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QuantiFERON-TB and tuberculin skin test in patients with active tuberculosis: the experience of a single medium-sized Italian University Hospital

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SUMMARY

Interferon-γ releasing assays (IGRAs) are currently widely employed in the initial work up of *Mycobacterium tuberculosis* infection, as well as in suspected tuberculosis (TB). These assays are commonly utilized over the Tuberculin Skin Test (TST) in high resource and low TB burden settings, despite the unclear benefits shown in such contexts. The debate on the use of TST and IGRAs is of current interest also in Italy due to the increasing presence of immigrants from countries with a high incidence of TB and the rising attention of health care institutions to economic costs. The aim of this study was to compare QuantiFERON-TB (QFT) and TST results in active TB. We evaluated QFT results and TST reactions from 245 consecutive patients having both tests, registered among 411 patients admitted for TB at the Infectious Disease Clinic, Department of Medicine of the University of Perugia (Italy). We compared the rates of positive QFT and TST tests and noted no statistically significant differences overall or in relation to age, gender, HIV status and TB localization. Among foreign-born patients with confirmed TB, we observed a lower rate of positive TST results. The results of our study indicated that both QFT and TST can be used in the work up of TB having special attention when evaluating foreign-born patients.

Keywords: Tuberculosis, QuantiFERON-TB, Tuberculin Skin Test, immunologic tests, interferon-gamma release assay.

INTRODUCTION

Worldwide, in 2018, an estimated 10 million persons were diagnosed with tuberculosis (TB) [1]. In Italy, in the same year, the incidence of TB was reported to be 6.5/100.000 inhabitants, with increasing numbers among foreign-born residents [2-7].

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Diagnostic tests for active TB include smear microscopy, molecular tests and culture-based methods. Sequencing methods that can simultaneously test for *Mycobacterium tuberculosis* (Mtb) identification and rifampin resistance are also commercially available [2]. However, according to the World Health Organization (WHO), culture remains the gold standard for diagnosing active TB. *M. tuberculosis* isolation is not only important for a definitive diagnosis, but also for assessing phenotypic drug susceptibility.

T-cell interferon-gamma assays (IGRAs) QuantiFERON-TB (QFT) and T-Spot-TB measure in-

terferon-gamma production following incubation with Mtb specific peptides. Tuberculin Skin Test (TST) is based on an immune reaction to intradermal injection of purified protein derivate [2, 8]. IGRAs and TST are recommended for the diagnosis of Latent TB Infection (LTBI); however, they are also utilized in the initial work up of suspected TB cases. Overall, there is no strong evidence that one test is more reliable than the other [8, 9].
The aim of our study was to compare the results of QFT and TST in patients with active TB admitted to the Infectious Disease Clinic, University of the Perugia, Perugia, Italy.

PATIENTS AND METHODS

Clinical setting
In the region of Umbria, Italy, as on January 1st, 2019 there were 882,015 registered residents of which 97,541 (11.1%) were foreign-born. Their countries of origin included Romania (27.2%), Albania (13.4%) and Morocco (9.8%). The remaining nationalities were present in rates ≤5%, mostly from other African and Eastern European countries [10]. There were no data available about irregular immigrants. The Italian National Healthcare System provides health care to all persons regardless of their legal status.
Infectious Disease Clinic (IDC), Department of Medicine, University of Perugia, Perugia, Italy, is the reference Center for TB in the region of Umbria. The hospital is an 800-bed university hospital and the IDC has 16 single-bed rooms with negative pressure for TB isolation.

Data source
The study was a retrospective analysis of QFT and TST results of patients with active TB diagnosed and admitted to our IDC between January 2006 and December 2018. The study design was in accordance with the Helsinki Declaration of the World Medical Association. On admission, every patient was required to sign an informed consent form for the permission to use patient data.
During the hospital stay, the following information was recorded: demographic characteristics, HIV status, clinical presentation of the TB, TST and QFT, Ziehl-Neelsen (ZN) stain of samples collected (sputum, bronchoalveolar lavage, feces, urine, tissue biopsy depending on the clinical presentation), results of the cultures and drug susceptibility.

