QuantiFERON TB Gold: a new method for latent tuberculosis infection

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--- **SUMMARY** ---

QuantiFERON-TB Gold obtained approval in 2003 by the Food and Drug Administration as a valid tool for the diagnosis of latent tuberculosis. In this report, we evaluated its potential use in the immunological diagnosis of *Mycobacterium tuberculosis* infections in different groups of subjects.
Our data indicate that QuantiFERON-TB Gold is specific for identifying subjects who have come into contact with *M. tuberculosis* and its use alongside traditional diagnostic techniques may be an important instrument for controlling tuberculosis.

KEY WORDS: QuantiFeron-TB Gold, Tuberculosis, Immunity, Esat-6, CFP-10

Tuberculosis (TB) remains a major global public health problem. More efficient controls should be implemented to better control the spread of TB and these should include the rapid identification of subjects with active and latent TB. The tuberculin skin test (TST) cannot be considered a gold standard because it has low sensitivity and specificity (Andersen *et al.,* 2000; Barnes PF, 2004). Recently, the Food and Drug Administration approved the use of the QuantiFERON-TB GOLD test (QFT-TB Gold) (produced by Cellestis Limited Australia). This is an in-vitro diagnostic test which measures the IFN-gamma released by T lymphocytes sensitised after incubation with the *M. tuberculosis*-specific antigens E-

SAT-6 and CFP-10 (Mazurek *et al.,* 2005). Both these antigens are absent from all BCG strains, are present in *M. tuberculosis*, *M. bovis* and in only a few non tubercular mycobacterial species such as *M. kansasii*, *M. szulgai* and *M. marinum* (Sorensen *et al.,* 1995). Several studies have demonstrated the specificity and sensitivity of this assay, and excellent results have been obtained both for patients with active TB (Mori *et al.,* 2004; Ravn *et al.,* 2005; Lee *et al.,* 2006) and those with latent TB (Arend *et al.,* 2001; Carvalho *et al.,* 2007; Higuchi *et al.,* 2007). In this paper we evaluate the use of QuantiFERON-TB Gold as an immunological diagnostic tool for *M. tuberculosis* infection. 135 blood samples obtained from different patients were divided up into 5 groups. Group I comprised 16 healthy subjects with negative TST.
Group II comprised 31 healthy subjects with positive TST, 11 of these were vaccinated with BCG. Group III comprised 30 patients with active pulmonary TB of *M. tuberculosis* who had been treated for less than 30 days. TB was demonstrated

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TABLE 1 - *Results for the QuantiFERON-TB test in the different groups examined.*

QuantiFERON TB-Gold	Group I	Group II	Group III	Group IV	Group V
Positive	0	15	23	22	3
Negative	16	16	2	16	14
Indeterminate	0	0	5	0	3
Total	16	31	30	38	20

with traditional tuberculosis diagnosis. Radiographic evidence disclosed unilateral and multilateral parenchymal lesions in TB patients. QFT was performed after the TB diagnosis. In this group 24 patients were also TST positive and 6 TST negative; 25 subjects developed a pulmonary TB and 5 developed extrapulmonary TB. Two patients were also HIV positive. Group IV was of 38 subjects with suspected TB (contacts, immigrants, highly suspected). For 10 subjects of this group TST was positive in 5 subjects. No patients developed active TB in this group.

Group V comprised 20 HIV+ subjects. The TST in this group was carried out in 11 subjects and the test was positive in 5 of these and negative in 6. The blood samples were taken at the Clinic of the Respiratory Diseases and the Infective Diseases Clinic of the University of Sassari. The patients enrolled in the study were 70 males and 65 females with a median age of 45 years (range 20-70 years). With the exception of group V subjects, the other patients no had immunodeficiency status. The majority of patients originated from Sardinia and only three were immigrants (n=2 from Africa and n=1 from Eastern Europe). Many of the patients enrolled were out-patients. In these subjects the TST was performed but they did not

undergo a follow-up visit or they came back after more of 72 hours. For these patients the TST was not taken into consideration. Each blood sample was incubated overnight with ESAT-6 and CFP-10 antigens, and with the negative control Nil (saline solution) and the positive control Mitogen (phytohemagglutinin). The plasma were collected the following day and stored at -20°C until immuneassay was carried out. IFN-γ was measured with a standard ELISA technique. Optical density was measured at 450 nm (with a reference filter of 650 nm) with the VERSA Tunable Max microplate reader (Molecular Devices). The data were processed using the calculation software supplied with the kit. The test was considered positive when: Mitogen was ≥ o <0.5 IU/ml and E-SAT-6 and CFP-10 <0.35 IU/ml. The test was considered negative when: Mitogen was <0.5 IU/ml and ESAT-6 and CFP-10 <0.35 IU/ml. The test was considered indeterminate when: Mitogen was <0.5 IU/ml and ESAT-6 and CFP-10 <0.35 IU/ml. In patients of group II and III, and in 97 subjects, where both QFT-Gold and TST data were available, results of two assays were compared using the χ^2 test.

