QuantiFERON®-TB Gold In-Tube test (QFT-GIT) for the screening of latent tuberculosis in recent immigrants to Italy

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SUMMARY

To evaluate the agreement between QuantiFERON®-TB Gold In-Tube test (QFT-GIT) and tuberculin skin test (TST) for the screening of latent tuberculosis infection (LTBI) in recent immigrants to Italy, 279 subjects were submitted to concomitant TST and QFT-GIT. The agreement was analyzed using *k* statistics. A total of 72/279 (25.8%) individuals were TST positive, while 107/279 (38.3%) were QFT-GIT positive. The overall agreement between QFT-GIT and TST was 70.9%, with *k* statistic of 0.35. Using different TST and QFT-GIT cut-offs, the best concordance value was obtained for QFT-GIT at >2.64 IU/ml and TST at >10mm (*k*=0.409). Discordant results were found for 58 subjects (21%) with QFT-GIT positive/TST negative and 23 (8%) with QFT-GIT negative/TST positive. A high amount of discordance QFT-GIT+/TST- was described. QFT-GIT might increase the identification of LTBI cases among recent immigrants.

KEY WORDS: QuantiFERON®-TB Gold In-Tube, Latent tuberculosis, TB screening, Immigrants

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INTRODUCTION

The tuberculosis (TB) notification rate in Italy is 7.5 cases/100,000 residents/year with a bimodal distribution of cases according to age: one peak appears between 55-64 years mostly represented by Italian subjects, another between 25-34 years represented by young patients of foreign origin (Euro, 2006). Throughout 2005 in Italy, 46.2% of TB cases were among immigrants (EuroTB, 2006). In fact, immigrants from countries with high tuberculosis incidence are at higher risk than the general population for developing active disease, particularly in the first two to five years after arrival (McKenna *et al.*, 1995; Diz *et al.*, 2007). Social discrimination, poverty and illegal status, which often characterize the existence of immigrants in industrialized countries and favor conditions of overcrowded housing, poor sanitation and malnutrition, are all factors facilitating reactivation and diffusion of TB during the initial years of residency after migration (McKenna *et al.*, 1995; Diz *et al.*, 2007). Therefore, the identification and treatment of subjects harbouring a latent tuberculosis infection (LTBI) are crucial for eradication of this disease.

The tuberculin skin test (TST), based on purified protein derivative (PPD), is inexpensive and easy to perform for identification of *Mycobacterium tuberculosis* (MT)-infected subjects, but the reading after 72 hours is subjective and requires a second visit, presenting a problem due to the high mobility of this population. TST cross-reactivity with the bacille Calmette-Guérin (BCG) and non-tuberculous mycobacteria (NTM) are responsi-

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ble for false-positive results, while anergy conditions are implicated in false-negatives. Certain alternative assays, based on the detection of interferon-γ (IFN-γ) produced by effector T-cells *in vitro*, have been developed in recent years.

In particular, a third generation assay, the QuantiFERON®-TB Gold In-Tube test (QFT-GIT) using three MT specific antigens, ESAT-6, CFP-10 and TB7.7(p4), which are not present in BCG or in most NTMs (Mazurek *et al.,* 2005; Pai *et al.,* 2004; Cardoso *et al.*, 2002), is now commercially licensed. Many recent studies have investigated the sensitivity and specificity of these IFN-γ release assays (IGRAs) compared to the traditional TST, even though a gold standard is still lacking (Brock *et al.*, 2004; Pai *et al.,* 2005; Kang *et al.*, 2005; Porsa *et al.*, 2006; Harada *et al.*, 2006; Ferrara *et al.*, 2006; Bua *et al.*, 2007; Dogra *et al.*, 2007; Mahomed *et al.*, 2006; Tsiouris *et al.*, 2006; Lee *et al.*, 2006; Nakaoka *et al.*, 2006; Connell *et al.*, 2006; Carvalho *et al.*, 2007; Mazurek *et al.*, 2007; Winje *et al.*, 2008; Sauzullo *et al.*, 2008). Moreover, evaluation of these methods is affected by differing levels of TB prevalence in the investigated populations and by other variables, such as BCG vaccination, immunosuppressive conditions and different versions of commercially-available tests (Menzies *et al.*, 2007).

