Journal of Infection 79 (2019) 15–23

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Characterization of QuantiFERON-TB-Plus results in latent tuberculosis infected patients with or without immune-mediated inflammatory diseases

Teresa Chiacchio[a,1], Elisa Petruccioli[a,1], Valentina Vanini[a], Gilda Cuzzi[a], Umberto Massafra[b], Gianpiero Baldi[c], Assunta Navarra[d], Rossana Scrivo[e], Claudio Mastroianni[f], Ilaria Sauzullo[f], Carmela Esposito[g], Fabrizio Palmieri[h], Fabrizio Cantini[g], Delia Goletti[a,*]

[a] Translational Research Unit, Department of Epidemiology and Preclinical Research, National Institute for Infectious Diseases Lazzaro Spallanzani–IRCCS, Via Portuense 292, 00149 Rome, Italy
[b] Department of Internal Medicine, S. Pietro Fatebenefratelli Hospital, Rome, Italy
[c] Rheumatology Unit, San Paolo Hospital, Civitavecchia, Rome, Italy
[d] Department of Epidemiology and Preclinical Research, National Institute for Infectious Diseases Lazzaro Spallanzani–IRCCS, Italy
[e] Rheumatology Unit, Department of Internal Medicine and Medical Specialties, Sapienza University, Rome, Italy
[f] Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy
[g] Department of Rheumatology, Hospital of Prato, Prato, Italy
[h] Department of Clinical and Clinical Research, National Institute for Infectious Diseases Lazzaro Spallanzani–IRCCS Rome, Italy

ARTICLE INFO

Article history:
Accepted 6 April 2019
Available online 11 April 2019

Keywords:
Tuberculosis
LTBI
IMID
IGRA
QuantiFERON-TB-Plus
Biological therapy
Anti-TNFα

SUMMARY

Objectives: Screening for latent tuberculosis infection (LTBI) diagnosis is mandatory in patients with immune-mediated inflammatory diseases (IMID) requiring biologics. QuantiFERON-TB-Plus (QFT-P), an LTBI diagnostic test, measures IFN-γ after *M. tuberculosis*-stimulation in TB1 and TB2 tubes in which a "CD4" or a "CD4 and CD8" response is respectively elicited. Aim of this study is to compare the response to QFT-P of IMID-LTBI patients candidates to a new biological therapy vs LTBI-subjects without IMID.
Methods: We prospectively enrolled 167 subjects: 61 IMID-LTBI and 106 NON-IMID-LTBI.
Results: All subjects were mitogen-responders. IFN-γ production was significantly lower in IMID-LTBI-patients compared to NON-IMID-LTBI-subjects. We observed discordant TB1 and TB2 results in 6.5% of IMID-LTBI-patients and in 8% of NON-IMID-LTBI-subjects. Applying a logistic regression analysis, we found that IMID-LTBI patients had a higher probability (TB1 stimulation OR 3.32; TB2 stimulation OR 4.33) to have IFNγ results $\leq$0.7 IU/mL compared to NON-IMID-LTBI-subjects. Interestingly, IMID-treatment did not interfere with the distribution of IFNγ-values.
Conclusions: These results indicate that IMID-LTBI-patients have a low IFN-γ response to QFT-P, a high proportion of results ranging in the grey zone and a distribution of IFNγ-values independent from the IMID-treatment. These results are important for the management of LTBI screening in IMID patients.

Introduction

M.tuberculosis (Mtb) was responsible for 10 million of new active tuberculosis (TB) disease cases and 1.6 million deaths in 2017,[1] and currently TB is the disease that generates the highest number of deaths among all the infectious diseases.[1] Recently, it has been estimated that 1.7 billion persons have a latent TB infection (LTBI), representing almost a quarter of the world population.[2] Only a small proportion of LTBI subjects (5-10%) will develop TB during their lifetime.

Immune-mediated inflammatory diseases (IMID) comprising rheumatic diseases (RD), chronic intestinal diseases and dermatological diseases (DD), may require treatment with immune suppressive drugs, including glucocorticoids and/or methotrexate (MTX). If these drugs are inefficacious or not tolerated, patients may be treated with biological agents targeting different cytokines and cells of immune response, such as TNFα, IL1, IL6, IL12/IL23, IL17, CD20 and CD28 lymphocytes. An increased risk to develop

* Corresponding author.
E-mail addresses: delia.goletti@inmi.it, d.goletti@tiscali.it (D. Goletti).
[1] Both authors contributed equally to the manuscript.

https://doi.org/10.1016/j.jinf.2019.04.010
0163-4453/

active TB disease has been reported in patients with rheumatic disorders taking biologic drugs, with the highest risk in anti-TNF exposed.[3–7] TNFα has an important role in the maintenance of granuloma integrity, and changes in TNFα effector functions induced by anti-TNF biologics, affect the containment of Mtb infection in the granuloma structure.[6] Differently, IL1, IL6, IL12/IL23, IL17 and CD28 lymphocytes are less implicated in this process.

No diagnostic gold standard exists for LTBI status. The tuberculin skin test (TST) and interferon-γ release assays (IGRAs) are the available commercial tests for detecting LTBI. IGRAs have several advantages: the results are not affected by Bacillus Calmette-Guérin (BCG)-vaccination and by the majority of environmental Mycobacteria; they are laboratory tests with internal positive and negative controls; moreover, only one patient-visit is required. However, since these assays are based on an immune response detection, they have a reduced sensitivity in children below 5 years of age and in immune-compromised subjects. Furthermore, they do not discriminate between active TB and LTBI and poorly correlate with the risk of developing active disease.[8–12]

In the last few years, several studies have described the role of CD8 T-cell response in TB. Mtb-specific CD8 T-cells are increased in patients with active TB, both in HIV-uninfected and -infected patients and in recent infections exposed to a smear-positive active TB case.[13–18] An increase of the CD8 T-cell responses associates with Mtb load, importantly, longitudinal studies have shown a decrease of the CD8 T-cell response during anti-TB treatment.[19]

QuantiFERON-TB-Plus (QFT-P) is an improved version of the previous QuantiFERON-TB Gold In-tube. It is now optimized with innovative Mtb-specific antigens that elicit both CD4 and CD8 T-cell responses, enabling a more accurate assessment of cell-mediated immune response to TB infection.[17,20] QFT-P is based on IFN-γ detection after stimulation by Mtb antigens included in TB1 and TB2 tubes in which a “CD4 only” or a “CD4 and CD8” response is elicited, respectively. Recently it has been demonstrated that QFT-P antigens could be also used to evaluate IP-10 as biomarker for the diagnosis of LTBI.[21,22]

Few data are available on the accuracy of the QFT-P in vulnerable populations. Recently, in Japan, an intermediate TB endemic country, QFT-P was shown to be more sensitive than T–SPOT.TB for LTBI detection in patients with rheumatoid arthritis (RA).[23] However, it is not available a detailed analysis of the response to QFT-P in IMID patients compared to NON-IMID subjects.

Therefore, the aim of this study is to compare the QFT-P response of IMID-LTBI patients candidates to a new biological therapy vs LTBI subjects without IMID in terms of amount of IFN-γ response, discordant results between TB1 and TB2, impact of the specific immune suppressive therapy and type of TB exposure.

