

Serial interferon- γ release assays for screening and monitoring of tuberculosis infection during treatment with biologic agents

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Abstract Screening for latent tuberculosis infection (LTBI) prior to the prescribing of anti-TNF agents and monitoring for infection during treatment are recommended. The feasibility of novel screening tools, including QuantiFERON-TB Gold In-Tube (QFT-GIT), remains unclear in the setting of immunosuppression. The aim of this study was to evaluate the usefulness of serial QFT-GIT during biologic therapy to assess whether dynamic changes in IFN- γ levels may be helpful in identifying reactivation of LTBI or newly acquired TB. We conducted a prospective study on patient candidates to TNF inhibitors. QFT-GIT was performed at baseline and after 3 and 6 months since biologic onset. A further follow-up period of 6 months was observed. Among patients enrolled ($n=119$; $F=69\%$; median age=47 years, range 18–80), 24 had at least 1 risk factor for LTBI. Ninety-six were taking immunosuppressants at the time of TB testing. At baseline, 5 patients displayed positive, 93 negative, and 21 indeterminate QFT-GIT results. We observed QFT-GIT conversions and reversions in 12 patients with

LTBI and in 73 without LTBI. QFT-GIT results changed of 28 % at month 3 and of 21 % at month 6; the greatest change was observed in patients with indeterminate results that became negative (15 %; $p<0.02$). No TB cases were detected. In conclusion, the routine use of both QFT-GIT and TST at screening seems not to give any advantage in the setting of patients awaiting biologics. In addition, the feasibility of serial QFT-GIT during biologic therapy needs definition since changes in IFN- γ levels may occur without a pathologic connotation.

Keywords Anti-TNF · QuantiFERON-TB Gold In-Tube · Rheumatoid arthritis · Tuberculin skin test

Introduction

Targeting tumor necrosis factor (TNF) proved to be a dramatic breakthrough in the treatment of rheumatoid arthritis (RA) and other chronic inflammatory rheumatic diseases resistant to conventional therapies [1]. While being highly effective, TNF blockers have raised concerns about the potential for an increased risk of reactivation of latent tuberculosis infection (LTBI) [2]. The results from the British Society for Rheumatology Biologics Register [3] and those from the French Research Axed on Tolerance of Biotherapies (RATIO) Register [4] indicate that the monoclonal antibodies infliximab and adalimumab exhibit a greater risk of inducing tuberculosis (TB) than the soluble TNF receptor etanercept when it stands for reactivation of LTBI. By contrast, using the hidden Markov modeling, both classes were equally unsafe on the outcome of newly acquired TB infection [5]. Consequently, screening for LTBI prior to anti-TNF therapy and a watchful monitoring during the treatment are recommended [1]. Until recently, in the

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absence of a gold standard for diagnosing LTBI, the screening comprised tuberculin skin test (TST), chest radiography, medical history focused on risk factors for TB, and physical examination. Since TST lacks sensitivity and specificity, especially in immunocompromised people [6], novel screening tools, the IFN- γ release assays (IGRAs), have been introduced, including QuantiFERON-TB Gold In-Tube (QFT-GIT), which quantify the in vitro release of IFN- γ following stimulation of T cells with *Mycobacterium tuberculosis*-specific antigens [7]. These tests are more specific than TST, as the antigens used are present in *M. tuberculosis*, but absent from bacille Calmette–Guérin (BCG) vaccine strains and most non-tuberculous *Mycobacteria* (NTM) [8]. Updated guidelines suggest the performance of both an IGRA and TST in patients with high risk for progression of TB infection [8]. However, the optimal screening strategy for LTBI is still questioned due to the uncertainty regarding the performance of IGRAs in immunosuppressed, the interpretation of indeterminate results, and the feasibility of serial testing. This latter point is of utmost importance since repeated TST tests should be avoided in patients with LTBI who have received prophylaxis therapy [9].

This study was undertaken to evaluate: (a) the feasibility of serial QFT-GIT testing during the treatment with anti-TNF agents to assess whether dynamic changes in IFN- γ plasma levels may allow the identification of cases of LTBI reactivation or newly acquired TB and (b) the agreement of QFT-GIT and TST in patients under consideration for biologics.