Overall, discordant TST and IGRAs results have been noted in previous studies (Brock *et al.*, 2004; Pai *et al.*, 2005; Kang *et al.*, 2005; Porsa *et al.*, 2006; Harada *et al.*, 2006; Ferrara *et al.*, 2006; Dogra *et al.*, 2007; Mahomed *et al.*, 2006; Tsiouris *et al.*, 2006; Lee *et al.*, 2006; Nakaoka *et al.*, 2006; Connell *et al.*, 2006; Carvalho *et al.*, 2007; Winje *et al.*, 2008; Mazurek *et al.*, 2007), but as the factors determining disagreement are still unclear the usefulness of these new methods in clinical practice, either as an addition/alternative to TST, is currently questionable.

As yet, Italian tuberculosis guidelines (Ministero della Sanità, 1998) have not been updated to include the use of QFT-GIT for routine diagnosis and clinical management of TB. Even if QFT-GIT cannot distinguish between latent and active tuberculosis disease, and has to be used in conjunction with risk assessment, radiography, and other clinical evaluations, it can become a very useful instrument for the screening of selected risk categories. In particular, little published literature is available on the use of IGRA's to screen immigrants within TB control programs (Carvalho *et al.*, 2007; Winje *et al.*, 2008). Our study aims to evaluate the agreement between QFT-GIT and TST for latent TB screening in a population of recent immigrants to Italy from high-incidence countries.

PATIENTS AND METHODS

Patients

Between September 2004 and December 2005, a population of 452 recent (less than two months) immigrants to Italy, all from high-incidence countries for tuberculosis and temporarily housed in a hospitality centre (Borgo Mezzanone, Foggia, Italy) while awaiting a legal residence permit were evaluated for tuberculosis (LTBI and active disease). The study was approved by the local ethical committee. After providing informed consent (written in four different languages and assisted by a cultural mediator), individuals were submitted to concomitant traditional TST and blood sampling for the QuantiFERON®-TB Gold In-Tube (QFT-GIT) test.

The main population characteristics (sex, age and country of origin) and the TB burden in the country of origin according to WHO statistics (World Health Organization, 2007) were included in the analysis. No subject reported previous active TB disease, recent known close contact, or prior positive TST. The vaccination status was unknown; as the identification of the BCG scar was often difficult, this variable was not considered in the analysis. All individuals were also screened for HIV infection. Immigrants resulting positive at TST and/or QFT-GIT were submitted to further investigation, including chest radiography and sputum examination for mycobacteria for the diagnosis of active TB.

Assays

TST was administered by injecting 0.1 mL of the standard test dose (5 tuberculin unit, TU) of PPD (BiocineTest-PPD®; Chiron S.r.l., Sovicille, Siena, Italy) according to the Mantoux method. Skin induration was evaluated after 72 hours and considered positive if ≥10 mm. Cut-off points of 5 mm and 15 mm, respectively, were also used for comparison. QFT-GIT (Cellestis, Carnegie, Australia) was performed, according to the man-

ufacturer's instructions, by collecting 1mL of whole heparinized blood in two tubes, one containing only heparin as negative control, and the other containing three MT specific antigens: ESAT-6, CFP-10 and TB 7.7 (p4). Tubes were kept at room temperature for a maximum of 16 hours and then incubated at 37°C for 16-24 hours; the tubes were then centrifuged, and the plasma removed and harvested to perform the ELISA. The IFN-γ value for TB-specific antigens was corrected by subtracting the value obtained for the respective negative controls; the test was considered positive if the IFN-γ level was above the cut-off test value (≥0.35 IU/mL).

Statistical analysis

Descriptive statistics were computed for demographic and clinical characteristics of all cases. Mean and standard deviation (SD) are presented for normally distributed variables, and median and interquartile range (IQR) for non-normally distributed variables.

Minimum-maximum ranges were also calculated. Agreement between QFT-GIT and TST was measured using *k* statistics. A *k* value of 1 implies perfect agreement; the following interpretation of agreement according to *k* values <1 can be considered: <0.20 = poor; 0.20 to 0.40 = fair; 0.40 to 0.60 = moderate; 0.60 to 0.80 =good; 0.80 to 1.00 = very good. Odd-ratio values and 95% confidence intervals are provided to evaluate the possible association of subject characteristics with TST and QFT-GIT positivity and the discordance between the two methods.

