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APLICABILITAT CLÍNICA DE LES TÈCNIQUES DE DETECCIÓ *IN VITRO* DE L'INTERFERÓ-GAMMA EN LA INFECCIÓ I LA MALALTIA TUBERCULOSA

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ANNEX II

Review

EXPERT REVIEWS

IFN-γ-release assays to diagnose TB infection in the immunocompromised individual

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The tuberculin skin test (TST) is used for diagnosing latent TB infection (LTBI). The main limitation of TST is its low sensitivity in populations with the highest risk of progression to active TB: immunosuppressed patients and young children. New IFN-γ-based tests appear as an alternative to the TST. IFN-γ-based tests seem more specific than the TST, being closely associated with LTBI factors, and not being affected by bacillus Calmette–Guérin vaccination. Indeterminate results are mainly related to immunosuppression. Looking at the available data, it seems prudent to recommend the utilization of IFN-γ-based tests after a negative TST result, in order to increase the sensitivity of detecting LTBI cases in severely immunosuppressed patients. In summary, IFN-γ-based tests appear to be a valuable tool, in combination with the TST, for diagnosing TB infection in immunosuppressed patients.

KEYWORDS: active TB • diagnosis • IFN-γ-release assays • immunocompromised patient • latent TB infection • *Mycobacterium tuberculosis* • TST • tuberculin skin test

TB is still a major cause of morbidity and mortality throughout the world. Indeed, there is an estimated global incidence of 8.8 million new cases, with a total of 1.6 million deaths annually [1]. The detection and treatment of active TB is crucial to control the global TB epidemic. The diagnosis of active TB is based on the study of compatible clinical and radiographic signs, combined with direct microscopic examination, culture of *Mycobacterium tuberculosis* and the *in vitro* amplification of mycobacterial target DNA by PCR-based methods. However, in order to better control the spread of TB, it is also necessary to identify and treat infected individuals before they become infectious to others through the progression to active TB.

Since the end of the 19th Century, the tuberculin skin test (TST) has been used for diagnosing latent TB infection (LTBI) and for assisting in the diagnosis of active TB. The TST attempts to measure cell-mediated immunity in the form of a delayed-type hypersensitivity response to the purified protein derivative (PPD) [2]. The PPD contains more than 200 antigens that are widely shared among mycobacteria other than *M. tuberculosis,* including the vaccinal strain of *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) and many nontuberculous mycobacteria (NTM) [3]. As a result, individuals sensitized by previous exposure to NTM or vaccinated with BCG respond immunologically to PPD. Consequently, unnecessary LTBI treatments are prescribed. In addition, errors in the administration of tuberculin and subjective reading of the results also confound accurate interpretation. Nevertheless, the main limitation of the TST is its low sensitivity in detecting LTBI in the group of individuals with a high risk of progression to active TB: immunosuppressed patients (especially with deficient cellular immunity) and young children [4].

Immunodiagnostic methods based on the *in vitro* quantification of the cellular immune response for diagnosing LTBI have been developed. The detection of IFN-γ released by sensitized T cells stimulated with specific *M. tuberculosis* antigens enables the identification of infected individuals. The main antigens used are the 6-kD *M. tuberculosis* early-secreted antigenic target (ESAT)-6 protein and the 10-kD culture filtrate protein (CFP-10), coded in the region of difference (RD) 1, which is present in *M. tuberculosis* but not in any BCG strain nor in the majority of NTM [5].

New *in vitro* diagnostic technology has been rapidly adapted from initial basic in-house methods to three commercially available techniques: QuantiFERON®-TB Gold (QFN-G) assay, QuantiFERON-TB Gold In Tube (QFN-G-IT)

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assay (Cellestis Ltd, Carnegie, Victoria, Australia) and T-SPOT.TB assay (Oxford Immunotec, Oxford, UK). The three tests have received final approval from the US FDA for use as an aid in diagnosing *M. tuberculosis* infection. There are some differences between the three tests. QFN tests are whole-blood assays that detect IFN-γ produced by T cells in response to ESAT-6 and CFP-10 using ELISA to measure IFN-γ concentrations in supernatants. The main differences between the QFN-G and QFN-G-IT assays are that, in the QFN-G assay the blood is stimulated in separate wells with ESAT-6 and CFP-10, respectively, and in the QFN-G-IT assay, both specific *M. tuberculosis* antigens are already included inside the same tube. Furthermore, in the QFN-G-IT assay, a third stimulating antigen has been included: TB7.7. This new antigen is encoded in RD11 and is not present in BCG strains and common NTM [6]. By contrast, the T-SPOT.TB assay detects the number of IFN-γ-producing T cells after stimulating a definite number of isolated peripheral blood mononuclear cells with ESAT-6 and CFP-10 by means of an enzyme-linked immunospot assay (ELISPOT). In the T-SPOT.TB assay, cells are also stimulated in separate wells. In all commercially available tests, the whole blood and isolated T cells were stimulated with the specific antigens over 16–24 h (overnight incubation) (Figure 1).

The three tests include a positive control (cells stimulated using phytohemagglutinin as mitogen) that detects the capacity of T cells to produce IFN-γ after stimulation. If no response against the mitogen is obtained, no negative result after specific

