Mycobacterial Interferon-γ Release Variations During Longterm Treatment with Tumor Necrosis Factor Blockers: Lack of Correlation with Clinical Outcome

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ABSTRACT. ***Objective.*** To assess the performance of serial QuantiFeron-TB Gold In-Tube (QFT-GIT) tests in patients with rheumatic diseases during longterm systemic treatment with biologic therapy, evaluating conversions and reversions in relation to the clinical outcome.

Methods. We conducted a prospective study on patients awaiting biologic agents. At baseline, they had chest radiographs, QFT-GIT tests, and tuberculin skin tests (TST); QFT-GIT was repeated at 3, 6, 12, and 18 months after onset of biologic therapy. In patients with no evidence of latent tuberculosis infection (LTBI) at baseline, TST was repeated at 12 months of biologic treatment.

Results. Among patients (n = 102; women 65.7%; median age 47 yrs, range 20–82), 14 (13.7%) were considered as having LTBI because of a minimum of 1 abnormal screening test. The agreement between QFT-GIT and TST was 88% (κ = 0.14). During biologic treatment, both patients with (n = 14) and those without (n = 88) evidence of LTBI at baseline showed conversions and reversions in QFT-GIT results at different timepoints. These fluctuations were not paralleled by significant clinical changes. The TST repeated at 12 months in patients with no evidence of LTBI at baseline continued to be negative. The median baseline interferon-γ (IFN-γ) concentration was not significantly different from that observed at each subsequent timepoint.

Conclusion. Dynamic changes occur with serial IFN-γ release assay testing in patients treated with biologic therapy that do not correlate with clinical outcome. A careful and integrated evaluation of the patient, including clinical information, should guide the treatment decision. This study was underpowered for definite conclusions and further studies are needed to determine the significance of these findings. (J Rheumatol First Release Dec 1 2012; doi:10.3899/jrheum.120688)

Key Indexing Terms:
TUMOR NECROSIS FACTOR BLOCKERS QUANTIFERON-TB GOLD IN-TUBE TESTS
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The development of interferon-γ (IFN-γ) release assays (IGRA) for the detection of tuberculosis (TB) infection aims to overcome the limitations of the tuberculin skin test (TST), the only available immunologic method before 2001[1,2]. Several reports have addressed the issue of the performance of IGRA in different settings. This performance is particularly important for patients initiating treatment with tumor necrosis factor (TNF) antagonists, who are at increased risk of reactivation of latent tuberculosis infection (LTBI)[3,4,5,6]. Studies comparing the performance of TST and IGRA prior to the onset of anti-TNF therapy have generally demonstrated a low concordance between these tests[7,8,9,10,11,12,13,14,15,16,17,18]. Therefore, even if IGRA are more closely associated with risk factors for LTBI than is TST[9,13,17,19], many authors suggest performing both tests prior to biologic treatment, to maximize the sensitivity for the detection of LTBI[7,8,18,19,20,21,22,23,24,25]. Another field of potential applicability of IGRA concerns serial testing to identify cases of TB reactivation or newly acquired TB during treatment with

TNF antagonists. Indeed, repeated TST should be avoided because of the possible booster effect[26], while blood assays may be repeated any number of times, even following a TST[27,28,29]. The utility of serial IGRA in patients undergoing treatment with anti-TNF has been evaluated in a few studies, whose interpretation was challenged by the finding of variability in IFN-γ plasma levels not paralleled by significant clinical change[23,30,31,32], except for one report in which persistently high levels of IFN-γ could predict the emergence of active TB[33]. To clarify this issue, we assessed, in a prospective study, the performance of serial QuantiFeron-TB Gold In-Tube (QFT-GIT; Cellestis Inc.), one of the commercially available IGRA tests, in patients with rheumatic diseases during longterm systemic treatment with anti-TNF therapy. The levels of IFN-γ were investigated and QFT-GIT conversions and reversions were evaluated in relation to the patients' clinical outcome.

MATERIALS AND METHODS

Between July 2008 and February 2010, patients with chronic inflammatory rheumatic diseases designated to start anti-TNF therapy were prospectively enrolled from the rheumatology outpatient clinic at Sapienza University of Rome, Italy. The study received Ethics Committee approval in accord with local requirements and written informed consent was obtained from each patient.

