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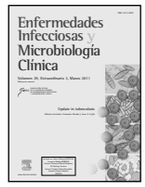

Diagnosis of tuberculosis infection using interferon-γ-based assays

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A B S T R A C T

Interferon-γ-based assays, collectively known as IFN-γ release assays (IGRAs), have emerged as a reliable alternative to the old tuberculin skin test (TST) for the immunodiagnosis of tuberculosis (TB) infection. The 2 commercially available tests, the enzyme-linked immunosorbent assay (ELISA), QuantiFERON-TB Gold In-tube (QFT-IT), and the enzyme-linked immunospot assay (ELISPOT), T-SPOT.TB, are more accurate than TST for the diagnosis of TB, since they are highly specific and correlate better with the existence of risk factors for the infection. According to the available data, T-SPOT.TB obtains a higher number of positive results than QFT-IT, while its specificity is lower. Although the sensitivity of the IFN-γ -based assays may be impaired to some extent by cellular immunosuppression and extreme ages of life, they perform better than TST in these situations. Data from longitudinal studies suggest that IFN-γ-based tests are better predictors of subsequent development of active TB than TST; however this prognostic value has not been consistently demonstrated. This review focuses on the clinical use of the IFN-γ -based tests in different risk TB groups, and notes the main limitations and areas for future development.

Diagnóstico de la tuberculosis mediante las técnicas basadas en la detección de interferón-γ

Palabras clave:
Determinación de liberación de interferón-γ
QuantiFERON-TB Gold In-Tube
T-SPOT.TB

R E S U M E N

Las técnicas de detección de la liberación de interferón-γ conocidas como IFN-γ *release assays* (IGRA), constituyen una alternativa fiable a la clásica prueba de la tuberculina (PT) para el inmunodiagnóstico de la infección tuberculosa. Las 2 pruebas comerciales disponibles, QuantiFERON-TB Gold In-tube (QFT-IT) y T-SPOT.TB, son más precisas que la PT para el diagnóstico de tuberculosis (TB), ya que son muy específicas y presentan una mejor correlación con la existencia de factores de riesgo para la infección tuberculosa. Según los datos disponibles, T-SPOT.TB detecta mayor número de positivos que QFT-IT, pero es menos específica. Aunque en determinadas situaciones, como en pacientes con inmunosupresión celular y en las edades extremas de la vida, estas técnicas siguen siendo superiores a la PT. Estudios longitudinales sugieren que las pruebas de liberación de IFN-γ son mejores predictores de la progresión a enfermedad tuberculosa; sin embargo, este hecho no ha sido demostrado completamente. Esta revisión trata el uso de los test de liberación de IFN-γ en diferentes grupos de riesgo de TB. Asimismo, remarca sus principales limitaciones y las áreas de desarrollo futuro.

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From the tuberculin skin test to the interferon-γ-based assays

The tuberculin skin test (TST), that recalls the delayed-type hypersensitivity response to the intradermal inoculation of purified protein derivate (PPD),[1] has been used to diagnose TB infection for the last hundred years. The PPD contains a mixture of more than 200 antigens that are widely shared by mycobacteria other than *Mycobacterium tuberculosis,* including the vaccinal strain of *Mycobacterium bovis* bacilli Calmette-Guérin (BCG) and many non-tuberculous mycobacteria (NTM). As a result, individuals sensitized by previous exposure to NTM or BCG vaccine may respond immunologically to PPD. The other main limitation of the TST is its low sensitivity in certain groups of individuals, such as immunosuppressed patients and young children.[2]