The chi-square test was used to compare categorical variables and a two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare quantitative variables. A p-value <0.05 was considered significant. Data were analyzed by SPSS 11.0 software for MacOs 10.4.

RESULTS

A flow-chart of all individuals participating in the study is presented in Figure 1. A total of 452 immigrants were tested with TST; these results were not available for 169 (37.4%) subjects because they did not return for the second visit. TST was positive (>10 mm) in 78 of the remaining 283 individuals (27.6%). At the same time, blood samples were obtained from immigrants for the QFT-GIT test. As the aim of the study was to compare the two tests, QFT-GIT was then analyzed only

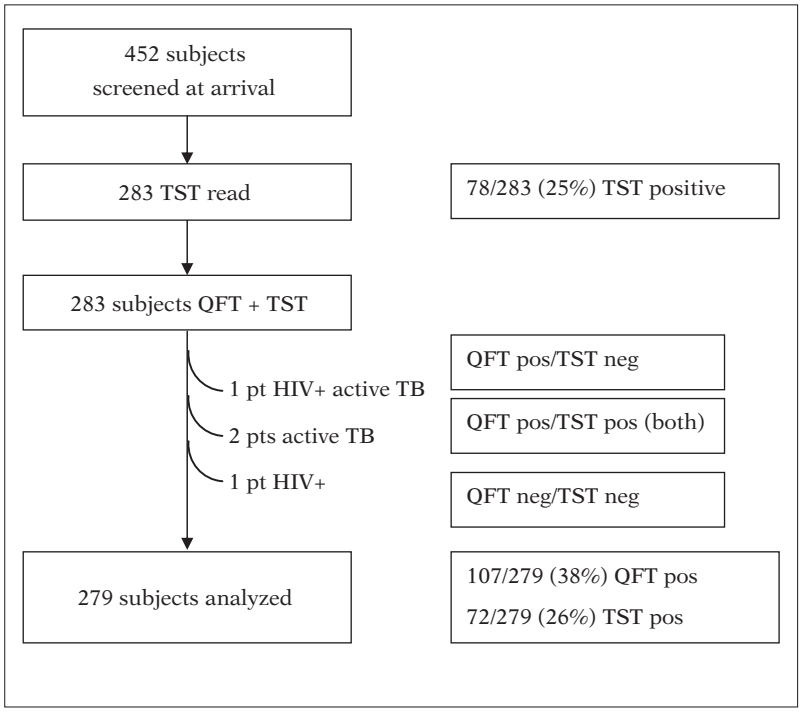


FIGURE 1 - *Diagram of participants in the study.*

TABLE 1 - *Demographic characteristics of the 279 immigrants included in the analysis compared to those of subjects who did not return for TST reading.*

Characteristic	*Valid TST and QFT-GIT N=279*	*TST not read N=169*	*p value*
Age			
Mean ± SD	27.1±6.2	27.1±6.1	0.5
Sex			
Male	268 (96%)	146(86.4%)	<0.001
Female	11 (4%)	23 (13.6%)	
Region of origin[1]			
African	135 (48.4%)	144 (85.2%)	<0.001
Eastern Mediterranean	131 (46.95%)	2 (1.2%)	<0.001
European	7 (2.5%)	14 (8.3%)	0.01
South-East Asian	6 (2.2%)	9 (5.3%)	0.07
Born in a country with a TB burden of[2]			
30-100 cases per 100,000	12 (4.3%)		
101-200 cases per 100,000	15 (5.4%)		
201-300 cases per 100,000	197 (70.9%)		
>301 cases per 100,000	54 (19.4%)		

[1]Countries of origin are grouped according to WHO: African Region (AFR): Côte d'Ivoire, Eritrea, Ethiopia, Ghana, Liberia, Niger, Nigeria, Togo. Eastern Mediterranean Region (EMR): Afghanistan, Egypt, Iraq, Morocco, Pakistan, Somalia, Sudan, Syrian Arab Republic. European Region (EUR): Armenia, Serbia and Montenegro, the former Yugoslav Republic of Macedonia, Israel (Gaza strip) Turkey. South-East Asia Region (SEAR): Bangladesh.
[2]No data available for one subject from Montenegro in the first group and 13 subjects from Montenegro, Serbia and Kashmir in the second group.

for those subjects (283) whose TST result was available.

