New immunodiagnostic tests for latent and active tuberculosis

Noi teste de imunodiagnostic pentru tuberculoza latentă şi activă

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Abstract

Latent tuberculosis infection diagnosis has relied for over 100 years on identification of a T-cell specific response by tuberculin skin test (TST). Recently blood-based in vitro interferon-γ release assays (IGRAs) have been developed to overcome the limitations of the skin test: an ELISA-based method (QuantiFERON-TB Gold In-tube) and an ELISPOT-based method (T.SPOT-TB). This paper describes the laboratory method and clinical interpretation of these two new immunodiagnostics, and also reviews the current literature related to their value in the diagnosis of latent tuberculosis infection and active tuberculosis. IGRAs have proved to be more specific than TST in diagnosis of latent TB infection in BCG-vaccinated individuals and have a better predictive value for progression to active tuberculosis. Blood-based IGRAs have limited value in diagnosis of active TB, but T-SPOT.TB in extrasanguinous fluids shows preliminary good diagnostic performance for smear-negative pulmonary and extrapulmonary TB. Skin testing with recombinant M. tuberculosis specific antigens might be an alternative for latent TB infection diagnosis in low-resource environments. There is a need for additional studies in immunocompromised individuals and in high-incidence countries for a better definition of the role of these new immunodiagnostics.

Keywords: *tuberculosis, latent tuberculosis infection, tuberculin skin test, interferon-gamma release assay*

Rezumat

Diagnosticul infecţiei tuberculoase latente s-a bazat timp de peste 100 de ani pe identificarea unui răspuns specific al celulei T prin testul cutanat la tuberculină (TCT). Recent au fost dezvoltate metode in vitro pe sânge periferic pentru măsurarea eliberării de interferon-γ la stimularea cu antigene micobacteriene (interferon-γ release assays, IGRAs): o metodă tip ELISA (QuantiFERON-TB Gold In-tube) şi una tip ELISPOT (T-SPOT.TB). Acest articol descrie metodele de laborator şi interpretarea clinică a acestor noi teste imunodiagnostice şi, de asemenea, sintetizează literatura medicală curentă asupra valorii lor în diagnosticul infecţiei tuberculoase latente şi a tuberculozei active. IGRAs s-au dovedit a fi mai specifice ca TCT în diagnosticul infecţiei TB latente la subiecţii vaccinaţi BCG şi au o valoare predictivă mai bună pentru progresia către tuberculoză activă. IGRAs din sânge au o valoare limitată în diagosticul tuberculozei active, dar T-

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SPOT.TB din lichidele extrasanguine a demonstrat rezultate preliminare bune ca metodă diagnostică în tuberculoza pulmonară negativă în microscopie şi tuberculoza extrapulmonară. Testarea cutanată cu antigene recombinante specifice pentru M. tuberculosis ar putea fi o alternativă pentru diagnosticul infecţiei TB latente într-un context cu resurse financiare limitate. Sunt necesare studii suplimentare la subiecţi imunodeprimaţi şi în ţări cu incidenţă mare a tuberculozei pentru o definire mai bună a rolului acestor teste imunodiagnostice.

Cuvinte-cheie: *tuberculoză, infecţie tuberculoasă latentă, testare cutanată tuberculinică, metode bazate pe eliberarea interferon-γ*

Introduction

Tuberculosis (TB) is a worldwide endemic infectious disease produced by *Mycobacterium tuberculosis* (Mtb) that has been present in man for over 3000 years (1). In 2009 around the world there were 9.4 million new TB cases and 1.7 millions died of TB (2). Tuberculosis elimination by 2050 (i.e. incidence of active TB of less than one case per 1 million population per year) is a goal of the World Health Organization (WHO) StopTB Partnership (3) and also of the Framework Action Plan to Fight TB in the European Union (4). Early diagnosis and effective treatment of active TB cases to interrupt transmission of infection within a population is the mainstay of a tuberculosis control program. However this approach will tackle only the tip of the iceberg, being known that around one third of the world population is infected with *M. tuberculosis*. Diagnosis and treatment of latent tuberculosis infection (LTBI) in individuals at risk to develop active disease is a second dimension of a strategy for elimination of tuberculosis.

Latent TB infection diagnosis has relied for over 100 years solely on the result of the tuberculin skin test (TST, also known as Mantoux test) (5). Blood-based *in vitro* interferon-γ release assays (IGRAs) have been developed as alternative tests to TST for diagnosis of latent TB infection. The purpose of this review is to evaluate the current status of IGRAs in the diagnosis of latent and active tuberculosis.