Materials and methods

Study participants

This study was conducted at the National Institute for Infectious Diseases (INMI) L. Spallanzani and approved by the INMI Ethical Committee (approval number 72/2015). Informed written consent was required to participate in the study. Patients with IMID candidates to a new biological treatment were prospectively enrolled from October 2015 until January 2019 if they had a concomitant condition of LTBI. As controls, 106 subjects with LTBI without IMID, in part recently described in another report,[17] were also enrolled.

IMID patients, having positive QFT-P results, were sent from the IMID physicians (FC, RS, GB, UM) to the infectious disease specialist (mainly to DG) because it is well known that IMID status associates with a higher risk to progress to active TB,[24] NON-IMID-LTBI were tested because contacts of active TB patients or screened for work requirements, immigration or adoption reasons. LTBI diagnosis were based on a positive QFT-P in the absence of radiological signs of active TB and clinical symptoms of TB disease. All subjects were interviewed regarding Mtb exposure. All subjects resulted negative for HIV infection. None of the enrolled patients developed active TB during the time of observation (at least 6 months after the enrolment). To perform this study, we followed the STROBE statement-checklist for case-control studies (https://www.strobe-statement.org/fileadmin/Strobe/uploads/checklists/STROBE_checklist_v4_case-control.pdf).

QuantiFERON-TB-Plus (QFT-P)

QFT-P tubes (a gift from Qiagen) were used and the assay was performed according to manufacturer’s instructions. Plasma supernatants were collected and IFN-γ was measured by ELISA. We defined as discordant responders the subjects responding only to the TB1 or TB2. The results were analyzed by a QFT-P Analysis Software (www.quantiFERON.com) and evaluated according to manufacturer’s criteria.

Statistical analysis

Data analysis was performed using STATA (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) and Prism 6 software (Graphpad Software 6.0, San Diego, USA). Categorical measures were compared using Chi-square test or Fisher’s exact test. For continuous measures medians and interquartile ranges (IQR) were reported and Kruskall-Wallis or Mann-Whitney U test were used for comparison across groups. We ran a logistic regression models to evaluate the association of distribution of IFN-γ results (dichotomized in $\leq$0.7 IU/mL and $>$0.7) with: (a) demographics characteristics, immunological parameters, IMID treatments; (b) IMID and NON-IMID status. For logistic regression analysis, the odds ratio (OR), 95% confidence intervals (CI) and p value have been reported.

Results

Demographic and epidemiological characteristics of the population

We enrolled 167 subjects: 106 were defined as NON-IMID-LTBI and 61 IMID-LTBI. LTBI was defined based on a positive QFT-P test. Women were 51% of the population, 62% of the enrolled subjects were from Western Europe (mainly Italy), while 22% were from Eastern Europe. Around 40% of the population was BCG-vaccinated. Significant differences in age, origin, BCG-vaccination and exposure were observed comparing all groups (Table 1). Regarding Mtb exposure, within the IMID-LTBI group, almost all subjects reported a remote contact (at least three years before) with smear-positive active TB patients (98%), while only one reported a recent infection (no more than 3 months before) (2%). All the IMID-LTBI patients were screened for Mtb infection because candidate to biological therapy. Conversely, among the NON-IMID-LTBI group, 72 reported a recent exposure (68%) whereas 34 reported a remote exposure to TB cases (32%) ($p < 0.0001$) (Table 1). Among the NON-IMID-LTBI subjects, 18 individuals have been screened for job reasons, 15 for immigration requirements, 1 person for adoption request and 72 because contacts of smear positive TB patients (Table 1). The majority of the IMID-patients had RA (31%) and psoriatic arthritis (23%) (Table 1). Information about disease activity were available only for 35 patients, and among them 83% had an active IMID (Table 1).

QFT-P response in LTBI subjects with or without IMID

QFT-P assay was performed for each patient and the IFN-γ production was evaluated by ELISA in response to QFT-

Table 1
Demographic and epidemiological characteristics of the subjects enrolled in the study.

Characteristics	IMID-LTBI	NON-IMID-LTBI	Total	*p* value
N. (%)	61 (36.5)	106 (63.5)	167	
Age, median (IQR)	64 (52.5-72)	42.5 (26–55)	49.5 (36–63.5)	<0.0001[a]
Female gender, *n* (%)	29 (47.5)	56 (52.8)	85 (50.9)	0.525[b]
Origin, *n* (%)				
Western Europe	49 (8.30)	55 (51.9)	104 (62.3)	0.008[c]
Eastern Europe	8 (13.1)	29 (27.4)	37 (22.2)	
Asia	2 (3.3)	10 (9.4)	12 (7.2)	
Africa	1 (1.6)	9 (8.5)	10 (5.9)	
South America	1 (1.6)	3 (2.8)	4 (2.4)	
BCG vaccinated, *n* (%)	12 (19.7)	52 (49.1)	64 (38.3)	<0.0001[b]
Exposure, *n* (%)				
Recent	1 (2)	72 (68)	73 (44)	<0.0001[b]
Remote	60 (98)	34 (32)	94 (56)	
Preventive therapy due to: *n* (%)				
Contact with pulmonary TB patient	0 (0)	72 (68)	72 (43)	NA
Contact with pulmonary TB patient and candidate to biological therapy	1 (2)	0 (0)	1 (0.5)	
Candidate to biological therapy	60 (98)	0 (0)	60 (36)	
Screening for job requirements		18 (17)	18 (11)	
Immigration screening		15 (14)	15 (9)	
Requirements for adoption		1 (1)	1 (0.5)	
IMID, N (%)				
Rheumatoid Arthritis	19 (31.1)	–		
Psoriatic Arthritis	14 (22.9)	–		
Ankylosing Spondylitis	11 (18.0)	–		
Horton Arteritis	3 (4.9)	–		-
Polymyalgia rheumatica	2 (3.3)	–		
Psoriasis	2 (3.3)	–		
Others IMID	10 (16.4)	–		
Disease activity of IMID LTBI, *n* (%)[d]				
Active disease	29 (83)	–		
Remittent disease	6 (17)	–		

IMID: immune-mediated inflammatory diseases; LTBI: latent tuberculosis infection; Others IMID: Rheumatoid Arthritis and Horton Arteritis; Rheumatoid Arthritis and Sjogren's syndrome; Undifferentiated spondyloarthritis; Non-radiographicSpondyloarthritis, Psoriasis and Spondyloarthritis; Lupus Erythematosus; Pemphigus; Undifferentiated connective tissue disease; Systemic vasculitis; Cryoglobulinemia HCV. N.: number; IQR: interquartile range; BCG: Bacillus Calmette et Guérin; NA: not applicable test.

[a] Kruskal–Wallis test.
[b] Fisher's exact test.
[c] Chi-square test.
[d] information available for only 35 IMID subjects, therefore the percentage is calculated on 35 subjects.

