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COMMENTARY

Serial QuantiFERON TB-Gold in-tube testing during LTBI therapy in candidates for TNFi treatment

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Summary *Objectives:* To evaluate the T-cell interferon (IFN)-γ response to *Mycobacterium tuberculosis*-specific antigens during latent tuberculosis infection (LTBI) therapy in candidates for tumor necrosis factor-α inhibitors (TNFi).
Methods: 1490 Patients were screened for LTBI. One-hundred and sixty-six of them were treated for LTBI and followed-up with QuantiFERON-TB Gold (QFT-IT) testing at baseline (T0) and therapy completion (T1); 92 subjects were also tested 3–6 months after therapy completion (T2).
Results: At T1 the QFT-IT reversion and conversion rates were 24% (27/111) and 18% (10/55), respectively. By multivariate analysis, the likelihood of reversion significantly decreased with older age (>50–60), larger TST size (>15 mm) and higher IFN-γ value at T0 (>1 IU/ml); the

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likelihood of conversion increased with higher IFN-γ levels at T0 (1 IU/ml) and in female patients. Quantitative data among those who scored QFT-IT-positive at T0 showed a decreasing trend of IFN-γ levels between T0 and T1 that reached statistical significance when T0 was compared to T2, and T1 to T2.

Conclusions: The data confirm the difficulty of interpreting the modulation of IFN-γ levels during LTBI therapy. Currently, there is no evidence to support the use of QFT-IT in the clinical practice of monitoring LTBI treatment in candidates for TNFi.

Introduction

Tumor necrosis factor-α inhibitors (TNFi) have been approved for treatment of immune-mediated inflammatory diseases (IMID) and also provide clinical benefits. However, patients treated with TNFi show an increased risk of serious life-threatening infections, including reactivation of *Mycobacterium tuberculosis* (MTB) infection.[1] Thus, screening for active tuberculosis (TB) and latent TB infection (LTBI) has become a mandatory procedure before initiating TNFi therapy.[2–5] In the past decade, several guidelines have been published on managing LTBI in individuals who are candidates for treatment with TNFi. However, the recommendations for use and the interpretation of the tuberculin skin test (TST) and interferon (IFN)-γ release assays (IGRAs) differ among these guidelines. For example, the US Centers for Disease Control and Prevention recommend performing either the TST or IGRA before starting any TNFi,[6] while the UK National Institute for Health and Clinical Excellence, for immunocompromised patients other than HIV-infected recommend performing an IGRA alone or an IGRA with a concurrent TST, and scoring positive for LTBI if either test is positive.[7] In our setting, characterized by a low prevalence of Bacillus Calmette–Guérin (BCG)-vaccination, we found that both TST and QuantiFERON-TB Gold (QFT-IT) (Cellestis Limited, Carnegie, Australia) are significantly associated with TB risk factors.[8]

QFT-IT measures antigen-specific IFN-γ secretion by peripheral blood $CD4^+$ T-lymphocytes, mainly effector T-cells, in response to *in vitro* MTB-specific stimulation.[9,10] It is hypothesized that the frequency of effector T-cells decreases as the mycobacterial antigen load (reflecting bacterial load) declines with treatment, and that measuring T-cell responses to MTB-specific antigens may thus be useful to monitor the treatment.[11–18] If this is correct, then these responses may be used as a tool to monitor the effect of treatment for LTBI or TB, and as surrogate markers of cure or predictors of relapse.

Few data are available on the modulation of IFN-γ response during LTBI therapy and most are from studies carried out on health care workers or close TB contacts.[17–25] Only a recent study involves subjects affected by IMID; however it focused on detecting the impact of TNFi therapy, rather than of LTBI treatment, on IFN-γ levels by serial IGRA and active TB progression.[26]

Therefore, the aim of our study was to evaluate the modulation of T-cell IFN-γ response to MTB-specific antigens during LTBI therapy in a large, screened population of IMID patients in Italy who were candidates for TNFi therapy.

Material and methods

Study population

Overall, the conditions of 1490 consecutive IMID patients were evaluated for LTBI at intervals between May 2006 and July 2011 in Florence, Italy by a predefined protocol.[8] The subjects were in care at three Florentine outpatient clinics for rheumatic diseases (rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis), psoriasis or other immunomediated chronic diseases which required the use of biological drugs (infliximab, etanercept, adalimumab and rituximab).[27] Informed consent was obtained from each patient.

Demographic information, data on BCG-vaccination, treatment regimens in the last 3 months and risk factors for LTBI were collected during a standardized interview. Treatments were classified into systemic corticosteroids, conventional disease-modifying anti-rheumatic drugs (DMARDs: including methotrexate, azathioprine, cyclosporine, leflunomide, cyclophosphamide and hydroxychloroquine), DMARDs mixed with systemic corticosteroids, and TNFi (infliximab, etanercept, or adalimumab). All subjects underwent a chest radiograph. Information regarding the risk factors for LTBI and active TB were also collected as described.[8,28] In particular, the following characteristics were considered to be LTBI risk factors: birth or residence for >6 months in a country with a high prevalence of TB (>20 cases per 100,000 inhabitants); history of household TB contact; health care workers in facilities following TB patients. Risk factors for active TB progression were: a medically-confirmed history of active TB; chest radiograph findings suggestive of previous TB (nodules, fibrotic scars, calcified granulomas or basal pleural thickening); being HIV-positive; body weight being 10% less than ideal body weight; presence of comorbidity (diabetes mellitus, silicosis, chronic renal failure/hemodialysis, neoplastic or hematological diseases); prolonged therapy with corticosteroids (>4 weeks) or other immunosuppressive therapy; injection drug use; and previous gastrectomy or jejunoileal bypass.

LTBI definition and therapy

Individuals who tested positive for either TST or QFT-IT were classified as LTBI[6–8] and offered LTBI treatment. The preferred treatment regimen used was rifampicin (RIF) plus isoniazid (INH) for 3 months at the standard dosage.[7] TNFi therapy for those who were not already being treated with TNFi was initiated at the end of LTBI treatment.

