IFN-γ release assay for tuberculosis in psoriasis patients treated with TNF antagonists: in vivo and in vitro analysis

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Running head: LTBI screening in psoriatic patients awaiting biologic treatment by IGRAs

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What's already known about this topic? Screening of latent tuberculosis (LTBI) is mandatory in patients with psoriasis prior to starting TNF blockers.

What does this study add? This paper evaluated the longitudinal changes of IFN-γ response to M. tuberculosis-specific antigens by serial QuantiFeron-TB Gold In-Tube (QFT-GIT) testing in patients with psoriasis during in vivo and in vitro treatment with biologic anti-TNF therapy.

ABSTRACT

Background: Screening of latent tuberculosis (LTBI) is mandatory in psoriatic patients prior to starting TNF blockers.

Objectives: To investigate the longitudinal changes of IFN-γ response to M. tuberculosis-specific antigens by serial QuantiFeron-TB Gold In-Tube (QFT-GIT) testing in psoriasis patients during long-term anti-TNF therapy. The direct in vitro effect of adalimumab on the IFN-γ secretion was also evaluated.

Methods: 148 psoriatic patients designated to start anti-TNF treatment were enrolled. We performed TST at screening and QFT-GIT at baseline and serially for 24 months after TNF-antagonist onset.

Results: At screening, QFT-GIT was positive in 22.3% of the patients, negative in 73.7% and indeterminate in 4%. The IFN-γ response following INH therapy declined and became QFT-GIT negative in 7.6% of 26 LTBI subjects; in 69.2% of LTBI subjects the QFT-GIT remained persistently positive with a significant increase of IFN-γ levels during the follow-up, even if no cases of active TB were found. Variations of IFN-γ levels were observed also in 7.4% of 27 patients without LTBI who switched to positive QFT-GIT after 12 or 18 months of biologic therapy suggesting a new or reactivation of LTBI. In vitro data showed that in the presence of adalimumab the IFN-γ levels were significantly reduced in a dose-dependent manner (p<0.05).

Conclusions: Fluctuations of IFN-γ release may occur in psoriasis patients treated with TNF antagonists. The clinical use of repeated blood tests and the correct interpretation of individual IFN-
γ changes could be useful in identifying possible cases of LTBI reactivation or newly acquired TB infection during long-term anti-TNF treatment.

INTRODUCTION
Psoriasis is a chronic inflammatory skin condition that is estimated to affect approximately 2% of the general population. Although standard therapies for psoriasis can be very effective, new biologic therapies specifically targeting tumor necrosis factor (TNF) have changed the landscape of treatment options for this disease. Since biologics disrupt the function of TNF involved in tuberculosis (TB) granuloma formation, they are connected with increased risk of TB reactivation. As screening methods prior to initiating biologic therapy, the National Psoriasis Foundation recommends using the tuberculin skin test (TST), considering chest radiography, obtaining an exposure history, and then continuing TB screening annually thereafter.

The TST is the traditional test for diagnosing latent tuberculosis infection (LTBI), but it lacks sensitivity and specificity, especially in patients with impaired immune function, and it may lead to high false-positive rates due to the inherently inflammatory skin of psoriasis patients. Novel screening tools, which measure the release of interferon-γ (IFN-γ) following stimulation of T cells with “Mycobacterium tuberculosis antigens” have been introduced for diagnosis of LTBI and show superior diagnostic accuracy in comparison to TST.

Recently, new data are emerging about the clinical use of the interferon-γ release assay (IGRA) for detection of LTBI in psoriatic patients requiring anti-TNF therapy, but prospective studies on the monitoring of TB reactivation in this risk group are scarce. The aim of this study was to investigate the performance of serial QuantiFERON-TB Gold In-Tube (QFT-GIT), one of the commercially available IGRA tests, in LTBI screening and monitoring of psoriasis patients undergoing anti-TNF treatment. In particular, we assessed the agreement of QFT-GIT with TST for LTBI screening in patients awaiting biologic treatment and we evaluated the longitudinal changes of IFN-γ response to M. tuberculosis-specific antigens during treatment with TNF-antagonist. Finally, the impact of anti-TNF therapy on QFT-GIT results was also investigated by assessing the direct in vitro effects of anti-TNF agents on the secretion of IFN-γ.
MATERIALS AND METHODS

Study population

We consecutively enrolled 148 patients with psoriasis designated to start anti-TNF treatment at the Dermatology Unit, Sapienza University of Rome, Polo Pontino, Italy, between September 2009-2011 and the follow-up was completed in February 2012.