At recruitment, data on demographics, bacillus Calmette-Guérin (BCG) vaccination status, previous and concomitant treatment regimens, and risk factors for LTBI (birth or prolonged residence in an area with a high prevalence of TB infection, history of household TB contact, previous diagnosis of TB that had been inadequately treated) were obtained by direct questioning and collected in a computerized form. All patients had a posteroanterior chest radiograph, which was reviewed by a radiologist aware that anti-TNF therapy was being considered and who was asked to search for signs suggesting LTBI[34]. On the same day, patients underwent TST and QFT-GIT: the first (Biocine Test PPD; Chiron) was performed according to the Mantoux method by the same experienced operator, and an induration ≥ 5 mm was considered positive[34]; the QFT-GIT (Cellestis) was carried out and interpreted by the same trained technicians, as per manufacturer's instructions. Briefly, 1 ml of whole blood was added to each of the 3 tubes: TB antigen (ESAT-6, CFP-10, and TB 7.7), mitogen positive control (phytohemagglutinin), and a negative control. Blood was incubated within 12 h of collection for 16 to 24 h at 37°C and then centrifuged. Plasma aliquots were harvested and stored in the cold until the amount of IFN-γ released (IU/ml) was determined using ELISA. The result obtained by the negative control was subtracted from the positive control and the antigen-stimulated samples. The cutoff value for a positive test was 0.35 IU/ml of IFN-γ in the sample after stimulation with the specific antigens, regardless of the result of the positive control. The result of the test was considered indeterminate if the antigen-stimulated sample was negative and if the value of the positive control was < 0.5 IU/ml after subtraction of the value of the negative control and/or if the negative control was > 8.0 IU/ml. Analysis of data was done with the QuantiFeron-TB Gold analysis software.

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 radiograph results were considered affected by LTBI after excluding active TB and received a 9-month course of isoniazid (INH) prophylaxis. In these patients, biologic treatment began following 4 weeks of chemoprophylaxis intake and QFT-GIT was performed again immediately prior to initiating biologics to determine whether INH may affect the test response. Although to date no prospective controlled trial has been done evaluating the optimal timeframe between INH and the start of anti-TNF therapy, observational data suggest that biologic treatment can be safely started 1 month after INH[35]. Hence, we decided to delay biologic therapy in patients needing INH, even if reports show success in preventing LTBI reactivation by starting antitubercular drugs in parallel with TNF blockers[36,37]. Patients with risk factors for LTBI who had negative screening results were questioned by an experienced infectious disease specialist who deemed that prophylaxis was not justified. In all the patients, QFT-GIT was repeated at 3, 6, 12, and 18 months after onset of TNF antagonist therapy, to evaluate its ability to identify possible cases of TB infection. In patients with no evidence of LTBI at baseline, TST was repeated at 12 months of biologic treatment. An additional followup period of 6 months was observed for all the patients, even for those ending biologic therapy prior to the 18th month of treatment.

Statistical analysis. SPSS version 13.0 for Windows (SPSS Inc.) was used. IFN-γ production in response to antigenic stimulation was expressed as continuous (IU/ml) measures. Medians and ranges of the different measurements were calculated. The analysis of concordance between QFT-GIT and TST was performed using Cohen's κ coefficient. Odds ratios and 95% 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 nonparametric Mann-Whitney U test. Data in the longitudinal analysis during the treatment course of individual patients were evaluated with the nonparametric Wilcoxon signed-rank test. All statistical analyses were 2-sided and considered significant in case of p values < 0.05.

RESULTS

Clinical data. The baseline demographics and disease characteristics of the patients included in the study (n = 102) are listed in Table 1. Patients were diagnosed with rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, or Behçet's disease based on standard criteria[38,39,40,41].

