Comparison of Quantiferon-TB Gold versus Tuberculin Skin Test for Tuberculosis Screening in Inflammatory Bowel Disease Patients

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INTRODUCTION

The reactivation of tuberculosis (TB) is a serious infectious complication in patients affected by inflammatory bowel disease (IBD) undergoing treatment with anti-tumor necrosis factor-alpha (TNF-α) agents. Noteworthy in the literature are documented cases of latent TB (LTB) reactivation and disseminated infections after the initiation of TNF-α therapy [1]. Animal models have shown the importance of TNF-α in controlling intracellular pathogens [2]. Therefore, latent TB (LTB) screening is strongly recommended before starting any anti-TNF-α therapy [3]. In Italy, incidence rates of TB have been fairly stable in the last two decades around 7 reported cases per 100,000 population [4] and the Bacille Calmette Guérin (BCG) vaccination rate is very low.

Traditionally, screening includes a thorough medical history, chest x-rays, and a tuberculin skin test (TST); however, the use of TST is controversial due to its high rate of false-negative results as a consequence of previous long-term immunosuppressive treatments (IST) [5]. The specificity of the TST is low because of the false positive results in patients who have been vaccinated with BCG and in those infected by...
non-tuberculosis mycobacteria [6, 7]. Advances in scientific knowledge have led to the development of innovative blood tests based on the detection of interferon (IFN)-γ release by memory effector T-cells that are stimulated in vitro with Mycobacterium tuberculosis-specific antigens, which offers new approaches for the diagnosis of TB infections [8-11]. Currently, three tests are commercially available in agency-approved formats. One test, the Quantiferon-TB Gold (QFT-G) method (Cellestis, Carnegie, Australia), uses an enzyme-linked immunosorbent assay to measure antigen-specific production of interferon (IFN)-γ by circulating T cells in whole blood and challenging them with the Mycobacterium tuberculosis-specific antigens ESAT-6 (early secretory antigen target -6) and CFP-10 (culture filtrate protein-10). The genes encoding these antigens are found in the region of difference 1 (RD1) of the Mycobacterium tuberculosis genome, which is deleted from the genome of Mycobacterium bovis, BCG, and certain non-tuberculosis mycobacteria, such as Mycobacterium avium. The second test, T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom) uses the Elispot technique to measure peripheral blood mononuclear cells that produce IFN-γ. The latest improvement in this technology is the Quantiferon-TB Gold InTube (QFT) test (Cellestis, Carnegie, Australia), which incorporates another specific TB antigen (TB 7.7) and involves a direct draw of whole blood into a vacutainer tube precoated with antigens, which is ready for incubation. The aim of our study was to evaluate the agreement between QFT and TST for LTB screening in Italian patients with IBD before starting the anti-TNF-α therapy.

**METHODS**

**Patients**

Between January 2008 and October 2010, 92 consecutive patients who were affected by IBD and candidates for anti-TNF-α therapy were prospectively enrolled, after approval by the local ethics committee and informed consent was obtained. In the outpatient clinic, we recorded the results of demographic, clinical, radiologic, and microbiological data for all IBD patients; every patient was asked about his or her history of vaccination with BCG, contact with patients affected by TB, and visits to countries with a high prevalence of TB. The diagnosis of IBD was established on the basis of clinical, laboratory, radiologic, endoscopic, and histological findings. The indications for anti-TNF-α therapy were in agreement with the Italian guidelines [12]. Immunosuppressive treatment was defined if steroids at any dose were taken for ≥ 2 weeks (prednisone ≥20 mg/daily or equivalents), thiopurines (2-2.5 mg/kg/daily) or methotrexate (10-15 mg/weekly) for ≥ 3 months, or TNF-α inhibitors administered during the last 12 weeks [13]. Every patient underwent a complete blood count analysis, and absolute lymphocyte counts were recorded. QFT and TST were performed.