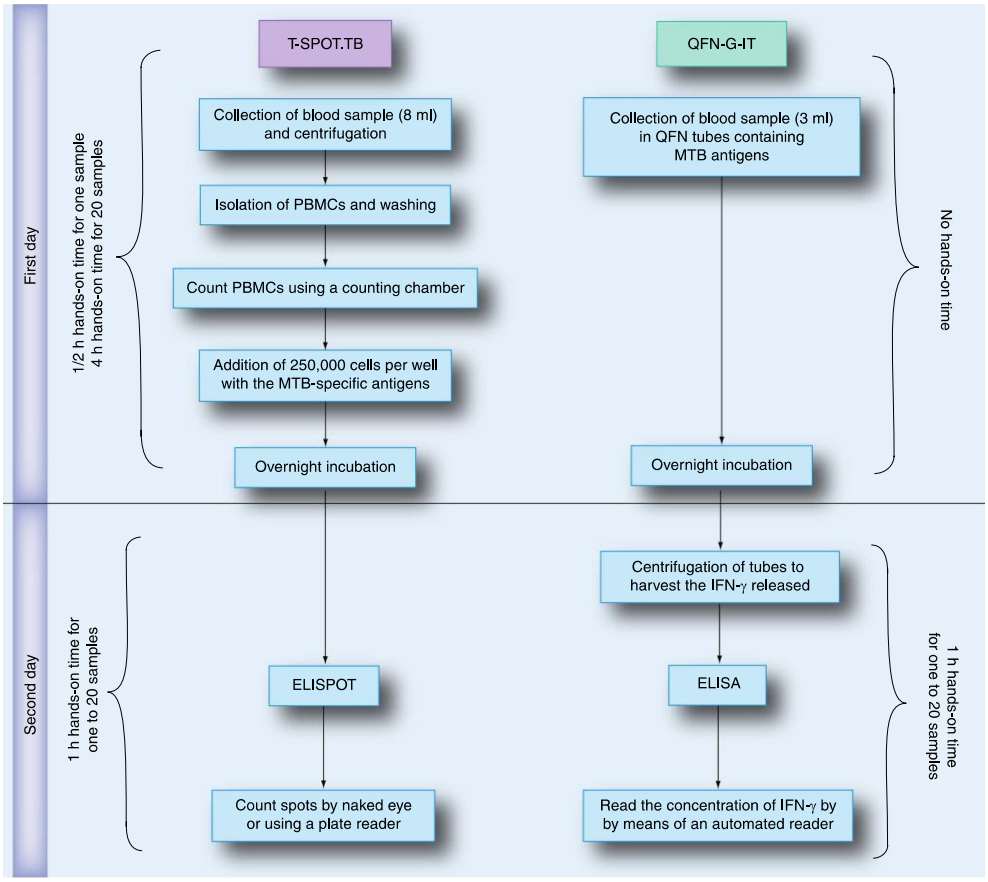

Figure 1. Comparison of T-SPOT.TB and QuantiFERON-TB Gold In Tube methodology.
ELISPOT: Enzyme-linked immunospot assay; MTB: *Mycobacterium tuberculosis*; PBMC: Peripheral blood mononuclear cell; QFN-G-IT: QuantiFERON-TB Gold In tube.

M. tuberculosis antigen stimulation could be considered. The test result is considered indeterminate if an antigen-stimulated sample is negative and if the value of the positive control is also negative after the subtraction of the value of the nil control. This positive control is especially useful for immunosuppressed patients, whose immunological response could be diminished, accounting for the detection of a lack of response after stimulation. However, if a specific lack of response occurs, response to TB antigens may not necessarily correlate with response the to the mitogen.

Given that there is an increased risk for progression from LTBI to active TB in immunosuppressed patients, it is crucial to identify and treat these infected patients. The risk of progression to active TB is higher in children, especially in very young children (<2 years of age) [7], suggesting T-cell response immaturity to contain TB infection. The sensitivity of TST in young children is unknown, but the existence of immature conditions indubitably induces a lower cutaneous response. To date, current guidelines recommend the use of IFN-γ-based tests for the diagnosis of LTBI in individuals with a high possibility of having a false-negative TST result, such as patients with suppressed cellular immunity, and also children [8–10]. This article discusses the potential utility of IFN-γ-based tests in the diagnosis of TB infection in immunocompromised patients: HIV-infected patients, patients receiving immunosuppressive therapies, patients with other immunocompromising conditions and children (not exclusively <2 years of age).

General studies including the immunosuppressed population

Although the sensitivity of IFN-γ-based tests in immunocompromised patients and the effect of immunosuppression in the management of the tests were identified as priority areas for research [11], it remains far from being clearly defined. Three studies have been carried out, including different groups of immunocompromised patients. In a prospective study conducted by the Modena group (Italy) [12], T-SPOT.TB and QFN-G tests were compared. A total of 393 patients were studied for suspected latent or active TB. A varied group of immunosuppressed patients made up 38% of the study population: patients with any form of cancer (independently if they were under chemotherapy), HIV infection, chronic renal failure, patients receiving immunosuppressant therapies (systemic steroids) or biological treatments, children under 5 years of age, elderly patients, and patients awaiting solid transplantation. Analyzing the overall results, the authors reported that IFN-γ-based tests were affected by factors potentially associated with reduced functioning of the cellular immune system, such as age or immunosuppressive treatments. Indeed, they noticed that indeterminate results for both IFN-γ-based tests were significantly more frequent in patients undergoing cancer chemotherapy than in participants not treated with chemotherapy. In general, indeterminate results were more frequent in QFN-G (11%) than in T-SPOT.TB (3%). In addition, QFN-G had a higher number of indeterminate results than T-SPOT.TB in all subgroups of immunosuppressed patients.

Similarly, a study by Kobashi *et al.* focused on patients with immunocompromising conditions (malignant diseases, immunosuppressive treatments, diabetes mellitus, chronic renal failure and HIV infection) described that QFN-G reached a rate of positive results for TB infection higher than the TST (78.1 versus 50%) [13]. They noted that indeterminate results (13%) were more frequent in patients receiving immunosuppressive therapy (particularly with lymphocytopenia in the peripheral blood) than in those who had other underlying diseases.

Recently, Richeldi *et al.* conducted a prospective study, which in a 1-year period enrolled 369 immunosuppressed patients (patients with end-stage chronic liver disease in the liver transplant candidacy period, individuals with chronic HIV infection and patients with hematologic malignancies) [14]. They observed that IFN-γ-based tests detected significantly more patients as being infected by *M. tuberculosis* than did the TST, although the results varied across the groups. In patients waiting for liver transplantation, the IFN-γ-based tests could replace the TST, but in HIV patients the low rate of positive results obtained by the TST and IFN-γ-based tests support an integrated diagnostic based on *in vivo* and *in vitro* assays. The authors concluded that, in accordance with the data, a combined approach to maximize the efficacy of LTBI infection should be recommended in severely immunocompromised patients.

HIV-infected patients

Patients co-infected with HIV and *M. tuberculosis* are more prone to a reactivation of LTBI and to the development of disseminated disease than immunocompetent individuals. TST sensitivity is low in HIV-infected patients. In one study developed in Zambia [15], only 30% of HIV-positive patients had a positive TST result compared with 62% of HIV-negative individuals. The presence of skin anergy means that false-negative TSTs have been reported in 26–41% of HIV-infected patients who are screened for LTBI [16]. Therefore, there is a need for an accurate test for LTBI detection that remains effective in HIV-co-infected individuals.