Latent tuberculosis infection

WHO has estimated that one third of the world's population is infected with *M. tuberculosis*, but this assumption has been withdrawn in recent reports (6). Only a minority of individuals infected with *M. tuberculosis* will ever develop active tuberculosis.

Persistence of live *M. tuberculosis* leading after a variable period of time in some individuals to active tuberculosis has long been considered as the typical natural history of Mtb infection. The risk of developing active tuberculosis is considered to be greater during the first years after infection (about half of the cases appear during the first 2 years (7)), but a molecular study has shown that a presumably primary infection with Mtb at the age of 7 resulted in active disease 33 years later (8), thus hugely extending the period at risk.

Latent tuberculosis infection (or LTBI) has been defined as the state in which an individual harbors live *M. tuberculosis* not revealing themselves through any clinical or other sign or symptom of active disease (9). T-cell immunity is considered crucial for containment of Mtb infection through granuloma formation, and T-cell responses are persistent throughout latency. However the physical and metabolic nature, and the location of persistent Mtb (so-called „dormant") is controversial (9).

Direct identification of *M. tuberculosis* in individuals with latent infection is not possible with current laboratory methods. As a result, LTBI diagnosis is actually based on the demonstration of an adaptive *M. tuberculosis* specific Thelper1-type cellular immune response. Consequently LTBI can be defined more realistically as the presence of a persistent Mtb-specific T-cell response in the absence of clinical evidence of tuberculosis.

However, it is not known to what extent the presence of such an immune response is a

Table 1. Criteria of positivity of a tuberculin skin test stratified by risk group (adapted after [11])

Reaction ≥ 5 mm (high-risk)	• Human Immunodeficiency Virus (HIV) infection • Fibrotic changes on chest X-ray consistent with prior tuberculosis (TB) • Recent close contact with pulmonary TB case • Recipients of organ transplantation • Immunosuppressive treatment including (but not limited to): systemic steroids (≥ 15 mg/day of equivalent prednisone for ≥ 1 month), tumor necrosis factor α antagonists
Reaction ≥ 10 mm (intermediate-risk)	• Recent immigrants from high-prevalence countries (or residents in high-prevalence countries) • Injection drug users • Residents and employees of: prisons, nursing homes, hospitals, homeless shelters • Mycobacteriology laboratory personnel • Persons with: silicosis, diabetes mellitus, chronic renal failure, leukemia, lymphoma, other malignancies, weight loss ≥ 10% of ideal body weight, gastrectomy, jejunoileal bypass • Children under 4 years old • Children and adolescents exposed to high-risk adults
Reaction ≥ 15 mm (low-risk)	• No risk factor

marker of an infection with viable (but dormant) bacilli, or rather a long-lasting immune marker of a previous infection that was completely eliminated (9). More important from a clinical and public health viewpoint is the fact that the presence of an Mtb-specific T-cell immune response carries a small, but significant risk of developing active tuberculosis in the future, and thus may be used as a tool to identify individuals that might benefit from preventive therapy.

Tuberculin skin test

Tuberculin skin test (TST) also known as Mantoux test, has been used for more than 100 years for the diagnosis of latent TB infection[5]. Tuberculin PPD (purified protein derivative) is a mixture of antigens, many of them being shared by other mycobacteria like *M. bovis, M. bovis* BCG and other species of mycobacteria. Tuberculin PPD RT 23 SSI (Statens Serum Institut, Copenhagen, Denmark, www.ssi.dk) is the standard recommended by the WHO and is the most widely used in Europe for Mantoux test; it contains 2 tuberculin units (T.U.) / 0.1 ml (0.04 mg of tuberculin PPD RT 23). In Romania there has been in use the Tuberculin PPD I.C. 65 (Institutul Cantacuzino, Bucharest, Romania, www.cantacuzino.ro); this tuberculin was found to be similar to the RT23 in a recent study (10).

TST consists of injection of 2 T.U. of Tuberculin PPD (0.1 ml) intradermally on one forearm. The test result is read 48-72 hours after antigen injection and the transverse maximal diameter of the induration formed at the injection level should be recorded in millimeters.

