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First evaluation of QuantiFERON-TB Gold Plus performance in contact screening

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Take home message: QFT-Plus improved the diagnostic accuracy for LTBI in the setting of contact screening

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ABSTRACT

Rationale: Identifying latently infected individuals is crucial for tuberculosis elimination. We evaluated for the first time the performance of a new type of interferon-γ release assays, QuantiFERON-TB Plus that includes an additional antigen tube (TB2) stimulating both $CD4^+$ and $CD8^+$ T-cells in contacts of tuberculosis patients.

Materials and Methods: Contacts were screened for latent tuberculosis infection by tuberculin-skin-test, QuantiFERON-TB Plus (QFT-Plus) and QuantiFERON-TB Gold in Tube (QFT-GIT).

Results: In 119 TB contacts, the overall agreement between QFT-Plus and QFT-GIT was high, with a Cohen's kappa of 0.8. Discordant results were found in 12 subjects with negative QFT-GIT and positive QFT-Plus results. In analyses of markers of tuberculosis exposure and tests results, the average time spent with the index case was the strongest risk factor for both tests' positivity. The difference in interferon-γ production between the two antigen tubes (TB2-TB1) was used as an estimate of $CD8^+$ stimulation provided by the TB2. TB2-TB1 values >0.6ml/IU were significantly associated with proximity to the index case and European origin.

Conclusion: QuantiFERON-Plus has a stronger association with surrogate measures of TB exposure than QFT-GIT in adults screened for LTBI. Interferon-γ response in the new antigen tube used an indirect estimate of specific $CD8^+$ response correlates with increased M. tuberculosis exposure suggesting a possible role in identifying individuals with recent infection.

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Key words: latent tuberculosis infection, Interferon-γ release assay**,** $CD8^+$ T-cell,

INTRODUCTION

Despite the progress recently made in tuberculosis (TB) control at a global level, the decline in TB incidence is much slower than that needed to achieve TB elimination by 2050[1]. Identifying and treating symptom-free people who are truly latently infected with *M.tuberculosis* (Mtb) is key to achieving this[2][3]. The current global burden of latent infection is uncertain, although it has been suggested that one third of the world's population may be latently infected with Mtb[4].

Although they show no sign of disease, individuals with latent tuberculosis infection (LTBI) are at risk of reactivating and up to 10% of them may develop active disease in their lifetime[5]. This risk is highest in the first 2 years following infection. Preventive treatment of recently-infected individuals reduces this[6]. However isoniazid preventive therapy is not optimal for a large-scale implementation program, and the current LTBI diagnostic tests – Tuberculin Skin Test (TST) and Interferon-γ release assay (IGRAs)- have significant limitations. TST may be falsely positive due to sensitization by environmental mycobacteria and BCG-vaccination[7]. In recent decades IGRAs measuring the INF-γ concentration after *in vitro* whole blood stimulation with peptides from the RD-1 region of the Mtb genome were developed to improve specificity of the diagnosis[8]. IGRAs are a useful indicator of Mtb exposure as their specificity is very high (97%)[9]. However, like TST, they lose sensitivity in immune-compromised individuals and children[10][11]; they identify both recent and past infection and they are poor at predicting LTBI subjects who are at greater risk of developing disease (positive predictive value IGRA 2,7% TST 1,5%)[12]. As a result when currently available diagnostic tests are used to guide the administration of preventive therapy, the number needed to treat to prevent one case of TB is too high to allow a large-scale preventive program. Different approaches have been described in the literature to help discriminate those at greater risk of active TB development. The use of INF-γ response to the latency antigen Heparin-Binding-Haemoagglutinin (HBHA)[13][14], immunoprofiling[15][16], gene expression pattern (i.e. IL-13 and AIRE)[17][18] and proportion of peripheral blood monocytes[19] have been studied as possible biomarkers for incipient TB. However all of these approaches are still confined to research fields and currently have minimal impact on patient management.

