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Authors

Pan, Sheng-Wei

Catanzaro, Donald G

Seifert, Marva

et al.

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Predicting stringent QuantiFERON-TB Gold Plus conversions in contacts of tuberculosis patients

Sheng-Wei Pan[a,b,c], **Donald G. Catanzaro**[d,*], **Marva Seifert**[c], **Rehan R. Syed**[e], **Naomi Hillery**[f], **Mei-Lin Ho**[g], **Valeriu Crudu**[h], **Elena Tudor**[h], **Nelly Ciobanu**[h], **Alexandru Codreanu**[h], **Antonino Catanzaro**[c], **Timothy C. Rodwell**[c]

[a]Department of Chest Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

[b]School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

[c]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of California San Diego, La Jolla, CA, USA

[d]Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA

[e]Division of Infectious Diseases and Global Public Health, Department of Medicine, University of California San Diego, La Jolla, CA, USA

[f]The Herbert Wertheim School of Public Health and Human Longevity Science, University of California San Diego, La Jolla, CA, USA

[g]Department of Chemistry, Fu Jen Catholic University, New Taipei City, Taiwan

[h]The Chiril Draganiuc Institute of Phthisiopneumology, Chisinau, Republic of Moldova

Abstract

Objectives: To assess associations between disease severity in index TB patients and QuantiFERON-TB Gold Plus (QFT-Plus) results in contacts, and predictors for QFT-Plus conversion in contacts over 6–12 months.

Methods: TB patients (n = 295) and the contacts (n = 1051) were enrolled during 2018–2021 with QFT-Plus performed at baseline and months 6 and 12. A strong CD8 response was defined as TB2 interferon gamma (IFN-γ) response minus TB1 >0.6 IU/ml and stringent conversion as change from QFT-plus negative to high-positive QFT-Plus (TB1 or TB2 IFN-γ responses >0.7 IU/ml).

Results: Contacts with index TB patients with sputum smear >1+ was associated with positive QFT-Plus compared to those without ($p < 0.001$). Contacts with index TB patients with bilateral lung disease were more likely to have strong CD8 responses than those without ($p = 0.038$). QFT-Plus stringent conversion occurred in 9.7% of contacts over 6–12 months.

*Corresponding author. dgcatanz@uark.edu (D.G. Catanzaro).

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A TB1 IFN-γ response ≥0.03 IU/ml combined with a TB2 ≥0.06 IU/ml was predictive of a 19-fold increased risk for QFT-Plus stringent conversion in contacts (odd ratio 19.565 [8.484–45.116], $p < 0.001$).

Conclusion: Bacterial burden and bilateral lung disease of index TB patients were associated with positive QFT-Plus and strong CD8 responses in contacts. TB1 and TB2 IFN-γ responses were synergistically predictive of stringent conversion in contacts.

Keywords

Interferon-gamma release assay; QuantiFERON-TB Gold plus; Stringent conversion; Tuberculosis contacts

Introduction

It is estimated that one-third of the world's population has been infected with *Mycobacterium tuberculosis* (MTB).[1] Although most people with MTB infection live healthy lives with a latent tuberculosis infection (LTBI), they carry a 5–10% lifelong probability of developing active tuberculosis (TB),[2] with an incidence of 0.84–3.3 per 1000 person-years, depending on exposure timeline and risk factors.[3,4] Half of that risk of TB disease occurs in the first few years after exposure, suggesting a clinical need for increasing prophylactic treatment for LTBI in contacts to decrease the probability of progressing to active TB.[5,6] Interferon-gamma (IFN-γ) release assays (IGRA) are the *in vitro* diagnostic standard for LTBI diagnosis, but due to limitations in their ability to differentiate LTBI from active TB disease, or to predict LTBI patients most likely to convert to active TB disease, their clinical potential has not been fully realized.

QuantiFERON-TB Gold Plus (QFT-Plus, Qiagen, Hilden, Germany) is the fourth generation of IGRA tests developed by Qiagen and uses a peptide cocktail of MTB ESAT-6 and CFP-10 proteins to stimulate T cells and report a quantitative IFN-γ response from patient blood *in vitro*. QFT-Plus measures the IFN-γ response from CD4+ T cells in its TB Antigen Tube 1 (TB1) and IFN-γ response from both CD4+ and CD8+ T cells in its TB Antigen Tube 2 (TB2).[7] It is thought that the difference of IFN-γ between TB2 and TB1 tube (TB2-TB1) can be used as a proxy for CD8 response.[8] A high CD8 IFN-γ response is broadly indicative of recent MTB infection and has been correlated with active TB disease.[8–11]