Among the 5 patients with a positive vaccination status, all had been inoculated with BCG in childhood except 1, who received the vaccine > 15 years prior to enrollment. Fourteen patients (13.7%) were considered as having LTBI because of 1 abnormal screening test, at a minimum. Chest radiograph appearances suggestive of LTBI were evidenced in 5 subjects (4.9%; 4 of them presented well-demarcated nodules in the upper pulmonary lobes and the last showed calcified hilar lymph nodes), 10 (9.8%) had a positive TST, and 2 (1.9%) a positive QFT-GIT. One patient had positive results for chest radiograph, TST, and QFT-GIT, while in another both TST and chest radiograph were positive. Of the 102 patients included, 64 (62.7%) initiated etanercept, 24 (23.5%) adalimumab, 8 (7.8%) infliximab, 4 (3.9%) abatacept, and 2 (1.9%) rituximab. The 2 latter patients had previously received an anti-TNF, while the other subjects were biologic-naive. During the first 18 months, biologic agents were withdrawn in 22 patients (8 after the third month, 9 after the sixth month, and 5 after the 12th month) because of non-TB-related side effects (n = 11), inefficacy (n = 4), or noncompliance (n = 7), but the additional observation period of 6 months was applied to all of them as well. Chemoprophylaxis was given to 13 of the 14 patients with LTBI; 1 with a positive TST declined to take INH. This patient, who had psoriatic arthritis, was given etanercept as monotherapy.

Table 1. Baseline demographic and disease characteristics of patients enrolled in the study. Data are n (%) unless otherwise indicated.

Characteristics	All, n = 102	QFT-GIT			TST	
		Positive, n = 2	Negative, n = 82	Indeterminate, n = 18	Positive, n = 10	Negative, n = 92
Women	67 (65.7)	2 (2.9)	51 (76.1)	14 (20.8)	7 (10.4)	60 (89.5)
Age, yrs, median (range)	47 (20–82)	33 (20–46)	47 (24–82)	47 (23–65)	62 (29–74)	46 (20–82)
Underlying disease						
Rheumatoid arthritis	54 (52.9)	2 (3.7)	39 (72.2)	13 (24.1)	7 (12.9)	47 (87)
Psoriatic arthritis	34 (33.3)	0	33 (97)	1 (2.9)	2 (5.8)	32 (94.1)
Ankylosing spondylitis	10 (9.8)	0	8 (80)	2 (20)	1 (10)	9 (90)
Behçet's disease	4 (3.9)	0	2 (50)	2 (50)	0	4 (100)
BCG-vaccinated	5 (4.9)	0	4 (80)	1 (20)	1 (20)	4 (80)
Risk factors for LTBI						
Birth or prolonged residence in a TB-endemic area*	12 (11.7)	1 (8.3)	8 (66.6)	3 (25)	2 (16.6)	10 (83.3)
History of household contact	4 (3.9)	0	2 (50)	2 (50)	0	4 (100)
Chest radiograph suggestive of LTBI	5 (4.9)	1 (20)	4 (80)	0	2 (40)	3 (60)
Previous diagnosis of TB	0	—	—	—	—	—
Concomitant treatment regimen						
Glucocorticoids	10 (9.8)	0	9 (90)	1 (10)	1 (10)	9 (90)
DMARD	19 (18.6)	2 (10.5)	16 (84.2)	1 (5.3)	1 (5.3)	18 (94.7)
DMARD and glucocorticoids	55 (53.9)	0	42 (76.4)	13 (23.6)	5 (9.1)	50 (90.9)
No immunosuppressants	18 (17.6)	0	15 (83.3)	3 (16.6)	3 (16.6)	15 (83.3)
Dose of immunosuppressants, mg; median/range†						
Glucocorticoids††	5 (0–50)	—	5 (0–50)	6.75 (0–25)	5 (0–25)	5 (0–50)
Methotrexate	0 (0–25)	8.75 (7.5–10)	7.5 (0–25)	0 (0–15)	12.5 (0–20)	0 (0–25)
Leflunomide	0 (0–20)	—	0 (0–20)	0 (0–20)	—	0 (0–20)
Cyclosporine	0 (0–250)	—	0 (0–200)	0 (0–250)	—	0 (0–250)
Sulfasalazine	0 (0–3000)	—	0 (0–3000)	0 (0–3000)	0 (0–2000)	0 (0–3000)
Azathioprine	0 (0–100)	—	0 (0–100)	0 (0–100)	—	0 (0–100)
Hydroxychloroquine	0 (0–400)	200 (0–400)	0 (0–400)	0 (0–400)	0 (0–400)	0 (0–400)