QuantiFERON-TB Plus (QFT-Plus) is a new generation of QTF-Gold In Tube (QFT-GIT) [5] that includes an additional antigen tube (TB2). The TB1 tube contains ESAT-6- and CFP-10-derived peptides (TB-7.7, present in QFT-GIT, has been removed), designed to elicit cell-mediated immune responses from $CD4^+$ T-helper lymphocytes. TB2 contains new peptides

able to stimulate IFN-γ production by both $CD4^+$ and $CD8^+$ T-cells [20].

Evidence supports the important contribution of $CD8^+$ T-cells in host defense against Mtb by both cytokine secretion and cytotoxic activity[21]. Firstly a positive correlation between specific $CD8^+$ T cells and increased mycobacterial load has been found in peripheral blood *ex vivo*[22]. Day et al. reported that more than 60% of individuals with smear-positive TB had detectable $CD8^+$ T cells response compared with 38% and 20% of smear-negative and LTBI respectively. Consistent with this paradigm a higher prevalence of Mtb-specific $CD8^+$ T cells have been reported in smear-positive versus smear-negative patients and in PTB compared with EPTB[23]. In addition, a positive correlation between the $CD8^+$ T cells response against TB antigens and a recent exposure to Mtb have been found. Recent contacts of active TB patients, independent of their response to QTF, have a greater $CD8^+$ T cell response compared to other study groups (active TB patients, health care workers, BCG-vaccinated healthy controls)[24]. This is in agreement with findings observed in a cattle model where a $CD8^+$ T cell response is present at the onset of infection.[25]

The INF-γ release assays currently in use primarily elicit a $CD4^+$ response, but emerging data provide a good rationale for also measuring specific $CD8^+$ T cell responses and in particular to further investigate the association between $CD8^+$ T cells and risk of disease progression.

In the present study we evaluate the performance characteristics of the new QFT-Plus assay in TST-positive contacts with recent exposure to people with confirmed active tuberculosis, assessing the use of QFT-Plus head-to-head with the previous QFT-GIT. In addition, we investigate for the first time the significance and the possible use of the $CD8^+$ INF-γ response provided by the second newly-added antigen tube.

MATERIAL AND METHOD

Study setting and participants

We conducted a cross-sectional study at Villa Marelli-Niguarda Hospital. TB incidence in Milan is of 16.6 new cases per 100.000 persons year (in 2011)[26], three-times higher than the Italian national average. From November 2014 to June 2015 we prospectively recruited TST-positive (TST≥5mm) contacts of notified active TB cases sent by the local public health services to be screened for LTBI.

Contacts were excluded if aged less than 18 years old, a previous positive TST was documented, preventive TB treatment was prescribed or past TB history was reported. Informed written consent was obtained from each study subject.

Contacts reporting mild or severe immunosuppression (diabetes mellitus, chronic kidney

disease, HIV, malignancy, immunosuppressive medications) were included.

The study was approved by the Ethics committee[*].

Contact screening strategy was based on the National Institute of Clinical Excellence TB guidelines 2011 [27] and Italian guidelines which recommends retesting those with positive TST results using an IGRA as confirmatory test. At the contact's first visit health status was established by clinical examination and chest X-ray. Further information on the country of birth, immigration status, nature of the contact to the source case, BCG-vaccination status (if details were unclear inspection of BCG-vaccination scar was performed by trained healthcare-assistants), and clinical history were obtained through personal interviews. When clinical suspicion persisted, chest CT scan and sputum sample analyses were requested.

All patients also underwent testing in line with recommended routine screening as part of contact investigation. Thus, TST-positive contacts who tested negative to a first QFT-GIT analysis were retested with QFT-GIT after 10-12 weeks to exclude delayed conversion.