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QFT-IT and TST

QFT-IT blood samples were obtained before TST performance. The samples were incubated at 37 °C, within 2–6 h of blood collection. QFT-IT was performed according to the manufacturer's instructions by a biologist who was blinded to the subjects' characteristics and TST results.

QFT-IT was repeated upon completion of LTBI therapy (T1), and again, in a subgroup, 3–6 months after the end of therapy (T2).

After blood collection, all participants were injected by a trained physician with using 5 international units (IU) of tuberculin (Biocine Test-PPD; Chiron, Siena, Italy) until May 2008. Then, in the remaining subjects we used 2 IU of tuberculin of PPD RT23 (Staten Serum Institute, Copenhagen, Denmark), that was shown to have an equivalent potency of the previous TST.[29] The transverse diameter of induration was measured in millimeters 72 h later using the ball pen method. TST induration was interpreted according to groups at risk and risk factors for MTB infection in accordance with published guidelines.[2,30]

QFT-IT: definition of conversion and reversion and interpretation of results

QFT-IT reversion

QFT-IT test reversion was arbitrarily defined as a change from a positive ($\geq$0.35 IU/ml) to a negative ($<$0.35 IU/ml) result.

QFT-IT conversion

QFT-IT test conversion was arbitrarily defined as a change from a negative ($<$0.35 IU/ml) to a positive ($\geq$0.35 IU/ml) result.

Uncertainty zone

Previous studies reported non-specific variations occurring during IGRA serial testing, defined as "uncertainty zone" (IFN-γ value in response to MTB antigen between 0.20 and 0.50 IU/ml), and "grey zone" (IFN-γ value in response to MTB antigen between 0.10 and 0.35 IU/ml).[31,32] Considering these reports, we also analyzed our data by using the more stringent definition of conversion and reversion, as indicated by Pai.[31] Results in the uncertainty zone were considered as 'uncertain'. A person whose IFN-γ result increased from $<$0.20 to $>$0.50 IU/ml on the repeated test was considered to have had a "conversion". Likewise, a person whose QFT-IT result decreased from a value of $>$0.50 to $<$0.20 IU/ml was considered to have had a "reversion". Results that fluctuated within the uncertainty zone during repeated testing were considered 'doubtful conversions' or 'doubtful reversions'.

Statistical analysis

Data was analyzed by GraphPad Prism 4.00 (GraphPad Software, San Diego, CA, USA) and STATA 11.0 (StataCorp, College Station, TX, USA). For continuous measures, median and interquartile ranges (IQR) were calculated. The significance of the differences between the groups was determined by using the Wilcoxon test for paired data. Non-parametric tests for paired data were used to compare the continuous measures of IFN-γ at the different time points. In particular Kruskal–Wallis test was used to compare medians at T0-T1, and the Friedman test to compare the medians at T0-T1-T2. For dichotomous measures, chi square was used. Differences were considered significant at p-values $\leq$0.05. Univariate and multivariate analysis were performed to analyze the association with the likelihood of reversion among those who scored positive to QFT-IT at baseline, and the association with the likelihood of conversion among those who scored negative to QFT-IT at baseline.

Results

Studied population

A total of 1490 consecutive subjects with IMID were evaluated in the outpatient service for LTBI screening. Indeterminate results to QFT-IT were found in 10 subjects (0.7%). Due to this low number, no significant associations with the listed IMID therapies were found, different from a recent report.[33] None of the screened subjects were found to have active TB (by clinical and radiological evaluations) at the time of screening and after a median follow-up time of 33 months (IQR 21–45 months). Based on our criteria, 249 subjects were classified as LTBI. Among them, 127 (51.0%) were $TST^{+}/QFT\text{-}IT^{+}$, 93 (37.3%) were $TST^{+}/QFT\text{-}IT^{-}$, 28 (11.2%) $TST^{-}/QFT\text{-}IT^{+}$ and one was indeterminate (0.4%) (Fig. 1). LTBI therapy was offered to all LTBI subjects. However, 42 subjects refused or were no longer candidates for biologic therapy for their IMID condition. Indeterminate results were excluded from further analysis.

We included 206 LTBI subjects who underwent therapy in the analysis. Among them, 166 were followed up from the screening time (T0) until completion of treatment (T1), and 92 were followed for an additional 3–6 months (T2) (Fig. 1). Characteristics of the LTBI population are described in Table 1. Our population was prevalently male, with a median age of 57.5 years and a low rate of previous BCG-vaccination. The most frequent underlying disease was rheumatoid arthritis and most of the subjects were on some kind of therapy for the rheumatic condition, with a limited number of subjects who had already started the TNFi (16–17.5%). No significant differences between the two subgroups were found (Table 1).

Evaluation of QFT-IT results over time

QFT-IT changes among the 166 LTBI treated-subjects who were followed-up until T1 are showed (Table 2). Amongst the 55 who scored QFT-IT-negative at baseline (T0), 10 subjects (18%) converted, and, amongst the 111 subjects who were scored QFT-IT-positive at baseline, 27 subjects (24%) reverted at T1. Of the 166 subjects evaluated, 92 were further tested at T2 showing a different and not homogeneous pattern of reversion and conversion (data showed in Table 2).

For further analysis from hereafter we consider the data reported at T0 and T1 unless differently specified. Using the "uncertainty zone" definition (Table 3), we identified 26 subjects with a "doubtful score", 17 at baseline and an