Patients and methods

We conducted a prospective study on patients with inflammatory rheumatic diseases designated to initiate biologics at the Rheumatology Division of Sapienza University of Rome, Italy. Patients were enrolled between July 2008 and February 2010 and the follow-up was completed in February 2011. At recruitment data on demographics, BCG vaccination, treatment regimens, and risk factors for LTBI were obtained by direct questioning and collected on a standardized electronic form. Concomitant treatment was registered focusing on assumption of systemic glucocorticoids and conventional disease modifying anti-rheumatic drugs. Birth or prolonged living in an area with a high prevalence of TB infection, history of household TB contact, and previous diagnosis of TB which had been inadequately treated were considered risk factors for LTBI. All patients were requested to execute a postero-anterior chest X-ray, which was reviewed by a radiologist aware that anti-TNF therapy was being considered and who was asked to search for signs suggestive of LTBI [6]. Patients underwent on the same day TST and QFT-GIT: TST (Biocine Test PPD, Chiron, Siena,

Italy) was performed according to the Mantoux method by the same experienced operator and an induration ≥ 5 mm was considered positive [6]; QFT-GIT (Cellestis Limited, Carnegie, Victoria, Australia) was performed and interpreted by the same trained technicians according to the manufacturer's instructions. A blinded interpretation for TST and QFT-GIT results was done. Patients with evidence of TB infection based on any of QFT-GIT, TST, or chest X-ray outcome were considered as affected by LTBI and received a 9-month course isoniazid (INH) prophylaxis after ruling out active TB. In these patients, biologics were commenced following at least 4 weeks of INH. QFT-GIT was repeated after 3 and 6 months since TNF antagonist onset; moreover, in patients in whom evidence for LTBI existed, the test was performed again prior to initiating biologic therapy to understand whether INH may affect QFT-GIT responses. In all the patients, an additional follow-up period of 6 months was observed. The study was approved by the local Ethics Committee and all patients gave written informed consent.

Statistical analysis

SPSS version 13.0 for Windows (SPSS Inc., Apache Software Foundation, Chicago, Illinois) was used. IFN- γ production in response to antigenic stimulation was expressed as continuous (IU/ml) measures. Median and ranges of the different parameters were calculated. Concordance between QFT-GIT and TST was performed using the Cohen's kappa coefficient with kappa values >0.75 representing excellent agreement beyond chance, values 0.40 – 0.75 representing fair to good agreement beyond chance, and values <0.40 representing poor agreement beyond chance. Odds ratios and their 95 % confidence intervals (CI) for factors associated with discordant results and indeterminate QFT-GIT results were estimated by univariate analysis. The differences of values between groups were analyzed using the non-parametric Mann–Whitney *U* test. Data in the longitudinal analysis were evaluated with the non-parametric Wilcoxon signed-rank test. All statistical analyses were two-sided and considered significant in case of *p* values <0.05 .

Results

Clinical data

The baseline demographics and disease characteristics of the patients ($n=119$) are listed in Table 1. Subjects enrolled were diagnosed with RA, psoriatic arthritis, ankylosing spondylitis, or Behcet's disease based on standard criteria [10–13]. Among the patients with a positive vaccination status, all had been inoculated with BCG in childhood, but

Table 1 Baseline demographics and disease characteristics of patients enrolled in the study