Blood samples were obtained for QFT-GIT, QFT-Plus and HIV testing from all subjects providing informed consent. QFT-GIT currently in use in clinical practice was performed at Niguarda Microbiology service while QFT-Plus was carried out in the Emerging Bacterial Pathogen Laboratory at San Raffaele Hospital. The QFT-Plus and QFT-GIT tests were performed according to the manufacturer's instructions. Peripheral blood samples for the two tests were obtained simultaneously directly into the QFT tubes and processed within 4 h. Test interpretation for both QFT-Plus and QFT-GIT was performed according to the manufacturer's instruction manual. QFT-GIT results were recorded positive if the antigen response were >0.35 ml/UI above the negative control response. Positivity (antigen response >0.35 ml/UI above the negative control response) of a single antigen tube (either TB1 or TB2) was sufficient to score the QFT-Plus test as positive.

Ascertainment of exposure

We assessed different factors as surrogate markers of Mtb exposure. The aggregate exposure time of contacts prior to the diagnosis of their respective source case was established by recording the extent of the contact during a typical week. TB contacts were categorized according to proximity to the index case[28]: we considered them to be "high proximity" if contacts and case patient were sharing routinely the same bedroom and lower proximity if contacts and case patient were sleeping in a different bedroom in the same house or in a different

[*] (GO/URC/ER/mm prot. n.82/DG, 26 Feb 2010 and successive amendments)

house. Sputum smear positivity of the index case was also assessed as TB case related risk factor.

Statistical Analysis

The agreement between QFT-GIT and QFT-Plus was evaluated by computing the overall percent of concordant results and Cohen's kappa coefficient with 95% Confidence Interval (CI). Univariate logistic regression and backward stepwise multivariate logistic regression models were used to identify factors associated with positive test results. The variables considered in the analyses were: gender, whether the country of birth was an endemic area of TB and whether it was European, BCG vaccination, immunocompromised status, smear status of index case, average time spent per week with the index case and place of sleeping with respect to the index case. The same analysis was performed for the variable denoting whether the differences between QFT-Plus TB2 and QFT-Plus TB1 was greater than the cut-off 0.6 IU/ml (as described in the Results). The level of significance considered was 5%. All statistical analyses were done using R statistical software (version 3.2.3).

RESULTS

A total of 119 Mtb-exposed individuals with positive TST (5mm) were investigated. Of these, 39 were contacts of a smear-negative culture-positive TB case, and 69 of a smear-positive culture positive index case. Participants had a median age of 38 years (25-75 percentile: 30-79), more than half (n=61, 51.26%) were non-European-born, 82 (78.85%) were BCG-vaccinated and 11 (9.24%) were immunocompromised subjects. Demographic characteristics of the cohort are shown in the Table 1.

Agreement between QFT-Plus and QFT-GIT

Sixty-eight out of 119 (57.1%) contacts were QFT-Plus positive. 64 subjects were positive in both antigen tubes, 2 were positive to TB1 only and 2 were positive to TB2 only. Fifty-six of 119 TST-positive contacts were positive to QFT-GIT. The overall agreement between the two IGRAs was high, with a Cohen’s kappa of 0.8 (95% CI 0.69-0.91). The two tests gave concordant results for 107 (89.9%) subjects (see Table 2). Discordant results were found in 12 subjects: they all scored negative to the QFT-GIT and positive to the QFT-Plus. Discordant results between the two IGRAs included the 4 contacts with a single tube QFT-Plus positivity. Moreover, contacts with IGRAs discordant results had overall low INF-γ responses but not as

low to be considered borderline results (median: TB1-Nil=0.83 IU/ml, TB2-Nil=0.73 IU/ml). The characteristics of subjects with discordant results are shown in Table 3. Only one of the 12 contacts with QFT-Plus positive and QFT-GIT negative results had a TST response less than 10mm (7mm). Globally, the median TB1 QFT-Plus antigen IFN-γ level (TB1-Nil) was 0.74 IU/ml, whereas the median TB2 QFT-Plus antigen IFN-γ level (TB2-Nil) was 0.67 IU/ml, as reported in Table 2.