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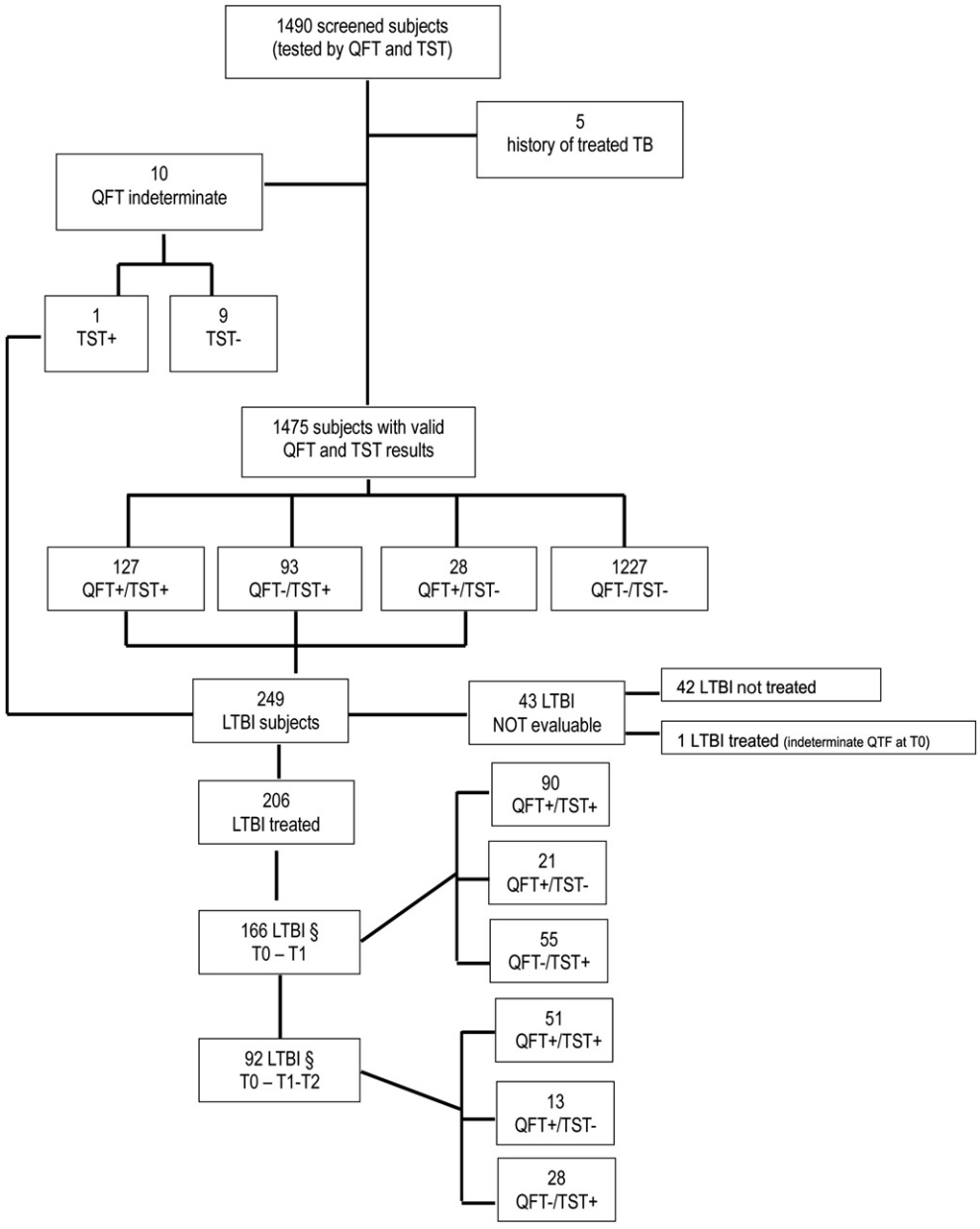

Figure 1 Flow diagram of the studied population. Definition of abbreviations: QFT-IT: QuantiFERON-TB Gold in-tube; TST: tuberculin skin test; LTBI: latent tuberculosis infection; T0: time of baseline QFT-IT testing; T1: time of LTBI therapy completion; T2: time 3—6 months after LTBI therapy completion. § Subjects evaluated with repeated QFT-IT at T0 and T1 or, for the subgroup, at T0, T1 and T2.

additional 9 at T1. This definition decreased the proportion of conversion from 18% to 13%, and reversion from 24% to 17%, while the proportion of subjects with stable results was similar using both definitions. The frequency of reversions and conversions did not differ significantly with either criteria ($p = 0.370$ and $p = 0.496$, respectively with standard and uncertainty zone definitions).

Factors associated with reversion and conversion

Reversion data

We analyzed the factors that may be associated with the probability of reversion and conversion of the QFT-IT score. Among the 111 subjects who scored QFT-IT-positive at enrollment, the likelihood of reversion decreased if age was between 50 and 60 years compared to subjects younger than 50 years ($p = 0.029$), if TST was larger than 15 mm with respect to sizes smaller than 5 mm ($p = 0.022$), if QFT-IT value at baseline was between 1.0 and 3.0 IU/ml ($p = 0.005$) or higher than 3.0 IU/ml ($p < 0.001$) with respect to results lower than 1 IU/ml (Table 4). No association was found between reversion and (i) IMID therapies (comparing by univariate analysis the frequency of this event among each group of subjects under different immune suppressive regimens to that of the group of the untreated subjects) (ii) the number of risk factors for TB-infection

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Table 1 Characteristics of the LTBI-treated population.

	Total LTBI-treated *N* (%)	LTBI T0—T1 *N* (%)	LTBI T0—T1—T2 *N* (%)
Total	206 (100)	166 (80.6)	92 (44.7)
Female gender	97 (47.1)	80 (48.2)	45 (48.9)
BCG-vaccinated	19 (9.2)	15 (9)	7 (7.6)
Birth/residence in countries with a high prevalence of TB	21 (10.2)	16 (9.6)	8 (8.7)
Median age (years) IQR	57.5 (20; 47—67)	58 (20; 47—67)	58 (19.5; 47.5—67)
Diagnosis			
Rheumatoid arthritis	64 (31.1)	56 (33.7)	31 (33.7)
Psoriasis	53 (25.7)	43 (25.9)	19 (20.7)
Psoriatic arthritis	49 (23.8)	36 (21.7)	25 (27.2)
Ankylosing spondylitis	21 (10.2)	17 (10.2)	7 (7.6)
Other[a]	19 (9.2)	14 (8.4)	10 (10.9)
Therapy			
No therapy (in last 3 months)	54 (26.2)	42 (25.3)	23 (25)
Therapy DMARDs and/or steroids	119 (57.8)	95 (57.2)	53 (57.6)
Therapy including TNFi	33 (16)	29 (17.5)	16 (17.4)

Definition of abbreviations: TNFi: tumor necrosis factor α inhibitors, DMARDs: disease-modifying anti-rheumatic drugs, TB: tuberculosis, BCG: bacillus Calmette—Guérin; IQR: interquartile range.