Test interpretation is dependent on the underlying risk of progressing to active tuberculosis and one recommendation for positivity criteria is described in *Table 1* (11). False-positive reactions can appear because of previous vaccination with bacillus of Calmette-Guerin (BCG) or exposure to nontuberculous mycobacteria. However recommendations for TST interpretation in BCG-vaccinated individuals are similar to those in non-BCG-vaccinated ones (11). False-negative reactions can appear in severe disease (including active TB) and immune suppression (including HIV infection) (12).

Recently it has been reported a first-in-man open clinical trial of a skin test with a *M. tuberculosis* specific reagent consisting of a 1:1 pro-

Table 2. Comparative characteristics of tuberculin skin test and interferon-γ release assays

	Tuberculin skin test	ELISPOT (T.SPOT-TB)	ELISA (QFT-G-IT)
Internal control	None	Nil and Positive (PHA)	Nil and Positive (PHA)
Antigens	PPD	ESAT-6 and CFP10 peptides	ESAT-6, CFP10 and TB7.7 peptides
Test substrate	skin	PBMCs	Whole blood
Time to result	72 h	20 h incubation and 3 h for final results	20 h incubation and 3-4 hours for final results
Outcome	Maximal transverse diameter of induration (millimetres)	Number of IFN-γ spot forming cells (T-cells)	Plasma concentration of IFN-γ produced by T-cells

PPD = purified protein derivative; ELISPOT = enzyme-linked immune spot; ELISA = enzyme-linked immunosorbent assay; QFT-G-IT = QuantiFERON-TB Gold In-tube; PHA = phytohemagglutinin; ESAT-6 = early secreted antigen target; CFP = culture filtrate protein; PBMCs = peripheral blood mononuclear cells; IFN = interferon

portion of a recombinant dimeric ESAT-6 (rdESAT-6) antigen and recombinant CFP10 (rCFP10) antigen in 41 adult healthy volunteers (13). Only one volunteer had a positive test consistent with a positive IGRA. No subject showed a sensitization on the second test performed. Thus this reagent seems promising for performing a more specific skin test for diagnosis of latent TB infection in low-resource environment where IGRAs cannot be performed. However there is a need for more clinical trials in different populations before it can be introduced in clinical practice.

In vitro interferon-γ release assays

Alternative methods have been developed to identify more accurately the T-cell response to Mtb antigens for diagnosis of latent TB infection. Several specific *M. tuberculosis* antigens have been identified for this purpose. These antigens are encoded by genes situated in the regions of difference RD1 (early secreted antigen target 6 – ESAT-6 – and culture filtrate protein 10 – CFP10 – antigens) and RD11 (TB7.7 antigens) of the genome of *M. tuberculosis* that are not found in the genomes of *M. bovis* BCG or many other nontuberculous mycobacteria like *M. avium* (14). However these antigens are not completely specific as their encoding genes are found in the genome of other mycobacteria: *M. kansasii, M. szulgai, M. marinum* and *M. riyadhense* (15).

Based on the IFN-γ T-cell response to stimulation with these specific antigens, two commercial methods have been developed in the last decade to overcome the limitations of tuberculin skin test in the diagnosis of LTBI: an enzyme-linked immunosorbent assay (ELISA) method, with a commercial name QuantiFERON TB Gold In-tube (latest version) (16) and an enzyme-linked immune spot (ELISPOT) method, with the commercial name T-SPOT.TB (17). Flow cytometry has also been used to measure IFN-γ release from stimulated T-cells in blood but only for research purposes, and will not be covered in this review. Several comparative characteristics of TST and IGRAs are shown in *Table 2*.

a. ELISA method (18)

QuantiFERON GOLD In-tube (Cellestis Ltd., Melbourne, Australia, www.cellestis.com) is an ELISA-based system that measures the IFN-γ release by T-cells from whole-blood after *ex-vivo* stimulation with Mtb-specific antigens: ESAT-6, CFP-10 and TB7.7.

Table 3. Criteria for interpretation of Quantiferon-TB Gold In-tube results (18)

Nil (IU/mL)	TB Antigen minus Nil (IU/mL)	Mitogen minus Nil (IU/mL)	Quantiferon-TB result	Interpretation
≤ 8.0	< 0.35	≥ 0.5	Negative	*M. tuberculosis* infection NOT likely
	≥ 0.35 and < 25% of Nil value	≥ 0.5		
	≥ 0.35 and ≥ 25% of Nil value	Any	Positive	*M. tuberculosis* infection likely
	< 0.35	< 0.5	Indeterminate	Results indeterminate for TB Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5		
> 8.0	Any	Any		

Responses to Mitogen positive control and occasionally TB antigen can be outside the range of the microplate reader, but this has no impact on test results.