P antigens (TB1 and TB2) (Fig. 1(A)) and mitogen (Fig. 1(B)). Interestingly, we found a significant lower IFN-γ response to both TB1 and TB2 antigens in IMID-LTBI patients compared to NON-IMID-LTBI group ($p = 0.0009$ and $p = 0.0012$, respectively) (Fig. 1(A)). To better evaluate the IFNγ responses to mitogen stimulation, we performed the analysis using the values without transforming the IFNγ values > 10 IU/mL in values = 10 (Fig. 1(B)) and the values converted in 10 IU/mL (Fig. 1(C)). Although all the subjects were mitogen-responders, the response of IMID-LTBI group was significantly lower compared to NON-IMID-LTBI (comparison with unconverted IFNγ values: $p < 0.0001$; comparison with converted IFNγ values: $p = 0.0003$) (Fig. 1(B) and (C)).

Considering that the majority of IMID-LTBI subjects had a remote exposure to Mtb whereas the NON-IMID-LTBI included both recently and remotely exposed subjects (Table 1), we compared the two groups stratifying the patients according to the time of exposure (Fig. 2). Interestingly, the IMID-remote LTBI subjects showed a lower amount of IFNγ compared to NON-IMID remote LTBI (TB1 stimulation: IMID 1.7 IU/mL, IQR 0.6-5.7; NON-IMID 4 IU/mL, IQR 0.8-10, $p = 0.1308$; TB2 stimulation: IMID 1.5 IU/mL IQR 0.6-6.6, NON-IMID 4.1 IU/mL IQR 0.9-10, $p = 0.05$). This difference was even more evident comparing the IMID-LTBI with the NON-IMID recent LTBI (TB1 stimulation: IMID 1.7 IU/mL IQR 0.6-5.7; NON-IMID 5.2 IU/mL IQR 1.8-10, $p = 0.0001$; TB2 stimulation: IMID 1.5 IU/mL IQR 0.6-6.6, NON-IMID 4.3 IU/mL IQR 1.7-10, $p = 0.0013$). Therefore, IMID-LTBI patients had a profile of response to Mtb antigen more similar to NON-IMID LTBI subjects with remote exposure.

Detailed analysis of the response to TB1 and TB2 in LTBI with or without IMID

Focusing on the 61 IMID-LTBI QFT-P-positive subjects, we found that all of them responded to TB1 and 93% responded to TB2. Similarly, we found that 95% of NON-IMID-LTBI subjects responded to TB1 and 96% responded to TB2 (Table 2). Furthermore, we did not find any significant difference, comparing the score of the QFT-P response to "TB1 and TB2", or "only TB1" or "only TB2", in LTBI with and without IMID (Table 2).

Selectively evaluating the QFT-P discordant responders, we found 4 IMID-LTBI (6.5%) with a "only TB1" response with IFN-γ values falling in the grey zone (Table 3). Similarly, in the NON-IMID-LTBI group, we found 9 discordant responders (8%), 4 with a "only TB1" response and 5 with a "only TB2" response, 6 out 8 subjects had IFN-γ values falling in the uncertain range of 0.2-0.7 IU/mL (Table 3). Nine out of the 13 subjects with discordant results reported a previous remote exposure to Mtb. Comparing by Fisher's exact test the number of discordant responders, we did not find any significant difference between the IMID-LTBI vs NON-IMID-LTBI group ($p = 0.6$) (Table 3).

Moreover, we evaluated the impact of IMID on the production of IFN-γ in response to QFT-P, stratifying the IFN-γ values in 4

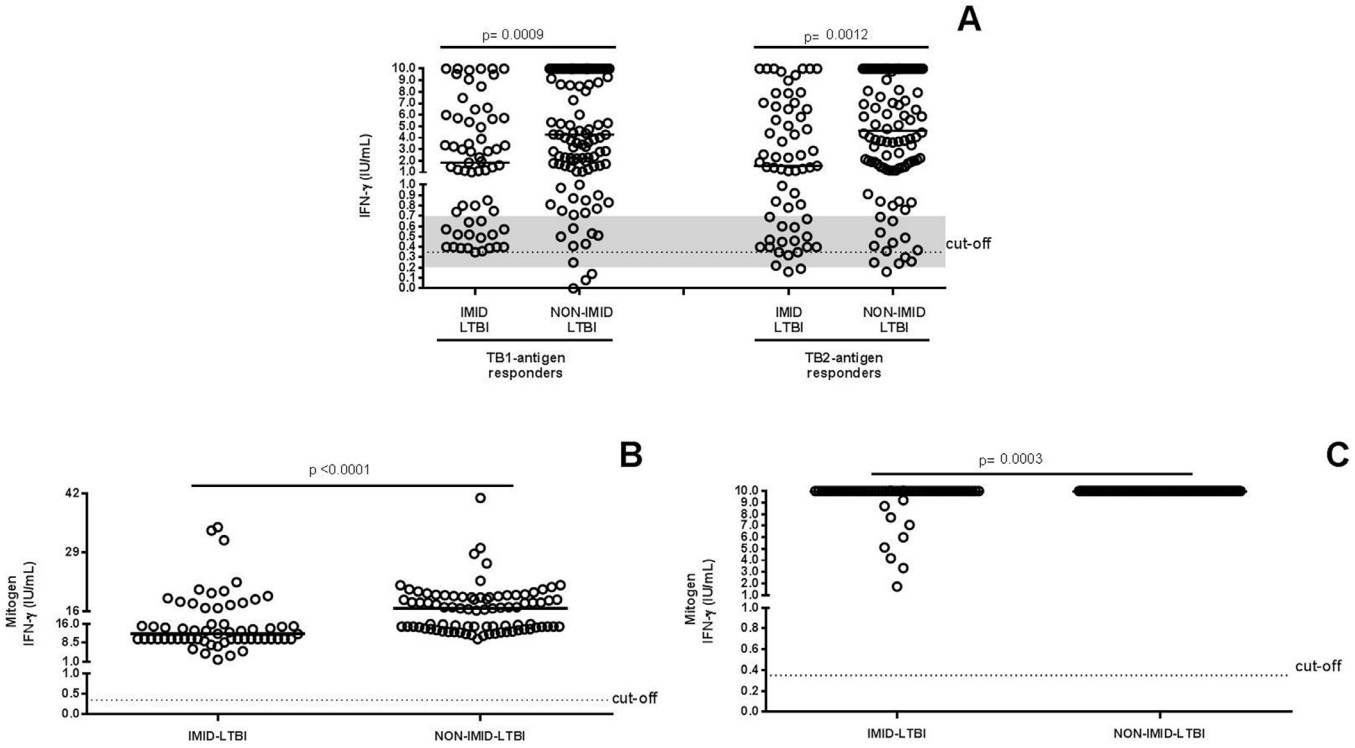

Fig. 1. Evaluation of IFN-γ production in response to QFT-P antigens and mitogen in LTBI subjects with or without IMID. The IFN-γ production was evaluated by ELISA in response to QFT-P antigens (A: TB1 and TB2 tubes) (B and C: mitogen). For 22 NON- IMID LTBI subjects the value of IFNg was not available. Horizontal lines indicate the median. The dotted line represents the cut-off value of 0.35 IU/mL. The gray section represents the IFN-γ values ranging in 0.2–0.7 IU/mL the so called "uncertainty zone". To better evaluate the IFNγ responses to mitogen stimulation, we performed the analysis using the values without transforming the IFNγ values $>$ 10 IU/mL in values $=$ 10 (B) and the values converted in 10 IU/mL (C). Statistical analysis was performed using the Mann–Whitney U test and the p value was considered significant if $\leq$0.05. TB: tuberculosis, LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; QFT-P: QuantiFERON-TB-Plus; TB1: tube 1; TB2: tube 2; IU: international unit; IFN: interferon.