As per the Italian guidelines, contacts of TB cases with initial positive TST results who tested negative to a first QFT-GIT analysis, were re-tested with QFT-GIT at 10-12 weeks. At the post-exposure follow-up, two contacts converted to QFT-GIT positive results. Both of them were part of the 12 contacts who initially showed QFT-plus-positive/QFT-GIT-negative discordant results (Table 2). One of them had a strong QFT-GIT positivity (>10 ml/IU) at 10 weeks post-exposure follow-up; while the second case reported a QFT-GIT of 0.5 ml/UI after 6 month of isoniazid preventive therapy (decision to treat was based on the strong TST positivity and the proximity of contact with the index case). In both cases the TB2 INF-γ response was greater than that found in TB1.

Independent predictors of QFT-Plus and QFT-GIT positivity

For both QFT-GIT and QFT Plus test, the univariate odds ratios of being positive for different possible surrogate markers of increasing exposure to Mtb is presented in Table 4. Contacts reporting that they had spent more than 12 hours per day with the index case were significantly more likely to be both QFT-GIT and QFT-Plus positive, compared to contacts spending 1-4 hours per day with the index case. For a subject with an exposure time > 12 hours, the odds of a positive test were 6 times higher by QFT-GIT and 14 times higher by QFT-Plus. Both test results were significantly more likely to be positive in subjects with closer sleeping proximity to the patient (same house versus different house). The odds of being QFT-GIT positive for subjects sleeping in the same house of the index case were approximately 4 times (different rooms: 3.79; same room: 3.98) higher than for those sleeping in a different house, whereas their odds of being QFT-Plus positive were approximately 6 times (different rooms: 5.78; same room: 5.65). The results of the backward stepwise multivariate logistic regression analysis are presented in Table 5. Only the variable indicating whether a contact spent on average more than 12h per day with the index case remained significantly associated with a positive QFT-GIT result (OR: 4.63; 95% CI: 2.05-10.47) and a positive QFT-Plus result (OR: 6.98; 95% CI: 2.86-17.02).

Predictors for CD8 T-cell stimulation

To assess the specific contribution of $CD8^+$ T cells, we subtracted the quantitative value of the first antigen tube expressed in IU/ml (TB1), which stimulates the $CD4^+$ population only, from the value provided by the second antigen tube (TB2), in which a combined $CD4^+$ and $CD8^+$ T cell stimulation occurred. We used a difference of 0.6 IU/ml to define positive results in order to reduce the bias of the intrinsic variability of the test[29]. Eighteen contacts out of 119 (15.13%) had a difference between TB2 and TB1 greater than 0.6 IU/ml. Univariate logistic regression was used to identify factors associated with differences between TB2 and TB1 > 0.6 IU/ml (Table 4). This method identified sleeping in the same room compared to sleeping in different houses (OR: 4.34; 95%CI: 1.37-13.81), and European origin (OR: 3.24; 95%CI: 1.07-9.75) to be to be significantly positively associated with a greater TB2 response. These associations persisted in the multivariate analysis, shown in Table 6.

DISCUSSION

We provide the first evaluation of QFT-Plus assay alongside the previous version QFT-GIT in a cohort of TST-positive contacts of active TB cases.

Positive results from QFT-Plus were associated with surrogate markers of increasing recent exposure to Mtb. Paired comparison between QFT-GIT and QFT-Plus shows an overall good, but not complete agreement. Furthermore the overall INF-γ response in QFT-Plus positive/QFT-GIT negative contacts was in the majority of cases out of the uncertainty zone for test interpretation[29], suggesting that differences between the tests are not due to test variability. Of note, the disagreement between the two tests all goes in the same direction, with a total of 12 TST-positive contacts positive with the new QFT-Plus and negative to QFT-GIT. With no gold standard for LTBI to refer to, it is difficult to assess whether the discordant results found during the contact screening are attributable to the higher sensitivity of the QFT-Plus test. If the TST were taken as the reference test for LTBI, this would mean that the proportion of TST-positive contacts confirmed by the IGRA test is increased by 17% when using the QFT-Plus compared to QFT-GIT. False positivity with TST is mainly due to sensitization by BCG-vaccination[7]. QFT-Plus specificity in a BCG-vaccinated population has not been investigated yet, however we found that QFT-Plus is not associated with BCG-vaccination both in univariate and multivariate analysis. Moreover only one of the 12 contacts with QFT-Plus positive and QFT-GIT negative result had a TST response less than 10mm while another showed an intense TST positivity which is less likely to be the result of previous vaccination[7]