Three patients, one of whom was also HIV-positive, presented radiological and microbiological signs of pulmonary active tuberculosis and were excluded from the study. An additional subject was excluded because of HIV infection.

Concordance between the two assays was then analyzed for 279 individuals whose demographic characteristics are shown in Table 1.

Interestingly, the features of people with available TST and QFT results differed from those of individuals who did not return for TST reading: in fact, women and African/European persons were less numerous in the group tested with both TST and QFT-GIT, while the number of subjects from Eastern Mediterranean countries was higher.

Of the 279 immigrants analyzed, 72 (25.8%) were TST positive with a skin induration of >10 mm diameter; in 19/72 subjects the diameter of skin induration was >15 mm. An additional 60 subjects presented a skin induration <10 mm and >5 mm. A total of 107/279 (38.3%) were QFT-GIT positive, with IFN-γ levels ranging from 0.35 to 31.34 IU/mL with a median value of 2.65 IU/mL.

The overall agreement between QFT-GIT and TST was 70.9%, with a *k* statistics of 0.35, including 49 subjects positive (18%) and 149 (53.4%) negative to both tests. On the contrary, a total of 81/279 subjects (29%) showed discordant results between the two assays; in particular, 58 were QFT-GIT positive/TST negative and 23 were QFT-GIT negative/TST positive (Tab. 2).

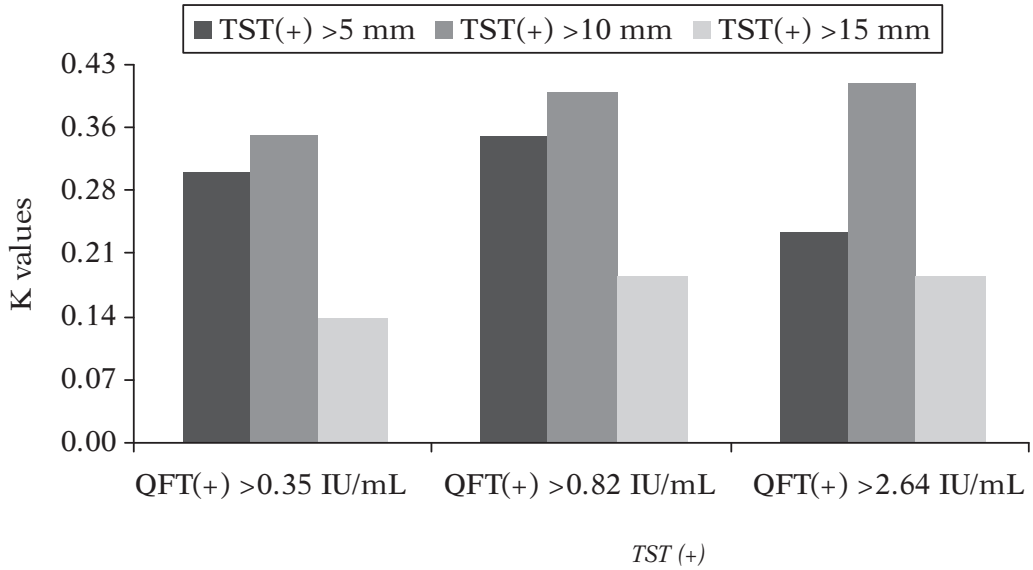
In order to better define the degree of concordance according to diameter of skin induration for TST and level of IFN-γ production, subjects were classified into three groups for TST response (>5 - ≤10 mm, >10 - ≤15 mm, and >15 mm) and into three groups for QFT-GIT response (≥0.35,

TABLE 2 - *Agreement between QFT-GIT and TST.*

QFT	*TST >10 Mm*		*Total*
	POS	*NEG*	
POS	49 (17.6%)	58 (20.8%)	107 (38.3%)
NEG	23 (8.2%)	149 (53.4%)	172 (61.7%)
	72 (25.8%)	207 (74.2%)	279