**Interferon-gamma release assay (IGRA)**

Peripheral blood samples were taken before TST was performed. The IGRA was performed according to the manufacturer’s recommendations [14, 15] (Quantiferon-TB Gold, Cellestis GmbH, Hannover, Germany). The concentration of IFN-γ was measured from three wells (negative control or nil well, antigen well containing CFP-10 and ESAT-6, and positive control or mitogen well). The test was considered positive if the concentration of IFN-γ in the antigen well minus that of the nil well was ≥ 0.35 IU/mL. The results were considered negative if the concentration of IFN-γ in the antigen well minus that of the nil well was < 0.35 IU/mL and the concentration in the mitogen well was ≥ 0.5 IU/mL. The results were considered indeterminate if the concentration of IFN-γ in the antigen well minus that of the nil well was < 0.35 IU/mL but the concentration in the mitogen well was < 0.5 IU/mL or if the concentration in the nil was > 0.7 IU/mL and the concentration in the antigen well was ≤50% above nil. When indeterminate IGRA was detected we repeated once the assay as suggested by a recent study in rheumatic patients [16].

**Tuberculin skin test (TST)**

The TST was performed, after IGRA, by injecting 0.1 ml of 5-TU PPD (Tuberculin Units of Purified Protein Derivated, Tubertest, Sanofi Pasteur, MSD, Lyon, France) intradermally (Mantoux method) into the volar or dorsal surface of the forearm, as recommended by the Official Statement of the American Thoracic Society [17]. The main diameter of the skin induration was measured 48-72 h after inoculation transversely to the long axis of the forearm and recorded in millimetres. TST was scored as positive if the diameter was ≥5 mm in patients under IST and ≥10 mm in patients without IST, respectively.

**Statistical analysis**

Proportions were compared by a two-sided Fisher’s exact test. Agreement between the tests was assessed by Cohen’s weighted k statistic. Correlations were analysed by Spearman’s rank test. The level of significance was set at 0.05.

**Objectives**

The primary objective of our study was to evaluate the performance and concordance of TST and IGRA for the screening of latent TB in IBD patients before starting anti-TNF-α therapy. A secondary objective was to evaluate the impact on the test results of variables including IST, risk factors for LTB and signs indicative of LTB in chest x-rays.

| Table I. Characteristics of the studied patients |
|-----------------|-----------------|
| Number of patients | 92                  |
| Women, n (%)       | 46 (50)           |
| Men, n (%)         | 46 (50)           |
| Crohn’s Disease, n (%) | 60 (65.2)       |
| Ulcerative colitis, n (%) | 32 (34.8)       |
| Age (years, mean, 95% confidence interval) | 39.6 (36.7-42.5) |
| IST at baseline, n (%) | 70 (76.1)       |
| AZA, n (%)         | 38 (41.3)         |
| MTX, n (%)         | 2 (2.1)           |
| Steroids, n (%)    | 30 (32.6)         |
| Non IST at baseline, n (%) | 22 (23.9)       |
| Chest X ray indicative of LTB, n | 0                  |
| BCG Vaccination, n (%) | 1 (1.1)          |