Immunodiagnostic methods have been developed based on the *in vitro* quantification of the cellular immune response, by detecting interferon-gamma (IFN-γ) released by sensitized T-cells stimulated with specific *M. tuberculosis* antigens. The two main antigens used are the 6-kD *M. tuberculosis* early-secreted antigenic target protein (ESAT-6) and the 10-kD culture filtrate protein (CFP-10), encoded in the region of difference 1 (RD1), which is present in *M. tuberculosis* but not in BCG or in most NTM.[3] This new *in vitro* technology has been rapidly adapted from initial *in-house* methods to the two commercially available techniques: QuantiFERON-TB Gold assays (QFT-G) (Cellestis Limited, Carnegie, Victoria, Australia) and T-SPOT. TB assay (Oxford Immunotec, Oxford, UK). Both tests, collectively known as IGRAs (Interferon-Gamma Release Assays), have been approved for sale in Europe and have received final approval from the U.S. Food and Drug Administration (FDA) as an aid for diagnosing *M. tuberculosis* infection. T-SPOT.TB detects the number of IFN-γ producing T-cells after stimulating a definite number of isolated peripheral blood mononuclear cells (PBMCs) with ESAT-6 and CFP-10 separately by means of enzyme-linked immunospot assay (ELISPOT). QFT-G tests are whole blood assays that use an enzyme-linked immunosorbent assay (ELISA) to detect IFN-γ produced in supernatants by stimulated T-cells. The QFT-G In Tube version (QFT-IT), includes a third antigen, TB7.7. This new antigen is encoded in RD11 and is missing from the BCG strains as well as most common environmental mycobacteria.[4] In the QFT-IT assay, the three specific *M. tuberculosis* antigens are already incorporated into the same tube (Fig. 1). Both *in vitro* tests include a positive control that detects the capacity of T cells to produce IFN-γ upon stimulation with a mitogen (phytohemagglutinin), in order to distinguish false-negatives from indeterminate results.

Interferon-γ-based assays for detecting latent infection in high-risk populations

This section will discuss the potential value of the IFN-γ-based tests in diagnosing latent TB infection (LTBI) in people at high risk of progression to active disease.

Contact tracing study

Between 5% and 10% of recently infected contacts will develop active TB within 2-5 years after exposure. The identification and treatment of these individuals constitutes an essential component of the TB control strategy in low-prevalence countries. Numerous studies have explored the utility of the IFN-γ-based tests in contact investigations.[5-9] In the absence of a gold standard test for the diagnosis of LTBI, the best approach to compare IFN-γ-based tests and TST consists of correlating their results with the degree of exposure to an infectious case. Positive results of IFN-γ-based tests were found to be more strongly associated with greater recent exposure than TST;[5,8] however, this association could not be demonstrated by others.[9] Besides, IFN-γ-based tests offer the advantage of high specificity, since they are not affected by prior BCG vaccination or by infection with most NTM.

Health care workers

Due to the risk of infection with *M. tuberculosis* through occupational exposure, periodical testing is recommended for all health care workers (HCWs). Serial TST testing may induce a boosting phenomenon, compromising its interpretation.[10] In a study performed in Barcelona (Spain),[11] prevalence of LTBI in HCWs without a previous positive TST was higher according to T-SPOT.TB (23.1%), and QFT-IT (17.3%) than according to TST (15.4%). Positive IFN-γ tests were associated with age and degree of occupational exposure, but not with BCG vaccination, a finding consistent with previous studies with QFT-G tests.[12,13] Although IFN-γ-based tests have became a good alternative to TST for serial testing of HCWs, factors such as reversions and conversions should be taken into account. Choi et al[14] described conversions of QFT-IT in HCWs 2-4 weeks after performing a TST test among TST reactors, but not among non-reactive individuals. Similarly, van Zyl-Smit et al[15] reported conversions a week after TST administration. However, when using a two-step screening strategy, IFN-γ test results were not influenced if TST was performed within three days.

Immunocompromised patients

The performance of the IFN-γ tests in immunocompromised patients and the effect of immunosuppression on these tests remains unclear.[16] Previous studies including different groups of immunocompromised patients found impaired performance of IFN-γ-based tests related to malfunction of cellular immune system, but they performed better than TST nonetheless.[17,18] In a prospective study including 369 immunosuppressed participants, Richeldi et al[19] found that IFN-γ tests detected more patients as being infected with *M. tuberculosis* than did TST.