Blood is collected in three 1mL tubes: Nil, Antigen (containing a mixture of ESAT-6, CFP10 and TB7.7 antigens) and Mitogen (containing phytohaemagglutinin as a cell functionality control). The tubes should be incubated at 37°C within 16 hours of collection. Following a 16 to 24 hour incubation period, the tubes should be centrifuged, the plasma is collected and the amount of IFN-γ (IU/mL) responses to the Mtb-specific antigens is measured by standard ELISA. Plasma is stable when refrigerated for up to 4 weeks or below -20°C (preferably less than -70°C) for extended periods.

Results are calculated using Analysis Software v1.5.1 (Cellestis Ltd., Melbourne, Australia, www.cellestis.com). Absorbances for the standards and test samples are entered into the computer and, based on the quality control acceptance criteria software, indicate pass or fail of the assay.

A test is considered "Positive" for an IFN-γ response to the Antigen tube that is significantly above the Nil IFN-γ value. The Nil sample adjusts for background, heterophile antibody effects, or nonspecific IFN-γ in blood samples. The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the Antigen tube and Mitogen tube. A high value in the Nil sample (> 8.0 IU/mL) yields an "Indeterminate" result and test should be repeated with another sample from the same patient. The Mitogen-stimulated plasma sample serves as an IFN-γ positive control for each specimen tested. A low response to Mitogen (Mitogen minus Nil <0.5 IU/mL) indicates an "Indeterminate" result, unless the Antigen minus Nil value is positive, in which case the result is valid. This pattern may occur with insufficient number of lymphocytes, reduced lymphocyte activity due to improper specimen handling, incorrect filling/mixing of the Mitogen tube, or the patient's lymphocytes anergy. Criteria of QuantiFERON-TB Gold In-tube results interpretation are described in *Table 3*.

b. ELISPOT method (19)

T-SPOT.TB (Oxford Immunotec Inc., Abingdon, UK, www.oxfordimmunotec.com) is an in vitro diagnostic assay which involves incubating peripheral blood mononuclear cells (PBMCs) with antigens specific for *Mycobacterium tuberculosis*. The assay involves four steps: sample collection and preparation, cell counting and dilution, plate set-up and incubation, and spot development and counting.

Blood samples can be collected in lithium heparin or sodium citrate blood collection tubes and then viable PBMCs are subsequently isolated using Ficoll-Paque^{TM} Plus density gradient centrifugation. Alternatively blood can be collected in Cell Preparation Tubes (CPT™), an evacuated BD Vacutainer tube containing

anticoagulant, separation gel and density gradient liquid which enables blood collection and PBMC separation to be conducted in one tube. Typically, for an immunocompetent patient, sufficient PBMCs to run the assay can be obtained from 4-8 ml venous blood; for immunocompromised patients, two 8 mL tubes should yield sufficient PBMCs to run the assay. Blood should be processed within 8 hours from venepuncture and should be stored at room temperature (18-25°C) until processed. After separation PBMCs should be washed twice in serum-free media (GIBCO^{TM}AIM-V or RPMI 1640 are suitable for this purpose) to remove endogenous cytokines. Serum-containing media should be avoided as variations in the background intensity may be observed.

T-SPOT.TB requires 250,000 viable white cells per well and four wells to be used for each patient sample. Cell counting and dilution should yield a concentration of 250,000 viable white cells per 100 µL.

Four wells are used for each patient sample: a Nil Control (only AIM-V medium), Panel A (ESAT-6 antigens), Panel B (CFP10 antigens) and Positive Control (phytohaemagglutinin as a cell functionality control). 100 µL cell suspension should be added to each well. The plate should be incubated for 16-20 h at 37ºC and 5% CO_2.