TABLE 3 - *Variables associated with positive TST and QFT-GIT results.*

		TST >10 mm			*QFT-GIT >0.35*		
		OR	*95% IC*	*p value*	*OR*	*95% IC*	*p value*
Sex (male)		0.6	0.1-2.9	0.48	0.5	0.1-2	0.25
Age	≤24 year old	0.6	0.3-1,1	0.07	0.8	0.5-1.4	0.5
	25-35 years old	1.2	0.7-2.1	2.1	1	0.6-1.7	0.9
	>35 year old	2.4	0.9-6.2	0.04	1.5	0.6-3.9	0.3
Region of origin	African	1.1	0.6-1.9	0.7	1	0.6-1.7	0.9
	Eastern Mediterranean	0.8	0.5-1.4	0.4	1	0.6-1.7	0.8
	European	4	0.7-27.8	0.7	1.2	0.2-7.3	0.8
	South-East Asian	0,6	0.1-5.2	1	0.3	0.01-2.9	0.3
TB burden	30-100 cases/100,000	3	0.8-11.8	0.5	1.2	0.3-4.3	0.8
	101-200 cases/100,000	1	0.2-3.7	1	0.8	0.2-2.6	0.7
	201-300 cases/100,000	0.8	0.4-1.4	0.4	1	0.6-1.8	1
	>300 cases/100,000	1	0.5-2.1	0.98	1	0.5-2	1



		>5 mm ≤10 mm		*>10 mm ≤15 mm*		*>15 mm*
QFT (+)	*N pts*	*K value*	*N pts*	*K value*	*N pts*	*K value*
>0.35 IU/mL	72	0.31	49	0.346	15	0.138
>0.82 IU/mL	60	0.35	43	0.404	14	0.194
>2.64 IU/mL	42	0.244	34	0.409	10	0.193

FIGURE 2 - *Degree of concordance according to diameter of skin induration for TST and the level of IFN-gamma production.*

≥0.82, ≥2.64 IU/mL). The *k* agreement ranged from 0.138 to 0.409, with the best value obtained for QFT-GIT >2.64 and TST >10 mm and ≤15 mm (Fig. 2).
No single demographic characteristic including sex, age, region of origin and TB burden in the country of origin, was associated with TST and/or QFT-GIT positivity (Table 3). A univariate analysis was then performed to identify factors associated with disagreement between the two tests; none of the above mentioned variables was significantly associated to the presence of discordance.