HIV infected patients

Patients co-infected with HIV and *M. tuberculosis* are particularly prone to a reactivation of LTBI and development of disseminated disease. In studies evaluating T-SPOT.TB and its ELISPOT pre-commercial version[20,21] or the QFT-G tests,[22,23] *in vitro* tests obtained higher rates of positive results than TST in diagnosing LTBI,[23,24] and a better association between positive results and presence of risk factors for LTBI.[22,23] In recent years, some studies in HIV-infected populations reported similar sensitivities for both IFN-γ tests.[21-25] As regards indeterminate results, a correlation between low $CD4^+$ cell counts and a low control positive response was found with QFT-IT,[22] while T-SPOT.TB and *in-house* ELISPOT appeared to be relatively unimpaired by low $CD4^+$ cell counts.[24,26,27] However, higher rates of indeterminate results with T-SPOT.TB and *in-house* ELISPOT have also been reported.[24,28]

Chronic immune-mediated inflammatory disease

Tumor necrosis factor (TNF)-α antagonists provide reliable treatment in patients with immune-mediated inflammatory diseases (IMID).[29] TNF-α is one of the key molecules involved in granuloma formation and containment of TB infection. Due to the increased risk of TB in patients receiving anti-TNF-α agents,[30] exclusion of active TB and screening for latent infection is mandatory before starting anti-TNF-α therapy.[31] However, cellular-mediated response to PPD is compromised by the corticosteroids and/or immunosuppressive drugs that most patients with IMID are already taking.[29,31] Experience with the IFN-γ-based tests in this population, although promising, is still limited.[32,33] Overall, agreement with the TST seems to be poor.[34-36] The discordant positive TST and negative IFN-γ-based test results have been attributed to false-positive TST results,[37,38] whereas the