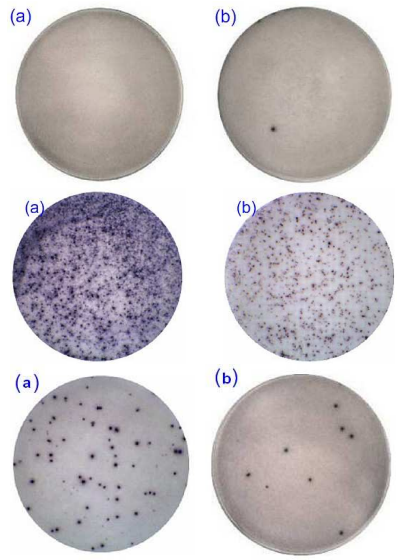

Figure 1. Typical T.SPOT-TB images: *top* Nil control (a) 0 spots, (b) 1 spot; *middle* Positive control (a) saturation, (b) > 20 spots; *bottom* Panel A or B (a) > 20 spots, (b) 8 spots

After the appropriate incubation time the wells should be washed thoroughly with PBS (Phosphate Buffer Saline). An appropriate volume of working strength Conjugate Reagent is prepared by making a 1/200 dilution in freshly prepared or sterile 1x PBS and added to each well. After incubation and thorough washing, substrate solution should be added and the spots should be allowed to develop for exactly 7 min. Reaction will stop by adding distilled water and the plate should be allowed to dry in the dark at RT. The number of distinct, dark blue spots on the membrane of each well should be counted either manually, using a magnifying glass or a suitable microscope, or using a dedicated ELISPOT plate reader instrument. Counting should be performed independently by two trained users and should be performed blind (i.e. without patient details). The results for all four wells (i.e. Nil Control, Panel A, Panel B and Positive Control) must be recorded.

A typical valid result will have few or no spots in the Nil Control (*Figure 1 top*) and more than 20 spots or saturated (too many to count) in the Positive Control (*Figure 1 middle*). The presence of more than 10 spots in the Nil Control should be considered as "Indeterminate" and the test should be repeated on another sample from the same patient. The presence of less than 20 spots in the Positive Control should also be considered "Indeterminate" unless either Panel A or Panel B responses are "Positive" (see below), in which case the result is valid. Weak responsiveness to PHA may reflect patient anergy.

T-SPOT.TB results are interpreted by subtracting the spot count in the Nil Control from the one in each of the Panels. The test result is "Positive" if either (Panel A minus Nil Control) or (Panel B minus Nil Control) is ≥ 6

spots and "Negative" if both of them are ≤ 5 spots (typical images can be seen in *Figure 1* bottom). Representative samples of positive and negative results (including controls) are shown in *Figure 2*. Significance of "Positive", "Negative" and "Indeterminate" is similar to those obtained after Quantiferon-TB test (see *Table 3*).

Performance of interferon-γ release assays for the diagnosis of latent tuberculosis infection

IGRAs have been developed specifically in order to overcome the limitations of TST for the diagnosis of latent TB infection. The diagnostic value of these tests has been evaluated in several systematic reviews (15, 20, 21). The following performance criteria have been evaluated (definitions are adapted from (22)):

- sensitivity (Sn) defined as the proportion of subjects with LTBI that have a positive test; because there is no gold standard for LTBI diagnosis, most studies measured sensitivity in microbiologically confirmed tuberculosis cases;

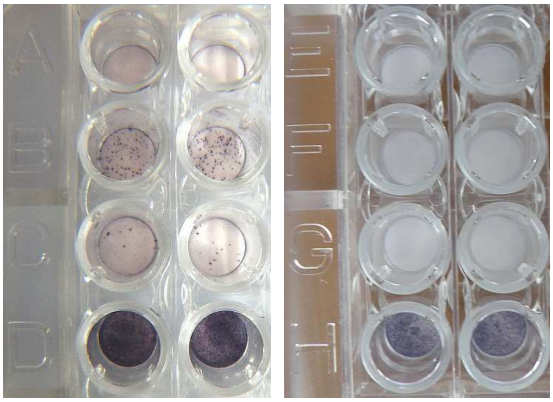

Figure 2. Representative images for T.SPOT-TB results including controls: *Left panel*: positive result (in duplicate) including A1,A2 – Nil control, B1,B2 – Panel A antigen, C1,C2 – Panel B antigen, D1,D2 – Positive control; *Right panel*: negative result (in duplicate) including E1,E2 – Nil control, F1,F2 – Panel A antigen, G1,G2 – Panel B antigen, H1,H2 – Positive control. Courtesy of Dr. Carmen C. Diaconu, Antiviral Treatment Unit, Stefan S. Nicolau Institute of Virology, Bucharest, Romania

- specificity (Sp) defined as the proportion of subjects without LTBI that have a negative test; because there is no gold standard for excluding LTBI diagnosis, all studies measured specificity in healthy individuals with no known TB exposure and from countries with a low tuberculosis incidence rate.