CHARACTERISTICS	ALL (n=119)	QFT-GIT			TST	
		POSITIVE (n=5)	NEGATIVE (n=93)	INDETERMINATE (n=21)	POSITIVE (n=14)	NEGATIVE (n=105)
Females (n/%)	82/68.9	5/6.1	61/74.4	16/19.5	9/10.9	73/89
Age (years; median/range)	47/18–80	59/18–80	49/20–80	38/21–63	60.5/27–73	46/18–80
Underlying disease (n/%)						
Rheumatoid arthritis	61/51.3	4/6.5	43/70.5	14/22.9	7/11.5	54/88.5
Psoriatic arthritis	40/33.6	1/2.5	37/92.5	2/5	6/15	34/85
Ankylosing spondylitis	13/10.9	0	10/76.9	3/23.1	1/7.7	12/92.3
Behçet's disease	5/4.2	0	3/60	2/40	0	5/100
BCG-vaccinated (n/%)	7/5.8	0	5/71.4	2/28.6	2/28.6	5/71.4
Risk factors for LTBI (n/%)						
Birth or prolonged living in a TB-endemic area ^a	13/10.9	1/7.7	8/61.5	4/30.7	2/15.4	11/84.6
History of household contact	5/4.2	0	3/60	2/40	0	5/100
Chest X-ray suggestive of LTBI	6/5	1/16.6	5/83.3	0	2/33.3	4/66.6
Previous diagnosis of TB	0	–	–	–	–	–
Concomitant treatment regimen (n/%)						
Glucocorticoids	12/10.1	1/8.3	10/83.3	1/8.3	1/8.3	11/91.6
DMARDs	19/16	2/10.5	16/84.2	1/5.3	1/5.3	18/94.7
DMARDs and glucocorticoids	65/54.6	1/1.5	49/75.4	15/23.1	7/10.7	58/89.2
No immunosuppressants	23/19.3	1/4.3	18/78.3	4/17.4	5/21.7	18/78.3
Dose of immunosuppressants (mg median/range) ^b						
Glucocorticoids ^c	5/0–50	0/0–25	2.5/0–50	5/0–25	0/0–25	5/0–50
Methotrexate	0/0–25	0/0–10	0/0–25	0/0–15	0/0–20	0/0–25
Leflunomide	0/0–20	0/0–20	0/0–20	0/0–20	0/0–20	0/0–20
Cyclosporin A	0/0–250	0	0/0–200	100/0–250	0	0/0–250
Sulfasalazine	0/0–3000	0	0/0–3000	0/0–3000	0/0–2000	0/0–3000
Azathioprine	0/0–100	0	0/0–100	0/0–100	0	0/0–100
Hydroxychloroquine	0/0–400	0/0–400	0/0–400	0/0–400	0/0–400	0/0–400

n number, DMARDs disease modifying anti-rheumatic drugs, QFT-GIT QuantiFERON®-TB Gold In-Tube, TST tuberculin skin test, BCG bacille Calmette–Guérin, LTBI latent tuberculosis infection, TB tuberculosis

DMARDs include methotrexate (56), leflunomide (12), cyclosporin A (6), sulfasalazine (25), azathioprine (5), hydroxychloroquine (14)

^a Includes Romania (5); Albania, Argentina, Peru (2 each); Brazil, Morocco (1 each)

^b Daily for all immunosuppressants listed, but methotrexate (weekly)

^c Prednisone equivalent

1 who received vaccine more than 15 years prior to the enrollment (an Italian patient who made the BCG when she was a medical student). Twenty-one patients (17.6 %) were considered as having LTBI due to the presence of at least 1 abnormal screening tool: chest X-ray suggestive of LTBI was evidenced in 6 (5 %) subjects, 14 (11.7 %) had a positive TST (median induration: 13 mm, range: 5–21), and 5 (4 %) a positive QFT-GIT (median: 1.21 IU/ml, range: 0.52–1.59). Since anti-TNF therapy was withheld in 10 patients (8.4 %), including 5 showing at least 1 screening tool positive, a total 109 patients received biologics: 71 (59.6 %) initiated etanercept, 24

(20.2 %) adalimumab, 8 (6.7 %) infliximab, 4 (3.4 %) abatacept, and 2 (1.7 %) rituximab. Only these 2 latter patients had previously received an anti-TNF, while the other subjects were biologic-naïve. Ruling out the 5 patients positive for at least 1 screening tool who did not commence treatment with anti-TNF, chemoprophylaxis for LTBI was given to 15 out of 21 patients, since 1 with a positive TST declined to assume INH. Patients with risk factors for LTBI with screening negative results were accurately visited by an experienced infectious disease specialist who finally deemed that prophylaxis was not justified.