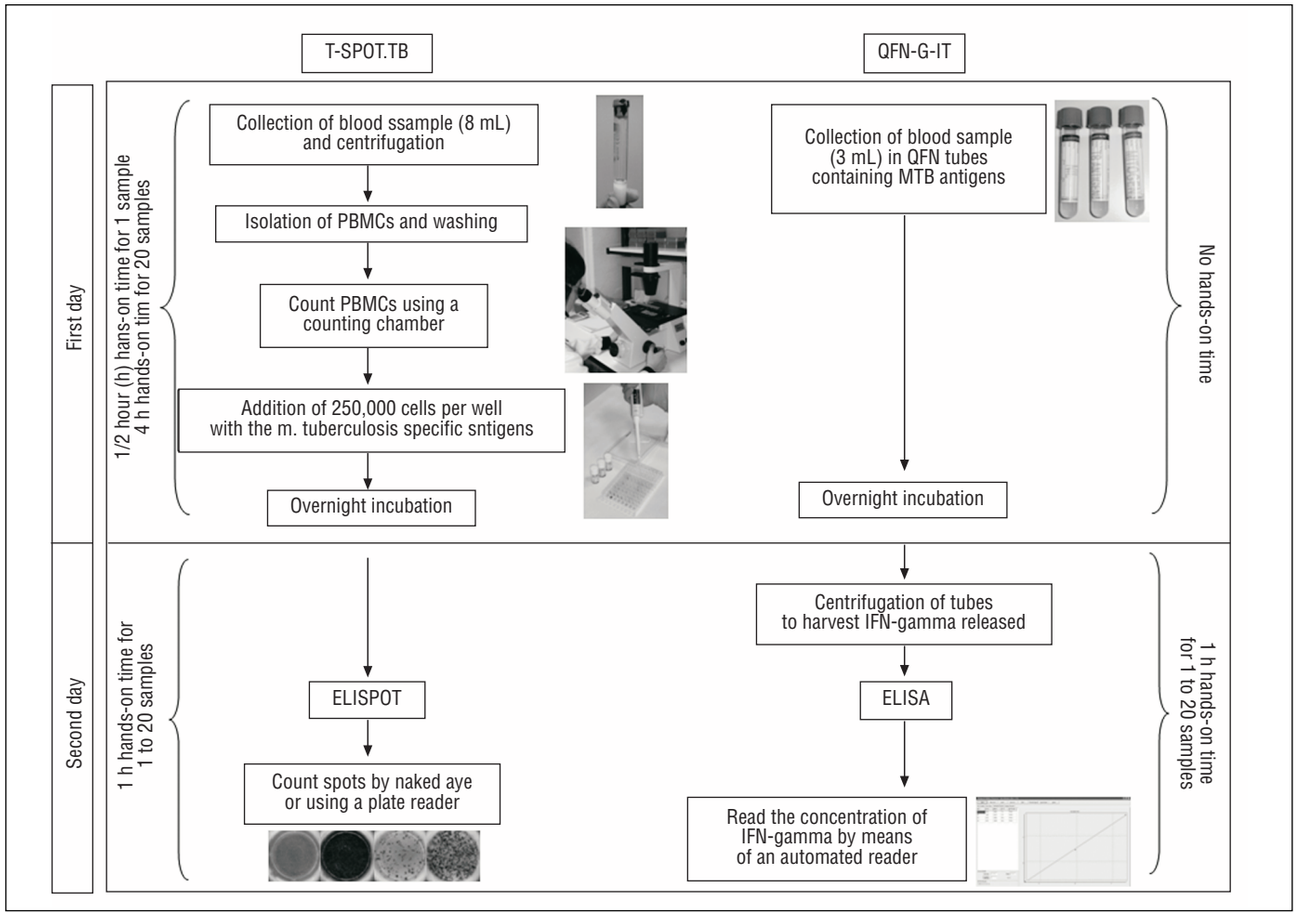

Figure 1. Comparison of T-SPOT.TB and QuantiFERON-TB Gold In Tube (QFT-IT) methodology. ELISA: enzyme-linked immunosorbent assay; ELISPOT: enzyme-linked immunospot assay; IFN: interferon; PBMCs: peripheral blood mononuclear cells.

discordant negative TST and positive IFN-γ-based test results have been considered false-negative TST results due to the immunosuppressive therapy being taken by these patients.[35,36,39] The available data show that the IFN-γ-based tests detect more cases of LTBI than TST does,[36,40] and a closer association with the presence of risk factors for TB infection.[34,35] The effect of DMID-associated immunosuppression on the performance of the IFN-γ-based tests has not been completely clarified. In a study involving 398 consecutive subjects, Bartalesi et al[41] did not find an association between results of the TST or QFT-IT and the use of conventional disease-modifying antirheumatic drugs, but reported an association of steroids with a lower likelihood of a positive result. In view of the high risk of TB in IMID patients receiving anti-TNF-α therapy, a strategy based on a simultaneous TST and one of the IFN-γ tests might maximize diagnostic sensitivity for the detection of LTBI.

Paediatric population

Children have a high risk of progression to active TB, especially infants under the age of two.[42] Early, specific diagnosis of LTBI is therefore crucial to prevent active disease. The sensitivity of TST in young children is unknown, but the existence of immaturity certainly induces a lower cutaneous response. In addition, BCG-vaccination, especially in TB endemic areas, reduces the test's specificity. Overall, T-SPOT.TB provides higher rates of positive results for LTBI than TST or the QFT-G tests. Recently, Davies et al[43] found that, in contrast to TST, ELISPOT results were not affected by young age or severe immunosuppression. Furthermore, a high correlation with the degree of exposure to *M. tuberculosis* with both IFN-γ-based tests has been demonstrated.[44] Results of both IFN-γ-based tests are unrelated to the BCG-vaccination status, which contributes to their high specificity.[4,7] Discordant results between these tests and TST are frequently found.[7,45-48] In a study conducted in Barcelona (Spain),[7] among BCG-unvaccinated children, 60% and 57% of those with positive TST had negative QF-IT and T-SPOT.TB respectively. Latorre et al[49] reported that 48% of children with TST positive and negative T-SPOT.TB had sensitized T cells against *Mycobacterium avium* sensitins. As regards the indeterminate results, a significantly lower IFN-γ release in response to mitogen (positive control) has been described in young children tested with QFT-IT, suggesting an age-dependent response.[47,50] As for T-SPOT.TB, it does not seem to be related to age,[17] except in the first weeks of life.[51] However, Nicol et al[48] reported a decline in positive T-SPOT.TB results in children less than 1 year of age, whereas TST results were unaffected.

