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ABSTRACT. An association between biologic agents and reactivation of active disease from latent tuberculosis infection (LTBI) has been established. Screening for LTBI is, therefore, now recommended for candidates for biologic drugs. The tuberculin skin test (TST) and interferon-γ release assays (IGRA) are the available commercial tests for detecting LTBI. We discuss their accuracy in immune-competent subjects and patients with autoimmune diseases, as well as potential new approaches to immune diagnosis. IGRA seem to be more accurate than TST in bacillus Calmette-Guerin vaccinated subjects and patients with autoimmune diseases. However, longitudinal studies are needed to estimate the risk of progression to TB after IGRA-based and/or TST-based diagnosis of LTBI in these vulnerable patients. New tests are needed to identify those patients with LTBI who will develop active TB and need prophylaxis. (J Rheumatol Suppl. 2014 May; 91:24–31; doi:10.3899/jrheum.140099)

Key Indexing Terms:
BIOLOGIC AGENTS ANTI-TUMOR NECROSIS FACTOR THERAPY TST
TUBERCULOSIS LATENT TUBERCULOSIS IGRA

Using a clinically pragmatic approach, one might define latent tuberculosis infection (LTBI) by the presence of a specific immune response detected by the tuberculin skin test (TST) or an interferon-γ release assay (IGRA), in the absence of active tuberculosis (TB). However, this response is not *per se* indicative of the risk of developing future active TB[1,2]. LTBI can be reactivated following a waning of the immune response, as in human immunodeficiency virus (HIV) infection, malnutrition, or after the use of immune-suppressant drugs in the context of transplantation or autoimmune diseases. In particular, it has been shown that treating autoimmune diseases with biologic drugs, such as tumor necrosis factor-α (TNF-α) inhibitors, increases the risk of TB development by 1.6- to 25-fold, depending on the clinical settings and the TNF antagonist used[3,4,5]. Here we discuss available commercial tests for measuring LTBI, their accuracy in immune-competent subjects and patients with autoimmune diseases, and potential new approaches to TB immune diagnosis.

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Tests for Measuring Latent TB Infection

There are 2 types of tests for measuring LTBI, the TST and IGRA, both of which of have advantages and disadvantages in particular circumstances (Table 1).

Tuberculin skin test. The TST has been available for the last 100 years. It consists of the intradermal injection of purified protein derivative (PPD), which induces a delayed-type hypersensitivity response. Tuberculin PPD is a crude mixture of antigens in which heat shock proteins predominate[6], many of which are shared by *Mycobacterium tuberculosis* (Mtb), *M. bovis*, *M. bovis* Bacillus Calmette Guérin (BCG), and several species of environmental mycobacteria. TST reactions are measured in mm of diameter of induration, 48–72 h after injection of the antigen.

From a histological point of view, the classic model of cellular infiltration during a delayed-type hypersensitivity response suggests that cell migration is biphasic, comprising an initial nonspecific infiltration (neutrophils) that also occurs in nonsensitized subjects, and a second specific peak (mainly CD4 T cells)[7,8,9,10,11]. The mechanism of this cellular infiltration is not completely clear, but it is likely that early after the injection, proinflammatory cytokines such as interferon-γ (IFN-γ), TNF-α, and TNF-β stimulate expression of adhesion molecules (e.g., E-selectin) on the endothelium and increase the permeability of the local blood

Table 1. Characteristics of immune diagnostic tests for tuberculosis.

Test Characteristics	TST	QFT-GIT	T-SPOT TB
Internal control	No	Yes	Yes
Mtb-specific antigen	No	Yes	Yes
Test substrate	skin	Whole blood	PBMC
Time required for results, h	72	16–20	16–20
Cells involved	Neutrophils, CD4, CD8 that transmigrate out of capillaries into the skin. Role of Treg [CD4+CD25^{high}FoxP3+] (10–12)	CD4 T cells *in vitro* with effector memory and central memory phenotype	CD4 T cells *in vitro* with effector memory and central memory phenotype
Cytokines involved	IFN-γ, TNF-α, TNF-β (10, 11)	IFN-γ	IFN-γ
Overall sensitivity for TB infection			
HIV-uninfected	77% (32)	70–80% (32)	80–91% (32)
Overall specificity for TB infection	59%/97% (32, 33)	96% (32)	93% (32)
Overall specificity for active TB	—	79% (33)	59% (33)
Criteria for conversion established by evidence	Yes	No	No
Cutoff established depending on age, immune suppression, BCG vaccination status	Yes	No	No
Ability to detect those at high risk of developing active TB	Weak (32, 37)	Weak (32, 37, 38)	Weak (32, 37, 38)