Performance of QFT-GIT and TST at baseline

Of the 119 patients enrolled, 5 (4 %) displayed positive, 93 (78 %) negative and 21 (18 %) indeterminate QFT-GIT results. The TST was positive in 2 patients (40 %) with a positive QFT-GIT, in 11 (12 %) with a negative QFT-GIT (2 of them had received BCG, while a third patient showed a chest X-ray suggestive of TB infection), and in 1 (5 %) with an indeterminate QFT-GIT (Fig. 1). Among 105 patients showing no reaction to TST, the QFT-GIT was positive in 3 (2.8 %), negative in 83 (79 %), and indeterminate in 19 (18.1 %). After excluding indeterminate results, the level of agreement between the 2 tests was 85.8 % ($\kappa=0.16$, 95 % CI, -0.0979 to 0.4199). QFT-GIT and TST results were not associated with the presence of risk factors, BCG vaccination, and any treatment; indeterminate QFT-GIT responses were not associated with concomitant immunosuppressants. In patients with indeterminate QFT-GIT results, we decided not to repeat the test since in 1 of them the TST was positive, while in the others both TST and chest X-ray were negative.

Follow-up during biologic treatment

Evaluation of longitudinal IFN- γ responses was limited to subjects completing a 6 month-period of treatment (12 with LTBI and 73 without LTBI at baseline). Conversions (test result changing from negative to positive: baseline IFN- $\gamma < 0.35$ IU/ml and follow-up IFN- $\gamma \geq 0.35$ IU/ml) and reversions (test result changing from positive to negative: baseline IFN- $\gamma \geq 0.35$ IU/ml and follow-up IFN- $\gamma < 0.35$ IU/ml) were registered. No TB cases were observed, not even during the additional follow-up period of 6 months.

Follow-up in patients with evidence of LTBI

At baseline the TST was positive in 8 patients (66.6 %) (median induration: 11 mm, range: 6–21) and negative in 4 (33 %); in this last group only 1 patient (8.3 %) had a positive QFT-GIT, the remaining 3 (25 %) showed signs

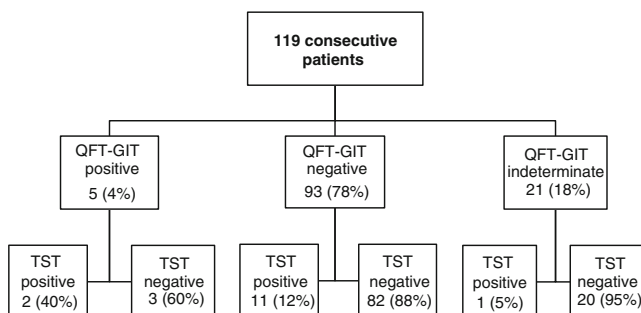


Fig. 1 Performance of QFT-GIT and TST at baseline, before starting anti-TNF treatment. *QFT-GIT* QuantiFERON-TB Gold In-Tube, *TST* tuberculin skin test

of previous TB infection at chest X-ray. After 1 month of anti-TB chemotherapy, 1 of the patients with QFT-GIT-negative/TST-positive profile displayed a QFT-GIT conversion that persisted until the sixth month. Following the beginning of biologics, the QFT-GIT was positive in 2 of 12 patients (16 %) at month 3 (of whom the previously described patient was 1) and both were still positive at month 6, when another patient displayed a positive result (3/12, 25 %). Only 1 assay was deemed indeterminate at month 3, while the only patient showing a positive QFT-GIT at baseline (IFN- γ level of 0.68 IU/ml) promptly reverted after 1 month of INH and continued to express a negative result both at month 3 and 6. No QFT-GIT displayed indeterminate at month 6. The individual IFN- γ response during follow-up is shown in Fig. 2. The median IFN- γ concentration observed at baseline in all 12 LTBI patients was not significantly different when compared with that at each time point (Table 2). In the 1 TST-positive patient (6 mm induration) who refused INH treatment, the QFT-GIT remained negative after 6 months of anti-TNF therapy.

Follow-up in patients without evidence of LTBI

Seventy-three patients who completed 6 months of anti-TNF treatment were TST negative at screening: among them, 59 (80.8 %) and 14 (19.2 %) showed a negative and an indeterminate QFT-GIT, respectively. After 3 months of TNF antagonists, the QFT-GIT was negative in 58 patients