[a] Includes undifferentiated spondyloarthropathy, sacroiliitis, ulcerative colitis, Crohn's disease, polymyalgia rheumatica, vasculitis (three each); systemic sclerosis (one).

or (iii) the number of risk factors for active TB-progression (data not shown).

Further, we stratified the data based on the IFN-γ values at baselines (Table 5). The majority of the subjects scored TST-positive, independent of the IFN-γ value at baseline. In terms of reversion, we found that amongst the 27 subjects who reverted, 16 (59%, 39—78) had IFN-γ values at baselines lower than 1.0 IU/ml and that they represented the majority (16/29, 55%, CI, 36—74) of those with IFN-γ values at baseline lower than 1.0 IU/ml. To note: although the rate

Table 2 QFT-IT score of the 166 LTBI subjects tested at T0 and T1, and the 92 LTBI subjects followed at T2.

QFT-IT at T0 *N*	QFT-IT at T1 *N* (%)	QFT-IT 3—6 months after T1 (T2) *N* (%)
55 Negative (<0.35 UI/ml)	45 (82) negative	22/23 (96) negative
		1/23 (4) positive
	10 (18) positive	1/5 (20) negative
		4/5 (80) positive
111 Positive (≥0.35 UI/ml)	27 (24) negative	13/17 (76) negative
		4/17 (24) positive
	84 (76) positive	4/47 (9) negative
		43/47 (91) positive

Definition of abbreviations: QFT-IT: QuantiFERON-TB Gold in-tube (CFP-10, ESAT-6 and TB7.7); LTBI: latent tuberculosis infection.

Table 3 QFT-IT reversion and conversion in 166 LTBI subjects at the end of therapy (T1) using two different cut-off criteria for interpreting QFT-IT results.

QFT at T0	QFT at T1	
	Positive — *N* (%)	Negative — *N* (%)
Standard definition[a]		
Negative (<0.35 IU/ml) (55 subjects)	10 (18)	45 (82)
Positive (≥0.35 IU/ml) (111 subjects)	84 (76)	27 (24)
"Uncertainty zone" definition[b]		
Negative (<0.20 IU/ml) (47 subjects)	6(13)	39 (83)
Positive (>0.50 IU/ml) (102 subjects)	78 (76)	17 (17)

[a] No significant difference between the likelihood of reversion and conversion (OR 0.80, $p = 0.370$).

[b] No significant difference between the likelihood of reversion and conversion (OR 0.46, $p = 0.496$).

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Table 4 Factors associated with the likelihood of reversion among the 111 subjects who scored positive to QFT-IT at baseline.

	Number (%)	Adjusted OR (95%CI)	*p*-Value
Age			
<50	20 (18)	1	
50–60	33 (30)	0.17 (0.03–0.83)	0.029
>60	58 (52)	0.30 (0.08–1.16)	0.081
Gender			
Male	61 (55)	1	
Female	50 (45)	1.82 (0.64–5.18)	0.260
TST classes			
<5 mm	21 (19)	1	
5–9 mm	7 (6)	0.54 (0.07–4.27)	0.557
10–14 mm	14 (13)	0.40 (0.07–2.24)	0.296
≥15 mm	69 (62)	0.23 (0.06–0.81)	0.022
QFT-IT classes			
0.35–1.0	29 (26)	1	
1.01–3	27 (24)	0.14 (0.03–0.54)	0.005
>3	55 (50)	0.10 (0.03–0.35)	<0.001

Definition of abbreviations: TST: tuberculin skin test; QFT-IT: QuantiFERON-TB Gold in-tube; OR: odds ratio; CI: confidence intervals.

was lower (13%, CI, 5–25), we also observed a considerable number of reversions (7/55) in the group of subjects with high IFN-γ values at baselines (>3.0 IU/ml). When the analysis was performed considering the more stringent definition of reversion, the results in these two subgroups of subjects (baseline IFN-γ values <1.0 IU/ml and >3.0 IU/ml) decreased, with a rate of reversion of 40% (CI, 19–64) and 11% (CI, 4–22), respectively.

Conversion data

Among the 55 subjects who scored QFT-IT-negative at enrollment, the likelihood of conversion increased significantly ($p = 0.014$), with higher QFT-IT values at baseline, whilst it decreased if the gender was female ($p = 0.019$). Differently however, no significant association was found with age and TST classes (Table 6). No association was found between conversion and (i) IMID therapies (comparing by univariate analysis the frequency of this event among each group of subjects under different immune suppressive regimens to that of the group of the untreated subjects) (ii) the number of risk factors for TB-infection or (iii) the number of risk factors for active TB-progression (data not shown). Among the 10 conversions observed, 6/10 had a baseline QFT-IT characterized by IFN-γ value above 0.1 UI/ml including 4 with a value above 0.2 that would have been scored as "doubtful" based on the "uncertainty zone" definition (data not shown).

Quantitative data of IFN-γ production over time

We evaluated the quantitative IFN-γ change over time among those who scored QFT-IT-positive at enrollment. As shown in Fig. 2A, amongst the 111 subjects evaluated at T0 and T1, the IFN-γ level at baseline was higher (median: 0.98; IQR: 0.1–8.8 IU/ml) than that reported at T1 (median: 0.7; IQR: 0.03–4.4), although not significant ($p = 0.20$). In the 92 subjects who were also evaluated at T2 (Fig. 2B), we confirm that the IFN-γ level at baseline was higher (median: 1.4; IQR: 0.19–10.0 IU/ml) although not significantly different than that reported at T1 (median: 1.01; IQR: 0.01–10.0 IU/ml) ($p = 0.15$). However, interestingly, the IFN-γ level was significantly higher at baseline (median: 0.6; IQR: 0.01–4.7 IU/ml) than at T2 ($p = 0.001$). Moreover, a significant difference was found between the IFN-γ levels at T1 and those at T2 ($p = 0.004$). When the values were analyzed considering

Table 5 Rate of reversion at the end of therapy (T1) by baseline IFN-γ value among the 111 subjects who scored QFT-IT-positive at baseline (T0), considering two different QFT-IT-positive scoring definitions.