Values in parentheses are reference numbers. TB: tuberculosis; BCG: bacillus Calmette Guerin; IFN: interferon; TNF: tumor necrosis factor; TST: tuberculin skin test; QFT-GIT: QuantiFERON TB Gold in tube; PBMC: peripheral blood mononuclear cells; HIV: human immunodeficiency virus.

vessels. Circulating CD4+CD25+FoxP3+ Treg cells influence the area of the TST induration[12]. Cutaneous CD4 T cells accumulating after PPD stimulation have a predominant CD45RO memory phenotype[12].

It is widely accepted that an induration > 5 mm is a positive reaction. Different cutoff sizes that allow for estimating the risk of developing TB, based on factors such as age, BCG-vaccination, and immune suppression diseases are also considered.

Although broadly used, TST has limitations. Sensitivity may be reduced by malnutrition, severe active TB disease, and immunodeficiency states, such as that related to HIV infection[13]. Decreased specificity is associated with exposure to nontuberculous mycobacteria and BCG-vaccination, although after 10 years or more, the effect of BCG-vaccination on TST reactions is limited if the vaccination was given in infancy[14]. Moreover, a TST necessitates 2 healthcare visits, one for the PPD injection and the other to measure the induration, leading to a loss of reading in around 10% of the cases[15]. Cutoff points have been defined to identify when preventive therapy is indicated for different age and risk groups[16]. Definitions of conversion and boosting have also been established; conversion is defined as an induration over 10 mm with an increase of at least 6 mm over the previous result[17]. The positive predictive value for TB development is low when considering high-prevalence and low- and middle-income settings[18], but increases in those with immunodeficiency[19,20,21].

IFN-γ release assays. IGRA have been available only for the past decade. Two licensed IGRA exist: QuantiFERON TB Gold in tubes (Cellestis, a QIAGEN Company; QFT-GIT) and T-SPOT.TB (Oxford Immunotec). Both tests measure *in vitro* IFN-γ production by a whole blood ELISA[22] or an enzyme-linked immunospot (ELISPOT) assay on peripheral blood mononuclear cells[23,24] (Table 1). The IFN-γ is produced by circulating T cells after 16–20 h stimulation in response to Mtb-specific antigens. The genes encoding these antigens are found in the regions of difference (RD), either RD1 (CFP-10 and ESAT-6) or RD11 (TB7.7), of the Mtb genome, which are deleted from the genome of *M. bovis* BCG and are not present in most environmental mycobacteria, including the *M. avium* complex[25,26,27]. The T cells that respond to the RD1 antigens are predominantly CD4. The phenotype of these cells can be characterized as being predominantly "effector memory" in patients with active disease (consistent with having recently encountered an antigen *in vivo*)[28] or as being predominantly, but not exclusively, "central memory" in those in whom Mtb replication is controlled either naturally (LTBI) or by drugs (patients successfully treated for active TB)[28,29,30].

A particular advantage of *in vitro* testing is that stimulation reactions with negative and positive controls (mitogen stimulus) are carried out in parallel, primarily to evaluate test performance with respect to background signals or general T cell responsiveness. In the setting of immunodeficiency, an impaired mitogen response may additionally be interpreted as a meaningful measure for assessing the overall extent of immunosuppression. Therefore, unlike TST, *in vitro* tests may be able to discriminate true negative responses from anergy. In general, the results of IGRA and TST correspond poorly, although agreement is stronger in countries with a low TB prevalence and low BCG-vacci-

nation coverage[31], as reported in detail below. Moreover, compared to the TST, positive IGRA responses are more closely associated with risk factors for LTBI.

Accuracy of TST and IFN-γ Release Assays in Immune Competent Subjects

IGRA have the same Achilles' heel as TST: there is no "gold standard" for LTBI. Therefore, as surrogate "standards," "active TB" status is used when assessing IGRA sensitivity, and subjects at extremely low risk when assessing specificity[22,32] (Table 1).