In the majority of studies, IFN-γ-based tests show a poor agreement with TST results [17–21]. The studies evaluating T-SPOT.TB and its precommercial version [18,19,22,23], and the QFN tests [23–26], seem to demonstrate that *in vitro* tests show a higher number of positive results than the TST in diagnosing LTBI [27].

In the last few years, some studies have compared IFN-γ-based tests in an HIV-infected population (Table 1) [17,18,21,23,27–29]. Rangaka *et al.* did not obtain significant differences in the proportion of positive T-SPOT.TB or QFN-G tests in HIV-infected persons [17], and Vincenti *et al.* found that the commercial tests reached a similar sensitivity [23]. By contrast, Mandalakas *et al.* conducted a cross-sectional study where they found that T-SPOT.TB reached a higher number of positive results for LTBI detection compared with the QFN-G test and TST [18], and in a prospective study Stephan *et al.* reported that the T-SPOT.TB (25.2%) and QFN-G (20.0%) assays showed more positive test results than the TST (12.8%) [27].

Some studies have found a correlation between QFN test results and risk factors for LTBI. Brock *et al.* found that 78% of HIV-infected patients with a positive QFN-G-IT (27/590) had risk factors such as long-term residence in a high TB-endemic area (odds ratio [OR]: 5.7; 95% CI: 2.6–12.5; p < 0.0001), known

Table 1. Recent studies analyzing the results of IFN-γ-based tests by comparison with the tuberculin skin test in HIV infection.

Study	Location	Population	Patients (n)	BCG (%)	Median CD4/mm³	Test	Number of positives (%)	% IFN-γ-based test versus TST concordance (κ)	% IFN-γ-based test concordance (κ)	Indeterminate results (%)	Ref.
Dheda *et al.* (2005)	UK	LTBI screening	29	NR	361	TST T-SPOT.TB	NR 6.9	- NR	- -	- 3.39	[29]
Brock *et al.* (2006)	Denmark	LTBI screening	590	NR	523	TST QFN-G-IT	ND 4.6	- -	- -	- 3.4	[24]
Rangaka *et al.* (2007)	South Africa	LTBI screening	74	51	392	TST (5 mm) T-SPOT.TB QFN-G	52 52 43	- 80 (0.60) 79 (0.58)	- 67 (0.34)	- 1 7	[17]
Jones *et al.* (2007)	NY, USA	LTBI screening	203	2	452.7	TST (5 mm) QFN-G	6.4 5.4	- 88 (0.38)	-	- 4.9	[26]
Luetkemeyer *et al.* (2007)	CA, USA	LTBI screening	294	6	363	TST (5 mm) QFN-G-IT	9.3 8.5	- 89.3 (0.37)	-	- 5.1	[20]
Clark *et al.* (2007)	UK	Active TB and LTBI screening	201	49	213	TST (5 mm) T-SPOT.TB	NR 24.9	- 89.06 (0.74)	-	- 2.5	[30]
Mandalakas *et al.* (2008)	OH, USA	LTBI screening	20	70	787.7	TST T-SPOT.TB QFN-G	62.5 72.2 35.3	- 0.43 0.46	- 64.3 (0.36)	- 10 15	[18]
Stephan *et al.* (2008)	Germany	LTBI screening	286	6.64	408	TST (5 mm) T-SPOT.TB QFN-G	12.8 25.2 20	- NR (0.201) 0.335	- 67.9 (0.15)	- 3.1 0.3	[27]
Karam *et al.* (2008)	Senegal	LTBI screening	285	72.6	179.5	TST (5 mm) In-house ELISPOT	21.5 50.6	- 61.1 (0.23)	-	- 13.3	[19]
Balcells *et al.* (2008)	Chile	LTBI screening	116	88.1	393	TST (5 mm) QFN-G-IT	10.9 14.8	- 90.8 (0.59)	-	- 0	[25]
Raby *et al.* (2008)	Zambia	Active TB	59	NR	212	TST (5 mm) QFN-G-IT	55 63	- NR	-	- 17	[34]
Aichelburg *et al.* (2009)	Austria	LTBI screening	830	NR	194	TST (5 mm) QFN-G-IT	ND 5.3	- NR	-	- 5.7	[37]
Talati *et al.* (2009)	GA, USA	LTBI screening	336	7.4	334	TST (5 mm) T-SPOT.TB QFN-G-IT	2.1 4.2 2.7	- 0.16 0.23	- 0.23	- 14 1.8	[21]
Aabye *et al.* (2009)	Tanzania	Active TB	93	NR	519	TST QFN-G-IT	ND 65	- -	-	- 22	[35]

BCG: Bacillus Calmette–Guérin vaccination; ELISPOT: Enzyme-linked immunosorbent spot; LTBI: Latent TB infection; ND: Not done; NR: Not reported; QFN-G: QuantiFERON®-TB Gold; QFN-G-IT: QuantiFERON-TB Gold In tube; TST: Tuberculin skin test.

TB exposure (OR: 4.9; 95% CI: 2.0–11.8; p = 0.001) or previous TB disease (OR: 4.9; 95% CI: 1.7–14.1; p = 0.007) [24]. Jones *et al.* evaluated the QFN-G assay and TST in 207 HIV-infected patients, obtaining a poor concordance between the TST and QFN-G assay, and the QFN-G assay results showed a statistically significant association between the number of risk factors for LTBI (TB exposure, homelessness, drug use, prison, healthcare worker, long-term care facility and foreign-born) and a positive test result, but not the TST (OR: 1.6; p = 0.039) [26].

Regarding the indeterminate results, in most studies the T-SPOT.TB and in-house ELISPOT assays appear relatively unimpaired by low CD4 cell counts [27,29–33]. On the contrary, when using the QFN-G-IT assay, a strong correlation between low CD4 T-cell count and a low mitogen response was detected [24].