Screening at enrollment included: clinical evaluation, chest radiograph, TST and QFT-GIT. All patients were questioned regarding their demographic details, history of Bacille Calmette-Guerin (BCG) vaccination, risk factors for LTBI (i.e. household TB contact, confirmed history of TB, birth or extended living in an area with a high prevalence of TB infection) and current treatments. For the purpose of this study, we classified as LTBI the subjects who tested positive for either TST or QFT-IT and had one of the following risk factor: chest X-ray suggestive of prior TB infection (apical pleural thickening, pulmonary nodules, upper lobe bronchiectasis, interstitial granulomatous calcification, cavitation, and lymph node or pericardial calcification), a history of exposure to a case of active TB, originating from an area with a high prevalence of TB infection. None of the individuals had clinical, radiologic and microbiological evidence of active TB, and none had received prior TB treatment. All patients classified as LTBI received a 9-month course of isoniazid (INH) prophylaxis, and after a minimum of 4 weeks of INH, biological therapy was commenced.

In all patients the TST was performed just at screening, while QFT-GIT was performed at baseline and repeated after 12 or 18 and 24 months since TNF antagonist onset. In addition, in LTBI patients the QFT-GIT was performed after 1 month of INH therapy immediately prior to initiating biologics to investigate whether INH may affect QFT-GIT responses. Evaluation of longitudinal IFN-γ responses was limited to subjects completing at least 18 months of treatment (26 patients with LTBI and 27 without infection at baseline). An additional clinical observation period of 6 months was done for all patients. The demographics and clinical characteristics of the patients are shown in Table 1.

Tuberculin skin test and Quantiferon TB Gold-In Tube (QFT-GIT)

After blood was drawn for the QFT-GIT assay, a TST (Biocine Test PPD, Chiron, Siena, Italy) was performed according to the Mantoux method. TST was scored as positive if the diameter was ≥5 mm in individual with concomitant immunosuppressants and ≥10 mm in subjects without
immunosuppressive treatment, respectively. In individuals with BCG vaccination the TST was considered positive if induration was ≥10 mm. The QFT-GIT was performed and interpreted by the same trained technicians according to the manufacturer’s instructions (Cellestis Ltd, Carnegie, Australia). Based on the definitions reported by Pai et al., we explored one definition for QFT-GIT conversions (result changing from negative to positive: baseline IFN-γ<0.35 IU/ml, follow-up IFN-γ≥0.35 IU/ml) and one definition for QFT-GIT reversions (result changing from positive to negative: baseline IFN-γ≥0.35 IU/ml, follow-up IFN-γ<0.35 IU/ml). A blinded interpretation for TST and QFT-GIT results was made.

**In vitro effect of anti-TNF on the IFN-γ release**

Cytokine production was assessed in whole-blood culture. In brief, aliquots of 0.5 ml of heparinized blood were stimulated with phytohemagglutinin A (PHA) (Sigma-Aldrich) at 5 μg/mL and adalimumab (kindly given by Dr. C Potenza) was added at different concentrations (0.8μg/ml, 1.6μg/ml, 3.2μg/ml, 6.4μg/ml, 12.8μg/ml, 25.6μg/ml) on the basis of therapeutically achievable levels and information provided by the manufacturer or published studies. Blood was cultured at 37°C in 5%CO₂ and after 16-20hrs supernatants were collected. All conditions were set up in triplicate wells. Positive control wells contained only PHA. The IFN-γ release (expressed as IU/ml) in the presence or absence of the drug was assessed by ELISA (QuantiFERON-CMI, Cellestis Ltd, Carnegie, Victoria, Australia). Cellular vitality was assessed with trypan blue.