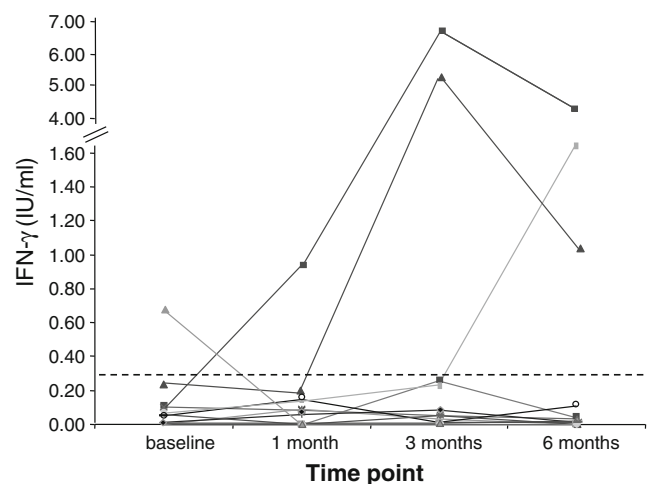


Fig. 2 Longitudinal changes of specific IFN- γ response following anti-tuberculous and anti-TNF treatment in LTBI patients. The IFN- γ response to *M. tuberculosis*-specific antigens was measured by QFT-GIT before and during the treatment in 12 patients with evidence of LTBI at baseline. The QFT-GIT was assessed at baseline (before the commencement of anti-TNF), after 1 month of isoniazid treatment, and after 3 and 6 months since the beginning of anti-TNF. No significant variations in IFN- γ concentrations were found during the follow-up ($p > 0.05$; Wilcoxon's signed-rank test, for the comparison of the results at baseline vs 6 months of therapy). The horizontal dashed line indicates the QFT-GIT assay cut-off value for a positive result (0.35 IU/ml)

Table 2 IFN- γ levels during prophylactic course with isoniazid and anti-TNF treatment in patients with evidence of latent tuberculosis infection (LTBI) at baseline

Patients ($n=12$)	Median (range) IFN- γ (IU/ml) release				<i>p</i> value
	Baseline	1 month ^a	3 months ^b	6 months ^b	
Group A ($n=4$)					
Antigens	0.17 (0.05–0.68)	0.31 (0–0.93)	2.68 (0–6.78)	1.33 (0–4.53)	0.252
PHA	12.22 (6–19)	6.57 (5–12.6)	7.81 (5–12)	4.52 (1.2–27)	0.774
Group B ($n=8$)					
Antigens	0.01 (0–0.10)	0.06 (0–0.15)	0.04 (0–0.31)	0.01 (0–0.11)	0.941
PHA	8.72 (2–20.45)	3.60 (0.55–9.12)	10.08 (0.19–16.49)	7.61 (0.96–23)	0.838

Group A: LTBI patients who had a QFT-GIT conversion (from negative to positive) and reversion (from positive to negative) during the follow-up. Group B: LTBI patients who remained QFT-GIT negative during the follow-up

PHA phytohemagglutinin

^a After the onset of isoniazid and before the beginning of anti-TNF treatment

^b After the beginning of anti-TNF treatment

(79.4 %), indeterminate in 10 (13.6 %), whereas 5 (6.8 %) turned positive. After 6 months of treatment, the QFT-GIT was negative in 57 patients (78 %), indeterminate in 13 (17.8 %), and remained positive in 3 of the 5 converters. The other 2 subjects, who at the third month showed a conversion with an IFN- γ response of 0.93 IU/ml and 0.63 IU/ml respectively, turned negative. Overall, after the beginning of anti-TNF treatment, the QFT-GIT results changed of 28 % at month 3 and of 21 % at month 6; the greatest change was observed in patients with indeterminate results turning to negative (15 %, $p<0.02$). The median IFN- γ concentrations during follow-up are reported in Table 3. As shown, there was no significant variation in IFN- γ concentrations during longitudinal QFT-GIT testing in patients who remained negative or indeterminate ($p=0.916$), and there was no significant increase in IFN- γ concentrations in patients who converted their response to

positive ($p=0.095$). No difference was seen in the baseline IFN- γ concentrations in response to antigens between the 2 groups of patients ($p>0.05$).

Discussion

The screening for LTBI in patients scheduled for biologics as well as the monitoring during the treatment are highly recommended [1]. In this study we investigated the feasibility of serial QFT-GIT testing during anti-TNF agents to assess whether dynamic changes in IFN- γ plasma levels may allow to identify cases of LTBI reactivation or newly acquired TB; furthermore, we evaluated the agreement of QFT-GIT and TST prior to anti-TNF onset.