It has been described that patients with a low CD4 cell count had more indeterminate QFN-G assay results. Luetkemeyer *et al.* found that patients with a CD4 count less than 100 cells/mm^3 had a relative risk ratio of indeterminate results of 4.24 compared with those with a CD4 count of 100 or more [20]. Jones *et al.* noticed that all indeterminate results occurred in patients with CD4 counts of less than 200 cells/mm^3 [26]. Furthermore, Raby *et al.* observed that with falling CD4 count there was a decrease in positive QFN-G-IT results, with a relative increase of negative and indeterminate results [34]. This was particularly marked at counts less than 100 cells/mm^3; in the study by Brock *et al.*, 24% (4/17) of patients with CD4 cell counts of less than 100 cells/mm^3 had indeterminate result compared with only 2.8% (16/573) of patients with CD4 cell counts of over 100 cells/mm^3 [24], and Aabye *et al.* reported that the number of indeterminate results using QFN-G-IT significantly increased with the decrease in the CD4 cell count [35]. Nevertheless, Balcells *et al.* described that, at least among subjects with a negative TST and a low CD4 cell count, the QFN-G test was positive in 8.2% of cases [25].

However, although indeterminate results have been more frequently described for QFN tests than for the T-SPOT.TB assay, Stephan *et al.* reported the opposite: that T-SPOT.TB provided significantly more indeterminate results than the QFN-G assay (eight vs one in 256 patients) [27]; Karam *et al.* found that the proportion of patients with a positive result for an in-house ELISPOT test decreased significantly with declining CD4 counts [19] and Talati *et al.* described a higher number of indeterminate results by the T-SPOT.TB than by the QFN-G-IT assay, being a CD4 count less than 200/µl associated with indeterminate results of the T-SPOT.TB assay, but not with the QFN-G-IT assay [21].

Interestingly, Raby *et al.* assessed QFN-G-IT utility in patients with active TB [34]. In the study, T-lymphocyte counts were estimated (CD3, CD4 and CD8). A total of 17% of the indeterminate results were obtained by the QFN-G-IT assay. Although a low CD4 count was associated with both negative and indeterminate results, CD8 count was high or normal in those with negative results but low in those with indeterminate results. Given that the overlapping peptides used as antigens in the QFN-G-IT assay, owing to their length, are essentially MHC class II-restricted, only CD4 cells will respond. Nevertheless, subjects with low CD4 counts in conjunction with high/normal CD8 counts react to phytoheamagglutinin but not to the specific antigens, generating negative results and consequently suppression of both cell lines, leading to indeterminate results. This observation adds further useful clinical information to an indeterminate result.

Regarding the possibility of using IFN-γ-based tests for diagnosing active TB, Clark *et al.* proposed that a combination of TB antigen-specific IFN-γ responses and CD4 T-cell counts could differentiate active TB from latent TB in HIV-infected patients [30]. The authors obtained a positive predictive value of 86 and 79% for diagnosing active TB when the ratio of the combined number of ESAT-6 and CFP-10 IFN-γ spot-forming cells per million of peripheral blood mononuclear cells for CD4 and CD8 T-cell count, respectively, was higher than the value of 1.5. Indeed, Rangaka *et al.* previously noticed that with in-house ELISPOT, the response to ESAT-6 and CFP-10 was higher in the group of HIV-infected subjects with TB, although this group had lower CD4 cell counts [36]. They concluded that a ratio of the ELISPOT response divided by the CD4 cell count higher than 1.0 had 88% sensitivity and 80% specificity for active pulmonary TB in HIV-infected individuals.

Aichelburg *et al.* conducted a prospective and longitudinal study involving 830 HIV-infected patients [37]. They screened LTBI by means of the QFN-G-IT assay at baseline and then followed patients up for a mean time of 19 months. They detected patients that developed active TB only among the patients with a positive QFN-G-IT in the baseline (3 out of 44). Any of the 44 patients accepted prophylaxis at the baseline. The authors concluded that the QFN-G-IT assay is a sensitive tool for the detection and prediction of active TB in HIV-infected individuals.

Until now, an association between positive results by IFN-γ-based tests and the presence of risk factors to LTBI has been described. Furthermore, in HIV-infected patients that have a high incidence of NTM infections, IFN-γ-based tests increase the specificity in diagnosing LTBI. Therefore, the accumulated evidence discussed here presents IFN-γ-based tests as useful tools for diagnosing LTBI in HIV-positive individuals. In highly immunocompromised patients, the QFN-G test seems impaired by low CD4 T-cell counts.

Chronic immune-mediated inflammatory disease

Biological agents, especially anti-TNF-α agents, have emerged as an effective treatment in patients with chronic inflammatory diseases such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis [38,39]. TNF-α is one of the key molecules involved in granuloma formation and the maintenance of TB infection. Consequently, patients undergoing TNF-α inhibition are at an increased risk (28–54 per 100,000 population) of developing active TB [40]. A strong association between anti-TNF-α antibody treatment (infliximab) and reactivation of LTBI has been described [39,41]. Before starting a treatment based on TNF-α inhibition, the appropriate screening for LTBI and exclusion of active TB has become mandatory [42]. However, most patients with chronic inflammatory diseases are already under corticosteroid and/or immunosuppressive drugs prior to anti-TNF-α therapy. It is well known that this group of patients may not be able to produce

an adequate delayed-type hypersensitivity reaction to the TST owing to their deficient cell-mediated immunity [38,42]. Therefore, in this population the utility of the IFN-γ-based tests, although promising, is still limited at present [43,44].