**Statistical analysis**

SPSS version 13.0 for windows (SPSS Inc., Apache Software Foundation, Chicago, Illinois) was used. IFN-γ production was expressed as continuous (IU/mL) measures. Median (ranges) of the different parameters was calculated. The differences of values between groups were analysed using the non-parametric Mann-Whitney U-test. For comparison of categorical variables or percentages, we used Fisher's exact and Chi square tests. Analysis of concordance between tests was performed using Cohen's kappa coefficient. Odds ratios (OR) and their 95% confidence intervals (CI) for factors associated with discordant and indeterminate QFT-GIT results were estimated by univariate analysis. Longitudinal analysis was evaluated with the non parametric Wilcoxon-signed-rank test. Student's t test was used for analysis of in vitro experiments. All statistical analyses were two-sided and considered significant in case of p values <0.05.
RESULTS

Performance of QFT-GIT and TST at baseline screening

Of the 148 patients screened for LTBI, 33 (22.3%) had a positive QFT-GIT result, 109 (73.7%) negative, and 6 (4%) had an indeterminate result. The TST was positive in 101 (68%) and negative in 47 (32%). The level of agreement between two tests was 54% (k=0.23). QFT-GIT and TST results were not associated with the presence of risk factors, BCG vaccination, and any treatment; the occurrence of indeterminate QFT-GIT was not associated with concomitant immunosuppressive treatment (OR= 0.16; 95% CI: 0.01-1.81, p<0.09).

A LTBI diagnosis was made in 44 patients, including 33 with TST+/QFT-GIT+, 3 with TST+/QFT-GIT indeterminate, and 8 with TST+/QFT-GIT-. In this last group, 2 patients showed a chest radiograph suggestive of previous TB, while in the remaining 6 patients other TB risk factors emerged. Their median TST reaction was 19mm (range 6-36), and the median of IFN-γ responses was 1.5 IU/mL (range: 0.01-30 IU/ml), which tended to be higher than the test cut-off. The individual IFN-γ responses of 44 LTBI patients toward antigens are shown in Figure 1.

Discordant results with the combination TST+/QFT-GIT- were found in 65 (43.9%) patients, of whom 27 (41.5%) had received BCG vaccination and 7 (10.7%) were farmers and potentially exposed to environmental non-tuberculous Mycobacteria. In these discordant subjects, the median TST was 8mm (range 5-20), which was significantly lower compared to patients with concordant results (median: 19mm; range 6-36; p<0.05), and the median IFN-γ response was 0.03 IU/mL (range 0-0.25). The variables significantly associated with discordant results were the BCG vaccination (OR = 9.16; 95% CI: 2.3-37.1; p<0.002), and working as a farmer (OR = 5.30; 95% CI: 1.8-47.1; p<0.005). Among the patients enrolled we did not find discordant results with the combination TST-/QFT-GIT+.

Serial QFT-GIT during biologic therapy in patients with evidence of LTBI

At baseline the TST was positive in all 26 LTBI patients (median induration: 11.5mm; range: 7-35), while the QFT-GIT was positive in 20 (77%), negative in 3 (11.5%) and indeterminate in 3 (11.5%) patients.

After 1 month of INH, the QFT-GIT was positive in 21 (80.7%), negative in 4 (15.3%) and indeterminate in 1 (4%) patient. After 12 or 18 months since TNF antagonist onset, the QFT-GIT was positive in 18 (85.7%), negative in 2 (9.5%), and indeterminate in 1 (4.8%) of 21 patients; 5
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patients were lost to follow-up. At month 24, the QFT-GIT was performed only in 4 subjects who showed a positive response. The median IFN-γ concentration at baseline in all 26 LTBI patients was significantly different when compared with that of longitudinal testing, showing a significant increase in IFN-γ concentrations [median (range) of IFN-γ in response to antigens: 1.5 (0-25) IU/ml at baseline, 2.37 (0.05-19.77) IU/ml at 1 month, 7.87 (0-32.91) IU/ml at 12/18 month, and 14.84 (0.69-19.72) IU/ml at 24 months; p<0.05]. The longitudinal change of specific IFN-γ response for each LTBI subject is shown in Figure 2.