* Includes Romania (5); Albania, Peru (2 each); Brazil, Argentina, Morocco (1 each). † Daily for all immunosuppressants listed except methotrexate (weekly). †† Prednisone equivalent. TST: tuberculin skin test; QFT-GIT: QuantiFeron-TB Gold In-Tube; BCG: bacille Calmette-Guérin; LTBI: latent tuberculosis infection; TB: tuberculosis; DMARD: disease-modifying antirheumatic drugs [includes methotrexate (50), leflunomide (9), cyclosporine (5), sulfasalazine (22), azathioprine (5), hydroxychloroquine (12)].

Performance of QFT-GIT and TST at baseline. Of the 102 patients enrolled, 2 (1.9%) showed positive QFT-GIT results at baseline, 82 (80.4%) showed negative results, and 18 (17.6%) were indeterminate. The TST was positive in 10 patients (9.8%) and negative in 92 (90.2%); only 1 patient with a positive TST had previously received BCG. After excluding indeterminate results, the agreement between the 2 tests was 88% ($\kappa = 0.14$).

QFT-GIT and TST results were not associated with the presence of risk factors, BCG vaccination, diagnosis, or any treatment. The occurrence of indeterminate QFT-GIT was not associated with concomitant immunosuppressive treatment. However, in our study, the percentage of indeterminate results was higher than expected. As shown in Table 1, those patients were taking a higher median dose of glucocorticoids. Thus this finding may be a consequence of immunosuppressive treatment, because some recent papers reported an association of indeterminate QFT-GIT results with steroid dosage[19,42,43].

Followup during biologic treatment. All the patients were followed longitudinally and, after baseline testing, QFT-GIT was serially performed at 3, 6, 12, and 18 months to assess whether variations in IFN-γ plasma levels may be useful in identifying cases of LTBI reactivation or newly acquired TB. In addition, patients with evidence of LTBI at baseline were reevaluated by QFT-GIT after 1 month of INH therapy and prior to initiating biologics, to understand whether INH may affect QFT-GIT responses.

Patients with evidence of LTBI at baseline. At baseline, the TST was positive in 10 patients (71.4%; median induration 12.5 mm, range 5–21 mm) and negative in 4 (28.6%), while the QFT-GIT was positive in 2 patients (14.3%). Thirteen of the 14 patients with LTBI started INH treatment 4 weeks before administration of biologic therapy and continued for 9 months.

We observed a conversion of QFT-GIT result from negative (at baseline) to positive (during the followup) in 4 patients (28.5%) and a reversion from positive to negative in 2 patients (14.3%), with no significant differences in the baseline IFN-γ levels between converters and reverters ($p >$

0.05). As shown in Table 2, these variations occurred at different timepoints over the treatment period and were not paralleled by clinical manifestations. Among the converters, 1 patient switched to a positive QFT-GIT result once (at Month 12), 2 patients maintained the conversion in 2 successive determinations, and in 1 patient the conversion observed after 1 month of INH persisted throughout the study period. Interestingly, 2 subjects switched to positive at the end of the INH treatment (1 after 6 months and the other after 12 months since biologic treatment). Finally, the 2 patients with positive QFT-GIT result at baseline reverted after 1 month of antituberculous chemotherapy; however, while in 1 of them the negative response persisted throughout the followup, in the second a conversion developed at Month 3.

The median IFN-γ concentration measured at baseline in the 14 patients with LTBI was not significantly different from that at each subsequent timepoint [median IFN-γ released in response to antigens: 0.06 IU/ml (range 0–1.35) at baseline, 0.09 IU/ml (range 0–0.93) at 1 month, 0.05 IU/ml (range 0–6.78) at 3 months, 0.03 IU/ml (range 0–4.53) at 6 months, 0.02 IU/ml (range 0–2.56) at 12 months, and 0.01 IU/ml (range 0–6.71) at 18 months; $p > 0.05$].

In the 1 patient who was TST-positive (6 mm induration) who refused INH treatment, the QFT-GIT remained negative during the study period, and the patient did not develop TB.

Patients with no evidence of LTBI at baseline. At screening, 88 patients displayed a negative TST result, and among them, 70 (79.5%) had a negative and 18 (20.4%) an indeterminate QFT-GIT result because of low response to mitogen.