In this type of population, poor agreement between the TST and IFN-γ-based tests [45–50] has been found. The discordant positive TST and negative IFN-γ-based test results in BCG-vaccinated populations have been attributed to the BCG vaccination effecting the TST result [46,48,51–54]. However, the discordant negative TST and positive IFN-γ-based test results have been related to the immunosuppressive therapy that patients are receiving [47–49,51]. In general (Table 2), the available data suggest that the IFN-γ-based tests show a higher number of positive results than the TST in this population [47,52,55]. It has been described that IFN-γ-based assays, but not the TST, are closely associated with the presence of LTBI risk factors [45,49]. In the study by Matulis *et al.*, the following factors were closely associated with QFN-G-IT: being born or a resident in a high-prevalence country (OR: 11.7; 95% CI: 2.11–65.0; $p < 0.001$), history of household contact (OR: 17.8; 95% CI: 2.06–154; $p < 0.001$), chest x-ray suggestive of previous active TB (OR: 66.8; 95% CI: 10.1–441; $p < 0.001$) and a history of active TB (OR: 179; 95% CI: 6.69–4787; $p < 0.001$) [49]. Bocchino *et al.*, in a group of 15 patients with risk factors for LTBI, found that the TST and IFN-γ-based tests were positive in eight cases, and in the remaining seven the TST was negative, but at least one of the IFN-γ-based tests (QFN-G-IT and T-SPOT.TB assays) were positive [45]. The rate of indeterminate results ranged from 1.9 to 11.5%, being higher than those observed in healthy controls [45,47,56]. Indeterminate results were obtained in all IFN-γ-based tests, being impossible to conclude, at the moment, if any of them are more or less affected by this specific category of immunosuppression. However, Murakami *et al.* found that the TST was more strongly attenuated than an in-house ELISPOT assay by corticosteroid therapy in patients with rheumatoid arthritis [53]. In a study involving 398 consecutive subjects with immunomediated inflammatory diseases, Bartalesi *et al.* described that, by multivariate analysis, the use of conventional disease-modifying antirheumatic drugs was not associated with the results of the TST or QFN-G-IT tests, while the use of steroids was associated with a lower probability of a positive result [57].

There is scarce information regarding the utility of IFN-γ-based tests for monitoring TB infection during anti-TNF-α therapy. Matulis *et al.* found that the OR for a positive IFN-γ test (QFN-G-IT) were lower in patients treated with TNF-α inhibitors [49]. Similarly, Hamdi *et al.* described that treatments with TNF-α inhibitors (infliximab and etanercept) decreased IFN-γ release [58]. Moreover, in the Bartalesi *et al.* study, treatment including TNF-α inhibitors significantly decreased the positive outcome of the TST (OR = 0.3; 95% CI: 0.1–0.6; $p = 0.004$) without affecting QFN-G-IT results (OR: 0.9; 95% CI: 0.4–2; $p = 0.8$) [57]. However, more data are required to define their role in this setting.

Another interesting issue would be to establish the prognostic value of a positive IFN-γ-based test result for the subsequent development of active TB for patients undergoing treatment with TNF-α inhibitors. In this sense, Chen *et al.* prospectively followed up 43 rheumatoid arthritis patients who received adalimumab therapy and underwent serial TST and QFT-G assays [59]. Among the 35 patients with negative TST results, two patients developed active TB after 12 months of treatment and both patients had initially had QFN-G test results. Pratt *et al.* screened 101 patients with rheumatoid arthritis and seven cases had a positive QFN-G-IT test result [60]. Four of them subsequently started anti-TNF-α treatment. Of the patients that were followed-up, none developed active TB within 6–30 months.

According to the available data, IFN-γ-based assays seem to be useful for LTBI screening in patients with chronic inflammatory disease before starting treatments. Studies have demonstrated a higher number of positive results for the IFN-γ-based tests than the TST. However, in these patients with a significant risk of progression to active TB, the combined utilization of TST and IFN-γ-based tests, for confirming a negative TST by an IFN-γ-based test, should be recommended to reduce the possibility of failure in the LTBI diagnosis. At the moment, there are not enough studies available for stating its utility in monitoring during anti-TNF-α therapy. In this sense, further studies are required to establish the exact role of specific corticosteroids and/or immunosuppressive drugs in the IFN-γ-based test results.

Other immunocompromised situations

Recipients of solid organ transplants

For transplant recipients, the incidence of active TB is 20–74-times higher when comparing with the general population. In this immunosuppressed population, owing to anergy, the TST is frequently negative [61]. Studies assessing the utility of the IFN-γ-based tests have only been performed in patients awaiting liver transplantation. In these patients, accurate diagnosis of TB infection is very important as active TB can cause severe complications, and because active and latent TB infection treatments are very hepatotoxic. Manuel *et al.* found a good sensitivity for QFN-G in detecting LTBI [62]. They studied 153 patients prior to liver transplantation, obtaining a similar number of positive results: 37 for the TST and 34 for the QFN-G assay. However, they described discordant results (12 TST-positive/QFN-G-negative; and nine TST-negative/QFN-G-positive) that were not associated with prior BCG vaccination. By contrast, indeterminate results for QFN-G test were obtained in 7.8% of patients, all of them with a negative TST. Codeluppi *et al.* reported a case of pulmonary TB 55 days after the liver transplant [63]. In this case, the TST was negative prior to the transplantation, and the QFN-G test was also negative before and after transplantation.

Recently, Lindemann *et al.* studied the performance of the T-SPOT.TB and TST tests in 48 patients awaiting liver transplantat [64]. In their experience, four patients had a positive T-SPOT.TB result. Given that all patients were TST-positive, the T-SPOT.TB was repeatedly positive and they reported TB exposures, all of which were considered TB infected. In one patient, shortly after the transplantation, the reactivity against the TB-specific antigens was lost. This scenario should be considered when regarding the possibility of using IFN-γ-based tests for monitoring patients after transplantation.

Table 2. Summary of studies analyzing the values of IFN-γ-based tests by comparison with the tuberculin skin test in patients with chronic immune-mediated inflammatory disease prior to anti-TNF-α therapy.