A major challenge of QFT-GIT is the interpretation of indeterminate results: at baseline, we observed indeterminate

Table 3 IFN- γ levels during anti-TNF treatment in patients with no evidence of tuberculosis infection at baseline

Patients ($n=73$)	Median (range) IFN- γ release (IU/mL)			<i>p</i> value
	Baseline ^a	3 months ^b	6 months ^b	
Group A ($n=5$)				
Antigens	0.01 (0–0.02)	0.93 (0.08–3.99)	0.53 (0.01–1.55)	0.095
PHA	2.74 (1.26–5.40)	14.33 (0.64–15.16)	25.28 (7.94–40)	0.017
Group B ($n=68$)				
Antigens	0.01 (0–0.28)	0.00 (0–0.27)	0.01 (0–0.24)	0.916
PHA	3.25 (0–20.15)	2.56 (0–52.8)	4.42 (0–48.33)	0.026

Group A: Patients who had a QFT-GIT conversion (from negative to positive) during the follow-up. Group B: Patients who remained QFT-GIT negative or indeterminate during the follow-up

PHA phytohemagglutinin

^a Before the beginning of anti-TNF treatment

^b After the beginning of anti-TNF treatment

results in 18 % of the patients, of whom only 1 had a positive TST. Previous studies reported indeterminate tests in percentages ranging from 1.9 % to 36 % of the patients, depending on the clinical setting [14–21]. In fact, rates of indeterminate results were especially high in subjects with severe immunosuppression showing low CD4+ T cell count [14, 20], and in those receiving chemotherapy for malignancy [22], while in patients treated with biologics the rate of indeterminate responses rarely exceeded 10 % [23–25]. We cannot exclude that our higher rates might be a consequence of immunosuppressive treatment since patients with indeterminate QFT-GIT responses were taking a higher median dose of glucocorticoids (Table 1) and, interestingly, some recent papers reported an association of indeterminate QFT-GIT results with steroid dosage [25, 26]. Among the patients with negative QFT-GIT results at baseline showing a conversion during the follow-up, there was a trend to a reduced dose of glucocorticoids at 3 and 6 months. Likewise, patients changing to indeterminate after 3 months were assuming a slightly reduced dose of glucocorticoids with respect to the baseline; while at 6 months, no clear trend was observed (Table 4).

In our study, a good concordance between QFT-GIT and TST was found (85.8 %; $\kappa=0.16$), resembling values obtained by other authors [16–19]. The discordance between the two tests may be explained by several factors, including false negative TST results caused by anergy, false positive TST results in BCG-vaccinated subjects (whose exposure varies from country to country) or because of NTM infection, and false negative QFT-GIT results in patients receiving steroid therapy or anti-TNF agents [27]. The profile of patients with QFT-GIT-positive/TST-negative tests remains largely unsolved. We considered the isolated QFT-GIT positivity as being LTBI since our patients were scheduled for treatment with biologics and hence at increased risk of reactivation of LTBI. When examining the discordance of the reverse type (QFT-GIT-negative/TST-positive), only two patients reported a history of BCG vaccination, another showed a chest X-ray suggestive of

previous TB; while in the remaining eight patients, no risk factor emerged, although we cannot exclude NTM infections or remote TB infections. Indeed, blood assays are quite sensitive for the detection of recently acquired LTBI since they mainly measure effector T cell responses, while TST measures both effector and memory T cell responses [28]. This point warrants the lower performance of blood assays in Western Europe where most cases of LTBI occurred in a population born before 1945 and a low sensitivity of these tests should be expected due to longer duration of LTBI [29]. In this remark, it is interesting to note that our 9 patients (after excluding the 2 who received vaccination) with a positive TST, which was discordant with respect to QFT-GIT, had a median age of 66 years (range 27–73) and were all Italians, except for the only young woman of this group, aged 27, who was born in Albania, a TB-endemic country.

