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Limited usefulness of QuantiFERON-TB Gold In-Tube® for monitoring anti-tuberculosis therapy

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Summary
The usefulness of IFN-γ release assays to monitor the efficacy of anti-tuberculosis (TB) treatment is controversial. Sixty patients affected by culture-confirmed pulmonary TB ($M = 36$; mean age: 39.2 yr; Italians = 28) were serially tested in a low prevalence setting by means of QuantiFERON-TB GOLD In-Tube (QFT-IT) at baseline and after a successful six-month therapy regimen (T6). A sub-group of 40 cases was also tested at 1 and 3 months. Overall, 88.3% of patients scored a QFT-IT positive result at baseline, with the higher proportion of TB-specific IFN-γ responses in foreign-born patients ($p = 0.04$). TB-specific responses were highly variable over time, the within-person variability being correlated with baseline IFN-γ levels ($r = 0.731$; $p < 0.001$). Overall, 61.6% of cases still tested QFT-IT positive at the completion of therapy. Average IFN-γ levels increased over time, being persistently significantly higher in Italian patients than in foreign-born cases both at baseline ($p = 0.03$) and at T6 ($p = 0.02$). Reversion mainly occurred in patients (26.6%) with baseline IFN-γ levels close to the conventional cut-off value. No indeterminate results were recorded at any study time point.

In conclusion, QFT-IT adds no significant information to clinicians for treatment monitoring when applied in routine clinical practice in a low prevalence setting. Kinetics of T cell responses upon TB treatment and reversion (and conversion) thresholds need to be addressed. Diversity of IFN-γ responses among patients of different geographic origin is an issue to be investigated further.

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Introduction

The recent development of *in vitro* innovative blood tests based on the principle of detecting the release of interferon (IFN)-γ by *M. tuberculosis* (Mtb)-specific circulating effector memory T cells has offered an attractive challenge to tuberculosis (TB) management. Overall, IFN-γ release assays (IGRAs), also referred as T cell based assays, are more specific than tuberculin skin test (TST), more sensitive in detecting active TB and correlate better with TB exposure in immune-competent patients, at least in low prevalence settings.[1,2] Actually there are two commercial IGRAs available and approved by the US Food and Drug Administration (FDA): the ELISpot-based assay T-SPOT.*TB* (Oxford Immunotech, Oxford, UK) and the whole blood ELISA-based format QuantiFERON-TB Gold In-Tube (QFT-IT), provided by Cellestis (Carniege, Victoria, Australia). Both tests rely on the use of two Mtb-specific antigens (Ags), that are early secretory antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. ESAT-6 and CFP-10 encoding genes have been mapped to the genomic *region of difference* (RD)-1, which is selectively restricted to Mtb while is absent in most environmental mycobacteria and in all BCG vaccine strains.[1,2] The QFT-IT format also includes a third TB-specific Ag, that is TB7.7. Since the promising spread of commercial IGRAs in clinical practice, international guidelines and statements have been published worldwide in order to assess their application and performance interpretation in different patient populations and epidemiological settings.[3–6] Although this has lead to the routinely use of IGRAs for latent TB infection (LTBI) diagnosis and surveillance of new infection cases, important issues concerning the management of active TB still remain unresolved and need to be thoroughly investigated.[7–9] First, the use of IGRAs for diagnostic purposes in this setting is not fully shared, most observations suggesting the usefulness of IGRAs to rule out active TB.[10–13] Secondly, it is unclear whether IGRAs can be used as surrogate tools for monitoring the clinical efficacy of anti-TB treatment. It is thought that effector memory T cell responses decline upon Ag (bacterial) clearance and are replaced by the pool of central memory T lymphocytes that mainly produce interleukin (IL)-2 instead of IFN-γ.[14,15] By the other, *in vitro* evidence has suggested that anti-TB drugs do not exert any inhibitory effect on IFN-γ production within the range of therapeutically achievable concentrations.[16] Recent studies addressing the dynamics of specific T cell responses with the use of IGRAs in patients affected by active TB undergoing specific treatment have provided quite controversial results. To date, some studies have shown decreasing or negative responses, while others have reported increased or persistently positive responses both during and after the completion of treatment.[16–25] Variability of IFN-γ responses was influenced by factors, including assay characteristics, antigen load and functional diversity of T cell subsets. In addition, to our knowledge there are limited data on the usefulness of commercial QFT-IT for anti-TB treatment monitoring purposes when applied in routine clinical practice in a low prevalence setting.

To further highlight the clinical relevance of measuring the T cell response to anti-TB treatment, we evaluated longitudinal changes of Mtb-specific IFN-γ release by means of QFT-IT in a selected population of patients affected by susceptible culture-confirmed TB who successfully completed a standard six-month therapy regimen in a low prevalence setting.