During the followup, the same immunologic response as in patients with LTBI was found; variations in IFN-γ levels were observed that were not associated with clinical manifestations. In particular, the QFT-GIT assay was negative in 73 (83%), indeterminate in 10 (11.4%), and positive in 4 (5.6%) of 88 patients after 3 months of biologic treatment. At Month 6, the QFT-GIT assay was negative in 64 (78%), indeterminate in 14 (17%), and positive in 4 (4.8%) of 82 subjects; while at Month 12 the test was negative in 61 (83.6%), indeterminate in 6 (8.2%), and positive in 6 (8.2%) of 73 patients. Finally, at the end of followup (Month 18), QFT-GIT was negative in 60 (88.2%), indeterminate in 4 (5.9%), and positive in 4 (5.9%) of 68 patients.

Overall, 76 patients remained QFT-GIT-negative or indeterminate, while 12 patients changed their negative baseline QFT-GIT response to positive. The individual characteristics of these converters during serial QFT-GIT testing are shown in Table 3. A marked increase in IFN-γ levels was observed after 12 months of biologic therapy in 6 patients who did not develop active TB subsequently (Figure 1). A comparison of the baseline IFN-γ quantitative measurements between the patients who remained QFT-GIT-negative or indeterminate during the followup and those who switched to positive results did not reveal any significant differences ($p > 0.05$). Further, the QFT-GIT conversions were not associated with age, sex, type of biologic treatment, or inflammatory disease.

In the entire cohort of subjects, the median baseline IFN-γ concentration was not significantly different from that observed at each subsequent timepoint [median IFN-γ released in response to antigens: 0.01 IU/ml (range 0–0.28)

Table 2. Demographics and individual TST/QFT-GIT results over the study period in patients with evidence of LTBI at baseline.

Patient	Age, yrs/ Sex	Diagnosis	TNF Inhibitor	Baseline TST/QFT-GIT (IU/ml) Results*	QFT-GIT (IU/ml) Responses over Study Period (months since biologic onset) 1†	3††	6††	12††	18††
1	72 F	RA	ETA	Neg/Neg	ND	Ind	Neg	Neg	Ind
2	68 M	RA	ETA	Pos/Neg	ND	Neg	Pos (1.23)	Pos (2.56)	Ind
3	72 F	RA	ETA	Pos/Neg	Neg	Pos (5.13)	Pos (1.02)	Neg	Neg
4	71 F	RA	ETA	Pos/Neg	Neg	Ind	Lost to followup	—	—
5	56 M	AS	ETA	Neg/Neg	Neg	Neg	Neg	Pos (0.69)	Neg
6	20 F	RA	ETA	Neg/Pos (0.68)	Neg	Neg	Neg	Neg	Neg
7	68 F	RA	ETA	Pos/Neg	Neg	Neg	Neg	Neg	Lost to followup
8	48 F	PsA	ETA	Pos/Neg	ND	Neg	Neg	Neg	Neg
9	46 F	RA	ADA	Pos/Pos (1.35)	Neg	Pos (16.3)	Pos (25.5)	Lost to followup	—
10	43 M	RA	ETA	Neg/Neg	Neg	Neg	Neg	Neg	Neg
11	55 M	RA	ETA	Pos/Neg	Neg	Neg	Neg	Neg	Neg
12	29 F	RA	ADA	Pos/Neg	ND	Neg	Neg	Neg	Neg
13	56 M	AS	ADA	Pos/Neg	Neg	Neg	Neg	Neg	Neg
14	74 F	PsA	ETA	Pos/Neg	Pos (0.93)	Pos (6.78)	Pos (4.53)	Pos (0.62)	Pos (6.71)

* Prior to onset of biologic treatment. † After 1 month of isoniazid therapy and prior to the onset of biologic treatment; †† months since the onset of biologic treatment. Chemoprophylaxis was given to all patients except no. 8, who declined to take isoniazid. LTBI: latent tuberculosis infection; TST: tuberculin skin test; QFT-GIT: QuantiFeron-TB Gold In-Tube; RA: rheumatoid arthritis; AS: ankylosing spondylitis; PSA: psoriatic arthritis; ETA: etanercept; ADA: adalimumab; ND: not done; Ind: indeterminate.