Sensitivity. Several metaanalyses report that the sensitivity of both IGRA in detecting active TB is higher (78–92%) than that of the TST (65–77%); however, the sensitivities of IGRA are not high enough to be used as tests to rule out active TB[33].

Specificity. The specificity for the detection of active TB is higher for IGRA than for TST when considering BCG-vaccinated subjects among those without active disease. Indeed, Pai, *et al* reported a pooled specificity of 99% among non-BCG-vaccinated and 96% among BCG-vaccinated low-risk groups[31]. However, as shown recently[33] by a metaanalysis conducted by TBNET[34], when assessed among controls including TB suspects, specificity decreased (59–79%) and thus must be considered as insufficient. These assays cannot, therefore, distinguish between active TB and LTBI, as also previously shown[24,35,36].

Negative predictive value. Studies performed in low-incidence countries showed that the negative predictive value for progression to TB within 2 years is high (98–99% for IGRA), whereas it is lower in an intermediate-burden country such as Thailand (88%)[37]. In the few studies in which IGRA and the TST were concomitantly performed to compare the negative predictive value estimates, it was shown that the negative predictive value for the TST was 99.7% compared to 100% for the QFT-GIT[37].

Positive predictive value. The strength of the association between positive IGRA results and development of active TB was reported as weak to moderate, with relative risks of about 2–3 when considering studies performed in high-prevalence, low- and middle-income settings; the analysis was done using in-house developed and commercial IGRA[18]. Differently, a metaanalysis of studies conducted on individuals from a low-prevalence setting using only commercial IGRA showed a relative risk varying between 8 and 15[37]. Recently an additional metaanalysis, involving only studies with a definite followup for the development of active TB, was performed. This analysis showed relative risks of up to 6.8 for IGRA and 2.4 for the TST[38]. Therefore up to now, no available tests for LTBI have been shown to have a high prognostic value. However, in some populations the proportion of IGRA-positive individuals might generally be lower than the proportion of TST-positive individuals. This characteristic of IGRA might be useful in settings in which TST specificity is compromised by cross-reactivity with environmental mycobacteria, BCG-vaccination after infancy, or multiple BCG-vaccinations.

Accuracy in Vulnerable Populations and Persons with Autoimmune Diseases

Patients with rheumatoid arthritis (RA) not undergoing immunosuppressive therapy are characterized by a reduced recall response, often called anergy, which leads to false-negative TST results[39,40]. This effect may be due to T cell abnormalities and to the reduced antigen presenting capacity of monocytes[41,42]; a decrease in memory CD4 T cells (CD4+CD45RA–) has also been found in anergic patients, which may also contribute to the decrease in antigen reactivity[43]. Immune-suppressive therapy may worsen this situation. In fact, it has been shown that high doses of prednisone (≥ 15 mg/day)[44] and methotrexate, both drugs used for the treatment of RA, can cause false-negative TST reactions[44]. Similarly, *in vivo*, TNF antagonists have been shown to decrease the frequency of the subpopulation of memory CD4 T cells, rapidly releasing IFN-γ upon challenge with mycobacterial antigens (PPD, CFP-10)[45]. Moreover, if TNF antagonists are added *in vitro*, they inhibit the activation of CD4 T cells by mycobacterial antigens[45].

The literature contains evidence that in subjects with autoimmune diseases screened for LTBI, IGRA may provide a higher proportion of positive responses than TST[46]; indeed, up to 50% of IGRA-positive patients are missed by the TST[47,48,49,50]. This is probably because IGRA are *in vitro* tests relying on rapid production of IFN-γ by circulating mononuclear cells in response to antigens, whereas the TST requires an intradermal infiltration of T-specific cells through the induction of several cytokines, including TNF, often reduced by drug administration. Moreover, the advantages of IGRA are that confounding factors related to BCG-vaccination are avoided, IGRA are closely associated with risk factors for LTBI[50], and they have a low rate of indeterminate results (0–10.3%)[47]. Current evidence suggests that IGRA and TST results correspond poorly, although agreement is stronger in countries with a low prevalence of TB and low BCG-vaccination coverage[47,51]. Ideally in the clinical setting, immune-based diagnosis for LTBI is performed to identify individuals at risk of developing TB, but up to now, the positive predictive value of IGRA responses for development of TB in candidates undergoing therapy with TNF antagonists is not known. IGRA have shown to have a high negative predictive value for active TB development[52,53,54] in immunocompromised patients other than those with autoimmune diseases, such as HIV-infected subjects. Currently, among those with previous TB successfully treated, it is unknown whether the individuals with persistent positive IGRA scores have a higher risk of reactivating TB than subjects scored as