Materials and methods

Patients and study design

Sixty patients affected by culture-confirmed pulmonary TB attending the Respiratory Medicine Divisions of two Italian tertiary care hospitals (S.Camillo-Forlanini hospital, Rome and Monaldi hospital, Naples) were prospectively enrolled between September 2007 and July 2008. Exclusion criteria were human immunodeficiency virus (HIV) infection, any drug-resistance, current receipt of immune-suppressive drugs and previous anti-TB treatment. Diagnostic work-up included clinical history (previous BCG vaccination), physical examination, routine blood tests, and standard chest X-ray. Sputum samples and, in some instances broncho-alveolar lavage fluids (BALF), were sent for conventional microbiology, i.e., acid fast bacilli (AFB)-staining and culture for mycobacteria. High resolution computed tomography (HRCT) of the thorax was performed in selected cases. TST was performed by trained hospital staff through the intradermal inoculation (Mantoux method) of 5 international units (IU) of purified protein derivative (Biocine PPD, Chiron, Siena, I). The main transverse diameter of skin induration was measured in millimetres (mm) at 72 h. Positive results were recorded according to the guidelines of the *American Thoracic Society* (ATS).[26] All patients received a standard six-month daily combination therapy with isoniazid (I), rifampicin (R), ethambutol (E) and pyrazinamide (Z) for 2 months followed by daily IR for additional 4 months. Treatment follow-up was performed on an out-patient basis by monitoring sputum microbiology (microscopy and culture) and the chest X-ray status. No drug-related side effects were recorded during the whole duration of treatment and no cases of TB relapse have occurred until nowadays. The local Ethics Committee approved the study and all patients gave their oral informed consent.

QFT-IT was serially performed at baseline (T0) and after 6 months (T6) of treatment. In a sub-group of 40 patients, QFT-IT was repeated also at 1 and 3 months after the start of therapy (T1 and T3, respectively). QFT-IT performance and data analysis were realized according to the manufactures' instructions.

Statistical analysis

Univariate analyses were used to compare the clinical and demographic characteristics among Italian and foreign-born patients. Quantitative variables were analyzed using Student *t* test. Percentages were compared using a Chi square test or Fisher's exact test for small numbers. Comparison between the frequency of QFT-IT and TST positive results was based on Mc-Nemar test for paired proportions. Student's *t* tests for paired data were used to

compare mean levels of TB-specific IFN-γ release between baseline and endpoint. The correlation between baseline TB-specific IFN-γ release and within subject variability of TB-specific IFN-γ release was estimated using the Spearman rank correlation coefficient. *P* values were considered as being statistically significant at or below the alpha value of 0.05. All computations were done with R v. 2.9.1.[27]

Results

The main demographic and clinical characteristics of the study patients are reported in Table 1. The study population was composed of 28 Italian patients and 32 foreign-born cases, 20 of them coming from Romania. Mean stay in Italy was 6.2 ± 3.5 yr. All cases successfully completed a six-month regimen of anti-TB treatment, as assessed by complete symptoms recovery, persistent negative culture examination and significant improvement of the chest X-ray status. Sputum conversion on microscopy was achieved in 82% of cases within the first 30 days of therapy. All participants were tested by means of QFT-IT at baseline (T0) and at 6 months (T6) after the start of anti-TB therapy. Table 2 shows the distribution of the proportion of QFT-IT positive results and the relative analytical data of TB-specific IFN-γ production in the whole population and in the two subgroups of patients separately analysed. Overall, QFT-IT scored a positive result in 88.3% of the total population at T0, with the higher proportion of TB-specific IFN-γ responses in foreign-born patients ($p = 0.04$). The baseline percentage of QFT-IT positive results also overcame that of TST positivity in the latter group of patients ($p = 0.04$), while there was no significant difference in test performance in the former. More than half of the whole population (66.3%) still scored a QFT-IT positive result at T6. Mean concentrations of TB-specific IFN-γ release increased along with time (mean fold increase of 5.5, p = ns), being persistently significantly higher in Italian patients both at baseline and at the completion of treatment. These findings were not correlated with any disease associated factor, including sputum bacterial load, X-ray evidence of cavitation or disease extension (monolateral vs bilateral), or sputum conversion time (less or more than 30 days). No differences in the absolute count of peripheral lymphocytes at baseline and at T6 were recorded comparing QFT-IT positive and QFT-IT negative patients. No indeterminate results were scored at any study time point. Table 3 summarizes within-person dichotomous changes in QFT-IT status. As shown, QFT-IT reversion was scored in less than one third of the whole population. Reversions mainly occurred in patients with low baseline IFN-γ levels (i.e., close to the cut-off value) and was associated with cavitary TB ($p = 0.041$). Conversely, the majority of cases had unchanged qualitative QFT-IT responses.