During the follow-up we found variations of IFN-γ levels. In particular, among the 26 LTBI patients, we observed that after 1 month of INH, 2 (7.6%) patients with TST+/QFT-GIT+ showed a QFT-GIT reversion from positive to negative and 3 (11.5%) patients with TST+/QFT-GIT- showed a QFT-GIT conversion from negative to positive. Both changes in T-cell response persisted until the end of follow-up in all 5 subjects, whose characteristics are shown in Table 2. 1 out of 5 subjects (20%) was treated with etanercept and 4 (80%) were receiving adalimumab.

Serial QFT-GIT during biologic therapy in patients with no evidence of LTBI

Of the 27 patients without evidence of LTBI, the TST at baseline was negative in 8 (30%) and positive in 19 (70%), of whom 11 (57.8%) patients were BCG vaccinated. The QFT-GIT was negative in 25 (92.5%) and indeterminate in 2 (7.5%) patients. After 12 or 18 months since TNF antagonist onset, the QFT-GIT assay was negative in 23 (85.2%), indeterminate in 2 (7.4%) and positive in 2 (7.4%) patients. Overall, during biologic therapy no significant variations of IFN-γ concentrations were observed in these patients [median (range) of IFN-γ in response to antigens: 0.01 (0-0.24) IU/ml at baseline, 0.01(0-1.10) IU/ml at 12 or 18 months; p>0.05].

Follow-up showed fluctuations in IFN-γ responses also in subjects without LTBI; in particular after 12 or 18 months of biologic treatment, 2 (7.4%) patients with TST-/QFT-GIT- switched to a positive QFT-GIT (1 with adalimumab and 1 with infliximab), and at the time of conversion both subjects started INH therapy. However, while in 1 of them the positive response persisted throughout 9 months of INH, in the other patient a reversion from positive to negative QFT-GIT was showed after 2 month of INH (Table 3).
In vitro effect of adalimumab drug on IFN-γ release

The effect of adalimumab (ADA), a TNF inhibitor, on the IFN-γ release by T cells was examined in whole-blood cultures. We decided to test adalimumab because in our study we observed that both IGRA conversion and reversion were present in most subjects treated with this anti-TNF agent (4/5 of LTBI patients and in 1/2 of patients without LTBI). The levels of IFN-γ [median IU/ml (range)] measured at the different drug concentrations were as follows: 4.05 IU/ml (2.06-9.16) with PHA (5μg/ml); 2.29 IU/ml (1.38-6.61) with ADA (0.8μg/ml); 2.7 IU/ml (1.96-4.63) with ADA (1.6μg/ml); 1.85 IU/ml (0.74-5.41) with ADA (3.2μg/ml); 1.32 IU/ml (0.88-5.38) with ADA (6.4μg/ml); 1.19 IU/ml (0.53-3.73) with ADA (12.8μg/ml); 0.67 IU/ml (0.54-2.82) with ADA (25.6μg/ml). When the blood was stimulated in vitro with PHA only, a pronounced production of IFN-γ was observed, but in the presence of ADA the levels of cytokine were significantly reduced in a dose-dependent manner (p<0.05; Fig.3). No evidence of toxic effect of the different drug concentrations on the cellular survival was found.