Although the sensitivity and specificity are important characteristics of a diagnostic test, they are difficult to use in clinical practice; consequently likelihood ratios (LR) are increasingly used:

- positive LR defined as the ratio of likelihood of a positive test in subjects with LTBI to likelihood of a positive test in subjects without LTBI;
- negative LR defined as the ratio of likelihood of a negative test in subjects without LTBI to likelihood of a negative test in subjects with LTBI.

Likelihood ratios have the same limitation in LTBI diagnosis as specificity and sensitivity. However they have several advantages (23): incorporate both Sp and Sn from the same study, are less likely to change with the prevalence of LTBI and can be used to calculate post-test probability for LTBI. Post-test odds of a condition are equal to pre-test odds multiply by the likelihood ratio. Positive LR tells how much the odds of a condition are increased by a positive test, and a negative LR tells how much the odds of a condition are decreased by a negative test. For example for a pre-test probability of 50% (or pre-test odds of 1:1) a positive LR of 10 will increase the post-test odds to 10:1 (equivalent to a post-test probability of 90%). For the same 50% pre-test probability, a negative LR of 0.1 will decrease the post-test odds to 1:10, equivalent to a post-test probability of 9% (adapted from (23)).

All these indicators are related to the present diagnosis of LTBI. Apart from the lack of a reference standard for LTBI diagnosis, the importance of LTBI lies rather in the future risk of developing active TB (i.e. progression to active disease) rather than just in its presence. Consequently two more parameters were evaluated in relation to IGRAs accuracy as diagnostic tests (15):

- negative predictive value (NPV) for progression defined as the proportion of subjects with a negative test that DO NOT develop active tuberculosis during follow-up;
- positive predictive value (PPV) for progression defined as the proportion of subjects with a positive test that DO develop active tuberculosis during follow-up.

Although both these parameters do not actually measure LTBI (as not all LTBI progress to active disease), they are however important from a clinical and epidemiological viewpoint, being useful in designing recommendations in national tuberculosis programs.

All parameters described below are for the current commercial versions of IGRAs: QuantiFERON TB Gold In-tube (QFT-G-IT), and T-SPOT.TB. Results for previous commercial and in-house versions are not shown, although they have been included in some systematic reviews. Most of the data presented below are in immunocompetent individuals, as data in immunocompromised ones are scarce. All parameters are described as value [95% confidence interval].

Sensitivity (Sn) was 70% [63% to 78%] for QFT-G-IT and 90% [86% to 93%] for T-SPOT.TB (20). Pooled Sn for TST was 77% [71% to 82%] (20). These results suggest that T-SPOT.TB is more sensitive than both QFT-G-IT and TST, and that there is no difference in terms of sensitivity between the last two.

Specificity (Sp) was high for both IGRAs in all reviews. When only studies in low incidence countries were taken into account, specificity was 98% [87% to 100%] for T-SPOT.TB (1 study) and pooled specificity was 99.4% [98% to 100%] for QFT-G-IT (15). Specificity was lower in a study performed in intermediate-incidence countries (85% for T-SPOT.TB and 92% for QFT-G-IT (24)), suggesting that some of the subjects were actually infected with *M. tuberculosis*. There was no significant difference in specificity of QFT-G-IT between BCG-vaccinated and non-BCG-vaccinated subjects (20). By contrast pooled specificity of TST was evaluated at 88.7% [85% to 92%], and ranged from 55% to 95% (15). TST pooled specificity was very high in non-BCG-vaccinated populations: 97% [95% to 99%], but low and variable in BCG-vaccinated populations (20). These results suggest that in low-incidence countries IGRAs have excellent specificity (especially QFT-G-IT), much better than TST in BCG-vaccinated populations. Specificity is slightly lower in studies in intermediate-incidence countries (and presumably even lower in high-incidence countries), but this is probably due to biased inclusion of control subjects infected with Mtb.