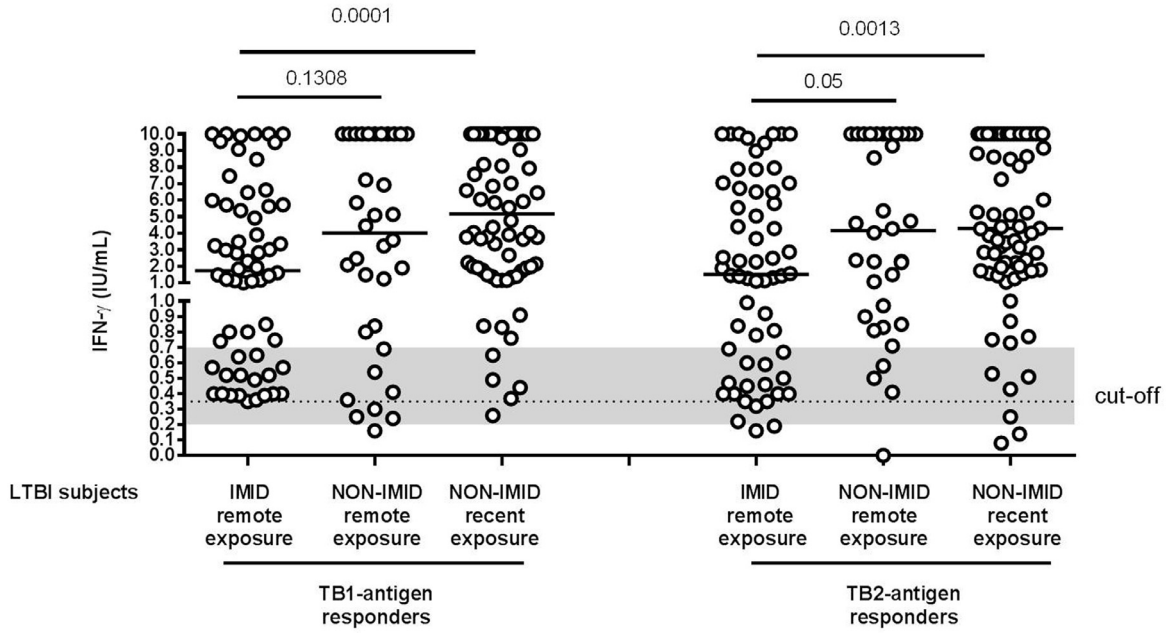

Fig. 2. Evaluation of IFN-γ production in response to QFT-P antigens and mitogen in LTBI subjects with or without IMID according to the Mtb exposure. The IFN-γ production was evaluated by ELISA in response to QFT-P antigens (A: TB1 and TB2 tubes). The NON-IMID LTBI subjects were stratified in recent (72) and remote (34) Mtb infection. For the IMID-LTBI group we considered only the remotely exposed subjects (60). Statistical analysis was performed using the Mann-Whitney U test and the p value was considered significant if $\leq$0.05. TB: tuberculosis, LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; QFT-P: QuantiFERON-TB-Plus; TB1: tube 1; TB2: tube 2; IU: international unit; IFN: interferon.

Table 2
Analytical evaluation of QFT-P results: lack of differences comparing IMID and NON-IMID LTBI subjects.

QFT-P response to:	IMID-LTBI (N. 61) Positive N. (%)	NON-IMID-LTBI (N. 106) Positive N. (%)	Comparison p^a value
TB1 and TB2	57 (93)	97 (91)	0.8
only TB1	4 (7)	4 (4)	0.5
only TB2	0 (0)	5 (5)	0.1
TB1	61 (100)	101 (95)	0.1
TB2	57 (93)	102 (96)	0.5

QFT-P: QuantiFERON-TB-Plus; LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; TB1: tube 1; TB2: tube 2; N.: number.
[a] Fisher's exact test.

Table 3
Proportion of QFT-P discordant-responders is similar in LTBI subjects with or without IMID.

	Discordant results over total (%)	Patient N.	Exposure	TB1 IFN-γ IU/mL	TB2 IFN-γ IU/mL	p^a
IMID-LTBI	4/61 (6.5)	Pt 1	Remote	0.52	0.16	0.6
		Pt 2	Remote	0.52	0.19	
		Pt 3	Remote	0.64	0.22	
		Pt 4	Remote	0.39	0.32	
NON-IMID-LTBI	9/106 (8)	Pt 1	Remote	0.84	0	
		Pt 2	Remote	0.25	0.83	
		Pt 3	Remote	0.30	0.41	
		Pt 4	Remote	0.24	0.97	
		Pt 5	Remote	0.16	0.58	
		Pt 6	Recent	1.41	0.08	
		Pt 7	Recent	0.37	0.25	
		Pt 8	Recent	1.49	0.14	
		Pt 9	Recent	0.26	0.43	

QFT-P: QuantiFERON-TB-Plus; LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; TB1: tube 1; TB2: tube 2; N.: number.
[a] Fisher's exact test comparison between number of QFT-P discordant responders in IMID-LTBI vs NON-IMID-LTBI.

Table 4
IMID LTBI patients have a significant higher proportion of QFT-P responders falling in the grey zone compared to NON-IMID subjects.

QFT-P results	IFN-γ value IU/mL	TB1			TB2		
		IMID-LTBI (N. 61) Number (%)	NON-IMID-LTBI (N. 106) Number (%)	Comparison p^a value	IMID-LTBI (N. 61) Number (%)	NON-IMID-LTBI (N. 106 Number (%)	Comparison p^a value
Positive	≥0.7	44 (72)	93 (88)	**0.02**	43 (70.5)	96 (90)	**0.001**
	0.35-0.69	17(28)	8 (7)	**0.0006**	14 (23)	6 (6)	**0.002**
Negative	0.20–0.34	0 (0)	4 (4)	0.3	2 (3.3)	1 (1)	0.5
	<0.2	0 (0)	1 (1)	>0.9	2 (3.2)	3 (3)	>0.9

QFT-P: QuantiFERON-TB-Plus; LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; TB1: tube 1; TB2: tube 2; N.: number; N.A.: not available.
[a] Fisher's exact test.

categories as previously reported:[25] ≥0.7 IU/mL or between 0.35 and 0.69 IU/mL (positive range); between 0.2 and 0.34 IU/mL or <0.2 IU/mL (negative range) (Table 4).