DISCUSSION

The present paper showed that, in psoriasis patients receiving both anti-TNF and INH therapy, serial IGRA testing is associated to dynamic changes of IFN-γ levels with QFT-GIT conversions and reversions. A decrease in IFN-γ levels with a QFT-GIT reversion was observed only in 2/26 (7.6%) of LTBI patients following INH therapy; on the other hand, in 18/26 (69.2%) of LTBI subjects the QFT-GIT remained persistently positive with a significant increase of IFN-γ levels during the follow-up, even if no cases of active TB were found. In addition, variations of IFN-γ levels were also observed in 2/27 (7.4%) of patients without LTBI who switched to positive QFT-GIT after 12 or 18 months of biologic therapy, suggesting possible cases of LTBI reactivation or newly acquired TB. Furthermore, in vitro study showed that in the presence of adalimumab the IFN-γ levels were significantly reduced in a dose-dependent manner. In this study the accuracy of the QFT-GIT for LTBI screening in psoriatic patients prior to starting anti-TNF was 75%, but the degree of agreement with TST was poor (54%) due to a significant discordance (43.9%) between the two tests, as reported in previous observations.22-24 The discordances (TST+/QFT-GIT-) observed among our patients were significantly associated with BCG vaccination and agricultural work in which the farmers are potentially exposed to environmental non-tuberculous Mycobacteria.

The screening and treatment of LTBI are highly recommended in patients scheduled for anti-TNF treatment. 2,26 The monitoring of patients who have already started the biologic also appears
important to detect possible cases of LTBI reactivation or newly acquired TB infection. The lack of a gold standard method for LTBI diagnosis requires a careful clinical management including not only TST, but also the evidence of history of untreated or partially treated TB, exposure to cases of active TB, presence of residual abnormalities on chest radiograph indicative of prior TB infection and residence in endemic areas. Recently, new data are emerging about the clinical use of IGRA for LTBI screening in psoriatic patients requiring anti-TNF therapy.\textsuperscript{11-16} Differently from TST, IGRA may be repeated any number of times with no risk of boosting or sensitization,\textsuperscript{27,28} and this supports their usefulness, when serially applied, in monitoring TB infection during long-term systemic treatment with anti-TNF, but the available data on the feasibility of repeated blood tests in this peculiar category of patients are scarce.\textsuperscript{13,16}

Our longitudinal follow-up revealed fluctuations in IFN-γ responses in individual patients. Previous studies reported a progressive decrease in IFN-γ after successful treatment for active TB\textsuperscript{29-31} and one would have expected a decrease in IFN-γ levels in LTBI subjects after INH treatment. However, we found an increase in IFN-γ concentrations in a high percentage of LTBI subjects, especially those receiving adalimumab. These data are consistent with the finding of Pai et al.\textsuperscript{32} showing that IFN-γ response is elevated even after completion of INH therapy. Even Higuchi et al.\textsuperscript{33} showed that only 25% of the contacts who were QFT-positive before INH became negative after treatment; 75% remained QFT-positive. In the setting of TB reactivation during biologic treatment, IFN-γ levels should rise but don't because of the fact that the biologics are on board. The increased IFN-γ response could reflect T-cell activity trying to destroy the organism.

Among the patients without LTBI at baseline, we observed a significant increase in IFN-γ levels in 2 patients who switched to positive QFT-GIT after 12 or 18 months of biologic therapy. These increased IFN-γ responses could be attributed to new infection or LTBI reactivation; in fact, after examination by an experienced infectious disease specialist, both subjects started INH therapy after the QFT-GIT conversion. However, while in one of them the high IFN-γ value, leading to a QFT-GIT conversion, persisted throughout 9 months of INH therapy, in the other subject a marginally positive QFT-GIT result became QFT-GIT negative after 2 months of INH. These QFT-GIT conversions and reversions observed in our patients with evidence of LTBI and in those without LTBI are consistent with the finding that Garcovich et al.\textsuperscript{16} observed in 50 Italian psoriasis patients treated with anti-TNF: 3 QFT conversions after 6 months and 2 after 12 months of treatment; nevertheless, no cases of active TB were reported.
We have extended the follow-up for clinical observation for a period of 6 months, since reactivation of LTBI mostly occurs during the first year of anti-TNF treatment, with a shorter median time to reactivation for infliximab (3–6months) compared to adalimumab (8–16months)\textsuperscript{34,35} and considering the discordance observed at baseline between a positive TST and a negative QFT-GIT, none of the patients who switched to positive QFT-GIT showed clinical progression towards active TB. However the risk of TB in those with recent IGRA conversion remains unknown, although Chen et al. observed in Taiwan that persistently high levels of IFN-\(\gamma\) or QFT-GIT conversion could predict the emergence of active TB in patients treated with anti-TNF\textsuperscript{36}.

