Role of interferon-gamma release assays (IGRAs) for the screening of latent tuberculosis infection in patients candidates for TNF-α antagonist

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Interferon-gamma release assay (IGRA) nello screening della tubercolosi latente nei pazienti candidati al trattamento con antagonisti del TNF-α

SUMMARY

Background: Tumor necrosis factor-α (TNF-a) inhibitors are associated with an increased risk of reactivation of latent tuberculosis infection (LTBI); thus, the guidelines recommends TB screening for all patients before starting anti-TNF-α therapy. The use of tuberculin skin test (TST) is controversial because of the immunosuppressive treatment may lead to false-negative TST results and previous BCG-vaccination produces false-positive. The introduction in clinical practice of the interferon-gamma release assays (IGRAs) has opened new perspectives for diagnosis of LTBI. The aim of the study was to investigate the performance of QuantiFERON-TB Gold In Tube assay (QFT-GIT) for the diagnosis of LTBI in patients with an immune-mediated inflammatory diseases candidates for anti-TNF-α therapy.

Methods: We enrolled 195 patients with rheumatoid arthritis (n=72), psoriatic arthritis (n=40), psoriasis (n=41), ankylosing spondylitis (n=10), Crohn's disease (n=18), and Behcet's disease (n=8). Screening included: clinical evaluation, chest X-ray, TST and QFT-GIT.

Results: Of the 195 patients, 32 (16.4%), 137 (70.2%) and 26 (13.3%) tested positive, negative and indeterminate with QFT-GIT test, respectively. The level of agreement between two tests was 81.6 % (k=0.55). Among the screened patients, 38 (19%) were considered to have LTBI and received isoniazid treatment, while 31 patients (16%) showed discordant results between two tests. Univariate analysis showed an association between BCG vaccination and discordant TST-positive/QFT-GIT-negative results (OR=6; 95%CI: 2.3-37.1; p<0.001); no association was observed between the immunosuppressive therapy and discordant TST-negative/QFT-GIT-positive results (OR=0.16; 95%CI: 0.01-1.8; p<0.09).

Conclusions: Our results suggest that QFT-GIT may be helpful for the diagnosis of LTBI in patients candidates for anti-TNF-α treatment because of its performance seems to not be affected by any type of immunosuppression.

BACKGROUND

Tumor necrosis factor-α inhibitors are approved for the treatment of immune-mediated inflammatory diseases (IMID) (3), but they are also associated with an increased risk of opportunistic infection, in particular several studies have reported that the patients with inflammatory diseases receiving TNF-α inhibitors have been shown to be at increased risk of developing severe disseminated tuberculosis (TB), some with fatal outcome (5, 15), so the reactivation of LTBI infection is the major complication of this therapy.

Thus, the screening for LTBI has become mandatory and the preventive treatment should be given to all patients with evidence of LTBI before starting anti-TNF-α therapy (8, 9).

The most widely used diagnostic tool to detect LTBI has been the tuberculin skin test (TST), but its main drawbacks are the presence of false positive results due to the cross-reactivity with BCG vaccine strains and nontuberculous mycobacteria, and low sensitivity because of it may be modified by concomitant immunosuppressive drugs and produce false negative results exposing the patients to the risk of TB reactivation when treated with anti-TNF-α therapy (10, 12).

The introduction in clinical practice of the Interferon-γ Release Assays (IGRAs), as the commercial QuantiFERON-TB Gold In-Tube (QFT-GIT) and T-SPOT.TB™ (TS-TB) assays, which measure IFN-γ released by T cells in response to *Mycobacterium tuberculosis*-specific antigens, has opened new perspectives for diagnosing LTBI (10, 12).

The aim of the study was to investigate the performance of QuantiFERON-TB Gold In Tube (QFT-GIT) assay and its agreement with TST for the diagnosis of LTBI in patients with an immune-mediated inflammatory diseases candidates

for biologic treatment with TNF-α blockers.

METHODS

Study population

A total of 195 patients with IMID candidates for anti-TNF-α therapy were enrolled in four outpatient clinics of a single referral centre (Sapienza University, Rome). Among the patients, the diagnosis was as follows: rheumatoid arthritis (n=72), psoriasis (n=47), psoriatic arthritis (n=40), ankylosing spondylitis (n=10), Crohn's disease (n=18), and Behcet's syndrome (n=8).

