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DIAGNOSTICS

Influence of previous tuberculin skin test on serial IFN-γ release assays

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SUMMARY

Tuberculin skin test (TST) and interferon-γ release assays (IGRAs) have been proposed for serial testing in tuberculosis. In the present study, we assessed the effect of TST on subsequent QuantiFERON-TB Gold In-Tube (QFT-GIT) results by monitoring the evolution of responses during a follow-up period of 6 weeks. One hundred and two subjects were initially tested with QFT-GIT and subsequently with TST; then the QFT-GIT was performed serially 1, 2, 4, and 6 weeks after the TST. A subgroup of 40 subjects was also assessed by older version of the QuantiFERON-TB Gold (QFT-G) assay. The results showed no significant variation in IFN-γ response over time in the tested patients, although two TST-positive subjects showed evidence of possible boosting effect. In addition, a direct comparison between the QFT-G and QFT-GIT test showed no significant differences at any time point with excellent agreement between two tests. No significant differences were seen in IFN-γ responses between BCG-unvaccinated and BCG-vaccinated patients at each time point.

In conclusion, our findings indicate that TST does not influence the outcome of subsequent IGRAs testing in individuals with negative TST results, but it can boost the IFN-γ response in subjects sensitized to TB antigens and not detected by IGRA.

1. Introduction

The interferon-γ release assays (IGRAs) are a new class of diagnostic tools that have revolutionized the diagnosis of latent tuberculosis (LTBI).[1–3] In a few years their use has expanded so rapidly that these tests now form an integral part of several national guidelines. IGRAs detect cellular immune response by measuring interferon-gamma (IFN-γ) released by sensitized T cells after stimulation with *Mycobacterium tuberculosis*-specific antigens, such as early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These proteins are present in all *M. tuberculosis* but they are absent from the Bacillus Calmette-Guerin (BCG) vaccine strains and from most nontuberculous mycobacteria (NTM), except for *Mycobacterium kansasii, Mycobacterium marinum, Mycobacterium szulgai, Mycobacterium flavescens,* and *Mycobacterium gastrii.*[4–6] Thus, as test antigens, these proteins offer greater specificity compared to tuberculin skin test (TST). The IGRA systems currently available for TB detection are the QuantiFERON-TB Gold (QFT-G) and its new variant QuantiFERON-TB Gold In-Tube (QFT-GIT) (Cellestis, Carnegie, Australia), in which a third antigen (TB 7.7) has been added, and the T-SPOT.TB™ (TS-TB, Oxford Immunotech, Abingdon, UK). IGRAs offer general advantages over the TST, including avoidance of cross-reactivity with NTM and BCG vaccination,[7] the completion of the test in a single visit and availability of results within 24 h; in addition, they are ex-vivo assays and can be repeated any number of times without sensitization and boosting.

In recent years a growing body of evidence supported the routine use of IGRAs in clinical practice. In high-income countries with low rates of TB, serial testing for LTBI is recommended for persons at increased risk of TB exposure.[8] In the United States, the Centers for Disease Control and Prevention (CDC, Atlanta, GA) have recommended that the QuantiFERON-TB Gold Test can be used in place of TST for all indications,[9] while in the United Kingdom, the National Institute for Health and Clinical Excellence (NICE) guidelines[10] and Italian Guidelines[11] recommend a two-step strategy for LTBI diagnosis: initial screening with TST followed by an IGRA in TST-positive cases. In the context of serial testing an important issue that needs to be better clarified is the potential boosting effect of the TST on IGRA responses.

The aim of the present study was to assess the effect of TST on subsequent IGRA results by monitoring the evolution of responses during a follow-up period of 6 weeks.

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2. Materials and methods

2.1. Study population

One hundred and two health care workers (57 males and 45 females) who were screened at the Department of Infectious and Tropical Diseases of the Sapienza University of Rome, Italy, were prospectively enrolled between 2007 and 2009. Their mean age was 40.2 years (range 20–67 years). 11 (10.8%) individuals were BCG-vaccinated.

At enrollment all patients were questioned regarding their demographic details and history of BCG vaccination, and were initially tested with QFT-GIT followed by TST: then the QFT-GIT was repeated at week 1, 2, 4, and 6 after the TST. A subgroup of 40 subjects was also assessed by the older version of the QFT-G assay. A blinded interpretation for TST and QFT-IT results was done. Exclusion criteria included: confirmed active TB, treatment of TB, HIV infection, and treatment with immunosuppressive drugs. Moreover, given the difficulty to discriminate between a true increase in IFN-γ and a boosting phenomenon, we also excluded from the study the individuals who were QFT-GIT positive at the screening.

To study the real testing performance on the routine clinical practice, no other exclusion criteria were defined. All participants underwent testing for the purpose of this study. For the analysis of results, the individuals were divided into the following two groups in according to their TST results: 1) patients with TST+/QFT-GIT- 2) patients with TST-/QFT-GIT-.