Definitions
European regions were defined according to WHO classification [2].
TB cases were defined as:
– Confirmed in presence of *M. tuberculosis* growth from cultures or positive results of both molecular techniques and Acid-Fast Bacilli (AFB) identification from clinical samples.
– Probable when met clinical criteria with the detection of AFB or positive molecular tests.
– Possible if met clinical criteria only [2, 11].
Tuberculosis was classified as pulmonary, extra-pulmonary or disseminated; the latter including the miliary lung pattern of tuberculosis, and TB involving two or more distant organs [2, 11].

Microbiology
Non-sterile specimens were processed with Na-Cl-NaOH prior to ZN stain and cultured using the Lowenstein-Jensen and the Middlebrook 7H9 broth base in the BACTEC MIGIT960 instrument (Becton-Dickinson, Diagnostic system, Sparks, Maryland, USA). Molecular techniques were used for Mtb identification on culture isolates and biologic samples [2, 11-13].

Tuberculin Skin Testing
PPD-5 U in 0.1 ml was inoculated in the volar forearm and the reaction was assessed after 48-72 h. Induration ≥10 mm was considered positive in HIV-negative patients, while a cut off ≥5 was required for positive reaction among HIV-positive patients [11, 14]. From 1995, our policy has been that all patients with a non-reactive TST undergo a second test one week later and the induration of the second test is considered the correct TST result [11, 13]

Interferon-gamma Releasing Assays (IGRAs)
Interferon-gamma releasing assays were performed using QuantiFERON-TB Gold In-Tube (QFT-GIT) (January 2006-July 2016), Cellestis Limited, Carnegie, Victoria, Australia, and QuantiFERON-TB Gold Plus (QFT-Plus) (August 2016-December 2018), Qiagen Biotechnology, Hilden, Germany. Blood for assays was collected prior to TST administration and the QFT was performed following the manufacturer's instructions [8, 9, 13].

Statistics

Data were recorded in a Microsoft Excel 2003 file and analyzed with SPSS version 20.0. The marginal homogeneity between QFT and TST was evaluated using the McNemar chi-square test with Yates correction. The analysis was stratified for gender, age, HIV status, TB localization and place of birth.

RESULTS

From January 1st, 2006 until December 31st, 2018, 411 patients with active TB were admitted to the IDC of the Perugia University Hospital: 270/411 (66%) were males, median age was 37 years (1-91), 9/411 (2.1%) were <18-years-old; 21/411 (5.1%) were HIV co-infected, while for 66/411 (16%) HIV testing results were not available because not performed. In this subgroup, 30/66 patients (45.5%) were Italians and 86.6% of these were ≥65-years-old, 15/66 (22.7%) were Africans, 8/66 (12.1%) Romanians and 13/66 (19.6%) from other countries. The 36/66 foreign-born patients had a median age of 40.5 years (18-82).

Regarding the nationalities of the 411 patients, we registered: 116/411 (28%) Italians, 107/411 (26%) Africans, 90/411 (21.9%) from other EU-European countries, mostly of the latter being from Romania (84/90, 93.3%). Patients from non-EU European countries made up 28/411 (7%) and 33/411 (8%) were from South America (Table 1).

Overall, 279/411 (67.8%) were diagnosed with pulmonary TB, 66/411 (16%) extra-pulmonary TB and 66/411 (16%) disseminated TB (Table 1). Mtb culture resulted positive in 297/411 (72.2%), AFB and molecular testing were both positive in 8/411 for a total of 305/411 (74.2%) confirmed TB cases. In the remaining 106/411 patients, TB diagnosis was considered probable in 31 (7.5%) and possible in 75 (18.2%), respectively (Table 1).