Interestingly, among those scored positive with IFN-γ values ≥0.7 IU/mL, we found a significant higher proportion of responders in NON-IMID-LTBI subjects compared to IMID-LTBI, (TB1 stimulation $p = 0.02$; TB2 stimulation $p = 0.001$) (Table 4). Differently, in those with a low range of IFNγ production (gray zone: values between 0.35 and 0.69 IU/mL), IMID-LTBI patients had a higher proportion of responders to both TB1 and TB2 antigens ($p = 0.0006$ and $p = 0.002$, respectively) compared to NON-IMID-LTBI subjects (Table 4).

Considering the different amount of IFNγ values in recent and remote NON-IMID LTBI subjects compared to IMID-LTBI patients (Fig. 2), we evaluated the distribution of IFNγ results according to the time of Mtb exposure (Table 5). We found that NON-IMID recent LTBI subjects had a significant higher number of TB1 responders with IFNγ ≥0.7 IU/mL compared to the IMID-remote LTBI patients ($p = 0.002$), similar results were found in response to TB2 stimulation ($p = 0.0015$). Differently, IMID-remote LTBI subjects had a higher number of responders falling in the IFNγ range 0.35–0.69 IU/mL (TB1 stimulation: $p = 0.0006$; TB2 stimulation: $p = 0.0013$).

Based on these findings, we stratified the IFNγ results as >0.7 IU/mL and ≤0.7 IU/mL and we calculated the odds ratio (OR), adjusted for age gender and origin (aOR), to have IFNγ results ≤0.7 IU/mL comparing the IMID-LTBI with the reference group of NON-IMID LTBI subjects (Table 6).

Interestingly, the probability to have IFNγ results ≤0.7 IU/mL was higher in IMID-LTBI patients compared to non IMID-LTBI subjects (TB1 stimulation: aOR: 3.32 and 95%CI: 1.24–8.90, $p = 0.017$; TB2 stimulation aOR: 4.33 and 95%CI: 1.53–12.24, $p = 0.006$) (Table 6).

Since the majority of IMID-LTBI patients had a Mtb remote exposure we performed the same analysis only in those with remote exposure (Table 6). Interestingly, we found that the high risk of IMID-LTBI patients to have IFNγ results ≤0.7 IU/mL was not confirmed for TB1 results (aOR 1.63, 95% CI: 0.47–5.60, $p = 0.439$). For TB2 results the higher risk was confirmed although the estimate was imprecise (aOR: 3.23, 95%CI: 0.80–13.05; $p = 0.099$).

Table 5
Distribution of positive IFN-γ values of QFT-P results in LTBI subjects with or without IMID according to the time of Mtb-exposure.

Time of exposure		TB1					TB2				
		Column A Remote	Column B Remote	Column C Recent	Columns-Comparison		Column A Remote	Column B Remote	Column C Recent	Columns-Comparison	
		IMID-LTBI (N. 60)	NON-IMID-LTBI (N. 34)	NON-IMID-LTBI (N. 72)	A vs B	A vs C	IMID-LTBI (N. 60)	NON-IMID-LTBI (N. 34)	NON-IMID-LTBI (N. 72)	A vs B	A vs C
QFT-P results	IFN-γ value IU/mL	Number (%)	Number (%)	Number (%)	p^a value	p^a value	Number (%)	Number (%)	Number (%)	p^a value	p^a value
Positive	≥0.7	43 (72)	26 (76)	67 (93)	0.8	**0.002**	42 (70)	30 (88)	66 (92)	0.07	**0.0015**
	0.35-0.69	17(28)	4 (12)	4 (6)	0.07	**0.0006**	14 (23.333)	3 (9)	3 (4)	0.098	**0.0013**

QFT-P: QuantiFERON-TB-Plus; LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; TB1: tube 1; TB2: tube 2; N.: number; N.A.: not available.
[a] Fisher's exact test.

Table 6
IMID-LTBI patients display a higher OR to have IFNγ results ≤0.7 IU/mL in response to QFT-P antigens, compared to NON-IMID-LTBI subjects.

		Results			Logistic Regression	
		≤0.70 N%	>0.70 N%	Total N%	(OR for IFNγ ≤0.70) aOR (95%CI)	p^a
TB1						
all patients	NON-IMID LTBI	13 (43.3)	93 (67.9)	106 (63.5)	1	
	IMID LTBI	17 (56.7)	44 (32.1)	61 (36.5)	3.32 (1.24–8.90)	**0.017**
Remote exposure	NON-IMID LTBI	8 (32.0)	26 (37.7)	34 (36.2)	1	
	IMID LTBI	17 (68.0)	43 (62.3)	60 (63.8)	1.63 (0.47–5.60)	0.439
TB2						
all patients	NON-IMID LTBI	10 (35.7)	96 (69.1)	106 (63.5)	1	
	IMID LTBI	18 (64.3)	43 (30.9)	61 (36.5)	4.33 (1.53–12.24)	**0.006**
Remote exposure	NON-IMID LTBI	4 (18.2)	30 (41.7)	34 (36.2)	1	
	IMID LTBI	18 (81.8)	42 (58.3)	60 (63.8)	3.23 (0.80–13.05)	0.099

QFT-P: QuantiFERON-TB-Plus; LTBI: latent tuberculosis infection; IMID: immune-mediated inflammatory diseases; TB1: tube 1; TB2: tube 2; N.: number; aOR: Odds ratio adjusted for age, gender and origin. CI: Confidence Interval.
[a] Wald Test.

In conclusion, these data suggest that the high risk of IMID-LTBI subjects to have IFNγ results ≤0.7 IU/mL is influenced by both, time of exposure to Mtb and IMID status.

Stratification of QFT-P response in ranges based on the type of immune suppressive therapy in IMID-LTBI

Applying a univariable logistic regression analysis we investigated, in the IMID-LTBI subjects, the association of TB1 and TB2 results with demographic data, immunological parameters and treatment (Table 7). We did not observe any association with any of the parameters considered. Interestingly, the type of IMID treatment and the absolute number of lymphocytes and monocytes did not have any effect on the distribution of the IFNγ values.

Discussion

In this study, we evaluated in a low TB endemic country such as Italy, the response of the QFT-P in LTBI individuals with or without IMID.

We reported a significant lower amount of IFNγ released in response to TB1 and TB2 stimulation in IMID-LTBI compared to NON-IMID-LTBI. Interestingly, the IMID-LTBI patients had a low mitogen response compared to NON-IMID LTBI subjects. However all IMID-LTBI patients had positive mitogen response with IFNγ values greater than 1 IU/mL, as previously showed in patients with long-term anti-TNFα treatment.[26] High QFT-P response is associated with a recent exposure to TB index cases.[16] Therefore, the low amount of IFNγ response found in this study in IMID-LTBI patients may be due to the remote exposure reported in all except one patient. Comparing the groups of IMID-remote LTBI and NON-IMID remote LTBI, we observed lower level of IFNγ in IMID subjects, even if this difference was significant only in response to TB2-peptides. On the contrary, the NON-IMID recent LTBI subjects had IFNγ values much higher than IMID-LTBI. Based on these findings, it is important to consider that a combination of parameters such as the IMID status and the time of exposure to Mtb, may modulate the amount of the Mtb-specific response.