Table 3. Demographics and individual TST/QFT-GIT results over the study period in 12 patients with no evidence of LTBI at baseline. All the patients showed a negative QFT-GIT at baseline except no. 6, who displayed an indeterminate result.

Patient	Age, yrs/ Sex	Diagnosis	TNF Inhibitor	QFT-GIT (IU/ml) Responses over Study Period (months since biologic onset) 3	6	12	18
1	68 M	PsA	ETA	Pos (1.03)	Pos (1.55)	Pos (0.57)	Pos (0.75)
2	37 F	RA	ADA	Neg	Neg	Pos (3.04)	Neg
3	42 M	AS	ADA	Neg	Neg	Neg	Pos (0.44)
4	44 F	RA	ADA	Pos (3.99)	Pos (1.16)	Neg	Neg
5	43 F	PsA	INF	Neg	Neg	Pos (0.53)	Neg
6	70 F	RA	ETA	Neg	Pos (0.53)	Pos (1.06)	Neg
7	40 M	AS	ADA	Neg	Neg	Pos (2.47)	Neg
8	47 F	PsA	ETA	Pos (0.93)	Neg	Neg	Neg
9	40 M	PsA	ETA	Pos (0.64)	Neg	Neg	Neg
10	34 M	PsA	ETA	Neg	Neg	Ind	Pos (1.53)
11	51 M	AS	ETA	Ind	Neg	Pos (4.64)	Pos (0.38)
12	45 M	PsA	ADA	Neg	Pos (0.50)	Neg	Neg

TST: tuberculin skin test; QFT-GIT: QuantiFeron-TB Gold In-Tube; TNF: tumor necrosis factor; TB: tuberculosis; AS: ankylosing spondylitis; PSA: psoriatic arthritis; RA: rheumatoid arthritis; ADA: adalimumab; ETA: etanercept; INF: infliximab; Ind: indeterminate.

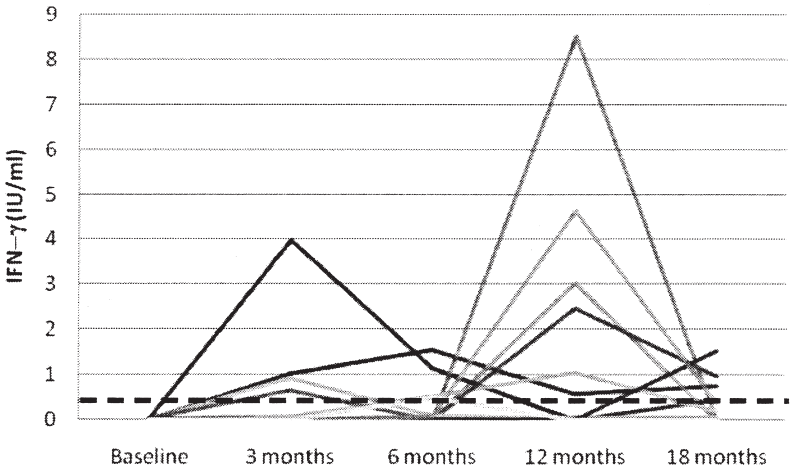

Figure 1. Longitudinal changes of specific interferon-γ (IFN-γ) response to *Mycobacterium tuberculosis*-specific antigens in 12 patients whose negative baseline QuantiFeron-TB Gold In-Tube (QFT-GIT) response converted to positive during the followup. After baseline testing, QFT-GIT was serially performed at 3, 6, 12, and 18 months after the beginning of biologic therapy. No significant variations in IFN-γ levels were found during the followup ($p > 0.05$; Wilcoxon's signed-rank test, for the comparison of the results at baseline vs 18 months of therapy). Horizontal broken line indicates the QFT-GIT assay cutoff value for a positive result (0.35 IU/ml).

at baseline, 0.01 IU/ml (range 0–3.99) at 3 months, 0.01 IU/ml (range 0–1.55) at 6 months, 0.01 IU/ml (range 0–8.53) at 12 months, and 0 IU/ml (range 0–1.53) at 18 months; $p > 0.05$]. The greatest statistically significant change of QFT-GIT was observed in patients with indeterminate results at baseline that became negative at the end of the followup (from 18% to 4%; $p < 0.05$). Taking into account the QFT-GIT conversions observed in this group of patients, at 12 months a second TST was performed, which continued to be negative in all of them.