Study	Location	Population	Patients (n)	BCG (%)	Test	Number of positives (%)	% IFN-γ-based test versus TST concordance (κ)	% IFN-γ-based test concordance (κ)	Indeterminate results (%)	Ref.
Cobanoglu *et al.* (2007)	Turkey	LTBI screening	68	100	TST (10 mm) QFN-G-IT	60.6 14.7	- 50.8	- -	- 10.3	[46]
Sellam *et al.* (2007)	France	LTBI screening	13	100	TST (10 mm) In-house ELISPOT	61.5 84.6	- 46.1	- -	- 0	[52]
Takahashi *et al.* (2007)	Japan	LTBI screening	14	NR	TST (20 mm) QFN-G	28.6 28.6	- 64.3	- -	- 5	[55]
Pratt *et al.* (2007)	UK	LTBI screening	101	78.5	TST QFN-G-IT	ND 7	- -	- -	- 9.9	[60]
Bocchino *et al.* (2008)	Italy	LTBI screening	69	3	TST (5 mm) T-SPOT.TB QFN-G-IT	26 30.4 31.8	- 78.4 (0.21) 80.5 (0.26)	- NR	- 5.8 2.8	[45]
Ponce de Leon *et al.* (2008)	Peru	LTBI screening	101	80	TST (5 mm) QFN-G-IT	26.7 44.6	- 70 (0.37)	- -	- 1.9	[47]
Vassilopoulos *et al.* (2008)	Greece	LTBI screening	70	40	TST (5 mm) T-SPOT.TB	38.6 22.8	- 72.8 (0.38)	- -	- 0	[48]
Matulis *et al.* (2008)	Switzerland	LTBI screening	142	83	TST (5 mm) QFN-G-IT	32.4 12	- 64 (0.17)	- -	- 6	[49]
Schoepfer *et al.* (2008)	Switzerland	LTBI screening	168	70.2	TST (5 mm) QFN-G-IT	18 8.3	- NR (-0.03)	- -	- 3	[51]
Greenberg *et al.* (2008)	NY, USA	LTBI screening	61	27.8	TST (5 mm) QFN-G	21.3 18	- NR	- -	- 11.5	[56]
Chen *et al.* (2008)	Taiwan	LTBI screening	43	100	TST (5 mm) QFN-G	18.6 13.9	- 60 (0.21)	- -	- 4.6	[59]
Murakami *et al.* (2009)	Japan	LTBI screening	71	100	TST (5 mm) In-house ELISPOT	21.4 14.1	- NR	- -	- NR	[53]
Bartalesi *et al.* (2009)	Italy	LTBI screening	398	4	TST (NR) QFN-G-IT	19 13	- 87.7 (0.55)	- -	- 1.2	[57]
Martin *et al.* (2009)	Ireland	LTBI screening	150	82	TST (5 mm) T-SPOT.TB QFN-G	18 9.8 7.1	- NR NR	- 98.2 (NR)	- 4.7 2.8	[54]
Behar *et al.* (2009)	MA, USA	LTBI screening	179	4.7	TST (5 mm) T-SPOT.TB	1.1 5.6	- 93.3 (-0.019)	- -	- 0	[50]

BCG: Bacillus Calmette–Guérin vaccination; ELISPOT: Enzyme-linked immunosorbent spot; LTBI: Latent TB infection; ND: Not done; NR: Not reported; QFN-G: QuantiFERON®-TB Gold; QFN-G-IT: QuantiFERON-TB Gold In tube; TST: Tuberculin skin test.

The IFN-γ-based tests appear reliable for diagnosing LTBI in patients awaiting liver transplants, being beneficial in detecting infected patients and for avoiding unnecessary anti-TB treatments. However, the presence of discordant results, failure in detecting LTBI in one patient that progressed to active TB and reversion after transplantation makes it necessary to interpret results with caution. Further studies are compulsory to establish the utility of the IFN-γ-based tests in other solid organ transplants. The specific immunosuppressant drugs used in each setting for avoiding organ rejection are likely to have a different effect on the performance of the IFN-γ-based tests.

End-stage renal disease

Regarding chronic renal conditions, current guidelines recommend LTBI screening for hemodialysis patients. In this sense, in contrast to a positive TST, positive IFN-γ-based tests have been associated with established TB risk factors. Passalent *et al.* compared the T-SPOT.TB assay with the TST in 203 patients with end-stage renal disease (ESRD) [65]. T-SPOT.TB was positive in 78.6% of patients with a history of active TB (OR: 7.24; 95% CI: 1.70–30.8; p = 0.007) and in 72.7% of patients with radiographic markers of previous infection (OR: 5.48; 95% CI: 1.20–25.1; p = 0.03); by contrast, the TST was only positive in 21.4% (OR: 2.73; 95% CI: 0.65–11.5; p = 0.17) and 18.2% (OR: 1.21; 95% CI: 0.24–6.21; p = 0.82), respectively. In a recently published study [66], enroling a total of 100 ESRD patients, the number of positive results for the TST, an in-house ELISPOT and QFN-G were similar (26, 27 and 21 positive results, respectively). Nevertheless, patients with contact to a TB case were more likely to have a positive ELISPOT (OR: 2.7; 95% CI: 1.0–7.2; p = 0.04) and QFN-G (OR: 2.8; 95% CI: 0.9–8.4; p = 0.02), whereas no association was found for a positive TST. By contrast, Triverio *et al.* found that, after adjusting for age and BCG administration, the OR of having a positive QFN-G-IT was 4.6-fold (p = 0.029) higher in patients with LTBI risk factors (chest x-ray suggestive of prior TB infection and/or contact with a patient with contagious active TB) than those without LTBI, but no association was found between LTBI risk factors and a positive TST or a positive T-SPOT.TB assay [67]. Similarly, Lee *et al.* described that the possibility of positive QFN-G increases in those patients with past TB disease and/or evidence of past TB disease on the chest x-ray [68]. None of the factors were associated with a positive TST or a positive ELISPOT result.

It has been reported that hemodialysis reduces the IFN-γ production level. Hursitoglu *et al.* described seven predialysis-positive and two indeterminate QFN-G-IT results in patients that became negative after hemodialysis [69]. This fact should be considered in order to schedule the IFN-γ-based test determination before and not after the hemodialysis process.

Malignant hematological disease

The T-SPOT.TB test has demonstrated its utility in diagnosing LTBI in a large contact study with immunosuppressed hematological patients [70]. T-SPOT.TB obtained a higher number of positive results (44.2%) than the TST (17.4%), and reached an overall rate of 4.3% of indeterminate results. Considering white blood cell counts, they did not detect differences in the number of indeterminate results by T-SPOT.TB, neither the number of positive results between patients with pathological white blood cell counts nor patients with normal counts. By contrast, for TST the level of positive results fell from 25.9 to 14.5%, although the difference was not significant. The study results suggest that the T-SPOT.TB assay was not affected by the immunosuppressive status.

A summary of the reported studies analyzing the value of IFN-γ-based tests by comparison with the TST in these immunocompromised conditions is shown in Table 3.