Sixteen subjects of Group I were negative both to TST and QFT. (Table 1, Figure 1). In this group
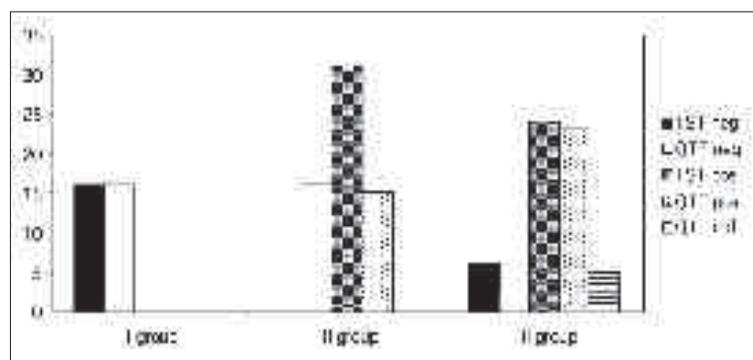

FIGURE 1 - *"Comparison between TST and QFT results in the I, II and III groups examined.*

we observed a total agreement between the two tests.

In the second group (31 patients), 15 TST positive subjects were positive to QFT, 16 TST positive subjects were instead negative to immunological assay. Results obtained from the two tests in this group were apparently controversial.

Nevertheless no statistical difference was observed between the two assays (p=0.799, odd ratio =1). Eleven subjects were TST positive because vaccinated with BCG, whereas 5 subjects according to QFT and TST results had a latent tubercular infection presumably after recent contact with active TB patients. The data obtained from this group indicated the higher specificity of QFT compared with TST. In fact, the immunological assay discriminates between healthy TST positive vaccinated BCG subjects from healthy TST positive subjects with latent TB infection. So the application of QFT is very important when TB infection is suspected in BCG vaccinated subjects. The five subjects TST+ but negative to QFT, have had no contact with TB patients. In these subjects, according to QFT results, prophylaxis was avoided and these data indicated that a definite diagnosis of latent tubercular infection based only on tuberculin test is not reliable, given that positive results may be found in subjects who have not been in contact with *M. tuberculosis*.

In TB patients of Group III, QFT was positive in 23 subjects (Table 1, Figure 1) negative in 2 and indeterminate in 5. Of the two negative subjects, one was HIV positive with disseminated TB. While of the five subjects with indeterminate values, one had tubercular meningitis and four had pulmonary TB. Of 23 QFT positive patients, 22 were TST positive and one TST negative. Of the two negative QFT patients, one was TST positive and one TST negative. The subject with tubercular meningitis, with indeterminate values to QFT was TST positive. Other people with indeterminate QFT were negative to TST.

These data indicated that no significant differences were observed between the performance of TST and QFT in TB patients (p=0.754 odd ratio=1.22). A high number of TB subjects were negative or indeterminate to immunological assay. This result indicated that the utility of QFT in the diagnosis of active TB may be low. In fact the capacity of the QFT assay to detect the production of IFN-gamma in subjects with active TB

depended on various factors, such as the stage of disease at the time the blood samples are taken and its association with other pathologies. It is clear that a negative or indeterminate QFT result in a subject with suspected active TB should be carefully evaluated, based on the patient's medical history. Consequently with the small number of extrapulmonary TB samples analyzed in this study it is impossible to check the sensitivity of immunological assay in this form of tuberculosis.

Of the 38 suspected subjects in Group IV, 16 were negative to QFT-TB Gold and 22 positive (Table 1). All subjects with a negative QFT were negative also at microbiological examinations and no patients developed TB, also the positive subjects did not develop disease. The TST was carried out only in ten subjects with positive QFT. This test was positive in five patients and negative in remaining five subjects. The high number of patients that failed the visit for TST reading demonstrates the poor compliance of the subjects with the TST and a better compliance with the QFT. Our results showed that immunological assay is useful in suspected subjects, such as a healthy person that had contact with active TB patients, because it specifically identified people to submit to preventive therapy.

Group V comprised 20 HIV+ subjects. Three of these had indeterminate results, fourteen negative and three positive (Table 1). The indeterminate subjects had very low levels of mitogen (0.01 IU/ml), while in the negative subjects the mitogen response was normal. In eleven of HIV+ patients the TST was carried out. For two HIV and QFT positive subjects the TST was also positive. In seven QFT negative patients the TST was negative in 6 of these, and positive in one subject. Two indeterminate QFT patients were positive to TST. The performance of immunological assay in HIV+ patients might depend on the stage of disease. Other studies are necessary to compare the sensitivity of TST and QFT in these immunodepressed subjects.

In 97 patients, where both results of QFT and TST were available, the statistical analysis indicated that no significant difference exists between the two assays (p=0.147, odd ratio =1,59).

Although we examined a small number of patients and no significant differences were observed between the two tests, our results indicate

that QuantiFERON-TB Gold is more specific than TST and so it could become an important instrument for diagnosing of TB especially in the latent form of disease. Another important advantage of the QFT to emerge from our study, is that no follow-up visit is needed for the immunologcal assay, as required after 72 hours to interpret the results by TST. Further studies are necessary to determine the utility of QFT-TB Gold in HIV+ subjects. Although more studies are also necessary to confirm the importance of the IFN-γ test as a diagnostic instrument for TB, our experience indicated that using QuantiFERON-TB Gold in conjunction with traditional diagnostic techniques in clinical practice may help to improve checks for TB infection.

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