The study was approved by the institutional review board (Department of Infectious and Tropical Diseases, Sapienza University of Rome) and informed consent was obtained.

2.2. QuantiFERON-TB Gold (QFT-G) and QuantiFERON-TB Gold in Tube (QFT-GIT) assays

The QFT-G and QFT-GIT tests were performed according to the manufacturer's instructions (Cellestis Limited, Carnegie, Australia). Briefly, for QFT-G, four aliquots of 1 ml of heparinized blood were seeded in a well plate in the presence of 3 drops of negative control (saline), phytohemagglutinin (PHA) as positive control and two *M. tuberculosis*-specific antigens: ESAT-6 and CFP-10 (overlapping peptides). For QFT-GIT, 1 ml of blood was drawn directly into three tubes coated with saline (negative control), PHA (positive control), and TB antigens (ESAT-6, CFP-10 and TB 7.7). The samples were incubated on the day of blood collection at 37 °C for 18 h. The amount of IFN-γ released (IU/ml) was determined using enzyme-linked immunosorbent assay (ELISA). For each subject, the negative control value was subtracted from TB antigen and PHA-stimulated samples. Analysis of data was done by the QuantiFERON-TB Gold Analysis Software.

2.3. Tuberculin skin test

TST was placed according to the Mantoux method using 5IU of purified protein derivative (PPD, Biocine, Chiron, Siena, Italy) by a trained physician.[12] Results were read within 48–72 h by the same individual. TST induration greater than or equal to 10 mm was considered positive.

2.4. 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 differences of values between the different groups were analyzed using the non-parametric Mann–Whitney *U*-test. For comparison of qualitative QFT-GIT results of each individual before and after the TST, McNemar's test was used and a Wilcoxon signed-rank test was performed to compare the IFN-γ responses at several time points. *P*-value < 0.05 was regarded as significant.

3. Results

3.1. Effect of TST on serial QFT-GIT testing

We evaluated the effect of TST on serial QFT-GIT responses in 102 patients who were initially tested with QFT-GIT just before TST and then followed-up longitudinally by QFT-GIT at week 1, 2, 4, and 6 after TST. For the analysis of results the individuals were divided into the following two groups in according to their TST results: 1) patients with TST-positive/QFT-GIT negative; 2) patients with TST-negative/QFT-GIT negative.

At the screening, among the 102 patients with QFT-GIT negative, 69 (67.4%) had a negative TST and 33 (32.3%) had a positive TST results; among them, 11 (10.7%) subjects were BCG-vaccinated. The agreement between TST and QFT-GIT was 67.6%.

After 1 week of antecedent TST, all 102 QFT-GIT-tested patients showed a negative response to *M. tuberculosis* specific-antigens. At the second time point (2 weeks after TST), the QFT-GIT results remained negative in 99 patients, but three unvaccinated subjects (2 TST-positive and 1 TST-negative) showed a conversion from negative to positive IFN-γ response. At third time point (4 weeks after TST), the QFT-GIT assay remained negative in all 99 patients, and was positive in 2 TST-positive converters subjects. In contrast, the same TST-negative subject, who at week 2 showed a QFT-GIT conversion with an IFN-γ response (0.40 IU/ml) just above the diagnostic cut-off of the test (0.35 IU/ml), turned QFT-GIT negative at week 4. Six weeks after the TST administration, the QFT-GIT assay continued to be negative in 100 patients, and remained positive in 2 TST-positive converters patients. The follow-up of IFN-γ responses is showed in Table 1. We observed that the median IFN-γ levels in the two groups of patients before and after TST did not show any significant variation at different weeks ($p > 0.5$ for each comparison, Wilcoxon signed-rank test), and QFT-GIT results over time were concordant in 97%. Similarly, no significant differences were seen in median IFN-γ responses between TST-negative BCG-unvaccinated and TST-positive BCG-vaccinated individuals at each time point ($p > 0.5$, data not showed).

The individual IFN-γ response to *M. tuberculosis* specific-antigens during serial QFT-GIT testing for each participant is shown in Figure 1. In the two converters TST-positive subjects, the median IFN-γ responses at each time point were significantly higher than cut-off of the assay (0.35 IU/ml) and remained elevated during the follow-up (median IFN-γ: 0.97 IU/ml at week 2; 1.19 IU/ml at week 4 and 1.09 IU/ml at week 6). In addition, we observed that the variability of the IFN-γ responses below the cut-off was different between TST-positive and TST-negative individuals.

3.2. Comparison of QFT-G and QFT-GIT assay

A subgroup of 40 subjects, who were enrolled at the beginning of the study, was tested with the older version of the QFT-G, which does not contain the additional antigen TB7.7 (Rv2654). In this subset, we assessed the effect of TST on mycobacterial-specific immune response by QFT-G assay. All individuals (29 TST-negative and 11 TST-positive) were QFT-G negative at baseline and remained negative for 6 weeks; no significant differences in IFN-γ responses were observed by quantitative QFT-G results