We demonstrated that the IMID status did not have any impact on the ability to respond to both TB1 and TB2 stimulation. We found a similar proportion of discordant TB1 and TB2 results, as previously reported in immunocompetent and immunocompromised LTBI subjects.[27] Moreover, discordant results were characterized by values falling in the uncertain range,[28] as already described.[25,27] In the present study, the number of total lymphocytes and monocytes did not affect the TB1 and TB2 response and all the subjects were mitogen-responders. Therefore, we cannot provide any specific reason to explain the false negative results among the discordant responders and, interestingly, we did not find an association between the discordant results and the IMID status. However, it is possible that the remote exposure, observed in the majority of LTBI subjects with discordant results, is a critical factor to determine the level of IFNγ production. In support of this, we found that the high risk of IMID-LTBI subjects to have IFNγ results ≤0.7 IU/mL is influenced by both exposure and IMID status.

Recently, a retrospective study performed in Netherland demonstrated a significant excess of borderline QFT results over random variability and an association between the results falling in the gray-zone range and relevant risk factors and/or evidence of Mtb infection.[29] Furthermore, a case report from the same authors showed a case of TB in a pregnant woman during infliximab therapy.[30] This patient had a negative borderline QFT result before starting the biological therapy and therefore the preventive TB treatment was not proposed.[30] According to the authors, a borderline QFT result in vulnerable populations, with or without any TB risk factor, should be considered as a TB-specific response that justifies the TB preventive therapy.[30] In our study, the IMID-LTBI

Table 7
Lack of association of IFNγ results obtained after TB1 and TB2 stimulation in IMID-LTBI subjects, with the demographic data, immunological parameters and IMID treatments.

Characteristics	Univariable Logistic Regression (OR for IFNγ ≤0.70)									
	TB1 results					TB2 results				
	≤0.70	>0.70	Total	OR (95%CI)	*p*	≤0.70	>0.70	Total	OR (95%CI)	***p***
Gender (N%)										
Male	8 (47.1)	24 (54.5)	32 (52.5)	1		9 (50)	23 (53.5)	32 (52.5)	1	
Female	9 (52.9)	20 (45.5)	29 (47.5)	1.35 (0.44–4.15)	0.600	9 (50)	20 (46.5)	29 (47.5)	1.15 (0.38–3.45)	0.804
Origin (N%)										
Other country	6 (35.3)	11 (25)	17 (27.9)	1		6 (33.3)	11 (25.6)	17 (27.9)	1	
Italy	11 (64.7)	33 (75)	44 (72.1)	0.61 (0.18–2.04)	0.424	12 (66.7)	32 (74.4)	44 (72.1)	0.68 (0.21–2.27)	0.539
Age (median IQR)	64 (53–68)	65 (53.5–73.5)	64 (53–72)	1.00 (0.96–1.04)	0.942	64 (53–68)	65 (52–73)	64 (53–72)	1.00 (0.96–1.05)	0.924
Lymph[a] (median IQR)	2.1 (1.6–2.6)	2.5 (1.7–2.5)	2.2 (1.7–2.5)	0.77 (0.32–1.84)	0.553	2.1 (1.4–2.7)	2.2 (1.8 –2.4)	2.2 (1.7–2.5)	0.76 (0.32–1.81)	0.542
Mono[b] (median IQR)	0.5 (0.4–0.6)	0.5 (0.4–0.7)	0.5 (0.4–0.7)	1.29 (0.51–3.26)	0.594	0.4 (0.3–0.6)	0.5 (0.4–0.8)	0.5 (0.4–0.7)	1.14 (0.44–2.93)	0.784
BCG vaccination (N%)										
No	12 (70.6)	37 (84.1)	49 (80.3)	1		13 (72.2)	36 (83.7)	49 (80.3)	1	
Yes	5 (29.4)	7 (15.9)	12 (19.7)	2.20 (0.59–8.24)	0.241	5 (27.8)	7 (16.3)	12 (19.7)	1.98 (0.53–7.34)	0.308
Immunosuppresive drugs (N%)										
No	8 (50)	22 (51.2)	30 (50.8)	1		9 (52.9)	21 (50)	30 (50.8)	1	
Yes	8 (50)	21 (48.8)	29 (49.2)	1.05 (0.33–3.30)	0.937	8 (47.1)	21 (50)	29 (49.2)	0.89 (0.29–2.75)	0.838
Cortisone therapy (N%)										
No	9 (56.3)	28 (65.1)	37 (62.7)	1		10 (58.8)	27 (64.3)	37 (62.7)	1	
Yes	7 (43.8)	15 (34.9)	22 (37.3)	1.45 (0.45–4.68)	0.532	7 (41.2)	15 (35.7)	22 (37.3)	1.26 (0.40–3.99)	0.695
Biologic therapy (N%)										
No	11 (68.8)	26 (60.5)	37 (62.7)	1		11 (64.7)	26 (61.9)	37 (62.7)	1	
Yes	5 (31.3)	17 (39.5)	22 (37.3)	0.70 (0.21–2.36)	0.560	6 (35.3)	16 (38.1)	22 (37.3)	0.89 (0.27–2.87)	0.840
FANS therapy (N%)										
No	12 (75)	29 (67.4)	41 (69.5)	1		13 (76.5)	28 (66.7)	41 (69.5)	1	
Yes	4 (25)	14 (32.6)	18 (30.5)	0.69 (0.19–2.53)	0.576	4 (23.5)	14 (33.3)	18 (30.5)	0.62 (0.17–2.24)	0.461
Nor biologic- nor cortisone- based therapy (N%)										
No	11 (68.8)	30 (69.8)	41 (69.5)	1		12 (70.6)	29 (69)	41 (69.5)	1	
Yes	5 (31.3)	13 (30.2)	18 (30.5)	1.05 (0.30–3.63)	0.940	5 (29.4)	13 (31)	18 (30.5)	0.93 (0.27–3.18)	0.907
Biologic vs combined therapy: (N%)										
• Biologic	4 (25.0)	10 (23.3)	14 (23.7)	1		5 (29.4)	9 (21.4)	14 (23.7)	1	
• Immunosuppressive drugs and cortisone	3 (18.7)	6 (13.9)	9 (15.2)	1.25 (0.20–7.61)	0.809	3 (17.6)	6 (14.3)	14 (23.7)	0.90 (0.15–5.25)	0.907
• Other drugs	9 (56.2)	27 (62.8)	36 (61.0)	0.83 (0.21-3.32)	0.796	9 (52.9)	27 (64.3)	36 (61.1)	0.60 (0.16–2.26)	0.451
Biologic vs combined therapy: (N%)										
• Biologic	4 (25.0)	10 (23.3)	14 (23.7)	1		5 (29.4)	9 (21.4)	14 (23.7)	1	
• Immunosuppressive drugs, cortisone and FANS	5 (31.2)	8 (18.6)	13 (22.0)	1.56 (0.31–7.82)	0.587	5 (29.4)	8 (19.1)	13 (22.0)	1.12 (0.23–5.37)	0.883
• Other drugs	7 (43.7)	25 (58.1)	32 (54.3)	0.70 (0.17–2.93)	0.625	7 (41.2)	25 (59.5)	32 (54.3)	0.50 (0.13–2.00)	0.330
Total (N%)	17 (27.9)	44 (72.1)	61 (100)			18 (29.5)	43 (70.5)	61 (100)		