Standard definition				"Uncertainty zone" definition			
Baseline IFN-γ response (IU/ml)	No. of subjects	T1 QFT-IT reversion *N* (%, C.I.)	TST+ at T0 *N* (%, C.I.)	Baseline IFN-γ response (IU/ml)	No. of subjects	T1 QFT-IT (IFN-γ >0.50 UI/ml) *N* (%, C.I.)	T1 QFT-IT "true" reversion *N* (%, C.I.)
0.35–1.0	29	16 (55, 36–74)	23 (79, 63–92)	0.5–1.0	20	8 (40, 19–64)	8 (40, 19–64)
1.01–3.0	27	4 (15, 4–34)	21 (78, 58–91)	1.01–3.0	27	22 (81, 62–94)	3 (11, 2–29)
>3	55	7 (13, 5–25)	46 (84, 71–92)	>3	55	48 (87, 76–95)	6 (11, 4–22)
Total	111	27 (24, 17–33)	90 (81, 73–88)	Total	102	78 (76, 67–84)	17 (17, 10–25)

Definition of abbreviations: TST: tuberculin skin test; QFT-IT: QuantiFERON-TB Gold in-tube; IFN: interferon; CI: 95% confidence intervals.

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Table 6 Factors associated with the likelihood of conversion among the 55 subjects who scored negative to QFT-IT at baseline.

	Number (%)	Adjusted OR	*p*-Value
Age			
<50	29 (53)	1	
50–60	15 (27)	0.40 (0.04–3.86)	0.427
>60	11 (20)	3.56 (0.41–30.6)	0.248
Gender			
Male	25 (45)	1	
Female	30 (55)	0.06 (0.01–0.63)	0.019
TST classes			
5–9 mm	9 (16)	1	
10–14 mm	9 (16)	0.92 (0.06–14.2)	0.951
≥15 mm	37 (67)	0.84 (0.09–7.73)	0.875
QFT-IT			
UI/ml at T0	55 (100)	1.10 (1.02–1.19)	0.014

Definition of abbreviations: TST: tuberculin skin test; QFT-IT: QuantiFERON-TB Gold in-tube; OR: odds ratio; CI: confidence intervals.

all the data simultaneously, a significant difference was found among the results obtained at the different time points (Friedman test $p = 0.002$).

Discussion

In recent years, a number of studies have been conducted to investigate the modulation of IFN-γ specific response during LTBI therapy, as measured by IGRAs. These studies were mainly carried out in health care workers or recent TB contacts and led to conflicting results, likely due to several confounding factors, including differences in LTBI treatment protocols, population of subjects studied (health care workers, recent close TB contacts, recent immigrants), settings in which repeated exposure to active TB during LTBI treatment could have occurred (eg, study conducted in India), and the TB endemic country where the study was performed (Western countries compared to Asian countries) (Table 7).[17–26]

To the best of our knowledge, this is the largest study (subjects were obtained after screening 1490 IMID patients who were candidates for TNFi treatment) aimed at evaluating the modulation of IFN-γ response during LTBI therapy. Among the 166 subjects followed up until T1, we observed a QFT-IT reversion rate of 24% and a conversion rate of 18%; using more stringent criteria,[31] 17% reverted and 13% converted.

Reversion of the IGRA scores after LTBI treatment was observed in several studies. In the largest cohort described so far by Chee et al., among 226 treated close contacts, the score of 38% reverted using the manufacturer's criteria for T-SPOT.*TB* assay.[20] More recently, in another cohort of 74 QFT-IT-positive TB contacts, Lee et al. found a similar rate of reversion (42%).[24] Although the results of these studies are different than ours, there are some similarities. In our study, the likelihood of achieving a QFT-IT reversion significantly decreased with older age, larger TST induration size and higher IFN-γ value at baseline QFT-IT. Chee also reported that CFP-10 reverters were significantly younger than non-reverters, while no correlation between TST size and change in CFP-10 responses were observed.[20]

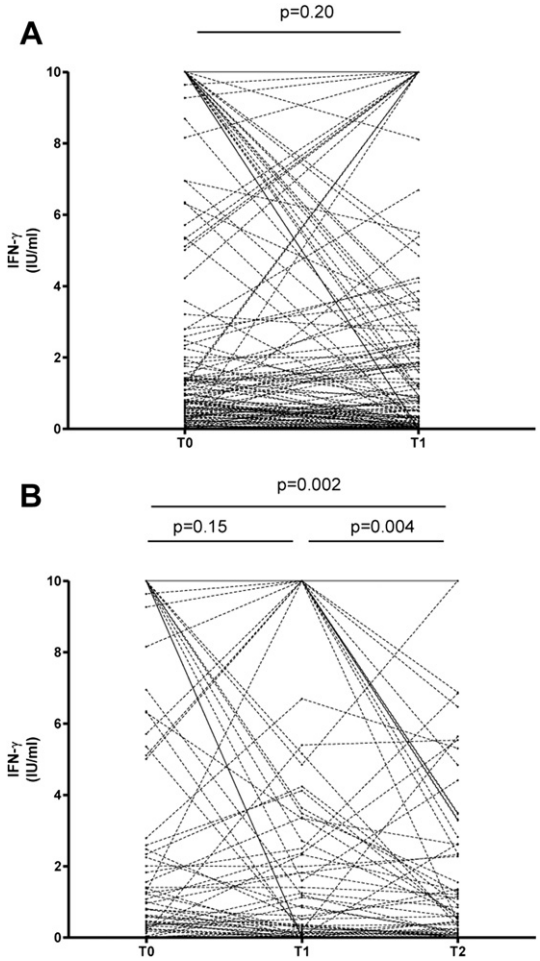

Figure 2 A–B. Longitudinal analysis of IFN-γ secretion in response to QFT-IT in the 111 subjects with LTBI with positive QFT-IT at T0, who were followed until T1 (A) and in the 92 subjects who were also evaluated at T2 (B). Definition of abbreviations: T0: start of therapy; T1: end of therapy; T2: 3–6 months after LTBI therapy completion; LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; IFN: interferon.