To address the dynamics of IFN-γ release along with treatment, QFT-IT was further performed after 1 and 3 months of therapy in a sub-group of 40 patients. To date, longitudinal changes in IFN-γ production in 40 patients respectively tested QFT-IT negative ($n = 6$) and QFT-IT positive ($n = 34$) at baseline. Three out 6 QFT-IT negative patients scored a transient positive response at T1 or T3. Definitive QFT-IT conversion to a positive response (1.2 IU/mL) was achieved only in one case at the end of therapy (T6). Monitoring of IFN-γ responses among QFT-IT positive patients provided highly variable results over time - some individuals showed increases of IFN-γ levels, while some others showed decreases or no variations. Overall, the ability to mount stronger, persistent or weaker IFN-γ responses over time was not associated with age, sex, origin, or with any disease related feature. Conversely, within-person variability was positively correlated with baseline IFN-γ production ($r = 0.731$; $p < 0.001$).

Discussion

The present longitudinal study was designed to evaluate the impact of anti-TB treatment on specific IFN-γ responses

Table 1 Demographics and clinical characteristics of the study population.

Parameter	Total	Italians	Foreign-born[a]	p
Patients, n (%)	60	28 (46.6)	32 (53.3)	—
Males, n (%)	36 (60)	14 (50)	22 (68.7)	ns
Age [b], yr	39.2 ± 14.3 (1.8)	42.7 ± 17.2 (3.2)	36 ± 10.5 (1.8)	ns
	36 [17—74]	41 [17—74]	33.5 [20—61]	
TST+, n (%)	45 (75.0)	23 (82.1)	22 (65.6)	ns
BCG vaccination, n (%)	26 (43.3)	0 (0)	26 (81.2)	—
AFB+, n (%)	39 (65)	15 (57.1)	24 (71.8)	ns
AFB score:				
1, n (%)	6 (15.3)	3 (18.7)	3 (13)	ns
2, n (%)	9 (23)	5 (31.2)	4 (17.3)	
3, n (%)	20 (51.2)	8 (50)	12 (52.1)	
4, n (%)	4 (10.2)	0 (0)	4 (17.3)	
Cavitary TB, n (%)	24 (40)	10 (35.7)	14 (43.7)	ns
Bilateral TB, n (%)	25 (41.6)	10 (35.7)	15 (46.8)	ns

Statistics: Chi square or Fisher exact test in case of qualitative variables; unpaired *t*-test in case of quantitative variables; ns = not significant.

[a] Countries of origin other than Romania included Columbia, Costa Rica, Croatia, Ethiopia, India, Moldavia, Morocco, Pakistan, Peru, Philippines and Ukraine.

[b] Data are expressed as mean ± SD (SEM); median [range].

Table 2 Distribution of analytical QFT-IT positive results at baseline (T0) and at 6 months (T6) after start of treatment.

Parameter	Total	Italians	Foreign-born	p
Tested cases at T0, *n*	60	28	32	—
QFT-IT+, n (%)	53 (88.3)	22 (78.5)	31 (96.8)	0.04
IFN-γ production [IU/mL] [a]	4.77 ± 6.6 (0.9)	7.36 ± 8.7 (1.9)	2.93 ± 3.8 (0.7)	0.03
	2.2 [0.36–28.1]	2.4 [0.42–28.1]	1.9 [0.36–17.6]	
Blood lymphocytes/mm^3 [a]	1598 ± 465 (60)	1609 ± 477 (90)	1587 ± 462 (81)	ns
	1565 [759–2845]	1588 [759–2442]	1532 [952–2845]	
Tested cases at T6, *n*	60	28	32	
QFT-IT+, n (%)	38 (63.3)	17 (60.7)	21 (65.6)	ns
IFN-γ production [IU/mL] [a]	7.29 ± 8.25 (1.3)	11.0 ± 9.6 (2.3)	4.3 ± 5.6 (1.2)	0.02
	4.2 [0.42–33.3]	9.9 [0.82–33.3]	1.6[0.4–24.2]	
Blood lymphocytes/mm^3 [a]	1834 ± 582 (75)	1781 ± 614 (116)	1881 ± 559 (98)	ns
	1786 [668–3313]	1760 [668–3260]	1825 [1050–3313]	

Statistical test: Chi square test in case of qualitative variables; unpaired *t*-test in case of quantitative variables; ns = not significant.
[a] Data are expressed as mean ± SD (SEM); median [range].