Interferon-γ-based assays for diagnosing active tuberculosis

Although the IFN-γ-based tests are widely used together with or in place of TST, their role in the diagnosis of active disease is still undefined. According to the results of two recent meta-analyses,[52,53] both commercial IFN-γ-based tests, performed in blood, have better sensitivity than TST for active TB. As expected, specificity for active disease was low, ranging from 59% for T-SPOT.TB to 79% for QFT-IT and 75% for TST.[53]

While a positive result of an IFN-γ-based assay does not distinguish between active and latent infection, in combination with the TST result it may help to exclude active TB.[54-56] A recent multicentre study[55] showed a very low likelihood of TB with a negative result on both TST and IFN-γ-based tests. Unfortunately, only 4% of patients were immunosuppressed, which precludes its generalization to the whole risk population. The usefulness of levels of IFN-γ, measured by QFT-IT, to predict clinical outcome was evaluated in two studies.[56,57] Although active TB was associated with higher IFN-γ levels, the benefit was presumed to be marginal in highly experienced centres.

Cellular immunosuppression and age, among other factors, may impair performance of IFN-γ-based tests.[58] In 4 studies that compared performance of QFT-IT in HIV-infected and non-infected adults with active TB, mean sensitivity was 64% in HIV-infected patients and 79% in non-HIV-infected patients.[59-62] Although the overall sensitivity of T-SPOT.TB is higher than that of QFT-IT in otherwise healthy people, data from head-to-head comparisons in HIV-infected patients are scarce.[60,63,64] In three studies, covering a total of 39 patients with culture-confirmed TB, QFT-IT and T-SPOT.TB detected 74% and 82% of cases respectively.[60,64] Despite impaired sensitivity, IFN-γ-based tests are clearly superior to TST for the diagnosis of active TB in these patients.[61,65-67]

IFN-γ-based tests may be of value in diagnosing TB in childhood, due to the absence of microbiological confirmation in a high proportion of cases. In a large prospective study, the sensitivity of T-SPOT.TB was 83%, and was not affected by HIV status.[20] A hospital-based study reported sensitivity rates of 100% for TST and 73% for T-SPOT.TB and QFT.IT, and specificity rates of 58%, 98% and 100% for TST, T-SPOT.TB and QFT-IT respectively.[68] A multicentre study comparing both IFN-γ-based tests with TST in 333 children aged 2 months to 16 years found that in 49 TB-confirmed cases, sensitivity was 82% for TST, 78% for QFT-IT and 66% for T-SPOT.TB, increasing to 96% and 91% when TST was combined with T-SPOT and QFT-IT respectively.[69]

As regards aged patients, the data available show that QFT-G is more sensitive than TST in patients older than 80 years with active TB.[70,71] Although the sensitivity of QFT-G decreased with age, it remained better than that of TST.[71,72]

Interferon-γ-based assays in fluids other than blood

IFN-gamma is predominantly produced by effector T-cells. The recruitment of specific T cells during active TB and the process via which antigen-specific cells clonally expand and migrate to the site of infection have been described.[73] Therefore, during active TB, it makes sense to apply IFN-based assays in samples collected directly from the site of infection. Data from a recent metanalysis[53] indicate that the T-SPOT.TB assay in extrasanguineous fluids is a promising tool for the diagnosis of active TB.