Pediatric population

Children represent 11–15% of the global TB burden [1]. In the pediatric population, TB infection is usually recent. In addition, BCG vaccination, especially in TB-endemic areas, affects the specificity of the TST. In this sense, a higher specificity has been reported for IFN-γ-based tests by comparison with the TST in children. However, the sensitivity of the IFN-γ-based tests diagnosing active TB in children has shown contradictory results among the different studies (Table 4) [22,71–77]. Nicol *et al.* reported, using in-house ELISPOT, that positive results obtained at diagnosis were higher in patients with a definite TB case (83.3%), than in patients with probable (72.3%) or possible TB cases (45.5%) [71]. Recently, Davies *et al.* also reported that a significantly higher proportion of HIV-infected children with definite or probable TB have a positive ELISPOT compared with a positive TST (p = 0.005) [78]. The authors noticed that, in contrast with TST, results from ELISPOT were not affected by young age or severe immunosuppression. In some cases it was impossible to obtain a microbiological diagnosis of active TB, often in developed countries, where the primary lesions are closed, small, with a low number of bacilli, being very difficult to get positive sputum, or gastric aspirate smears and cultures. This made it difficult to establish definite conclusions about the real IFN-γ-based test sensitivity and specificity.

Similarly, IFN-γ-based tests have also obtained discordant results in studies for diagnosing LTBI (Table 4). For interpretation of the results, especially the discordant ones, it is important to take into account the millimetres of induration considered positive in the TST, but also factors that could increase the false-positive immune response of the PPD, such as the number of BCG-vaccinations or the number of previous TSTs performed. ELISPOT has correlated more closely with measures of exposure to *M. tuberculosis*, such as duration and proximity to an active TB index case, than the TST [79–85]. However, in some studies, positive IFN-γ-based test and TST results were both equally frequent when exposure to the index case increased [86], or when the index case was a smear-positive patient [87]. In general, results seem to indicate that the ELISPOT test reaches a higher number of positive results than the TST or the QFN assay when detecting LTBI. Nevertheless, both IFN-γ-based test results have demonstrated independence regardless of the BCG-vaccination status [76,79,82,88]. Surprisingly, some studies have detected no influential impact of BCG in TST results [75,86]. However, these results have been obtained in countries with a high TB prevalence and

Table 3. Studies on number of positives, concordance with tuberculin skin test and number of indeterminate results of IFN-γ-based tests in different immunocompromised situations.

Study	Location	Population	Patients (n)	BCG (%)	Test	Number of positives (%)	% IFN-γ-based test versus TST concordance (κ)	% IFN-γ-based test concordance (κ)	Indeterminate results (%)	Ref.
Liver transplantation										
Manuel *et al.* (2007)	Canada	LTBI screening	153	82	TST 5 mm TST 10 mm QFN-G	24.2 17.6 22.2	TST 5 mm: - 85.1 (0.60) TST 10 mm: - 82.2 (0.48)	- - -	- - 7.8	[62]
Lindemann *et al.* (2008)	Germany	LTBI screening	48	29.2	TST (5 mm) T-SPOT.TB	12.8 8.3	- NR	- -	- NR	[64]
End-stage renal disease										
Passalent *et al.* (2007)	Canada	LTBI screening	203	NR	TST (10 mm) T-SPOT.TB	12.8 35.5	- 70.4 (0.25)	- -	- 5.4	[65]
Winthrop *et al.* (2008)	GA, USA	Contact study	100	NR	TST (5 mm) In-house ELISPOT QFN-G	26 27 21	- 71 (NR) 79 (NR)	- 87 (NR)	- NR NR	[66]
Hursitoglu *et al.* (2008)	Turkey	LTBI screening	56	NR	TST QFN-G-IT	NR 58.9	- NR	- -	- 13.6	[69]
Triverio *et al.* (2009)	Switzerland	LTBI screening	62	22.6	TST (5 mm) T-SPOT.TB QFN-G-IT	19 29 21	- NR (0.32) NR (0.16)	- NR (0.60)	- 11 8	[67]
Lee *et al.* (2009)	Taiwan	LTBI screening	32	71.9	TST (10 mm) T-SPOT.TB QFN-G	62.5 46.9 40	- 65.5 (0.32) 60 (0.25)	- 76.7 (0.53)	- 0 6.3	[68]
Hematological patients										
Piana *et al.* (2006)	Italy	Contact study	138	1.5	TST (5 mm) T-SPOT.TB	17.4 44.2	- 67.8	- -	- 4.3	[70]

BCG: Bacillus Calmette–Guérin vaccination; ELISPOT: Enzyme-linked immunosorbent spot; LTBI: Latent TB infection; NR: Not reported; QFN-G: QuantiFERON®-TB Gold; QFN-G-IT: QuantiFERON-TB Gold In tube; TST: Tuberculin skin test.

Table 4. Published studies comparing the number of positive results, the concordance and the number of indeterminate results between tuberculin skin test and IFN-γ-based tests in children with latent TB infection and active TB.

Study	Location	Population and mean or median years of age (range)	Patients (n)	BCG (%)	Test	Number of positives (%)	% of IFN-γ-based test versus TST concordance (κ)	% of IFN-γ-based test concordance (κ)	Indeterminate results (%)	Ref.
Ewer *et al.* (2003)	UK	Contact study Mean age 13.1 (11–15)	535	87.3	Heaf Test In-house ELISPOT	38.7 27.5	- 89 (0.72)	- -	- NR	[79]
Richeldi *et al.* (2004)	Italy	Contact study Neonates	41	0	TST (5 mm) In-house ELISPOT	0 4.9	- 0	- -	- NR	[81]
Liebeschuetz *et al.* (2004)	South Africa	Suspect TB Median 3 (3–6)	262	NR	TST (5 mm) In-house ELISPOT	46.8 68.6	- 64.2 (NR)	- -	- NR	[22]
Nicol *et al.* (2005)	South Africa	Active TB Median 2.6 (0.25–13)	70	NR	TST (NR) In-house ELISPOT	NR 70	- NR	- -	- NR	[71]
Nakaoka *et al.* (2006)	Nigeria	Contact study Mean 7.4 (1–14)	161	36	TST (5 mm) QFN-G-IT	31.6 39.8	- NR	- -	- 0	[87]
Hill *et al.* (2006)	The Gambia	Contact study Median 7.0 (0.5–14)	718	46	TST (10 mm) In-house ELISPOT	32.5 32.3	- 83 (0.62)	- -	- NR	[86]
Connell *et al.* (2006)	Australia	Suspected LTBI or active TB Median 9.2 (0.6–18)	101	49	TST (5 mm) QFN-G	50.5 19.8	- 57.4 (0.3)	- - -	- 17	[72]
Dogra *et al.* (2007)	India	Suspected TB or LTBI Median 6 (1–12)	105	82	TST (10 mm) QFN-G-IT	9.5 10.5	- 95.2 (0.73)	- -	- 0	[75]
Detjen *et al.* (2007)	Germany	Active TB Median 2.3 (0.3–7)	28	14.2	TST (5 mm) T-SPOT.TB QFN-G-IT	100 93 93	- 92.9 (0.00) 92.9 (0.00)	- 95.6 (0.91)	- 0 0	[90]
Lewinsohn *et al.* (2008)	Uganda	Contact study <5 years of age	296	79.4	TST (5 mm) ELISA	65.2 61.5	- 72 (0.39)	- -	- NR	[85]
Bakir *et al.* (2008)	Turkey	Contact study Mean 7.5 (0.08–16)	908	80	TST (5 mm) In-house ELISPOT	60.6 42	- 71.7 (NR)	- -	- 0	[98]