Likelihood ratios (LR) were computed in only one systematic review (21). Positive LR for LTBI were 7.9 [3.6 to 17.3] for T-SPOT.TB, 10.8 [5.3 to 21.8] for QFT-G (including the QFT-G-IT studies) and 2.2 [1.4 to 3.4] for TST $\geq$ 10mm. Negative LR for LTBI were 0.10 [0.06 to 0.18], 0.23 [0.16 to 0.32], and 0.51 [0.36 to 0.73] respectively. These results suggest that QFT-G-IT is slightly better than T-SPOT.TB in diagnosing LTBI, while the opposite is true for excluding LTBI. These considerations are in consonance with the Sn and Sp characteristics described above.

Positive and negative predictive values for progression to active tuberculosis are probably more clinically relevant than previous parameters. They were reported only in one systematic review (15).

Pooled negative predictive values for studies performed in low-incidence countries were 99.8% [99.4% to 100%] for QFT-G-IT and 97.8% [94.5% to 99.4%] for T-SPOT.TB. NPV in one study performed in intermediate-incidence country (Thailand) was 88% (25). NPV could be computed for TST in only one study, and the value was 99.7% [98.5% to 100%]. Thus a negative test (either IGRA or TST) in a low-incidence country would accurately predict a very low risk of developing active tuberculosis. This prediction would be less accurate in intermediate-incidence country, and probably

even less accurate in high-incidence country, although there have been no studies performed in such countries up to now.

Only four studies have evaluated the positive predictive value (PPV) for progression to active tuberculosis in subjects with positive IGRAs. Two studies were performed in HIV-positive individuals and showed a PPV for progression to active tuberculosis of 10% in 12 months for T-SPOT.TB (26) and 8.3% in 19 months for QFT-G-IT (27). Two more studies were performed in close contacts of infectious pulmonary TB cases. One showed a PPV for progression to active TB of 14.6% in two years for QFT-G-IT compared with 2.3% for a TST ≥ 5 mm and 5.6% for a TST ≥ 10 mm (28). In another study IGRAs were performed only in TST-positive individuals, and the PPV for progression was 3.1% for TST ≥ 10 mm, 2.8% for QFT-G-IT and 3.3% for T-SPOT.TB (29). The number of studies (and of subjects included) is still low, and there is a clear need for additional studies on the PPV for developing active tuberculosis in different settings (low-, intermediate- and high-burden countries). However, from data that we have until now, when excluding the study in which IGRA testing was restricted to TST-positive individuals, the PPV for progression was relatively high for IGRAs, between 8 and 15%, suggesting a better performance of IGRAs when compared to TST.

Performance of interferon-γ release assays for the diagnosis of active tuberculosis

Prompt diagnosis of active tuberculosis is needed for an effective treatment to be administered as soon as possible. However currently available techniques are inadequate for a rapid diagnosis of smear-negative pulmonary TB cases. The diagnostic performance of IGRAs performed on blood, but also in extrasanguinous fluids in the diagnosis of active tuberculosis was evaluated in two systematic reviews (21, 30).

All parameters described below refer to the current commercial versions of IGRAs: QuantiFERON TB Gold In-tube (QFT-G-IT), and T-SPOT.TB. Results for previous commercial and in-house versions are not shown. Apart from specificity and sensitivity (defined above), diagnostic odds ratio was also reported. Diagnostic odds ratio (DOR) is defined as the product of true classified cases (true positives and true negatives) divided by the product of false classified cases (false negative and false positive). Most parameters are expressed as pooled value [95% confidence interval].

TST and IGRAs performed in blood

Tuberculin skin test (TST) using tuberculin PPD RT23 had a pooled sensitivity of 65% [61-68%], a pooled specificity of 75% [72-78%] and a pooled DOR of 5.72 (30). For TST ≥ 10mm, the mean positive LR was 1.8 [1.0 to 3.3] and the negative LR was 0.63 [0.39 to 1.03] (21).

QFT-G-IT performed in blood had a pooled sensitivity of 80% [75-84%], a pooled specificity of 79% [75-82%] and a pooled DOR of 11.5 (30). Positive LR was 2.1 [1.1 to 4.0] and negative LR was 0.38 [0.22 to 0.68] (21).

T-SPOT.TB performed in blood had a pooled sensitivity of 81% [95%CI, 78-84%], a pooled specificity of 59% [95%CI, 56-62%] and a pooled DOR of 18.9 (30). LRs were calculated separately for subjects aged > 47 years old and < 47 years old. Positive LR was 4.7 [95%CI, 2.4 to 9.1] and negative LR was 0.11 [95%CI, 0.06 to 0.20] for subjects > 47 years old and respectively 2.3 [95%CI, 1.3 to 4.0] and 0.20 [95%CI, 0.10 to 0.40] for those < 47 years old (21).