IST: immunosuppressive treatment; AZA: azathioprine; MTX: methotrexate
RESULTS

Patient characteristics are summarised in Table I. All 92 patients who were candidates for anti-TNF-α therapy were tested with TST and QFT-G. At the time of the LTB screening, 76% (70/92) of patients were under immunosuppressive therapy. Chest X-rays indicative of LTB were negative in all patients. Only one patient had been vaccinated with the BCG vaccine. All patients were residents of Italy, and none of them came from geographic areas with a high prevalence of TB. The QFT-G test was positive in 14.1% of patients and indeterminate in 0.9%, while the TST was positive in 15.2% of the studied patients. The results of the concomitant TST and QFT-G tests are shown in Table II. QFT-G+/TST+ was detected in 9.8% of patients. The agreement QFT-G-/TST– was observed in 73 patients, QFT-/TST- was observed in 5 patients (AZA: 1; Steroids: 4), all on immunosuppressive treatment. The disagreement QFT-G+/TST- was observed in 7 patients. QFT-/TST+ was observed in 5 patients (AZA: 1; Steroids: 2), all of whom was previously vaccinated. The disagreement QFT-G+/TST- was observed in 4 patients (AZA: 2; Steroids: 2), all on immunosuppressive treatment. Indeterminate QFT, after repeating the test in 5 patients, was confirmed in only one patient, who was under treatment with AZA and had negative TST. Globally, 10.8% of the patients showed a variance between the tests; however, the variance between the tests was detected only in 14.3% of the patients on IST therapy, while none of the patients who were not under IST therapy obtained this result.

The analysis of the agreement between the two tests, assessed by k statistics, showed only moderate strength in our population, with a k of 0.508 (Table III). However, when analyzing the subgroups of patients on IST and without IST separately, the agreement was very good (k=1) in the second subgroup but only fair (k=0.388) for patients on IST.

The correlation analysis, presented in Table IV, indicates a good correlation between the two tests for latent TB. TST results were positively correlated with patients’ age. The 18 patients showing positive for latent TB by QFT-G, TST, or both tests were treated with isoniazide (INHA) (300 mg/day/9 months) and, after a median of 2 months (range 1-3), started an anti-TNF-α treatment [18]. None of these patients showed evidence of TB reactivation during the follow-up period.

DISCUSSION

Tuberculosis screening tests are highly recommended for IBD patients before starting anti-TNF-α therapy, as the reactivation of LTB infection represents one of the main complications that can occur during the course of therapy. Tuberculin skin test, QFT-G and chest x-rays are the most prevalent tests currently used for LTB screening. Several studies have compared TST and QFT-G for the screening of LTB, but only a limited number of them have evaluated their role on IBD patients; therefore, in the literature, the indications for the use of these two tests in the clinical practice for IBD patients are still missing. In a meta-analysis [19] performed on an unselected population, the specificity of QFT-G was 99% (95% CI: 98-100%) in non-BCG-vaccinated populations and 96% (95% CI: 94-98%) in BCG-vaccinated populations. The specificity of the TST was 97% (95% CI: 95-99%) in non-BCG-vaccinated populations but dropped to 59% (95% CI: 46-73%) in BCG-vaccinated populations.

Our study, performed on an Italian population of IBD patients (only one of which was BCG-vaccinated), screened for anti-TNF-α therapy showed a disagreement between QFT-G and TST in 10.8% of patients (k value of 0.508). This discordance was confined to IST-treated patients (14.3% of patients, k value of 0.388), while the non-IST-treated patients had a 100% rate of concordance. These results can be compared to the published studies performed in other countries where TB has a different prevalence and there is a high number of BCG-vaccinated subjects. Schoepfer et al [5], in a Swiss IBD cohort of 212 patients (114 Crohn’s disease, 44 ulcerative colitis, 10 indeterminate colitis, 44 controls), 71% of whom were BCG-vaccinated and 81% of whom were under IST, found...
lower rates of positive TST in IST patients compared to those without IST (14% vs. 34%, p=0.0007). At the same time, the rate of positive QFT-G was similar in the two groups (9% vs. 6%). When looking at the correlation between the two tests, the k value was -0.0297 (-0.0314 in BCG-vaccinated and -0.0538 in non-vaccinated patients), indicating a negative correlation of the two tests in IBD patients. The agreement between QFT-G and TST was better in the controls (k=0.13). However, these results are hardly comparable to our data because of the large difference in the rate of BCG vaccination (almost absent in our population) and its impact on TST results. The study concluded that QFT-G is a better screening tool in immunocompromised IBD patients because of the increased rate of false-negative results of TST in this group. Qumseya et al [20] performed a retrospective, observational American study of 340 IBD patients tested with QFT-G and 85 tested with TST, with 40% on IST and 40% before beginning anti-TNF-α treatment, and found the rate of positivity for QFT-G in their patients to be low (1.3%) compared to the European study. The rate of indeterminate results, however, was found to be 2.7%, which is close to the 3% found by Schoepfer et al [5]. The agreement between the TST and QFT-G was found to be moderate (k=0.4152, P=0.0041), similar to our study. However, no data on BCG vaccination was reported. In a French multicentre prospective study [21] including 93 IBD patients, the correspondence between TST and QFT-G tests was poor (k=0.218; 95% CI: 0.118-0.554). In the subgroup of patients (n=18) treated with anti-TNF, the concordance between two tests was lower (k= -0.0485; 95% CI: 0.734-0.637) compared with the subgroup without anti-TNF (k= 0.324; 95% CI:0.045-0.693). Furthermore, the discrepancy in the TST+/QFT-G- results was associated with the anti-TNF treatment (p=0.05). However, the concomitant use of immunosuppressive therapy did not affect the results of either test (P>0.05). These results agree with those of the study by Matulis et al [22], which was performed on an immunosuppressed population and showed that neither corticosteroids nor conventional immunosuppressants significantly affected IGRA.