TB1: tube 1; TB2: tube 2; OR: Odds Ratio. CI: Confidence Interval. Drugs association have been evaluated in 59 IMID-LTBI, because 3 patients were not in therapy at the time of enrollment; OR for one-unit increase.
[a] 3 missing values.
[b] 4 missing values.

patients had a higher probability to have results falling in the grey-zone range compared to NON-IMID group. Stratifying the patients according to the IMID treatment, we demonstrated that the type of IMID treatment did not have any impact on the distribution of IFNγ values in IMID-LTBI patients. Although the treatment seems not interfering with the distribution of IFNγ values, it may be important to consider with caution the QFT-P borderline results, in order to exclude false negative results due to the IMID status. A potential approach to avoid borderline results, is to perform long-term Mtb antigens-stimulation to recall a specific T memory response and consequently a higher IFNγ production, as previously shown in LTBI patients.[31–33]

The present study concerns only IMID patients scored positive to QFT-P and sent to infectious disease specialists because IMID associates with a high risk of progression to active TB.[24,34–36] Since we could not evaluate the prevalence of LTBI in the IMID population, our study is not in contradiction with a recent meta-analysis reporting that anti-TNFα drugs significantly reduce the rate of positive score to IGRA.[37]

Limitations of this prospective study are the relatively low number of patients enrolled; the variety of the IMID considered and the different regimens of therapy for IMID. However, this study is well designed, with inclusion of LTBI subjects from a multicenter study whose results answer, and at the same time opens, important questions on the use of IGRA in patients undergoing biologic therapy.

In conclusion, we evaluated in a low TB endemic country such as Italy, the response to QFT-P in IMID patients diagnosed with LTBI and candidates to a new biological therapy. Comparing IMID-LTBI with NON-IMID-LTBI, we showed that IMID-LTBI had a lower amount of IFN-γ response to QFT-P with a higher proportion of results in the borderline zone and this was not dependent on the type of IMID treatment.

Future studies will help to characterize the real meaning of the borderline zone of QFT-P, especially in the vulnerable populations, to better define the accuracy of the assay in terms of sensitivity, specificity and prediction to active TB development. These results will help in the management of patients taking lifelong immune-suppressive drugs.

Declarations of interest

- Rossana Scrivo has been a consultant for AbbVie, Grünenthal, Janssen Cilag, Novartis, UCB Pharma in 2018; for UCB Pharma in 2019.
- Claudio Mastroianni has been a consultant and presented talks for MSD, AbbVie, GILEAD, ViiV, Janssen Cilag and Angelini in 2018.
- Delia Goletti has been a consultant and presented talks for Quidel, Janssen Cilag and Qiagen in 2018; for Quidel and Diasorin in 2019.
- The other authors do not report any conflict of interest.

Funding

The study was supported by: the Italian Ministry of Health “Ricerca Corrente, linea di ricerca N4, progetto 2 Identificazione e validazione di biomarcatori per la diagnosi e la gestione della infezione/malattia tubercolare” ; GR-2018-12367178; the European Commission (grant number 643381-TBVAC2020-H2020-PHC-2014-2015). The funders as well as the Qiagen company had no role in-study design, analyzing the data, decision to publish, or drafting themanuscript.

Acknowledgments

The authors are grateful to all the patients, nurses (in particular Sara Pantanella, Daniela Milordo, Lorella Tinari, Luciana Dell’Arciprete) and physicians who helped to conduct this study.

Author contributions

TC analyzed, interpreted data and wrote the manuscript; EP analyzed the data, participated to the interpretation of data and to drafting the work; VV processed and analyzed the patient samples, GC and IS participated to the enrollment of IMID patients at different TB stage and LTBI subjects; UM, GB, RS, CM, FP, CE, and FC selected the IMID patients with LTBI candidates to a new biological therapy that were sent to DG and participated to drafting the work; AN performed the statistical analysis, DG designed the study, coordinated and supervised the project, enrolled patients, interpreted data, contributed to the analysis of the data and to the writing of the manuscript. All authors discussed the results and approved the final version of the manuscript.

References

1. WHO. Global *tuberculo*sis report 2018. www.who.int/tb/publications/global_report/en/. 2018.
2. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. *PLoS Med* 2016 25;**13**(10):e1002152.
3. Goletti D, Petrone L, Ippolito G, Niccoli L, Nannini C, Cantini F. Preventive therapy for tuberculosis in rheumatological patients undergoing therapy with biological drugs. *Expert Rev Anti Infect Ther* 2018;**16**(6):501–12.
4. Cantini F, Nannini C, Niccoli L, Petrone L, Ippolito G, Goletti D. risk of tuberculosis reactivation in patients with rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis receiving non-anti-TNF-targeted Biologics. *Mediators Inflamm* 2017;**2017**:8909834.
5. Cantini F, Niccoli L, Goletti D. Tuberculosis risk in patients treated with non-anti-tumor necrosis factor-alpha (TNF-alpha) targeted biologics and recently licensed TNF-alpha inhibitors: data from clinical trials and national registries. *J Rheumatol Suppl* 2014;**91**:56–64.
6. Baddley JW, Cantini F, Goletti D, Gomez-Reino JJ, Mylonakis E, San-Juan R, et al. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective (Soluble immune effector molecules [I]: anti-tumor necrosis factor-alpha agents). *Clin Microbiol Infect* 2018;**24**(Suppl 2):S10–20.
7. Cantini F, Nannini C, Niccoli L, Iannone F, Delogu G, Garlaschi G, et al. Guidance for the management of patients with latent tuberculosis infection requiring biologic therapy in rheumatology and dermatology clinical practice. *Autoimmun Rev* 2015;**14**(6):503–9.
8. Goletti D, Carrara S, Vincenti D, Saltini C, Rizzi EB, Schinina V, et al. Accuracy of an immune diagnostic assay based on RD1 selected epitopes for active tuberculosis in a clinical setting: a pilot study. *Clin Microbiol Infect* 2006;**12**(6):544–50.
9. Goletti D, Carrara S, Butera O, Amicosante M, Ernst M, Sauzullo I, et al. Accuracy of immunodiagnostic tests for active tuberculosis using single and combined results: a multicenter TBNET-Study. *PLoS One* 2008;**3**(10):e3417.
10. Goletti D, Lee MR, Wang JY, Walter N, Ottenhoff THM. Update on tuberculosis biomarkers: from correlates of risk, to correlates of active disease and of cure from disease. *Respirology* 2018;**23**(5):455–66.
11. Abubakar I, Drobniewski F, Southern J, Sitch AJ, Jackson C, Lipman M, et al. Prognostic value of interferon-gamma release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. *Lancet Infect Dis* 2018;**18**(10):1077–87.
12. Kik SV, Schumacher S, Maria Cirillo D, Churchyard G, Boehme C, Goletti D, et al. An evaluation framework for new tests that predict progression from tuberculosis infection to clinical disease. *Eur Respir J* 2018;**52**(4) pii: 1800946. doi:10.1183/13993003.00946-2018.
13. Riou C, Tanko RF, Soares AP, Masson L, Werner L, Garrett NJ, et al. Restoration of CD4+ Responses to Copathogens in HIV-Infected Individuals on Antiretroviral Therapy Is Dependent on T Cell Memory Phenotype. *J Immunol* 2015;**195**(5):2273–81.
14. Chiacchio T, Petruccioli E, Vanini V, Cuzzi G, La Manna MP, Orlando V, et al. Impact of antiretroviral and tuberculosis therapies on CD4(+) and CD8(+) HIV/M. tuberculosis-specific T-cell in co-infected subjects. *Immunol Lett* 2018;**198**:33–43.
15. Chiacchio T, Petruccioli E, Vanini V, Cuzzi G, Pinnetti C, Sampaolesi A, et al. Polyfunctional T-cells and effector memory phenotype are associated with active TB in HIV-infected patients. *J Infect* 2014;**69**(6):533–45.
16. Barcellini L, Borroni E, Brown J, Brunetti E, Campisi D, Castellotti PF, et al. First evaluation of QuantiFERON-TB Gold Plus performance in contact screening. *Eur Respir J* 2016;**48**(5):1411–19.