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Table 7 IGRA trends in LTBI treated subjects who scored IGRA-positive at baseline.

First author	Country	IGRA	Studied population	Number of subjects who scored IGRA-positive at baseline	LTBI therapy	Trends of IGRA	Trends of IGRA	Reversion at T1	Factors associated with reversion or decreasing trend
						Short term	T1	% (95%CI)	
Pai, 2006	India	QFT-GIT	Health care workers	10 IGRA positive	INH 6 months	na[a]	Decrease[c]	10 (2.5—44.5)	na[a]
Ewer, 2006	UK	ELISpot	Contacts	38 IGRA positive 25 controls	$RIF^{+}INH$ 3 months	Increase	Decrease	8 (1.7—21.4)	na[a]
Wilkinson, 2006	UK	ELISpot	Immigrants	16 IGRA positive 8 controls	$RIF^{+}INH$ 3 months	Increase	Decrease[c]	[b]	na[a]
Chee, 2007	Singapore	T-SPOT.*TB*	Close contacts	226 IGRA positive	INH 6 months	na[a]	Decrease	38 (31.3—44.3)	Younger age
Goletti, 2007	Italy	QFT-G	Contacts	28 IGRA positive 11 controls	INH 6 months	na[a]	Decrease	3.6 (0.1—18.3)	No past MTB exposure
Higuchi, 2008	Japan	QFT-G	Contacts	28 IGRA positive 5 controls	INH 6 months	na[a]	Decrease	25 (10.7—45)	na[a]
Herrmann, 2009	France	QFT-GIT	Children contacts	25 IGRA positive	$RIF^{+}INH$ 3 months	Increase	Decrease	[b]	na[a]
Lee, 2010	South Korea	QFT-GIT	Contacts	74 IGRA positive	RIF 4 months	na[a]	Decrease	42 (30.5—54)	Smaller TST size, lower IFN-γ value
Dyrhol-Riise, 2010	Norway	QFT-GIT	Contacts, immigrants and others	40 IGRA positive	$RIF^{+}INH$ 3 months	na[a]	No change	12,5	Low IFN-γ values at baseline
Chen, 2012	Taiwan	QFT-G	RA patients	37 IGRA positive	INH 9 months	Decrease	Decrease	na[a]	Further decline of IFN-γ levels after TNFi onset
Our data	Italy	QFT-GIT	IMID population	111 IGRA positive	$RIF^{+}INH$ 3 months	na[a]	Decrease	24 (16—32)	Younger age, smaller TST size, lower IFN-γ value

Definition of abbreviations: IGRA: interferon-γ release assay; QFT-G: QuantiFERON-TB Gold (CFP-10 and ESAT-6); QFT-G IT: QuantiFERON-TB Gold in-tube (CFP-10, ESAT-6 and TB7.7); T1: end of therapy; IMID: immune-mediated inflammatory diseases; INH: isoniazid; RIF: rifampin; RA: rheumatoid arthritis.

[a] na = Not available.

[b] No percentage of reversion was reported.

[c] Decreasing trend observed, but not statistically significant.

This result may be due to the fact that TB infection in younger subjects is more likely to be recently acquired. Moreover, the decline of IFN-γ response is more easily and rapidly achieved after treatment of recent contacts without previous MTB exposure.[17] Our results are in agreement with those reported by Lee, who observed a significantly larger baseline TST induration size and higher IFN-γ levels among subjects without QFT-IT reversion.[24] Similar findings were reported by Franken et al., in a cohort of TB contacts in whom a very high IFN-γ value (above 4 IU/ml) at baseline QFT-IT response was associated with the absence of QFT-IT modulation over a 24-month follow-up period, with or without INH preventive treatment.[34]

To note: the 12 month reversion after the baseline assay was also observed in absence of LTBI therapy in 6.4% of 109 TB contacts in an high-burden TB setting (India).[31] In that study, reversion was more likely to occur in those with QFT-IT results close to the cut-off, while no reversion was observed in the subgroup that had >3 UI/ml IFN-γ levels at enrollment. In the present report, when we stratified our data by baseline IFN-γ values (Table 5), we found a significantly higher reversion rate among the group with 0.35—1.0 UI/ml IFN-γ levels than the group with >3 UI/ml IFN-γ levels (16/29 *vs* 7/55, $p < 0.001$), but even in the latter group the proportion of reversions was noticeable (13%). The comparison with the Pai et al. study[31] is relevant because it shows two important aspects: first, the assay *per se* may have limits of reproducibility or may detect subtle changes that, at present, are difficult to interpret; second, it shows the potential impact of LTBI therapy on the dynamics of IFN-γ response, as also suggested by the quantitative analysis (see below).

We found that a sizeable proportion of subjects (18%, or 13% if using more stringent criteria) converted at T1. In the above-mentioned study, Pai et al.,[31] using the same definitions, reported a QFT-IT conversion rate of 21.2% and 11.8%, respectively, and suggested that this was probably due to exposure to MTB that occurred during the follow-up period. As far as our study is concerned, the low TB endemic setting and the shorter follow-up period make it unlikely that subsequent MTB exposure would contribute to affecting conversion results. By multivariate analysis, conversion was significantly associated with higher IFN-γ levels at baseline. In particular, among 10 conversions observed, 6 had IFN-γ values above 0.10 UI/ml at baseline including 4 that were above 0.2 and considered "doubtful results". Moreover, as previously reported, TST may affect subsequent IGRA results in terms of increasing the levels of IFN-γ release appearing from 3 days to some months after a TST. This effect is more likely in IGRA-positive subjects, but it has been observed in IGRA-negative individuals (2—12%).[35]

In this study, none of the screened subjects, neither IGRA-score positive, negative or indeterminate subjects, developed active TB within a 33-month median time of follow-up observation. This differs from recent reports,[26] probably due to the different TB endemic countries (Taiwan *vs* Italy) in which the studies were performed.