Pulmonary tuberculosis

Rapid diagnosis of pulmonary TB relies on the detection of acid-fast bacilli (AFB). However, this can be difficult due to the low sensitivity of the sputum smear. In addition, a significant proportion of cases cannot be confirmed by culture. In a prospective study by Jafari et al,[74] all 12 patients with smear-negative pulmonary TB, but none of the 25 controls, had positive T-SPOT.TB test from the bronchoalveolar lavage (BAL). TB-specific T cells were more concentrated in BAL than in peripheral blood, indicating a highly selective compartmentalization at the site of infection.[75] A recent large study carried out by the TBNET confirmed the high sensitivity, specificity and predictive values of T-SPOT.TB from the BAL.[76]

Pleural tuberculosis

The diagnosis of pleural TB is often difficult due to the limitations of conventional tests.[77] Wilkinson et al[73] found a 15-fold greater concentration of ESAT-6-specific spot-forming T cells in pleural fluid than of PBMCs in 10 patients with pleural TB. These cells were not found in the pleural fluid of 8 patients with nontuberculous pleuritis.[73] In a TBNET study,[78] T-SPOT.TB was performed on mononuclear cells from blood and pleural fluid in 20 patients with pleural TB and in 21 with pleural effusion of other causes. T-SPOT.TB was positive in 90% of cases on blood samples and in 95% of cases pleural fluid. Specificity was 67% for blood and 76% for pleural fluid. In another study of 28 patients with pleural TB, results in pleural fluid were inconclusive in 52% of cases, due to high background IFN-γ production.[79] Commercial IFN-γ tests, T-SPOT.TB and QFT-IT in pleural fluid were compared to unstimulated IFN-γ for the diagnosis of pleural TB in 74 patients.[80] In 11 (15%) cases, the cell counts were not large enough to perform the tests. In the 63 remaining patients, sensitivity, specificity, positive predictive value and negative predictive value were: for T-SPOT.TB, 86, 60, 84 and 64% respectively; for QFT-IT, 57, 80, 87 and 44% respectively, and for unstimulated IFN-γ, 97, 100, 100 and 94% respectively. The authors concluded that the IFN-γ-based assays had suboptimal accuracy for the diagnosis of pleural TB.

Tuberculous meningitis

Tuberculous meningitis (TBM) is a challenge for clinicians because of the frequent absence of microbiological confirmation and high mortality if not promptly treated. In one study including 10 patients with a diagnosis of TBM, T-SPOT.TB detected *M. tuberculosis* antigen-specific IFN-γ in CSF from nine patients (90%), but in none of the seven controls (specificity 100%).[81] In a study with 12 patients with TB of the central nervous system and 25 without TB, T-SPOT's sensitivity and specificity in CSF were 75%.[82] Recently, in a prospective observational study of 31 patients with confirmed or probable TBM, the same group of investigators[83] reported a sensitivity of 59% and a specificity of 89% for T-SPOT.TB in CSF mononuclear cells. However, since the diagnosis was not confirmed microbiologically in 21 of these patients, the sensitivity may have been underestimated. Similarly, in a study of 140 patients with meningitis (81% HIV-infected), using ≥46 spot-forming cells as cut-off point and after excluding bacterial and cryptococcal meningitis, the positive and negative predictive values of T-SPOT.TB in CSF were 100% and 68% respectively.[84]

Interferon-γ-based assays for predicting subsequent active tuberculosis

The ability to predict subsequent active TB among latently infected people is essential in order to select those who would benefit from chemoprophylaxis and to avoid unnecessary treatment for low-risk persons. Doherty et al[85] demonstrated a strong association of reactivity to ESAT-6 and progression to active TB in twenty-four household contacts of smear-positive TB patients. In a study involving 601 close contacts of sputum smear-positive TB, Diel et al[86] found that while 14.6% of contacts with positive QFT-IT who declined treatment developed TB within the 2-year follow-up, only 2.3% of those with positive TST did. This difference between TST and QFT-IT disappeared when only unvaccinated contacts were considered.[87] More recently, the same group of investigators[88] extended the original study and reported progression to active TB for up to four years for a cohort of 954 close contacts of smear-positive index cases. Of 147 untreated contacts with a positive QFT-IT test 19 (12.9%) developed active TB, whereas only 17 of 155 (3.1%) with TST >5 mm did. The progression rate was higher among children (28.6%). In addition, none of 824 untreated contacts with negative QFT-IT developed active TB, confirming the high negative predictive value of the test.[88] In the study by Kik et al[89] of 339 close contacts of sputum smear-positive TB, TST, QFT-IT and T-SPOT.TB were comparable in predicting development of TB during a two-year period. Positive predictive values were 3.1% for TST ≥10 mm, 3.8% for TST ≥15 mm, 2.8% for positive QFT-IT and 3.3% for T-SPOT.TB. In a cohort of 308