Interferon-gamma responses were evaluated using the QFT-GIT for 262/336 (77.9%) patients and the QFT-Plus assay for 74/336 (22%). Of the latter 74 patients, 4 (5.5%) were HIV co-infected, while HIV was not tested in 19/72 (26.3%). The QFT-Plus resulted indeterminate for 2 patients, who were both HIV-negative. Overall, QFT results were evaluable for 334/411 (81.2%), resulting positive in 305 (91.3%). Of the 334 QFT results registered being positive: 243/264 (92.0%) were HIV-negative, 14/17 (82.3%) HIV-positive, 48/53 (90.5%) not tested for HIV; 203/218 (93.1%) pulmonary, 53/62 (85.4%) extra-pulmonary, and 49/54 (90.7%) disseminated TB.

TST results were available for 299/411 (72.7%) and tested positive in 265 (88.6%). Specifically, 230/259 (88.8%) HIV-negative, 12/15 (80.0%) HIV-positive and 23/25 (92.0%) HIV-not tested patients; 183/201 (91.0%) pulmonary TB, 38/44 (86.3%) extra-pulmonary TB and 44/54 (81.4%) disseminated TB.

In Table 2, the results from the QFT and TST tests were compared considering the subgroup of patients with both tests available. In one patient, QFT resulted indeterminate, therefore data are shown for 245 patients. Overall, 205/245 (83.6%) QFT and TST resulted positive, 18/245 (7.3%) QFT positive and TST negative (15/18 confirmed TB), 7/245 (2.8%) both IGRA and TST negative, 15/245 (6.1%) QFT negative and TST positive. QFT and TST results were also compared for age, gender,

Table 1 - Characteristics of the study population.

Characteristics		*All patients*	*Patients with TST and QFT*
Gender (Male)		270 (66%)	166 (67.8%)
Median age (IQR)		37 (29)	35 (26)
<18 year-old[1]		9 (2.1%)	6 (2.5%)
Place of birth	Italy	116 (28.2%)	56 (22.9%)
	Africa	107 (26.0%)	67 (27.3%)
	Europe (EU)[2]	90 (21.9%)	56 (22.9%)
	Europe (Non-EU)[3]	28 (6.8%)	17 (6.9%)
	South America	33 (8.0%)	26 (10.6%)
	Asia	37 (9.1%)	23 (9.4%)
HIV status	Negative	324 (78.8%)	212 (86.5%)
	Positive	21 (5.1%)	12 (4.9%)
	Not tested	66 (16.1%)	21 (8.6%)
TB classification	Confirmed	305 (74.2%)	173 (70.6%)
	Probable	31 (7.5%)	20 (8.2%)
	Possible	75 (18.2%)	52 (21.2%)
TB localization	Pulmonary	279 (67.8%)	161 (65.7%)
	Extra-pulmonary	66 (16.1%)	40 (16.3%)
	Disseminated	66 (16.1%)	44 (18%)

[1]Italy 4, Africa 3, South America 1, Albania 1;
[2]European-Union: Romania 84, Poland 2, Spain 2, France 1, Greece 1;
[3]Non-European Union: Albania 13, Macedonia 5, Moldova 4, The Ukraine 4, Former Yugoslavia 1, Kosovo 1.
TST = Tuberculin Skin Test; QFT = QuantiFERON-TB test; TB = tuberculosis.

HIV status, TB localization and place of birth. The performances of both tests were analysed and any statistically significant differences were evidenced (Table 2).

In Table 3, the results are shown including only the subgroup of patients with confirmed TB having both QFT and TST results. Among the 173 patients, QFT and TST resulted positive for 149/173

Table 2 - Comparison of QFT and TST in 245 TB cases according to age, gender, HIV status, TB localization and place of birth. Data are presented as absolute numbers and % (in brackets).