Recent findings suggest that the discordance between IGRAs and TST in recently-exposed

individuals may be related to delayed conversion of IGRAs relative to TST[30][31]. In this study we find that most of the discordant cases (QFT-GIT negative/QFT-Plus positive) show intense TST positivity; moreover, we reported a shorter period of conversion for QFT-Plus compared to QFT-GIT at least in two individuals of our cohort. These results suggest that QFT-Plus may be more sensitive in detecting new or recent infection with Mtb than the QFT-GIT.

Our data demonstrate that risk factors for test positivity were the same for both IGRAs. QFT-Plus showed stronger associations with surrogate measure of recent exposure than QFT-GIT both in univariate and multivariate analysis The average time spent per day with the index case had the strongest association with test positivity.

We investigated for the first time the difference in INF-γ production between the two QFT-Plus tubes and surrogate markers of increasing exposure. TB2-TB1 differential values were used as an indirect estimate of specific $CD8^+$ stimulation with the newly added antigens. A cut-off value was set at 0.6 ml /IU in order to exclude small variations due to inter-test variability[29]. Positive TB2-TB1 differences (>0.6ml/IU) were significantly associated with sleeping proximity to the index case with an odds ratio comparable to the one obtained in the analysis of QFT-GIT and QFT-Plus (sleeping in the same room compared to sleeping in different houses OR: 4.34; 95%CI: 1.37-13.81). Moreover, European origin (OR: 3.24; 95%CI: 1.07-9.75) was significantly associated with TB2-TB1 > 0.6ml/IU, while it was not statistically significant for the QFT-GIT and the QFT-Plus results.

As individuals from European countries have a low risk for Mtb exposure, these findings are consistent with the hypothesis that the difference in response between the TB1 and TB2 tubes could be used as a surrogate marker of recent exposure (linked to the specific index case exposure), and not to previous cumulative Mtb exposure. A recent flow-cytometry study reported a positive correlation between the $CD8^+$ T cells response against the QFT-GIT antigens and recent exposure to Mtb in contacts of active TB patients compared to controls (active TB patients, health care workers, BCG-vaccinated healthy controls)[24].

Tests currently used for Mtb infection diagnosis do not reflect CD8+ T cell cytokine production[32], however results reported in previous flow-cytometry studies and our own findings provide a strong rationale for measurement of Mtb-specific CD8+ T cell response. If validated, this may prove to be a surrogate marker of recent infection which, having the highest risk of progression to active TB, may enable QFT-Plus to distinguish recent infection from long lasting reactivity and hence allow better targeted delivery of preventive therapy.

Mtb-specific $CD8^+$ T cell have been more frequently detected in individuals with active TB when compared with LTBI and correlated with increasing antigenic burden[21][23][22][33]

[34], suggesting that the presence of $CD8^+$ T cells in a small proportion of latently infected individuals may be predictive of Mtb active replication and more likely disease progression[22]. Consistent with these results, in a previous study we found that the difference in responses between the QFT-Plus tubes may positively correlate with increasing antigenic load in active TB patients, as it was significantly more common in smear-positive versus smear-negative active TB patients[35]. In the present study, we observed a greater TB2 antigen response (TB2-TB1 difference >0.6ml/UI) in 18 (15.13%) individuals, all QFT-Plus positive. We speculate that the small subgroup of latently infected contacts with TB2-TB1 difference >0.6ml/UI have higher antigenic burden. However, to date, we do not have the tools to directly assess Mtb antigenic burden, as current LTBI tests rely on the (indirect) measurement of a specific immune response.