DISCUSSION

A high prevalence of LTBI and elevated risk for TB reactivation was observed in immigrants within the first two-five years after arrival (McKenna *et al.*, 1995; Diz *et al.*, 2007). Hence, the correct selection of LTBI patients for chemoprophylaxis is one of the most important public health problems. Obviously, the availability of a new rapid test, such as QFT-GIT, would be extremely useful for such subjects since the low rate of TST readings (more than 35%) is due to the lack of reappearance for the second visit. After exclusion of active TB cases, the LTBI prevalence in our population ranged from 26% with TST to 38% with QFT-GIT. When considering subjects positive to at least one test, this percentage increased to 46%, confirming the high LTBI risk in recent immigrants and corresponding to the high rate of TB cases observed among immigrants throughout 2006 in Italy (46.2%) (EuroTB, 2006). The peculiar aspect of this comparative study is the population screened, consisting of recent immigrants residing in Italy for no longer than two months, even though most African immigrants spend six months to one year in nations other than their native country (e.g. in Libya) before reaching Italy by boat.
As expected, a rather low grade of concordance (71%, k=0.35) between the two tests was disclosed, firstly because the two assays evaluate different mechanisms of immunological response against MT elicited by different antigens, and a low degree of concordance had already been described in previous studies (Menzies *et al.*, 2007; Kang *et al.*, 2005; Porsa *et al.*, 2006; Ferrara *et al.*, 2006; Mahomed *et al.*, 2006; Tsiouris *et al.*, 2006; Lee *et al.*, 2006; Nakaoka *et al.*, 2006; Connell *et al.*, 2006; Carvalho *et al.*, 2007; Winje *et al.*, 2008; Mazurek *et al.*, 2007), even if only a few (Menzies *et al.*, 2007; Mahomed *et al.*, 2006; Tsiouris *et al.*, 2006; Nakaoka *et al.*, 2006; Mazurek *et al.*, 2007) were based on the latest *in-tube* version of QFT. However, our most interesting finding was that QFT-GIT permitted the identification of more LTBI cases than TST, which is in contrast to earlier reports (Mazurek *et al.*, 2007; Menzies *et al.*, 2007). Considering all versions of QuantiFERON® in different studies, discordant positive QFT and negative TST results have been reported at a range varying from 1% to 11% of all tested subjects (Menzies D. *et al.*, 2007).
It is not clear which factors are responsible for the discordant results in our population, as none of the considered variables (including age, sex, country of origin and relative TB burden) was significantly associated with disagreement between the two tests. The presence of TST positive/QFT-GIT negative results can be easily justified by NTM interference or previous BCG vaccinations, thus emphasizing the greater specificity of QFT-GIT. One limitation of our study is the lack of data regarding BCG status. However, it is more difficult to explain why there are 58 (21%) QFT-GIT+/TST- subjects, and if this result might imply a higher sensitivity for QFT-GIT than that previously estimated for QFT-G (Pai *et al.*, 2007; Pai *et al.*, 2008). A study by Mahomed *et al.* comparing QFT-G with QFT-GIT disclosed a moderate (k=0.5) concordance between the two tests, with a greater prevalence of positive results for QFT-GIT perhaps accounting for the enhanced sensitivity of this latest test version containing the additional third antigen, TB7.7 and a greater technical simplicity (Mahomed H. *et al.*, 2006). In addition, according to Nakaoka *et al.* children at high risk for TB had discordant TST negative/QFT-GIT positive results in 11% of cases, showing a greater sensitivity for QFT-GIT (Nakaoka H. *et al.*, 2006).
Errors in the administration and reading of TST cannot be excluded as this technique was performed "in the field" and not in a hospital setting, even though by trained personnel, and subjectivity is one of TST's main limitations. The presence of immunosuppression could also influence TST and QFT results, even if only a few studies are

available (Luetkemeyer *et al.,* 2007). Our results were not influenced by HIV-related immunosuppression as all HIV-positive subjects were excluded from the analysis, but other causes of immunodeficiency, such as malnutrition and fatigue due to the very difficult migratory journey, cannot be excluded.

Some reports have suggested that QFT assays might be affected by a boosting effect when performed after TST (Igari *et al.,* 2007). This was not the case in our study as the two tests were administered at the same time.

The effect of changes in the TST and QFT-GIT cut-off points was also investigated. Our best *k* agreement was obtained for QFT-GIT IFN- values >2.64 IU/ml and TST skin induration >10 mm; therefore, a higher cut-off for QFT-GIT could be considered, at least for high risk populations, such as immigrants, as also suggested by Arend *et al.* (Arend SM *et al.*, 2007).

On the contrary, concordance was not increased if a cut-off >15 mm for TST was utilized. Even if a >15 mm skin induration is more likely a consequence of MT infection than due to NTM or BCG (Menzies *et al.*, 2007; Mazurek *et al.*, 2007; Wang *et al.*, 2002), as proposed by some authors, other studies (Mahomed *et al.*, 2006) found a poorer agreement between a TST induration >15 mm and QFT-GIT (Pai *et al.*, 2007; Pai *et al.*, 2008; Luetkemeyer *et al.,* 2007; Igari *et al.*, 2007; Arend *et al.*, 2007).

In conclusion, our findings indicate that QFT-GIT could be useful for screening recent immigrants with a high rate of unavailable TST results. As laboratory organization is not a problem in developed countries, currently the only limit remains the relatively high cost of IGRAs'. However, preliminary analyses appear to demonstrate that the use of QFT-GIT for identifying subjects requiring anti-TB chemoprophylaxis might be considered a better cost/effective long-term strategy (Diel *et al.*, 2007). If these new IFN-γ assays were less expensive and technically simplified with reduced times for obtaining test results, they would represent an true innovation in clinical practice regarding TB control and prevention.

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