DISCUSSION

IGRA were developed as a new tool for detecting TB infection, with the advantage of using more specific antigens than TST, because these are not shared with any of the BCG vaccine strains and most nontuberculous mycobacteria[44]. Further, unlike TST, IGRA may be repeated any number of times with no risk of boosting or sensitization[27,28,29], and some suggest their use only or as a substitute for TST for serial healthcare worker screening[45]. However, in these subjects, interpretation of repeated IGRA

results was complicated by the lack of data on optimal cutoffs for serial testing and unclear explanation of conversions and reversions[46]. An association between IGRA conversion and TB occurrence has not been demonstrated; in addition, along with conversions, some studies report rates of subsequent reversions that are similarly challenging to interpret[46]. Uncertainty on this issue also exists in patients treated with TNF blockers, who are at increased risk of developing TB during therapy[47]. Hence, the feasibility of repeated blood tests in this unusual category of patients is of utmost relevance, but the available data are scarce[23,30,31,33].

In our study, we performed serial QFT-GIT in 102 patients with inflammatory rheumatic diseases during longterm systemic biologic treatment to analyze whether dynamic changes in the IFN-γ levels could identify possible cases of reactivation of LTBI or newly acquired TB. Although a careful clinical evaluation and physical examination remain the best means of detecting TB, it is important to know how to optimize the use of the available tests, especially in people who are at higher risk for developing active TB. This need is vital during treatment with biologics, regardless of TB screening status at baseline. Therefore, in all the patients, after the baseline evaluation, QFT-GIT was repeated at 3, 6, 12, and 18 months after the beginning of biologics; and in those with evidence of LTBI at baseline, the test was also performed after 1 month of INH treatment, prior to initiation of biologics. We decided to extend the followup until the 18th month with a subsequent observation period of 6 months, based on the reported median time to onset of TB presented by the 3 TNF antagonists (6 weeks for infliximab, 3–8 months for adalimumab, and 11.2 months for etanercept)[48]. At baseline, agreement between TST and QFT-GIT, as measured by the κ coefficient, was poor (κ = 0.14), owing to a sizeable discordance in the rate of positive results between the 2 tests. This is in accord with other observations[43], and the routine use of both QFT-GIT and TST at screening should be reserved for selected situations[49]. Overall, these findings support our limitations in specifically identifying a population most likely to benefit from therapy for LTBI prior to anti-TNF treatment.