17. Petruccioli E, Chiacchio T, Pepponi I, Vanini V, Urso R, Cuzzi G, et al. First characterization of the CD4 and CD8 T-cell responses to QuantiFERON-TB Plus. *J Infect* 2016;**73**(6):588–97.
18. Petruccioli E, Vanini V, Chiacchio T, Cuzzi G, Cirillo DM, Palmieri F, et al. Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold In-tube assays in subjects with or without tuberculosis. *Tuberculosis (Edinb)* 2017;**106**:38–43.
19. Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'rie T, et al. Functional capacity of Mycobacterium tuberculosis-specific T cell responses in humans is associated with mycobacterial load. *J Immunol* 2011;**187**(5):2222–32.
20. Sauzullo I, Mengoni F, Mascia C, Pavone P, Savelloni G, Massetti AP, et al. Diagnostic performance in active TB of QFT-Plus assay and co-expression of CD25/CD134 in response to new antigens of Mycobacterium tuberculosis. *Med Microbiol Immunol* 2019;**208**(2):171–83.
21. Petrone L, Vanini V, Chiacchio T, Petruccioli E, Cuzzi G, Schinina V, et al. Evaluation of IP-10 in Quantiferon-Plus as biomarker for the diagnosis of latent tuberculosis infection. *Tuberculosis (Edinb)* 2018;**111**:147–53.
22. Goletti D, Lindestam Arlehamn CS, Scriba TJ, Anthony R, Cirillo DM, Alonzi T, et al. Can we predict tuberculosis cure? What tools are available? *Eur Respir J* 2018;**52**(5) Print 2018 Nov. doi:10.1183/13993003.01089-2018.
23. Igari H, Ishikawa S, Nakazawa T, Oya Y, Futami H, Tsuyuzaki M, et al. Lymphocyte subset analysis in QuantiFERON-TB Gold Plus and T-Spot.TB for latent tuberculosis infection in rheumatoid arthritis. *J Infect Chemother* 2018;**24**(2):110–16.
24. Minozzi S, Bonovas S, Lytras T, Pecoraro V, Gonzalez-Lorenzo M, Bastiampillai AJ, et al. Risk of infections using anti-TNF agents in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis: a systematic review and meta–analysis. *Expert Opin Drug Saf* 2016;**15**(sup1):11–34.
25. Petruccioli E, Chiacchio T, Vanini V, Cuzzi G, Codecasa LR, Ferrarese M, et al. Effect of therapy on Quantiferon-Plus response in patients with active and latent tuberculosis infection. *Sci Rep* 2018;**8**(1) 15626-018-33825-w.
26. Sauzullo I, Scrivo R, Sessa P, Mengoni F, Vullo V, Valesini G, et al. Changes in T cell effector functions over an 8-year period with TNF antagonists in patients with chronic inflammatory rheumatic diseases. *Sci Rep* 2018;**8**(1) 7881-018-26097-x.
27. Pieterman ED, Liqui Lung FG, Verbon A, Bax HI, Ang CW, Berkhout J, et al. A multicentre verification study of the QuantiFERON((R))-TB Gold Plus assay. *Tuberculosis (Edinb)* 2018;**108**:136–42.
28. Nemes E, Rozot V, Geldenhuys H, Bilek N, Mabwe S, Abrahams D, et al. Optimization and interpretation of serial QuantiFERON testing to measure acquisition of mycobacterium tuberculosis infection. *Am J Respir Crit Care Med* 2017;**196**(5):638–48.
29. Uzorka JW, Kroft LJM, Bakker JA, van Zwet EW, Huisman E, Knetsch-Prins C, et al. Proof of concept that most borderline Quantiferon results are true antigen-specific responses. *Eur Respir J* 2017;**50**(5) Print 2017 Nov. doi:10.1183/13993003.01630-2017.
30. Uzorka JW, Delfos NM, Witte AMC, Scheper H, van Soolingen D, Arend SM. Tuberculosis after a borderline QuantiFERON result during screening before infliximab. *Eur Respir J* 2018;**52**(2) Print 2018 Aug. doi:10.1183/13993003.00913-2018.
31. de Paus RA, van Meijgaarden KE, Prins C, Kamphorst MH, Arend SM, Ottenhoff THM, et al. Immunological characterization of latent tuberculosis infection in a low endemic country. *Tuberculosis (Edinb)* 2017;**106**:62–72.
32. Leyten EM, Arend SM, Prins C, Cobelens FG, Ottenhoff TH, van Dissel JT. Discrepancy between Mycobacterium tuberculosis-specific gamma interferon release assays using short and prolonged in vitro incubation. *Clin Vaccine Immunol* 2007;**14**(7):880–5.
33. Butera O, Chiacchio T, Carrara S, Casetti R, Vanini V, Meraviglia S, et al. New tools for detecting latent tuberculosis infection: evaluation of RD1-specific long-term response. *BMC Infect Dis* 2009;**9** 182-2334-9-182.
34. Grzybowsky S. Tuberculosis among patients with various radiologic abnormalities, followed by the chest clinic service. *Am Rev Respir Dis* 1971;**104**(4):605–8.
35. Goletti D, Sanduzzi A, Delogu G. Performance of the tuberculin skin test and interferon-gamma release assays: an update on the accuracy, cutoff stratification, and new potential immune-based approaches. *J Rheumatol Suppl* 2014;**91**:24–31.
36. Goletti D, Petrone L, Ippolito G, Niccoli L, Nannini C, Cantini F. Preventive therapy for tuberculosis in rheumatological patients undergoing therapy with biological drugs. *Expert Rev Anti Infect Ther* 2018;**16**(6):501–12.
37. Wong SH, Gao Q, Tsoi KK, Wu WK, Tam LS, Lee N, et al. Effect of immunosuppressive therapy on interferon gamma release assay for latent tuberculosis screening in patients with autoimmune diseases: a systematic review and meta-analysis. *Thorax* 2016;**71**(1):64–72.