IGRA-negative or subjects who reverted from a positive to a negative IGRA. Moreover, it is still unclear whether IGRA testing may also be more accurate than TST for screening patients who have already received TNF antagonists, as results vary from one study to another[47,48,49,50]. The potential superiority of the IGRA over TST is reasonable; however, to prove it, we need studies that evaluate the positive predictive value of different immunodiagnostic tests in these patients.

Serial IFN-γ Assays

Serial testing for LTBI is performed every 1–2 years in highly exposed groups, such as healthcare workers and prisoners, as well as patients at high risk of disease, such as HIV-infected subjects or subjects with autoimmune diseases who are candidates for biologic agents. Although it has been reported that TST may boost IGRA responses, this effect is not seen if IGRA are done within 3 days of performing the TST[6,55].

Unlike the TST, which should only be repeated if previously negative, IGRA tests may be repeated. However, high rates of spontaneous reversions and conversions are found in those untreated with anti-TB drugs, although it is always difficult to know whether conversion is spontaneous or a consequence of real Mtb infection or laboratory inaccuracy[32,56].

Although QFT-GIT conversion has been reported to be related to higher risk of progression to TB[57], especially in particular circumstances such as when children are exposed to Mtb as newborns[58], or in patients with autoimmune diseases receiving biologic therapies[59], the predictive value of IGRA (QFT-GIT) conversion for development of TB disease is still debated, and fluctuations in IFN-γ responses among serially tested individuals reported in longitudinal studies remain unexplained and nonspecific.

Importantly, spontaneous reversions and conversions are more frequent in subjects with borderline results close to cutoff values and are more likely if scored negative in the TST[51,60]. In such cases, it has been interpreted as self-clearance of infection, but there is not enough evidence to draw such a conclusion[56,61]. For these reasons, serial testing with IGRA should be considered unreliable and is not recommended, at least until the conversion and reversion phenomena are better understood in both immune-suppressed and immune-competent patients[32,51].

Potential Improvement of Immune-based Tests

Potential improvement of immune-based tests can be achieved using different cutoff points and formats.

Different cutoff points and the proposed concept of a "gray zone." Using the TST, subjects are classified as positive or negative for LTBI using cutoff points depending upon the presence or absence of key comorbidities, such as HIV, autoimmune diseases, as well as a given epidemiological situation.

As shown above, IGRA promises to be more specific than the TST. However, no "gold standard" for LTBI is available. In addition, the intensity of IFN-γ responses may vary with the underlying condition, i.e., TB or LTBI: for a fixed cutoff point, sensitivity may be different if the IGRA is used to detect active TB as opposed to LTBI. It has recently been shown that test specificity for LTBI and test sensitivity for active TB actually depend upon the proportion of active TB and LTBI subjects in the study population and that there is a tradeoff between maximizing the specificity of IGRA to LTBI and sensitivity to active TB. Different cutoff points for IGRA use in suspected TB and suspected LTBI subjects in relationship to the prevalence of TB in that country will probably be needed[62,63].

Moreover, during QFT-GIT serial testing, previous studies found nonspecific variations, defined as an "uncertainty zone" (IFN-γ value in response to Mtb antigen between 0.20 and 0.50 IU/ml) and a "gray zone" (IFN-γ value in response to Mtb antigen between 0.10 and 0.35 IU/ml)[32,61,64]. Recent studies on subjects with autoimmune diseases also considered the use of different cutoff values for LTBI diagnosis but did not reach any final conclusions[51,65].

Different Formats

Neither of the 2 IGRA can currently distinguish active TB from LTBI. Consequently, novel concepts that include the use of different antigens and readouts have been investigated to develop better assays.

Antigens. To design new diagnostics, it is necessary to extend our knowledge of potential immunogenic Mtb antigens[66,67]. Ideally, antigens should represent the different stages of Mtb infection and should include Mtb antigens expressed during the early onset of infection (growth stage), the latent/dormancy stage, and resuscitation of the dormancy stage[68,69].