Categories		*QFT +*	*QFT -*	*P*[1]
Total Population n=245	TST+	205 (83.6)	15 (6.1)	0.73
	TST -	18 (7.3)	7 (2.8)	
Age groups				
<30 years n=81	TST+	73 (90.1)	0	NA[2]
	TST -	8 (9.9)	0	
30-59 years n=117	TST+	96 (82.1)	11 (9.4)	0.82
	TST -	9 (7.7)	1 (0.9)	
≥60 years n=47	TST+	36 (76.6)	4 (8.5)	0.38
	TST -	1 (2.1)	6 (12.8)	
Gender				
Females n=79	TST+	66 (83.5)	3 (3.8)	0.34
	TST -	7 (8.9)	3 (3.8)	
Males n=166	TST+	139 (83.7)	12 (7.2)	1
	TST -	11 (6.6)	4 (2.4)	
HIV status				
Negative n=212	TST+	179 (84.4)	11 (5.1)	0.44
	TST -	16 (7.5)	6 (2.8)	
Positive n=12	TST+	7 (58.3)	3 (25.0)	1
	TST -	2 (16.6)	0 (0)	
TB localization				
Pulmonary n=161	TST+	139 (86.3)	9 (5.6)	1
	TST -	9 (5.6)	4 (2.5)	
Extra-pulmonary n=40	TST+	33 (82.5)	2 (5)	1
	TST-	2 (5)	3 (7.5)	
Disseminated n=44	TST+	33 (75.0)	4 (9.0)	0.55
	TST -	7 (15.9)	0 (0)	
Place of birth				
Italy n=56	TST+	44 (78.6)	5 (8.9)	0.73
	TST -	3 (5.4)	4 (7.1)	
Foreign-born n=189	TST+	161 (85.2)	10 (5.3)	0.42
	TST -	15 (7.9)	3 (1.6)	

[1]McNemar chi-square test with Yates correction;
[2]McNemar test not calculable.
TST = Tuberculin Skin Test; QFT = QuantiFERON-TB test; NA = not applicable; TB = tuberculosis.

Table 3 - Comparison of QFT and TST in 173 patients with confirmed TB according to age, gender, HIV status, TB localization and place of birth. Data are presented as absolute numbers and % (in brackets).

Categories		*QFT +*	*QFT -*	*p*[1]
Total Population n=173	TST+	149 (86.1)	6 (3.5)	0.12
	TST -	14 (8.1)	4 (2.3)	
Age groups				
<30 years n=56	TST+	50 (89.3)	0 (0)	NA[2]
	TST -	6 (10.7)	0 (0)	
30-59 years n=88	TST+	74 (84.1)	5 (5.7)	0.58
	TST -	8 (9.1)	1 (1.1)	
≥60 years n=29	TST+	25 (86.2)	1 (3.5)	1
	TST -	0 (0)	3 (10.3)	
Gender				
Females n=58	TST+	50 (86.2)	2 (3.5)	0.45
	TST -	5 (8.6)	1 (1.7)	
Males n=115	TST+	99 (86.1)	4 (3.5)	0.27
	TST -	9 (7.8)	3 (2.6)	
HIV status				
Negative n=151	TST+	131 (86.8)	4 (2.6)	0.08
	TST -	12 (8)	4 (2.6)	
Positive n=10	TST+	6 (60)	2 (20)	1
	TST -	2 (20)	0 (0)	
TB localization				
Pulmonary n=114	TST+	103 (90.4)	2 (1.8)	0.18
	TST -	7 (6)	2 (1.8)	
Extra-pulmonary n=22	TST+	20 (90.9)	0 (0)	1
	TST -	0 (0)	2 (9.1)	
Disseminated n=37	TST+	26 (70.3)	4 (18.9)	0.55
	TST -	7 (10.8)	0 (0)	
Place of birth				
Italy n=41	TST+	33 (80.5)	3 (7.3)	1
	TST -	2 (4.9)	3 (7.3)	
Foreign-born n=132	TST+	116 (87.9)	3 (2.3)	0.035
	TST -	12 (9.1)	1 (0.8)	

[1]McNemar chi-square test with Yates correction;
[2]McNemar test not calculable.
TST = Tuberculin Skin Test; QFT = QuantiFERON-TB test; NA = not applicable; TB = tuberculosis.