The main aim of our study was to assess whether dynamic changes in IFN- γ plasma levels through serial QFT-GIT may allow the identification of reactivation of LTBI or newly acquired TB. Therefore, we tested our patients after 3 and 6 months since the beginning of biologics as evidence exists that LTBI reactivation may occur starting from the second month of anti-TNF therapy [30]. Some papers to date report on the performance of QFT in patients on anti-TNF agents [17, 18, 31, 32]. In 1 of these studies, 2 patients with positive QFT results at 12 months of adalimumab therapy developed active TB [18]; while in another, the odds for a positive IFN- γ assay were decreased in patients treated with TNF inhibitors [17]. Very recently, other papers have been published which tested patients both before and after the onset of biologics [24, 33–35]. Chen and the colleagues observed that persistently high levels of IFN- γ or QFT conversion could predict the emergence of active TB in patients treated with anti-TNFs [24], confirming their previous findings [18]. However, these results, obtained in Taiwan, an intermediate TB-burden area, do not seem to be consistent in the setting of low TB-burden countries, including Italy, where the prevalence of LTBI and overt disease is low. Indeed, both Garcovich and the colleagues [33] and ourselves observed fluctuations in QFT-GIT results with no cases of active TB. In particular, in our study, conversions and reversions of QFT-GIT responses developed during the 6-month follow-up in patients with and without evidence of LTBI. In 25 % of LTBI patients, we observed a conversion of QFT-GIT and a persistent positivity during the follow-up, which confirmed the positive TST at baseline even if no apparent TB exposure emerged nor did the patients develop the disease during the follow-up.

Table 4 Daily dose of glucocorticoids (mg median/range) in patients with a negative QFT-GIT at baseline changing to positive or indeterminate during the follow-up

Daily dose of glucocorticoids (mg median/range)				
Patients	Baseline	3 months	Baseline	6 months
Group A (<i>n</i> =13) ^a	0 (0–18)	0 (0)	0 (0–10)	0 (0–5)
Group B (<i>n</i> =13) ^b	5 (0–50)	0 (0–12.5)	5.5 (2.5–6)	5 (2.5–12.5)

^a Group A: patients who had a QFT-GIT conversion from negative (baseline) to positive (*n*=7 at 3 months; *n*=6 at 6 months)

^b Group B: patients who had a QFT-GIT change from negative (baseline) to indeterminate (*n*=7 at 3 months; *n*=6 at 6 months)

Furthermore, the presence of risk factors at baseline was not associated with the conversion of QFT-GIT. The reversion observed after prophylaxis in one LTBI patient suggests that this changing may be useful in the evaluation of INH therapeutic response in LTBI subjects receiving anti-TNFs. Obviously, more observations are needed to confirm this hypothesis, but previous studies reported a progressive decrease in IFN- γ response after successful treatment for active TB [36–38]. Among the patients without LTBI, the changes in IFN- γ concentrations during the follow-up might be due to variations in laboratory procedures, nonspecific biological and environmental causes, and/or within-subject fluctuations [39, 40]. Indeed, all the patients showing a conversion in QFT-GIT response were deeply questioned and examined by an experienced infectious disease specialist, but neither worrisome features emerged, nor did the patients show symptoms or signs of active TB. Hence, starting chemoprophylaxis was deemed not appropriate and the patients did not develop the disease later. Whereas it has been reported that the IFN- γ response is significantly reduced in subjects on anti-TNFs [17, 41]; in our patients, no treatment significantly affected the QFT-GIT response, although in the 6-month follow-up we noticed a reduced number of indeterminate results.

In conclusion, the high risk for progression of infection to active TB of patients awaiting biologics should not be disregarded. The routine use of both QFT-GIT and TST at screening seems not to give any advantage; however, it may be reserved for selected situations since they measure different aspects of the immune response and use different antigens and interpretation criteria, hence different results may be expected [8]. In this respect, to date, a good history considering the place of birth, travel or residence in regions with increased prevalence of TB, and personal and family exposure to TB is still the most useful screening tool available.

Moreover, the feasibility of serial QFT-GIT testing during treatment with biologics to identify LTBI reactivation or newly acquired TB still needs definition since dynamic changes in IFN- γ plasma levels may occur without a pathologic connotation. Hence, these fluctuations may rather confuse the picture than assisting physicians in the management of patients. Although the lack of boosting of T cell IFN- γ responses following a TST [42–44] implies that blood assays may be repeated any number of times, we need studies with long-term follow-up to allow a proper interpretation of the fluctuations in serial testing responses. In the meantime, we suggest that taking an accurate medical history and the judgment of an experienced physician should guide the decision of management of patients treated with anti-TNFs showing variations in serial blood testing.

Disclosures None.

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