Our study has limitations. The foremost of these was the sample size, which comprises 119 subjects. Moreover because of the lack of gold standard tests for LTBI, we were unable to adequately resolve the discordance between QFT-GIT and QFT-Plus. In addition, TST-negative contacts were not recruited in our sample and a full evaluation of the test would benefit of their presence. Finally, the positive predictive value of the test and of the new parameter, the difference between the two antigen tubes, needs to be properly assessed in a longitudinal cohort. However, this would require follow-up of a large cohort (as incident TB is an uncommon event) and could only be performed in groups who are not eligible for chemoprophylaxis.

To our knowledge, our study is the first evaluation of QFT-Plus assay among recent contacts of TB cases. Although limited by the small sample size, our data show that QFT-Plus in contact screening has an improved performance compared to QFT-GIT and suggests a role for the differential value between the two tubes as a proxy for recent infection. Larger prospective studies are needed to assess the positive predictive value of the test and the possible role of the differential value between the two antigens tube as marker for recent infection.

In conclusion, the difference between the two antigen tubes, used as an indirect estimate of specific $CD8^+$ activation, is associated with factors indicating increased Mtb exposure, suggesting that this might identify individuals at greater risk of progression to active TB.

QFT-plus shows stronger association with surrogate measures of exposure compared to QFT-GIT and therefore seems at least as accurate as QFT-GIT in the setting of contact screening.

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Table1: Demographic characteristics

	Number	%
Sex (n=119)		
Male	63	52.9%
Female	56	47.1%
Estimated incidence of TB in country of birth° (n=119)		
0-50 per 100000 person-year	48	40.4%
>50 per 100000 person-year	71	59.7%
Country of birth (n=119)		
European	58	48.7%
NON European	61	51.3
BCG* vaccination (n=104)		
No	22	21.1%
Yes	82	78.8%
Smear status of index case (n=108)		
Negative	39	36.1%
Positive	69	63.9%
Time spent with the index case (hours per day) (n=108)		
1-4	27	25%
5-8	25	23.1%
9-12	9	8.3%
>12	47	43.5%
Sleeping proximity to the index case (n=108)		
Different house	61	56.5%
Different rooms	19	17.6%
Same room	28	25.9%
Immunocompromised[§] (n=119)		
No	108	90.8%
Yes	11	9.2%

° As per WHO Report 2014

*BCG bacilli Clamette-Guérin

[§] Causes of immunosuppression: diabetes mellitus (6), chronic kidney disease (0), HIV (2), malignancy (2), immunosuppressive medications (1)

Table 2: Test results

QFT-GIT results	QFT Plus results		Positive results per tube		QTF Plus IFN- γ concentrations (IU/ml)*	
	Negative	Positive	TB1	TB2	TB1-Nil	TB2-Nil
Negative (n=63)	51 (80.95%)	12 (19.05%)	10°	10§	0.01 (-0.01;0.17)	0.04 (0;0.23)
Positive (n=56)	0	56 (100%)	56	56	10.60 (2.94;16.57)	11.00 (3.32;17.75)
Total (n=119)	51 (42.86%)	68 (57.14%)	66	66	0.74 (0.01;9.65)	0.67 (0.04;8.94)

*median (25-75 percentile)
° 2 were positive to TB1 only
§ 2 were positive to TB2 only