Epitopes of CFP-10 and ESAT-6. Different epitopes of CFP-10 and ESAT-6 selected by computational analysis to be multiepitopic, called "RD1 selected peptides"[24,70], have been shown to be associated with the active phase of Mtb replication as in active TB[30,71,73] and recent infection[73]. Unlike QFT-GIT, the RD1-selected peptide response decreases significantly after therapy for active disease[74,75,76,77] or prophylaxis in recent infections[73]. The difference might be related to the amount and composition of epitopes covered by the peptides used in the 2 different tests: QTF-GIT peptides cover all the CFP-10 and ESAT-6 intact proteins[22] (in addition to having TB7.7 peptide from the RD11 region) whereas the RD1-selected peptides are few and selected to be highly immunogenic[24,70]. Therefore, an oligoclonal response (rather than a polyclonal one against all RD1 epitopes) appears to be a sensitive tool for monitoring Mtb replication[73] as well as active disease[74,75].

Secreted antigens other than CFP-10 and ESAT-6. Rv3615c encoded outside RD1, similar in size and sequence

homology to CFP-10 and ESAT-6, has been shown to be highly immunogenic and associated with Mtb-specific responses[78].

Antigens of latency. During LTBI, persisting tubercle bacilli are deprived of nutrients and oxygen[10,11,79,80] and, as part of the adaptive response of Mtb to hypoxia, expression of the dormancy survival regulator (DosR) regulon is observed. The functions of most DosR-regulon encoded proteins, hereafter referred to as "latency antigens," are unknown[81,82]. However, it has recently been shown that IFN-γ responses to certain latency antigens are associated with LTBI in peripheral blood[66,83,84,85], and not at the site of TB disease[9,86].

Similarly, an IFN-γ response to heparin-binding hemagglutinin has been associated with LTBI[87,88,89], whereas a low response was found in patients with active TB, because of the suppressive capacity of the Treg cells in the periphery[88].

Immune Factors Other Than IFN-γ

Immune factors other than IFN-γ have been proposed as alternative markers to detect Mtb-specific responses using the QFT-GIT. There is strong evidence that IFN-γ-inducible protein-10 is an alternative marker of Mtb-specific responses[71,90,91] with less dependence on the CD4 T cell counts in HIV-infected subjects[72,92]. Simultaneous detection of IFN-γ and interleukin-2 profiles of RD1-specific T cells using a modified QFT-GIT has enabled identification of subjects with TB, distinguishing LTBI from active TB[93]. Polyfunctional T lymphocytes, cells simultaneously producing a range of cytokines, have been associated with superior functional capacity and are correlated with TB control[28,94,95,96,97,98]; moreover, an effector memory phenotype has been associated with active disease whereas a central memory phenotype has been associated with cured TB or LTBI stages in HIV-uninfected[28] or HIV-infected subjects[98].

TST measures *in vivo* PPD-specific cell-mediated immunity. Because of its poor specificity, this test may be inadequate to assess evidence of LTBI in BCG-vaccinated patients. IGRA are *in vitro* tests that rely on the rapid production of IFN-γ by circulating mononuclear cells in response to Mtb-specific antigens. IGRA testing in vulnerable patients such as individuals with immune-mediated inflammatory diseases is feasible because of a strong correlation with risk factors for TB.

Longitudinal studies are needed to estimate the risk of progression to TB after IGRA-based and/or TST-based diagnosis of LTBI in these vulnerable patients. Serial IGRA testing is not recommended for those already scored positive. As previously established for TST, IGRA cutoff stratification based on age and immune suppression is probably needed. New immune-based tests are needed to distinguish active TB from LTBI and as tools that may help to predict which subjects with LTBI will develop active TB, thereby improving the identification of subjects needing prophylaxis.

The above discussion on performance of assays can be summarized as follows:

- Among patients with autoimmune diseases, up to 50% of IGRA-positive patients are missed by TST, suggesting that IGRA are accurate tests for LTBI diagnosis in these patients
- Longitudinal studies are needed to estimate the risk of progression to TB after an IGRA/TST-based diagnosis of LTBI, because of the low estimated risk found in immune-competent subjects
- Owing to scarce evidence, it is safer to suggest both the TST and IGRA as screening tests for LTBI in patients with autoimmune diseases
- Based on the present evidence, serial tests are not recommended for IGRA-positive subjects.

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