Screening at enrollment included: clinical evaluation, chest X-ray, TST, QFT-GIT. The demographic data, information regarding BCG vaccination status, the patients' TB history and risk factors for *M. tuberculosis* infection were collected, along with information on current immunosuppressive treatment. Overall, 59% of patients were male and the median age (range) of enrolled patients was 52 years (18-80). A definite history of BCG vaccination was available for 20 participants (10%). At the time of enrolment, 106 patients (54%) were under treatment with corticosteroids and/or immunosuppressive drugs, including methotrexate, cyclosporine, azathioprine and hydroxychloroquine.

QuantiFERON-TB Gold In-Tube (QFT-GIT)

The QFT-GIT was performed according to the manufacturer's instructions (Cellestis Limited, Australia). Briefly, 1 ml of heparinized blood was drawn directly into each of 3 evacuated blood collection tubes: one containing heparin alone (as negative control), the second containing the phytohemagglutinin (PHA) as positive control, and the third with peptides of

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ESAT-6, CFP-10 and TB 7.7 dried on the inside of the tube. The samples were incubated on the day of blood collection at 37°C in a humidified atmosphere. After 24 hours incubation, plasma aliquots were harvested, and frozen at -70° C, until the assay was performed. The amount of IFN-γ released (IU/ml) was determined using enzyme-linked immunosorbent assay (ELISA). Analysis of data was done by the QuantiFERON-TB Gold Analysis Software.

Tuberculin skin test
After blood was drawn for the QFT-GIT assay, a TST was placed according to the Mantoux method using 5IU of purified protein derivative (PPD) (Chiron, Siena, Italy). Results were read within 48-72 hours by the same individual. Induration >5mm was considered positive.

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 (range) of the different analyzed parameters was calculated. The analysis of concordance between QFT-GIT test and TST was performed using the kappa statistics measure (k, Cohen test), with a k-value of 0.75 representing excellent agreement beyond chance, 0.40–0.75 representing fair to good agreement beyond chance, and 0.40 representing poor agreement beyond chance (1). Odds ratios (OR) and their 95% confidence intervals (CI) for factors associated with discordant results, and indeterminate QFT-GIT test results, were estimated by univariate analysis. All statistical analyses were two-sided and considered significant in case of P-value less than 0.05.

RESULTS
A total of 195 consecutive patients with IMID were included in the study, and summary of their characteristics is shown in Table 1. Overall, 59% of patients were male and the median age (range) of enrolled patients was 52 years (18-80). A definite history of BCG vaccination was available for 20 participants (10%).
Among the 195 IMID patients candidates for biologic therapy, 32 (16.4%) had a positive QFT-GIT result, 137 (70.2%) had a negative and 26 (13.3%) had an indeterminate QFT-GIT test result (Figure 1). The overall agreement between two tests was 81.6 % (k=0.55). The 111 (57%) patients concordant in their negative results were classified as no TB patients and thus they started anti-TNF-α therapy. Regarding

the indeterminate results, these were overrepresented in patients with negative TST, this observation should be useful for clinicians as clinical information on immunological status of the patients. By univariate analysis we found an association between indeterminate results and immunosuppressive treatment, but no differences were found between corticosteroids and various immunosuppressive drugs.
Latent TB was diagnosed in 38 (19%) individuals and all received isoniazid treatment one month before the first dose of anti-TNF-therapy. The QFT-GIT was positive in 34 patients and negative in 7. Among them 5 individuals had abnormal chest-x ray suggestive of latent TB, and 1 individual was a household contact of TB. The median IFN-α responses (2.60 IU/mL; range: 0.35-19.16) of these 38 LTBI patients toward specific antigens was higher than cut-off of the test (0.35 IU/mL), suggesting a good performance of QFT-GIT for latent TB infection.
In the present study we found a 16% of discordant results between the two tests, which was similar to that reported by other authors [15-16]. By univariate analysis the BCG vaccination was the only variables associated with discordant TST-positive/QFT-GIT-negative (OR=6; 95%CI: 2.3-37.1; p<0.001), and no association was found between the immunosuppressive therapy and discordant TST-negative/QFT-GIT-positive results (OR=0.16; 95%CI: 0.01-1.8; p<0.09).