(86.1%). There were no significant differences observed for positive rates overall, age, gender, HIV status and TB localization. Lower rate of TST positive results among foreign-born individuals was observed.

DISCUSSION

In vitro immunologic IGRA tests were first made available in 2001 to overcome the limitations of TST. Subsequently, improved generations of IGRAs were made available [9, 13, 15-24]. Compared with TST, IGRA assays have been reported to be more specific for LTBI diagnosis in low-TB-burden settings and are more widely used in high-income countries [9]. Both IGRAs like TST have limitations, due to their cross reactivity with a few non-tuberculosis mycobacteria, their reduced sensitivity in immunocompromised patients and children, and are also unable to distinguish between recent and remote infection [9, 11, 13, 15-24]. Study results to date have suggested that TCD8+ is a component of the host immunity in TB and reacts at an early phase of Mtb infection and at a stage of LTBI reactivation. This allowed for the release of a new generation of QFT, the QFT-Plus that includes a fourth tube with peptides that also stimulate CD8+ lymphocytes [17, 20, 21, 23, 24]. Several studies have evaluated QFT-Plus performance in adult and pediatric patients with active TB, LTBI or recently exposed subjects and some authors have demonstrated that QFT-Plus performs better when compared to QFT-GIT for detecting Mtb infection due to the TB2 response [17, 20, 21, 23, 24]. In our laboratory, QFT-GIT was used between 2005 and 2016, therein the QFT-Plus was utilized. For our analysis both QFT-GIT and QFT-Plus results were pooled, as only 2% of our patients were younger than 18 years of age and only 5% were HIV co-infected. For both groups, studies have reported a greater sensitivity for QFT-Plus [17, 19, 23].

TST is commonly used in low-income countries due to its being more economical, but is operator dependent and limited by the risk of false positive and false negative results [17, 18, 25-27]. On the other hand, in high-income and low TB incidence countries, its specificity increases and both IGRAs and TST tests can be utilized [11, 22].

Regarding comparisons of QFT and TST results in active TB, similar sensitivity rates have been reported for the two tests, whereas other results have reported lower sensitivity rates for TST [9, 17, 22, 29]. Overall, in our study, QFT and TST had similar rates of positivity. Statistically significant differences were not observed either for all the 245 patients or the 173 patients with confirmed TB diagnosis. Moreover, no differences were observed between the two tests for age, gender, HIV status and TB localization with the only exception of foreign-born persons with confirmed TB in which TST showed lower positive results compared to QFT. The latter finding appeared not related to difference in age, gender, HIV status or TB localization between Italian and foreign-born patients. Worse general conditions and malnourishment of this subgroup can be factors associated with false negative TST results [11, 17, 23, 29, 30].

With regard to the high rates of TST positive responses in our study, it may be due to our reading TST after a boosting dose in patients with initial negative reaction and utilizing different cut-off for HIV co-infected patients [9, 11, 14, 17, 19, 28]. The booster dose has been suggested to reduce the rate of false negative results because of waned cell-mediated immunity. Furthermore, it does not imply false positive reactions in unexposed individuals [11].

The limitations of this study included being a retrospective single center study, lack of QFT and TST results for all of the patients, low number of HIV co-infected individuals, as well as the fact that testing standard changed over the 13-year course of study.

In conclusion, in agreement with past publications, our study indicated that QFT and TST lead to similar results and both can be utilized in the work up of TB, having special attention when evaluating foreign-born patients [17, 18, 21, 22].

Conflict of interest

The authors declare no conflicts of interest.

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