Uncertainty exists regarding the clinical significance of fluctuations in IFN-\(\gamma\) responses with serial testing which remain, for the most part, unexplained and nonspecific\textsuperscript{23,24,37-44}. The magnitude of these fluctuations can be of sufficient size to cause test interpretations to change from negative to positive (conversion) or from positive to negative (reversion), especially when the IFN-\(\gamma\) responses are near cut points separating positive and negative results. Currently it is difficult to understand these fluctuations, but we believe it is important to find a tool to monitor the individual IFN-\(\gamma\) changes that allows discriminating non-specific variations from those associated with new infection or LTBI reactivation, particularly in patients at increased risk of TB infection. In this respect, we cannot also exclude that, at least in LTBI patients at baseline, treatment with INH may reduce the possibility of TB reactivation during anti-TNF therapy and consequently our ability to assess the risk of active TB when a positive IGRA occurs in the course of follow-up. Considering the dynamic nature of the immune response to \textit{M. tuberculosis} infection, it is not surprising the very early conversion and reversion of QFT-GIT results. Accurate interpretation and clinical guidance is needed in the setting of serial testing\textsuperscript{45}. LTBI should be considered clinically but not immunologically latent; in this respect in high risk individuals surveillance of specific IFN-\(\gamma\) production is of great relevance.

Fluctuations also regarded the indeterminate results which were in low percentage (4%) at the screening and present in patients taking a higher median dose of glucocorticoids. Some papers reported that the odds for a positive IFN-\(\gamma\) response were decreased in patients on anti-TNF\textsuperscript{46,47}. For this reason we also assessed the in vitro effect of anti-TNF antagonists on the IFN-\(\gamma\) release by T cells. The in vitro experiments were performed with adalimumab because the majority of our patients (80%) who had IGRA conversion and reversion were receiving this drug.
Although in vitro data showed that the IFN-γ production was down-regulated by addition of adalimumab in a dose-dependent fashion, we observed during follow-up a decrease in the frequency of indeterminate results suggesting that neither glucocorticoids, nor anti-TNF affected the performance of IGRAs in vivo.

In conclusion, we suggest that IGRAs should be considered as first-line diagnostic procedure in screening for LTBI in all patients with extensive skin disease. The feasibility of repeated blood tests in this peculiar subset of patients is of great relevance in identifying possible cases of LTBI reactivation or newly acquired TB during long-term anti-TNF treatment. Further studies are needed for the correct interpretation of the IFN-γ fluctuations in serial testing and in such situations clinical judgment should guide the appropriate management of psoriasis patients treated with anti-TNF.

ACKNOWLEDGEMENTS

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the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. *Am J Respir Crit Care Med* 2000;161:S221-47.


**FIGURE LEGENDS**

**Figure 1.** Individual IFN-γ response to *M. tuberculosis* specific-antigens in 44 individuals awaiting anti-TNF treatment and latently infected by *M. tuberculosis*. The short solid lines represent the median value for TB antigens (1.5 IU/mL). The horizontal dashed line indicates the QFT-GIT assay cut-off value for a positive result (0.35 IU/ml).

**Figure 2.** The IFN-γ response to *M. tuberculosis*-specific antigens was measured in 26 LTBI patients with psoriasis by QFT-GIT during the treatment. The QFT-GIT was assessed at baseline (before the started of TNF antagonist), after 1 month of isoniazid prophylaxis and serially after 12 or 18 and 24 months since TNF antagonist onset. 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 the end of the follow-up). The horizontal dashed line indicates the QFT-GIT assay cut-off value for a positive result (0.35 IU/ml).