An Austrian study [23] was performed on 208 IBD patients, all of whom were BCG-vaccinated and 71.6% of whom were under IST therapy. The correlation between the IGRA and TST results was fair (84.9%, k=0.21) in this population with a high proportion of IST (71.6%) and a BCG vaccination rate of 100%. The presence of risk factors for LTBI showed an association with positive results from TST (OR 3.7, 95% CI: 1.5-9.6) and IGRA (OR 3.5, 95% CI: 1.2-11.3). TST positivity was associated furthermore with age (OR 1.06, 95% CI: 1.02-1.10), as in our study, and with signs indicative of LTBI in a chest x-ray (OR 4.9, 95% CI: 1.1-19.9). IGRA results (but not TST) were affected by IST (OR 0.3, 95% CI: 0.1-0.9).

In a recent systematic review and meta-analysis of the performance of IGRA [24] in nine studies with IBD patients, the correspondence between the TST and the QTF-G/ QFT-G In-Tube was 85% (95% CI: 77-90), while the agreement of TST and T-SPOT.TB was 72% (95% CI: 64-78). The percentage of indeterminate results was 5% (95% CI: 2-9) for all QFT-tests. Both positive QFT-TB Gold/QFT-TB Gold In-Tube results (OR 0.37, 95% CI: 0.16-0.87) and positive TST results (OR 0.28, 95% CI: 0.10-0.80) were significantly influenced by IST (both P=0.02). The populations analyzed in these studies were highly heterogeneous, and the only study reporting a low prevalence of BCG-vaccinated subjects was our preliminary report [25].

Our data are the first obtained in Italian IBD patients, where the prevalence of TB and the rate of BCG vaccination are low, that compared the performance of LTBI screening tests in IBD patients and evaluated them before the start of anti-TNF-α treatment. In clinical practice a gold standard test for the LTBI screening is not available. Both QFT and TST have advantages and disadvantages. TST has low direct cost but needs two visits of patients to evaluate the skin reaction at 48 hours and it can be affected by previous BCG vaccination. Quantiferon has higher cost and both tests seem to be influenced by IST (although in a different way).

CONCLUSIONS

Our very preliminary results, although limited by the small number of studied subjects, might suggest that in our population, with low TB rate and very low BCG vaccination rate, both tests could be employed. However, the results obtained in IST patients suggest the possibility of a variance between TST and QFT. In these cases, strategies should be implemented to improve the efficacy of the LTBI screening. Further studies are needed to better clarify different drug effects, to investigate the reasons for the discrepancies between the two tests and to monitor the outcome, during the anti-TNF therapy, of the different LTBI screening strategies.

Conflicts of interest: none to declare

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