The median proportion of indeterminate results was 7% for QFT-G-IT and 3.4% for T-SPOT.TB.

All this data show that either IGRA has a better performance as a diagnostic test for active tuberculosis than TST; however neither one is accurate enough to be used as a routine method for diagnosis of active tuberculosis, mainly because it cannot differentiate from latent infection. Consequently the use of IGRAs for active TB diagnosis would be particularly inaccurate in high-burden countries where latent infection (and though false-positive results) would be more frequent. However in one review, T-SPOT.TB seems to be effective in rul-

ing out active TB in subjects older than 47 years, although this awaits further confirmation.

IGRAs performed in extrasanguinous fluids

The most promising potential application of IGRAs in the diagnosis of active TB would be in the identification of a localized immune response at the site of disease. This would yield an alternative method of diagnosis in smear-negative pulmonary TB and also in some forms of extrapulmonary TB where a fluid sample can be obtained. Potential clinical samples would be: bronchoalveolar lavage fluid (smear-negative pulmonary TB), pleural fluid, ascitic fluid, cerebrospinal fluid, synovial fluid. IGRAs have been performed in first three of these and their diagnostic performances have been recently summarized (30).

64 patients have undergone QFT-G-IT and 128 patients have undergone T-SPOT.TB in extrasanguinous samples. Indeterminate results were 23.1% for QFT-G-IT and 5% for T-SPOT.TB. Pooled sensitivity was 48% [39% to 58%] for QFT-G-IT and 88% [82% to 92%] for T-SPOT.TB. Pooled specificity was 82% [70% to 91%] for QFT-G-IT and 82% [78% to 86%] for T-SPOT.TB. Finally pooled diagnostic odds ratio were 3.84 [1.73 to 8.5] for QFT-G-IT and 35.83 [15.6 to 82.4] for T-SPOT.TB.

Although the numbers of patients included in these studies were small, the results suggest that T-SPOT.TB in extrasanguinous fluids has fewer indeterminate results and better diagnostic performance than QFT-G-IT. More studies are needed however before recommendations can be made for clinical use of IGRAs in extrasanguinous fluids for diagnosis of active TB.

Conclusions

Interferon-γ release assays have better diagnostic performance than tuberculin skin test in latent TB infection diagnosis. T-SPOT.TB is better in excluding LTBI and QFT-G-IT is better in confirming it. Both IGRAs are valuable tools in predicting progression to active tuberculosis. A negative IGRA (and also a negative TST) in a low-incidence country is associated with a very low risk of progression to active tuberculosis. Conversely a positive IGRA is much better than a positive TST in predicting the risk of progression to active disease (8-15% versus 2-3%) in immunocompetent individuals and also in HIV-infected ones. Skin testing with recombinant Mtb-specific antigens is a promising (although still experimental) tool for LTBI diagnosis in low-resource environments.

There is still a need for additional studies regarding diagnostic characteristics of IGRAs in other categories of immunocompromised individuals and in high-incidence countries.

Blood-based IGRAs are slightly better diagnostic tools for active tuberculosis than TST, but their accuracy is not high enough to be used in clinical practice. However T-SPOT.TB in extrasanguinous fluids (and to a lesser extent QFT-G-IT) is a promising method for diagnosis of smear-negative pulmonary and some forms of extrapulmonary tuberculosis.

Abbreviations

BCG – bacillus of Calmette-Guerin
CFP – culture filtrate protein
DOR – diagnostic odds ratio
ELISA – enzyme-linked immunosorbent assay
ELISPOT – enzyme-linked immune spot
ESAT – early secreted antigen target
HIV – human immunodeficiency virus
IFN – interferon
IGRAs – interferon-γ release assays
LR – likelihood ratio
LTBI – latent tuberculosis infection
Mtb – *Mycobacterium tuberculosis*
NPV – negative predictive value
PBMCs – peripheral blood mononuclear cells
PBS – phosphate-buffered saline
PHA – phytohaemagglutinin
PPD – purified protein derivative
PPV – positive predictive value
QFT-G-IT – Quantiferon-TB Gold In-tube
RD – region of difference
Sn – sensitivity
Sp – specificity
SSI – Statens Serum Institut

TB – tuberculosis
TNF – tumour necrosis factor
TST – tuberculin skin test
WHO – World Health Organization

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