In summary, it appears unlikely that the observed QFT-IT variations, at least in qualitative terms, are a real effect of LTBI treatment in the IMID population studied; they are probably the effect of the fluctuation of results close to the cut-off,[35] probably due to the limitations of the QFT-IT.[36] Moreover it is important to consider that IGRA have a lower sensitivity in immune-compromised subjects and that the fluctuations may also reflect the impact of the immune suppressive drugs on the immune system.[33,37—39]

Based on the available evidence, it is not possible to define to what extent this variability may be due to host-biological factors rather than to laboratory or test-related errors. However, interpreting the reversion or conversion of IGRAs remains an open issue.[35] Probably, assays based on different antigens and/or biomarkers may be more appropriate to evaluate Mb-specific response changes over time.[40—43]

We showed that among those who were QFT-IT-positive at baseline, there was a decrease of IFN-γ levels from baseline to T1, which become highly significant among the 92 subjects followed until T2. This result is in agreement with that reported in the literature (Table 7), in which an overall decline of the IFN-γ levels after LTBI therapy was observed, despite the discrepancies in treatment strategies, the IGRAs used, and the country and populations of subjects studied. Altogether, the quantitative data suggest that LTBI therapy may reduce IFN-γ-specific response, albeit a control group was not present in most of these studies, including the present one. However, in the few reports in which a control group was included, no quantitative changes occurred among the untreated subjects.[17,21—23]

Taking our and other studies into account, there is currently no evidence to support the use of QFT-IT in the clinical practice of monitoring LTBI treatment response. To further address this issue, larger, longitudinal studies are needed to evaluate the variation of IGRA scores with the risk of TB progression.[44]

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Conflict of interest

FB, DG, MS, AC, LA, JM, GF, FLG, MB, FP, NP, MMC, EG declare they have no conflict of interest. Institution of AB, AM, received in 2010 and in 2011 an unrestricted educational grant from A.D.A. srl, representative of Cellestis in Italy for the QuantiFERON-TB Gold test.

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References

1. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, et al. Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* 1999;**354**: 1932—9.

2. Gardam MA, Keystone EC, Menzies R, Manners S, Skamene E, Long R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. *Lancet Infect Dis* 2003;**3**(3):148–55.
3. Wallis RS. Tumour necrosis factor antagonists: structure, function, and tuberculosis risks. *Lancet Infect Dis* 2008;**8**(10): 601–11.
4. Valesini G, Montecucco C, Cutolo M. Recommendations for the use of biologic (TNF-alpha blocking) agents in the treatment of rheumatoid arthritis in Italy. *Clin Exp Rheumatol* 2006;**24**(4): 413–23.
5. Solovic I, Sester M, Gomez-Reino JJ, Rieder HL, Ehlers S, Milburn HJ, et al. The risk of tuberculosis related to TNF antagonist therapies: a TBNET consensus statement. *Eur Respir J* 2010;**36**:1185–206.
6. Mazurek M, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K, et al. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection – United States, 2010. *MMWR Recomm Rep* 2010;**59**(RR-5): 1–25.
7. National Institute for Health and Clinical Excellence. *Tuberculosis. Clinical diagnosis and management of tuberculosis, and measures for its prevention and control* [last accessed 30.07.12], www.nice.org.uk/guidance/CG117; July 24 2012.
8. Bartalesi F, Vicidomini S, Goletti D, Fiorelli C, Fiori G, Melchiorre D, et al. QuantiFERON-TB gold and TST are both useful for latent TB screening in autoimmune diseases. *Eur Respir J* 2009;**33**:1–8.
9. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008;**149**(3):177–84.
10. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000;**356**: 1099–104.
11. Chee CBE, Barkham TMS, KhinMar KW, Gan SH, Wang YT. Quantitative T-cell interferon-gamma responses to *Mycobacterium tuberculosis*-specific antigens in active and latent tuberculosis. *Eur J Clin Microbiol Infect Dis* 2009;**28**:667–70.
12. Sauzullo I, Mengoni F, Lichtner M, Massetti AP, Rossi R, Iannetta M, et al. *In vivo* and *in vitro* effects of anti-tuberculosis treatment on mycobacterial interferon-gamma T-cell response. *PLoS One* 2009;**4**(4):e5187.
13. Aiken AM, Hill PC, Fox A, McAdam KP, Jackson-Sillah D, Lugos MD, et al. Reversion of the ELISPOT test after treatment in Gambian tuberculosis cases. *BMC Infect Dis* 2006;**6**:66.
14. Carrara S, Vincenti D, Petrosillo N, Amicosante M, Girardi E, Goletti D. Use of a T-cell based assay for monitoring efficacy of anti-tuberculosis therapy. *Clin Infect Dis* 2004;**38**:754–6.
15. Butera O, Chiacchio T, Carrara S, Casetti R, Vanini V, Meraviglia S, et al. New tools for detecting latent tuberculosis infection: evaluation of RD1-specific long-term response. *BMC Infect Dis* 2009;**9**:182.
16. Kabeer BS, Raja A, Raman B, Thangaraj S, Leportier M, Ippolito G, et al. IP-10 response to RD1 antigens might be a useful biomarker for monitoring tuberculosis therapy. *BMC Infect Dis* 2011;**11**:135.
17. Goletti D, Parracino MP, Butera O, Bizzoni F, Casetti R, Dainotto D, et al. Isoniazid prophylaxis differently modulates T-cell responses to RD1-epitopes in contacts recently exposed to *Mycobacterium tuberculosis*: a pilot study. *Respir Res*.
18. Herrmann JL, Belloy M, Porcher R, Simonney N, Aboutaam R, Lebourgeois M, et al. Temporal dynamics of interferon gamma responses in children evaluated for tuberculosis. *PLoS One*.
19. Pai M, Joshi R, Dogra S, Mendiratta DK, Narang P, Dheda K, et al. Persistently elevated T-cell interferon-gamma responses after treatment for latent tuberculosis infection among health care workers in India: a preliminary report. *J Occup Med Toxicol*.
20. Chee CB, KhinMar KW, Gan SH, Barkham TM, Pushparani M, Wang YT. Latent tuberculosis infection treatment and T-cell responses to *Mycobacterium tuberculosis*-specific antigens. *Am J Respir Crit Care Med*.
21. Ewer K, Millington KA, Deeks JJ, Alvarez L, Bryant G, Lalvani A. Dynamic antigen-specific T-cell responses after point-source exposure to *Mycobacterium tuberculosis*. *Am J Respir Crit Care Med*.
22. Wilkinson KA, Kon OM, Newton SM, Meintjes G, Davidson RN, Pasvol G, et al. Effect of treatment of latent tuberculosis infection on the T-cell response to *Mycobacterium tuberculosis* antigens. *J Infect Dis*.
23. Higuchi K, Harada N, Mori T. Interferon-gamma responses after isoniazid chemotherapy for latent tuberculosis. *Respirology*.
24. Lee SH, Lew WJ, Kim HJ, Lee HK, Lee YM, Cho CH, et al. Serial interferon-gamma release assays after rifampicin prophylaxis in a tuberculosis outbreak. *Respir Med* 2010;**104**(3):448–53.
25. Dyrhol-Riise AM, Gran G, Wentzel-Larsen T, Blomberg B, Haanshuus CG, Mørkve O. Diagnosis and follow-up of treatment of latent tuberculosis; the utility of the QuantiFERON-TB gold in-tube assay in outpatients from a tuberculosis low-endemic country. *BMC Infect Dis* 2010;**10**:57.
26. Chen DY, Shen GH, Chen YM, Chen HH, Hsieh CW, Lan JL. Biphasic emergence of active tuberculosis in rheumatoid arthritis patients receiving TNFα inhibitors: the utility of IFNγ assay. *Ann Rheum Dis* 2012 Feb;**71**(2):231–7.
27. Furst DE, Keystone EC, Braun J, Smolen JS, Burmester GR, Emery P, et al. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2011. *Ann Rheum Dis* 2012;**71**(Suppl. 2):i2–45.
28. Jasmer RM, Nahid P, Hopewell PC. Clinical practice. Latent tuberculosis infection. *N Engl J Med* 2002;**347**(23):1860–6.
29. Sgountzos V, Simopoulou S, Kretsou S, Sakayianni K, Pavlerou S, Gourgoulianis K, et al. Comparative study of RT23 and Merieux tuberculin tested among healthy volunteers. *Int J Tuberc Lung Dis* 2009;**13**(3):312–6.
30. American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med* 2000;**161**(4 Pt 2):S221–47.
31. Pai M, Joshi R, Dogra S, Zwerling AA, Gajalakshmi D, Goswami, et al. T-cell assay conversions and reversions among household contacts of tuberculosis patients in rural India. *Int J Tuberc Lung Dis* 2009;**13**(1):84–92.
32. Harada N, Higuchi K, Sekiya Y, Rothel J, Kitoh T, Mori T. Basic characteristics of a novel diagnostic method (QuantiFERON TB-2G) for latent tuberculosis infection with the use of *Mycobacterium tuberculosis*-specific antigens, ESAT-6 and CFP-10. *Kekkaku* 2004;**79**:725–35 [Japanese].
33. Bélard E, Semb S, Ruhwald M, Werlinrud AM, Soborg B, Jensen FK, et al. Prednisolone treatment affects the performance of the QuantiFERON gold in-tube test and the tuberculin skin test in patients with autoimmune disorders screened for latent tuberculosis infection. *Inflamm Bowel Dis* 2011;**17**(11): 2340–9.
34. Franken WP, Arend SM, Thijsen SF, Bouwman JJ, Koster BF, van Dissel JT, et al. Interferon-gamma release assays during follow-up of tuberculin skin test-positive contacts. *Int J Tuberc Lung Dis* 2008;**12**(11):1286–94.
35. van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. *PLoS One* 2009;**4**(12):e8517.
36. Perry S, Sanchez L, Yang S, Agarwal Z, Hurst P, Parsonnet. Reproducibility of QuantiFERON-TB gold in-tube assay. *J Clin Vaccine Immunol* 2008;**15**(3):425–32.
37. Goletti D, Carrara S, Mayanja-Kizza H, Baseke J, Mugerwa MA, Girardi E, et al. Response to *M. tuberculosis* selected RD1 peptides in Ugandan HIV-infected patients with smear positive