The TST was positive in 10 patients (9.8%) and negative in 92 (90.2%); only 1 patient with a positive TST had previously received BCG. This finding is consistent with other reports showing that most BCG-vaccinated patients had a negative TST result[36,43], thus challenging the assumption that BCG vaccination may confound TST results. We separately analyzed patients with (n = 14) and those without (n = 88) evidence of TB infection at baseline, and in both groups a dynamic response profile was evident, with conversions and reversions during anti-TNF treatment and after INH chemoprophylaxis. Despite the high specificity of the test, these fluctuations were not paralleled by significant clinical changes, as observed in 2 recent studies[30,31]. In the first, 66 Korean patients with rheumatic inflammatory diseases underwent serial QFT-GIT, with a changed result rate of 30.3%, although 47 patients were treated with anti-TNF therapy. However, the unusual finding, which remained unexplained, was that conversions from the baseline negative test were observed only in patients with ankylosing spondylitis[30] and not in other patients. The other study was performed in 50 Italian patients with psoriasis continuously treated with TNF inhibitors: QFT conversions from baseline occurred in 3 QFT/TST concordant cases after 6 months and in 2 more QFT/TST discordant cases after 12 months of treatment[31]. Nevertheless, no case of active TB was reported, not even in the population from Korea, an intermediate TB burden country. Similarly, a retrospective study of 460 immune-compromised individuals showed a very low incidence of progression to active TB after positive IGRA[23]. The challenge in interpreting such results appears more complex when considering the study from Chen, *et al*, in Taiwan, a high/intermediate TB burden area, where persistently high levels of IFN-γ or QFT conversion could predict the emergence of active TB in patients treated with anti-TNF[33]. To date, this remains the only report indicating the possibility of predicting the emergence of TB through the monitoring of IFN-γ release levels by serial IGRA assays. Indeed, the other reports were not able to associate the kinetics of IFN-γ plasma measurements to the development of active disease in the different clinical settings[23,30,31,50,51,52,53]. Therefore, currently there is no consensus on how to interpret IGRA conversions and reversions, although an approach has been attempted based on the choice of a different QFT cutoff[52]. This considers a "zone of uncertainty" arbitrarily defined in the range of 0.20–0.50 IU/ml IFN-γ plasma levels: any value < 0.20 IU/ml was considered "definitely negative" and any value > 0.50 IU/ml "definitely positive," while results fluctuating within the uncertainty zone during repeated testing were considered "doubtful conversions" or "doubtful reversions." Accordingly, we analyzed our 12 patients without evidence of LTBI at baseline who converted their QFT-GIT results during the followup, observing that "true conversions" occurred in 10 of 12, although in only 4 cases did IFN-γ levels change in at least 2 consecutive QFT-GIT tests (Table 3). In patients with evidence of LTBI at baseline, all positive QFT-GIT results that emerged during the followup turned out to be "true conversions" (Table 2). Therefore, as suggested[54], the finding of a positive QFT-GIT with low IFN-γ levels, especially in patients with a negative screening at baseline, may be considered a false-positive result. Nonetheless, this hypothesis may apply as well to the higher IFN-γ levels found in patients with no apparent signs or symptoms of TB. In all our patients with no evidence of LTBI at baseline, including the 12 converters, the second TST performed at 12 months since biologic treatment remained negative. Overall, patients with negative QFT-GIT at baseline who converted

during the followup were carefully investigated and examined by an experienced infectious disease specialist. However, no risk factor for TB or exposure to active TB cases emerged, and the chest radiograph repeated at the time of QFT-GIT conversion did not show any changes compared to baseline. Further, considering the patients with evidence of LTBI at baseline, no cases of TB were diagnosed nor did the disease develop during the entire followup of 24 months in the QFT-GIT converter group.

In previous studies, a progressive decrease in IFN-γ levels after successful treatment for active TB was reported[55,56,57]. Here, we observed a persistent QFT-GIT reversion in only 1 LTBI subject following initial INH therapy, whereas in another case, after initial reversion, the test again turned positive during followup. Thus, more data are needed to assess the usefulness of QFT-GIT in monitoring the INH treatment response in candidates for anti-TNF therapy.

Finally, apart from conversions and reversions, the fluctuations also involved indeterminate results. This is relevant because this type of response may offer some clues about the effect of biologic treatment on the performance of IGRA. In our patients with no evidence of LTBI at baseline, we observed the greatest statistically significant QFT-GIT change in subjects with indeterminate results at baseline that changed to negative at the end of the followup (from 18% to 4%; $p < 0.05$). However, neither glucocorticoids, nor disease-modifying antirheumatic drugs, nor anti-TNF affected the QFT-GIT response, although it has been reported that the IFN-γ response is significantly reduced in patients taking anti-TNF[9,58]. Interestingly, it has recently been shown that repeating an initially indeterminate IGRA may drastically reduce the indeterminate rate[37,43], suggesting a role for possible human sample handling error that could simply be reduced by repeating the test. Yet, in our study, QFT-GIT was repeated at 3- to 6-month followup intervals, not soon after the first test[37,43], hence these findings are not strictly comparable.

Our data demonstrate that dynamic changes occur with serial IGRA testing in patients treated with anti-TNF therapy and, most importantly, these fluctuations do not correlate with clinical outcome. We cannot exclude that, at least in patients with evidence of LTBI at baseline, treatment with INH may reduce the possibility of TB reactivation in patients taking biologics and consequently our ability to quantify the risk of active TB when a positive IGRA occurs in the followup. Hence, a careful and integrated evaluation of the patients, including clinical information, should guide the appropriate management and treatment decision. However, our study was underpowered for definite conclusions, and further studies are needed to determine the significance of these findings.

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