CONCLUSION
Patients with inflammatory diseases need sensitive and specific tests for detection of *M. tuberculosis* infection prior to the initiation of IFN-γ-inhibitor therapy, because of the high risk of reactivation of LTBI when on therapy. We report the comparison between the QFT-GIT and the TST for diagnosing LTBI among patients with inflammatory diseases who are candidates for anti-TNF-α therapy. Establishing sensitivity and specificity of either test in this setting is difficult due to the absence of a gold standard for diagnosis of LTBI, but as a low incidence of indeterminate results was found, the present study confirms the feasibility of QFT-GIT for latent TB screening in patients candidates for anti-TNF-α therapy not only to confirm a positive TST result, but also to rule out false-negative TST results due to previous long-term immunosuppressive treatments.
In conclusion, at the screening a combination of TST and QFT-GIT should maximize the sensitivity for the diagnosis of LTBI in immunosuppressed patients.
At the moment the discordant results are an unresolved question and so the patients with discordant results should be
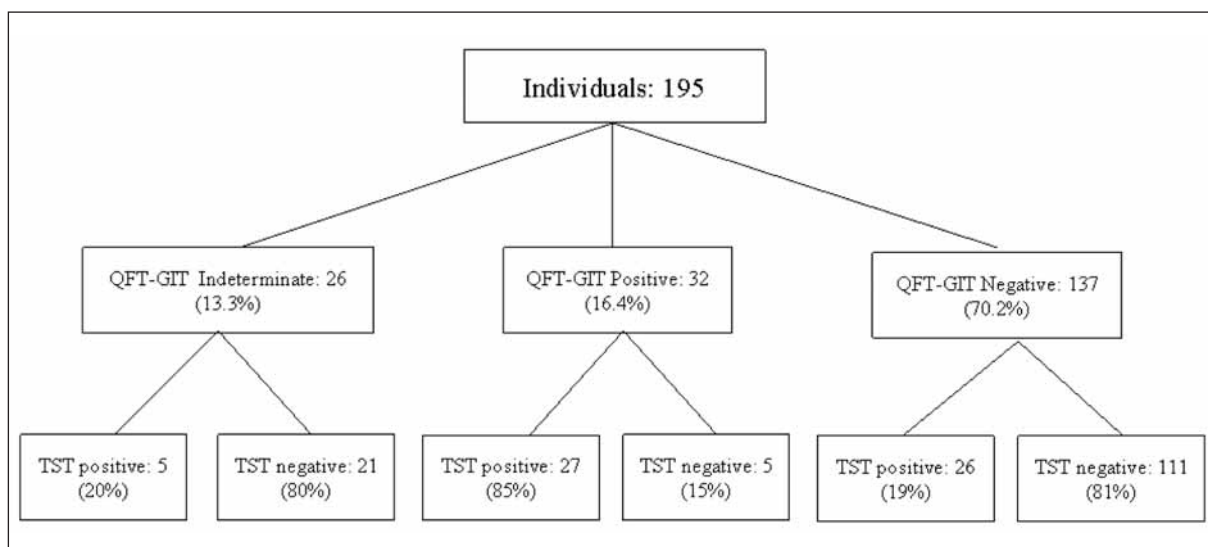

Figure 1. *QFT-GIT assay and TST results in the 195 study patients. QFT-GIT test >0.35 IU/mL was considered as positive. TST induration >5mm was considered as positive.*

closely monitored and longitudinal studies are required to elucidate the clinical significance of these discordant results.

Table 1. *Baseline characteristics of study population*

Characteristics	n (%)
N° of patients	195
Age (range)	52 (18-80 years)
Sex	
Male	115 (59%)
Female	80 (41%)
Immigrant	11 (6%)
low TB incidence	10 (90%)
high TB incidence	1 (10%)
BCG-vaccinated	20 (10%)
Current Treatments*	130 (61%)
glucocorticoids	19 (15%)
methotrexate + glucocorticoids	98 (75%)
cyclosporine + methotrexate + glucocorticoids	13 (10%)
Diagnosis	
Rheumatoid arthritis	72 (37%)
Psoriasis	47 (24%)
Psoriatic arthritis	40 (20%)
Ankylosing spondylitis	10 (48%)
Crohn's disease	18 (5%)
Behcet's syndrome	8 (4%)

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