[a]Results in casual contacts, control subjects (not history of exposure) and children with suspected TB are also reported in the study.
[b]5 mm in children with suspected active TB or recent TB contact, and 10 mm in children who were younger than 4 years of age or were tested because of recent immigration from an area of high TB prevalence, regardless of BCG status.
[c]In-paired tests with QFN-G-IT (1.5%) and QFN-G (2.3).
[d]In-paired tests with T-SPOT.TB.
BCG: Bacillus Calmette–Guérin vaccination; ELISPOT: Enzyme-linked immunosorbent spot; LTBI: Latent TB infection; NR: Not reported; QFN-G: QuantiFERON®-TB Gold; QFN-G-IT: QuantiFERON-TB Gold In tube; TST: Tuberculin skin test.

Table 4. Published studies comparing the number of positive results, the concordance and the number of indeterminate results between tuberculin skin test and IFN-γ-based tests in children with latent TB infection and active TB.

Study	Location	Population and mean or median years of age (range)	Patients (n)	BCG (%)	Test	Number of positives (%)	% of IFN-γ-based test versus TST concordance (κ)	% of IFN-γ-based test concordance (κ)	Indeterminate results (%)	Ref.
Connell *et al.* (2008)	Australia	Suspected latent TB Mean 10.2 (0.7–18.8) Active TB Mean 8.2 (1.8–13.6)	100	47	TST (5 mm) T-SPOT.TB QFN-G-IT	60 25 29	- 75 (0.51) 75 (0.50)	- 93 (0.83)	- 14 3	[73]
Hesseling *et al.* (2008)	South Africa	Contact study Mean 2.9 (NR)	29	100	TST (10 mm) T-SPOT.TB QFN-G	54 89 39.6	- 46.1 (0.15) 88.9 (0.78)	- 56.2 (0.03) -	- 3.6 14.3	[83]
Winje *et al.* (2008)	Norway	LTBI screening (positive TST) Born in 1991 (14–15)	511	46.2	TST (5 mm) QFN-G-IT	- 9	- -	- -	- 0	[84]
Dominguez *et al.* (2008)	Spain	LTBI screening; contact study Median 9 (1–17)	125	68	TST (5 mm) T-SPOT.TB QFN-G-IT	85.8 38.1 37.3	- 51.2 (0.18) 47.2 (0.12)	- 87.1 (0.71)	- 2.2 0	[76]
Mandalakas *et al.* (2008)	OH, USA	HIV infected. LTBI screening Mean 4.4 (NR)	23	91.3	TST (5 mm) T-SPOT.TB QFN-G-IT	26.1 52.2 16.7	- NR (-0.02) NR (0.44)	- 66.4 (0.33)	- 0 0	[18]
Chun *et al.* (2008)*	Korea	Close contacts Median 1.7 (0–12.8)	42	100	TST (5 mm) QFN-G-IT	62 19	- 57.1 (0.19)	- -	- 0	[88]
Lighter *et al.* (2009)	Finland	LTBI screening and active TB Mean 9 (1–5)	207	36	TST (10 mm) QFN-G-IT	56 15.2	- 55 (0.17)	- -	- 1.4	[92]
Nicol *et al.* (2009)	South Africa	Suspected latent or active TB Median 1.5 (1–2)	243	100	TST (10 mm) T-SPOT.TB	38.8 25.2	- 79.9 (0.55)	- -	- -	[93]
Bergamini *et al.* (2009)	Italy	Suspected latent or active TB Mean 11.1 (0–19)	496	38.9	TST (5/10 mm)‡ T-SPOT.TB QFN-G QFN-G-IT	NR 14.3 (22/154) 18.8 (34/181) 17.1 (54/315)	- NR (0.52) NR (0.43) NR (0.35)	- - NR -	- 1.5 and 2.3§ 12.6¶ 16.4¶	[94]
Kampmann *et al.* (2009)	UK	LTBI screening Mean 6.9 (0.25–16) Active TB Mean 9.2 (0.5–15)	118 91	74 60.4	TST (5 mm) T-SPOT.TB QFN-G-IT TST (5 mm) T-SPOT.TB QFN-G-IT	68.4 31.03 32.2 37.4 37.8 46.2	- 75 (0.49) 77 (0.53 - 71.6 (0.42) 80.5 (0.61)	- 92 (0.82) - 82.7 (0.66)	- 8.6 5.1 - 8.9 8.8	[77]

*Results in casual contacts, control subjects (not history of exposure) and children with suspected TB are also reported in the study.
‡5 mm in children with suspected active TB or recent TB contact, and 10 mm in children who were younger than 4 years of age or were tested because of recent immigration from an area of high TB prevalence, regardless of BCG status.
§In-paired tests with QFN-G-IT (1.5%) and QFN-G (2.3).
¶In-paired tests with T-SPOT.TB.
BCG: Bacillus Calmette–Guérin vaccination; ELISPOT: Enzyme-linked immunosorbent spot; LTBI: Latent TB infection; NR: Not reported; QFN-G: QuantiFERON®-TB Gold; QFN-G-IT: QuantiFERON-TB Gold In tube; TST: Tuberculin skin test.