *Mycobacterium riyadhense* (Diel *et al.*, 2011; van Ingen *et al.*, 2009; Hermansen *et al.*, 2016). The presence of similar antigens in NTM theoretically lowers the specificity of the IGRAs in diagnosing LTBI (van Ingen *et al.*, 2009). Compared to TST, IGRAs have better specificity, positive and negative controls, clear interpretation criteria and require only one visit in the clinic (Pai *et al.*, 2014).

Sensitivity and specificity TST, QFT, T-SPOT.TB

There is no method to truly confirm the diagnosis of LTBI, because we do not have a gold standard for diagnosing LTBI. The sensitivity of IGRAs or TST for LTBI diagnosis is typically assessed in patients with active TB, treating this group as a surrogate for LTBI (ECDC, 2011). The specificity of TST and IGRAs reflects the true negative rate of patients tested for LTBI. Populations with a recognizable low risk of *M. tuberculosis* infection introduce a surrogate for a group, free of *M. tuberculosis* infection (ECDC, 2011). Menzies *et al.* (2007) calculated the sensitivity and specificity of 3 tests based on 56 studies. Pooled sensitivity was lowest for the TST (70%), higher for QFT (76%) and the highest for T-SPOT.TB (88%). Pooled specificity was the lowest

for the TST (66%), higher for T-SPOT.TB (92%) and the highest for QFT (97%). The meta-analysis proved that no tests had high sensitivity. IGRAs were more specific than the TST in populations vaccinated with BCG (Menzies *et al*., 2007). The meta-analysis of Diel *et al*. (2010) showed that the pooled sensitivity of TST was 70% compared to 81% for the QFT and 88% for the T-SPOT.TB. The specificity of the QFT was 99% and 86% for the T-SPOT.TB. This meta-analysis included 25 studies (Diel *et al*., 2010). Both meta-analyses had similar results. Hoffman *et al*. (2016) prepared the first evaluation of the new test generation called QuantiFeron TB Gold Plus (QFT-Plus) in comparison with the older version of QuantiFeron TB Gold In Tube (QFT). QFT analyses IFN-γ released only by CD4+ T-helper cells after stimulation with *M. tuberculosis* antigens while QFT-Plus analyses the response of CD8+ cytotoxic T lymphocytes. Hoffman *et al*. (2016) counted the sensitivity of the new method based on data from 163 patients, including 77 health care workers and 86 suspected cases of TB. QFT-Plus produced 87.9% true-positive results, which was interpreted as demonstrating increased sensitivity compared to 80% for QFT in the meta-analysis (Hoffmann *et al*., 2016; Sester *et al*., 2011; Barcellini *et al*., 2016). This is one of the first such studies and therefore has some limitations, so further studies are needed to confirm these findings (Hoffmann *et al*., 2016).

Predictive value of IGRAs

A clear understanding of the predictive value of IGRAs for the development of active TB disease is necessary (Lim, 2016). The positive predictive value (PPV) for the progression of LTBI is the probability that in the case of an individual with a positive test, there is real a risk of developing active TB disease later in their life. The negative predictive value (NPV) is the probability that a patient with a negative test does not have LTBI and therefore will not develop active TB (Hermansen *et al*., 2016). The NPV is high in low-endemic countries, whereas the PPV of both the TST and IGRAs is low in these countries. Therefore, currently, only targeted testing in specific high-risk groups is recommended. Studies assessing the PPV of IGRAs show heterogeneous results (Hermansen *et al*., 2016). In 2015, Tuberculosis Network European Trials Group (TBNET) calculated the PPV and NPV of the QFT test, noting results of 1.9% and 99.9%, respectively (Zellweger *et al*., 2015) while Hermansen *et al*. (2016) in Denmark, a TB low endemic country, showed a high NPV (99.85%) and a low PPV (1.32%) for the same test. Their study included a 5-year retrospective cohort study assessing the risk of TB among patients with positive and negative QFT results (Hermansen *et al*., 2016). Lim (2016) analysed 3 studies in a low-TB- incidence countries and confirmed that IGRA has a very high NPV (99.5%) and a low PPV (about 4%) for future active TB (Lim, 2016; Hermansen *et al*., 2016; Zellweger *et al*., 2015; Sloot *et al*., 2014). Detecting LTBI and the need for treatment in specific cases should focus on patients with the highest risk of reactivation of TB. Current diagnostics of LTBI are deficient with limited PPV for the development of active TB (Turetz and Ma, 2016). There are a limited number of studies of the predictive value of IGRAs in countries with intermediate or high incidence of TB.

Summary

Standard diagnostic methods for an active TB diagnosis are known and have clear guidelines. A number of studies concerning the issue have been published. The diagnosis of LTBI however lacks gold standard. There are indirect tests: TST, QFT and T-SPOT.TB for detection of *M. tuberculosis* infection that must be used in conjunction with the patient's epidemiological history, risk assessment, current medical status, radiography and microbiological methods. The sensitivity, specificity and predictive values of IGRAs for the diagnosis of LTBI in low, intermediate and high-TB incidence settings should be the subject of further studies. New studies are also needed to explore the use of the new generation assay of QFT-Plus for the diagnosis of LTBI and active TB in various populations. QuantiFERON – Plus can be used as an adjunct tool in the diagnosis of active TB, but certainly cannot be used solely and indiscriminately, separate from other clinical epidemiological and radiological factors.

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