by means of QFT-IT in patients affected by culture-confirmed pulmonary TB who successfully completed a standard therapy regimen. Although increasing efforts have been focused on IGRAs use in clinical monitoring of anti-TB therapy,[16–25] to our knowledge only a limited number of studies has addressed the usefulness of commercial last generation ELISA-based QFT-IT in this scenario. Despite our study was realized in a low prevalence area, ethnicity of the study population was quite heterogeneous as it was composed both of Italian patients and of foreign-born cases mainly coming from high TB burden countries. This is an important issue as differences in QFT-IT performance were recorded when patients were separately analysed. Baseline QFT-IT assessment had a sensitivity of 88.3%, which significantly ranged from 78.5% in Italian patients to 96.8% among those foreign-born. Overall, sensitivity of commercial ELISA-based IGRAs varies across studies ranging from 64% with QFT-Gold (the predecessor of QFT-IT)[23] to 95% by means of QFT-IT,[25] the assay characteristics, local epidemiology and disease features accounting for these differences.

Unexpectedly, baseline levels of IFN-γ were significantly higher in Italian cases than in foreign-born patients. It is unclear whether the intensity of IGRA responses may be detecting proxy recent TB exposure. Unfortunately, this was not so easy to be addressed in our setting. This finding was even more surprising as the majority of foreign-born patients were living in Italy for a mean time of more than 5 years suggesting no substantial differences at least in environmental TB exposure in comparison with resident patients. Recent observations suggest that quantitative IFN-γ responses are correlated with disease activity.[28–30] This was not the case in our setting, while additional factors including the influence of prior TB exposure in the country of origin,[31] immunological and genetic factors, and strain differences cannot be excluded as well.

Although QFT-IT is a robust and highly reproducible assay,[32] we found that TB-specific responses were highly variable over time, the within-person variability being correlated with baseline IFN-γ levels. Intra-individual variability has been reported across different studies, ranging from 16 to 80%.[33] It is widely thought that this is an issue of concern having implications for the interpretation of results close to the cut-off point and the definition of test conversion and reversion in serial testing,[34,35] as the optimal threshold to distinguish these events from non specific variations is actually not known. Recently, Veerapathran et al. have recommended a 30% increase in the cut-off level in the response as a true conversion looking at active TB patients.[36] In addition, Pai et al. have found that the rate of QFT conversions may significantly change upon different-cut-off values when looking at LTBI cases.[37,38]

In agreement with previous observations in cured TB cases,[25,39–41] a consistent proportion of patients (66.3%) still scored a positive QFT-IT response, which occurred along with an increase of IFN-γ levels, at the completion of therapy. Conversely, Sauzzullo et al. have found that QFT-IT positive responses became negative in 27 out of 38 cured patients while disease resolution was not achieved in 5 out 11 patients who had a persistent IFN-γ response.[16] There is evidence that cured TB patients can retain strong ESAT-6 responses for several years after the completion of

Table 3 Monitoring of QFT-IT status at baseline and at 6 months after start of treatment.[a]

QFT-IT status	Total (*n* = 60)	Italians (*n* = 28)	Foreign-born (*n* = 32)	p
Positive > positive	37 (61.6)	16 (57)	21 (65.6)	ns
Negative > negative	6 (10)	5 (17.8)	1 (3)	
Positive > negative	16 (26.6)	6 (21.4)	10 (31.2)	
Negative > positive	1 (1.6)	1 (3.5)	0 (0)	

Statistics: Fisher exact test.
[a] Data are expressed as n (%).

therapy.[18] Chee et al. first proposed that differential T cell responses to ESAT-6 and CFP-10 may suggest different function properties. To date, T cell responses to ESAT-6 may persist as a scar of previously treated or quiescent infection, whereas those to CFP-10 may be indicative of active disease as decrease over time upon successful treatment.[42] By this point of view, QFT-IT, which represents the latest improvement of the previous in plate format, is critically of limited usefulness as all TB antigens are tested simultaneously in a single tube. In our setting, 63% of 15 patients randomly re-tested after further 6 months from the completion of therapy still scored a QFT-IT response in the absence of any clinical sign of TB relapse (mean IFN-γ levels $\pm$ SD: 7.11 $\pm$ 7.5 IU/mL; range: 0.4—20.7). Finally, reversion to a negative QFT-IT response was achieved in about one third of the study population (26.6%). In agreement with previous observations[37,39] most of cases who became negative on QFT-IT had baseline IFN-γ levels close to the conventional cut-off point. Conversion to a positive QFT-IT result at T6 was not a concern in our series as it was recorded in only one patient (1.6%).

In conclusion, our results suggest that QFT-IT adds no significant information to clinicians for treatment monitoring as long lasting IFN-γ responses may persist despite the infection has been successfully cured.

Conflict of interest

None of the authors have any conflict of interest to declare.

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