**Figure 3.** The effect of adalimumab on the IFN-γ release was examined in whole-blood cultures. The IFN-γ production was evaluated after overnight incubation with different concentrations of adalimumab (0.8μg/ml, 1.6μg/ml, 3.2μg/ml, 6.4μg/ml, 12.8μg/ml, 25.6μg/ml). Controls wells contained only PHA at 5 μg/mL. In the presence of adalimumab the levels of IFN-γ were significantly reduced in a dose-dependent manner (p<0.05). Student’s t test was used for statistical analysis.
Table 1. Demographic and clinical characteristics of the patients with psoriasis

<table>
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<tr>
<th>Characteristics</th>
<th>Patients (n=148)</th>
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<tr>
<td>Age (years), mean (range)</td>
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<tr>
<td>Gender</td>
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<td>Male, n (%)</td>
<td>51 (34.4)</td>
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<td>Female, n (%)</td>
<td>97 (65.6)</td>
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<td>Psoriasis, n (%)</td>
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<td>Psoriatic arthritis, n (%)</td>
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<td>Risk factors for LTBI</td>
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<td>Born or resident in high-prevalence country, n (%)</td>
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<td>Etanercept, n (%)</td>
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<td>Infliximab, n (%)</td>
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LTBI: latent tuberculosis infection; TB: tuberculosis; BCG: Bacillus Calmette-Guerin; TNF: tumor necrosis factor
*includes: methotrexate (10-25 mg/week for psoriasis, 7.5-20 mg/week for psoriatic arthritis), leflunomide (10-20 mg/day), cyclosporine (2.5-5 mg/kg of body weight/day), systemic corticosteroids (5 mg/day).
Table 2. Individual results among LTBI patients who showed changes of QFT-GIT results during biologic treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>TNF antagonist</th>
<th>TST/QFT-GIT (IU/ml) results</th>
<th>QFT-GIT (IU/ml) results during biologic treatment</th>
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<td>Baseline*</td>
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<td>Pos (2.28)</td>
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<tr>
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<td>ADA</td>
<td>Pos/ Neg (0.16)</td>
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<td>ADA</td>
<td>Pos/ Neg (0.16)</td>
<td>Pos (0.46)</td>
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<td>ADA</td>
<td>Pos/ Pos (1.5)</td>
<td>Neg (0.22)</td>
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<tr>
<td>5</td>
<td>ADA</td>
<td>Pos/ Pos (0.76)</td>
<td>Neg (0.2)</td>
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LTBI: latent tuberculosis infection; TST: tuberculin skin test; QFT-GIT: QuantiFERON-TB Gold In-Tube; TNF: tumor necrosis factor.
ETA: Etanercept; ADA: adalimumab; Neg: negative; Pos: positive;
* Prior to onset of biologic treatment
** After 1 month of INH therapy and immediately prior to initiating biologics

Table 3. Individual results among patients without LTBI who showed changes of QFT-GIT results during biologic treatment

<table>
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<tr>
<th>Patient</th>
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<th>TST/QFT-GIT (IU/ml) results</th>
<th>QFT-GIT (IU/ml) results during biologic treatment</th>
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<td>ADA</td>
<td>Neg/Neg (0.23)</td>
<td>Pos (1.10)</td>
</tr>
<tr>
<td>2</td>
<td>INF</td>
<td>Neg/Neg (0.05)</td>
<td>Pos (0.59)</td>
</tr>
</tbody>
</table>

LTBI: latent tuberculosis infection; TST: tuberculin skin test; QFT-GIT: QuantiFERON-TB Gold In-Tube; TNF: tumor necrosis factor; ADA: adalimumab; INF: Infliximab; INH: isoniazid therapy; Neg: negative; Pos: positive;
* Prior to onset of biologic treatment
** At the time of QFT-GIT conversion (after 12 or 18 months since TNF antagonist onset) the patients started INH therapy