Table 3: QFT-Plus and QFT-GIT discordant results

Sample no	BCG scar	TST**	QFT-GIT	QFT-Plus TB1°	QFT-Plus TB2§	Index case smear status	Relation to Index case	Immunosuppression
C1	Yes	20	Neg	1.83	0.51	Pos	Household, primary caregiver	Prednisone treatment
C11	Yes	7	Neg*	0.49	0.83	Pos	Boyfriend	No
C15	No	21	Neg*	0.11	0.48	Pos	Employer (index case: house-made)	No
C17	Yes	10	Neg	0.38	0.41	Neg	Household, sister	No
C39	Yes	20	Neg	0.83	0.88	Pos	Hospital close contact (sharing the same room)	Cancer
C53	Yes	20	Neg	0.3	0.58	Pos	Colleague, every day ride at work	No
C63	Yes	16	Neg	0.74	0.67	Neg	Household	No
C69	Yes	14	Neg	0.52	0.29	Pos	Household	No
C75	Yes	11	Neg	0.81	0.9	Pos	Household	No
C78	No	11	Neg	1.88	1.93	Pos	Colleague (sharing the same room)	No
C91	Yes	14	Neg	0.36	0.1	Neg	Household	No
C98	Yes	20	Neg	1.65	1.14	Pos	Household	Pregnant

° TB1-Nil
§ TB2-Nil
* Repeated test by QFT-IT at follow up converted to positive
** Diameter of induration in mm

Table 4: Univariate logistic regressions

	QFT Positive		QFT Plus Positive		TB2-TB1>0.6	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.02 (1.00;1.05)	0.09	1.02 (1.00;1.05)	0.101	1.03 (1.00;1.07)	0.053
Sex						
Male	1		1		1	
Female	0.73 (0.35;1.5)	0.387	0.76 (0.37;1.57)	0.458	1.49 (0.55;4.10)	0.435
Estimated incidence of TB per 100000 person-year in country of birth°						
0-50	1		1		1	
>50	1.44 (0.69;3.01)	0.333	1.63 (0.78;3.42)	0.197	0.82 (0.30;2.25)	0.7
Country of birth						
NON European	1		1		1	
European	0.84(0.41;1.73)	0.635	0.74 (0.36;1.54)	0.428	3.24 (1.07;9.75)	0.037
BCG* vaccination						
No	1		1		1	
Yes	2.04 (0.75;5.53)	0.161	2.26 (0.87;5.89)	0.096	0.98 (0.25;3.87)	0.978
Smear status of index case						
Negative	1		1		1	
Positive	1.12 (0.51;2.47)	0.780	1.39 (0.63;3.07)	0.413	1.29 (0.41;4.03)	0.662
Time spent with the index case (hours per day)						
1-4	1		1		1	
5-8	1.65 (0.48;5.67)	0429	3.23 (0.97;10.72)	0.055	3.55 (0.34;36.53)	0.288
9-12	2.8 (0.57;13.83)	0.206	4.37 (0.89;21.61)	0.070	7.43 (0.59;94.26)	0.122
>12	6.78 (2.28;20.16)	0.0006	14.78(4.62;47.25)	5.6e-06	7.03 (0.85;58.2)	0.071
Sleeping proximity to the index case						
Different house	1		1		1	
Different rooms	3.79 (1.29;11.14)	0.015	5.78 (1.71;19.52)	0.005	0.51 (0.06;4.52)	0.545
Same room	3.98 (1.55;10.23)	0.004	5.65 (2.00;15.97)	0.001	4.34 (1.37;13.81)	0.013
Immunocompromised						
No	1		1		1	
Yes	0.62 (0.17;2.22)	0.459	1.35 (0.37;4.88)	0.649	0.54 (0.06;4.46)	0.563

° As per WHO Report 2014

*BCG bacilli Clamette-Guérin

Table 5. Backward stepwise multivariate logistic regressions for predicting QFT-GIT or QFT Plus Positivity

	QFT-GIT Positive		QFT Plus Positive	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Time spent with the index case (hours per day)				
1-12	1		1	
>12	4.63 (2.05; 10.47)	0.0002	6.98 (2.86; 17.02)	1.98e-05

° As per WHO Report 2014

Table 6. Backward stepwise multivariate logistic regression for predicting TB2-TB1>0.6

	OR (95% CI)	p-value
Country of birth		
NON European	1	
European	3.46 (1.03;11.69)	0.0453
Sleeping proximity to the index case		
No same room	1	
Same room	5.90 (1.83;18.97)	0.0029