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pulmonary tuberculosis: a pilot study. *BMC Infect Dis* 2008; **28**(8):11.

38. Leidl L, Mayanja-Kizza H, Sotgiu G, Baseke J, Ernst M, Hirsch C, et al. Relationship of immunodiagnostic assays for tuberculosis and numbers of circulating CD4+ T-cells in HIV infection. *Eur Respir J* 2010;**35**(3):619—26.
39. Goletti D, Raja A, Syed Ahamed Kabeer B, Rodrigues C, Sodha A, Carrara S, et al. Is IP-10 an accurate marker for detecting *M. tuberculosis*-specific response in HIV-infected persons? *PLoS One* 2010;**5**(9):e12577.
40. Delogu G, Chiacchio T, Vanini V, Butera O, Cuzzi G, Bua A, et al. Methylated HBHA produced in *M. smegmatis* discriminates between active and non-active tuberculosis disease among RD1-responders. *PLoS One* 2011;**6**(3):e18315.
41. Goletti D, Butera O, Vanini V, Lauria FN, Lange C, Franken KL, et al. Response to Rv2628 latency antigen associates with cured tuberculosis and remote infection. *Eur Respir J* 2010; **36**(1):135—42.
42. Chiacchio T, Petruccioli E, Vanini V, Butera O, Cuzzi G, Petrone L, et al. Higher frequency of T-cell response to *M. tuberculosis* latency antigen Rv2628 at the site of active tuberculosis disease than in peripheral blood. *PLoS One* 2011;**6**(11): e27539.
43. Vanini V, Petruccioli E, Gioia C, Cuzzi G, Orchi N, Rianda A, et al. IP-10 is an additional marker for tuberculosis (TB) detection in HIV-infected persons in a low-TB endemic country. *J Infect* 2012;**65**(1):49—59.
44. Diel R, Goletti D, Ferrara G, Bothamley G, Cirillo D, Kampmann B, et al. Interferon-γ release assays for the diagnosis of latent *Mycobacterium tuberculosis* infection: a systematic review and meta-analysis. *Eur Respir J* 2011;**37**(1): 88S—99S.

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