Table 1
Summary of 9 studies evaluating the predictive value of the interferon-γ based tests for the development of tuberculosis

Study (reference)	Country	Study population	Period of follow-up (years)	Test	Positive test n/N (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Doherty et al[85]	Ethiopia	Household contact adults	2	ELISA (PPD)	21/24 (88)	100	18	33	100
				ELISA (ESAT-6)	9/24 (38)	86	83	67	93
Leung et al[87]	Hong Kong	Silicosis patients	2.5[a]	TST (10 mm)	203/308 (66)	77	35	6.4	96
				T-SPOT.TB	204/308 (66)	88	36	7.4	98
Diel et al[88]	Germany	Close contact adults	4	TST	555/903 (60)	89	61	3.1	99
				QFT-IT	147/903 (16)	100	86	12.9	100
Kik et al[89]	Netherlands	Contact immigrant adults	2	TST	339[b]	100	16	3.1	100
				QFT-IT	178/324 (55)[b]	63	45	2.8	98
				T-SPOT.TB	181/299 (61)[b]	75	40	3.3	98
Hill et al[90]	Gambia	Household contact childre & adults	2	TST	843/2230 (38)	54	62	1.7	99
		(HIV +ve and -ve)		T-SPOT.TB	649/1736 (37)	42	63	1.7	99
Aichelburg et al[91]	Austria	HIV (+) adults	1.6	QFT-IT	44/783 (5.6)	100	95	8.1	100
Santin et al[92]	Spain	HIV (+) adults	1.6[a]	TST	7/120 (6)	–	94[c]	–	100[c]
				QFT-IT	13/120 (11)	–	89[c]	–	100[c]
Bakir et al[93]	Turkey	Contact children	1.3	TST	550/908 (61)	80	40	2	99
				T-SPOT.TB	381/908 (42)	73	59	3	99
Higuchi et al[94]	Japan	Contact adolescents	3.5	TST	95	–	73	–	100
				QFT-G	4/88[d]	–	96[e]	–	100[e]

NPV: negative predictive value; PPV: positive predictive value.
[a]Mean follow-up.
[b]Only subjects with positive TST were tested with QFT-IT and T-SPOT.TB.
[c]Calculated with 120 non-treated patients (those with negative TST and negative/indeterminate QFT-IT).
[d]Only subjects with positive TST were tested with QFT-G.
[e]Calculated with 84 non-treated subjects (those with negative QFT-G).

silicosis patients, Leung et al[87] found that a positive T-SPOT.TB significantly predicted development of active TB during a follow-up of more than 2 years (RR 7.80; 95%CI 1.02-59.6). Unexpectedly, TST was not predictive of TB, regardless of the cut-off point used. In a study with 2348 household contacts in Gambia, Hill et al[90] found that neither the TST nor the T-SPOT.TB predicted development of TB. The lack of predictive value was attributed to the high-burden of TB and recent transmission.

Two studies assessed QFT-IT and progression to active disease in HIV-seropositive individuals.[91,92] In a large study in a low-prevalence country, 8.1% of HIV-seropositive patients with a positive QFT-IT result at baseline, and left untreated, developed TB during a median follow-up of 19 months. None of the 738 patients with negative results had TB.[91] In a study in Spain of 135 HIV-infected individuals without active disease, none of the 103 patients who had a negative or indeterminate QFT-IT result at baseline had TB after a median follow-up of 20 months.[92]

Development of TB was also assessed in child and adolescent contacts. Bakir et al[93] studied 908 children with recent household exposure to TB, most of whom received preventive therapy. During a follow-up of 1.3 years, children with positive T-SPOT.TB had a 3- to 4-fold higher risk of developing active TB than those with negative T-SPOT.TB. However, rates of progression were similar in children with positive T-SPOT.TB and TST reactors. Since a high proportion of children were treated, the true incidence rates may have been underestimated. In the study by Higuchi et al,[94] 349 students underwent QFT-G and TST simultaneously, but only those with positive QFT-G were given chemoprophylaxis. Follow-up of the 91 students with positive TST but negative QFT-G showed no cases of active TB.

Although IFN-γ-based tests seem to predict subsequent active TB better than TST, the majority of high-risk people with positive tests will not develop active TB. Conversely, subsequent active TB in the next two to three years seems to be extremely low among people with a negative result.[85-87,89-94] Table 1 summarizes the nine studies assessing development of active TB with IFN-γ-based tests.

Final remarks and areas of future development

IFN-γ-based assays have become a reliable alternative to the old TST for the diagnosis of TB infection. Both commercial tests, QFT-IT and T-SPOT.TB, have a higher specificity than TST, and a better correlation with risk factors for TB and the degree of contact with an infectious case. Although their sensitivity may be affected to some extent by immunosuppression and extreme ages of life, they perform better than TST in these situations. Besides, IFN-γ-based tests do not induce boosting, and no additional visits are required for reading.

A great deal is now known about IFN-γ-based assays, and their use has expanded considerably. However, the prognostic value of a positive/negative result for the development of active TB, the significance of discordant results, the cut-off points to use in immunosuppressed people, the conversion/reversion phenomenon, and their role in the diagnosis of paucibacillary forms of TB, are some of the important questions that remain unresolved.

The actual prognostic value of a positive IFN-γ result needs to be clarified. Although the available data suggest that IFN-γ tests predict progression to active disease better than TST, most people with a positive result will not develop TB. Large prospective studies are urgently needed. Furthermore, the question of whether quantification of IFN-γ release may be of help in this situation, as has been previously suggested,[85,86] should also be addressed. Because of the discordance between IFN-γ tests and TST results, practitioners are reluctant to use them in everyday clinical practice. Trials focusing specifically on understanding the discordant results between IFN-γ tests and the TST, and between IFN-γ tests themselves, are required. This issue is especially relevant in childhood, where the effect of NTM infection may play an important role.[49] Since *in vitro* assays rely on the secretion of IFN-γ, which is largely produced by $CD4^+$ T cells, determining the CD4 threshold at which the performance of these assays declines is of particular importance. In addition, studies exploring the effect of the different immunosuppressor drugs on the response, as well as the accuracy of new cut-offs for diagnosing LTBI in immunosuppressed patients, are needed. Detection of *M. tuberculosis* specific T cells in samples other than blood with ELISPOT is a promising tool for the diagnosis of smear-negative pulmonary TB[76] and other paucibacillary forms of TB.[78,81] The methodological procedures and appropriate cut-offs should be established.

While awaiting answers to these questions, the use of the IFN-γ-based tests in clinical practice should be guided by clinical judgement and evidence-based guidelines for different groups of patients must be developed.

Finally, technical modifications of IFN-γ-based tests are being explored.[95] The attempts to improve IFN-γ-based tests include the study of alternative readouts to measure IFN-γ release.[95,96] the use of alternative *M. tuberculosis* specific antigens,[4,97] and the simultaneous measurement of chemokines[98] and interleukins.[99] The next generation of IFN-γ-based tests will significantly enhance diagnostic sensitivity without diminishing specificity, and will also reduce the rate of indeterminate results, especially in immunosuppressed patients and children.

Conflict of interest

The authors have no 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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