Previous versions of the QFT assay have been used to assess the risk of active TB in contacts and while the QuantiFERON Gold In-Tube (QFT-GIT) did not evaluate CD8 response, smear-positivity and severe lung disease of index TB patients was associated with the risk of TB in contacts[10,12–14] and high QFT-GIT results in contacts at baseline were moderately predictive of incident TB disease.[15,16] Additionally, contacts have been shown convert from QFT-GIT negative at baseline to strong positive during follow-up,[17] suggesting the potential of monitoring LTBI status to prioritize contacts. A large longitudinal study assessing serial IFN-γ responses using the QFT-GIT test in a high TB burden area, determined that a IFN-γ conversion from extremely negative to high-positive QFT-GIT (IFN-γ >0.70 IU/ml) at day 360 was predictive of a 10-fold increased risk of developing TB over 2 years.[18] Similar studies have not been completed by using QFT-Plus to assess stringent conversions during

follow-up and risk factors of conversion have not been explored comprehensively. Filling in this knowledge gaps on QFT-Plus is clinically relevant because appropriate interpretation of IGRA results may help guide precision management for contacts, especially for those with initial QFT-Plus negative (IFN-γ <0.35 IU/ml), suggesting no initial mandatory therapy.

In our study, we used a prospective clinical study in TB patients and their contacts to examine the association between the disease severity of index TB patients and the QFT-Plus IFN-γ responses in contacts, with a specific focus on the contributions of the CD8 response. In addition, we evaluated the QFT-Plus results from contacts over 12-month to examine the temporal correlations between baseline factors and QFT-Plus stringent conversion among contacts who initially tested QFT-Plus negative.

Methods

Study design and enrollment

This prospective cohort study was conducted in partnership with the Republic of Moldova's National TB Program (NTP) encompassing their TB clinics, contact tracing program, and the National TB Reference Laboratory (NTRL). This study was approved by the Ethics Committee of the Chiril Draganiuc Institute of Phthisiopneumology in the Republic of Moldova, the University of California San Diego Human Research Protections Program (Project #180068), the University of Arkansas Institutional Review Board (Protocol #1806126401R002), and the Department of Defense Office of Human Research Oversight (Project #E00212.1a) in the US.

Patients with active pulmonary TB and their contacts were enrolled from October 2018 through October 2021. New TB patient identification was carried out by staff monitoring Moldova's electronic NTP System of Information for Monitoring and Evaluation of TB (SIME-TB) and contact investigations were carried out by district TB nurses. New TB patients identified for public health contact tracing activities and their contacts were approached to determine their interest in the study and enrolled following informed consented.

The inclusion criteria for participants with pulmonary TB were (1) sputum acid-fast bacilli (AFB) smear-positive or nucleic acid amplification test (NAAT)-positive within the previous four weeks, (2) ≥18 years old, and (3) willing to identify contacts in the past three months, whether living with the contacts in the same house or not. TB patients were excluded from the study if they had received any TB treatment for more than 4 weeks prior to screening and 12 weeks prior to testing. The inclusion criteria for contacts were (1) exposure to a smear-positive or NAAT-positive TB patient, (2) ≥5 years old and ≥16 kg, (3) no evidence of TB on clinical evaluation which includes chest x-ray. Contacts were excluded if they (1) were pregnant, (2) were ever treated to prevent TB (therapy for LTBI is not routinely provided in the Republic of Moldova), (3) had a history of prior TB disease, (4) had their QFT-Plus tests after 12 weeks post exposure, or (5) had no QFT-Plus at baseline. Contacts identified to have active TB disease within 3 months after presumed exposure were classified as co-prevalent TB patients in the analysis.

QFT-Plus assay and measurement

Ten milliliters of whole blood were drawn from each participant into a Lithium Heparin tube upon enrollment and at 6 and 12-months following enrollment. All blood samples were transported daily by dedicated vehicle to the NTRL within 8 h at room temperature, where blood was transferred into the four QFT-Plus tubes and IFN-γ levels were measured per the manufacturer's instruction. QFT-Plus results were classified according to definitions in Table 1, defining stringent conversion as QFT-Plus change from QFT-Plus negative (both TB1 and TB2 IFN-γ responses <0.35 IU/ml) to high-positive (either TB1 or TB2 IFN-γ responses ≥0.70 IU/ml) and strong CD8 response as TB2-TB1 IFN-γ >0.6 IU/ml. Demographic data on age, sex and comorbidities were collected. For TB patients, sputum AFB smear grade, culture results, and the presence of bilateral lung disease and/or cavitary lung lesion were recorded.

Statistical analysis

Student's *t* test and Mann–Whitney *U* test were used to compare group means and medians as appropriate and the χ^2 test or Fisher's exact test was used to compare proportions. A paired t test was performed to compare TB1 and TB2 IFN-γ responses within groups. Receiver operating characteristic (ROC) analysis was used to analyze the ability of TB1 and TB2 to predict stringent conversion of QFT-Plus, areas under the ROC curve (AUCs) and the maximum value of the Youden index were calculated to determine the optimal cut-off values.[19] Logistic regression analysis was used to identify factors associated with QFT-Plus stringent conversion at months 6 and 12. Odds ratios (ORs) and 95% confidence intervals (CIs) were generated. Kaplan–Meier survival curve with log-rank test was used to examine the differences in probability of QFT-Plus conversion between groups stratified by baseline TB1 and TB2 IFN-γ responses. Analyses were conducted by SPSS (v18.0; SPSS Inc, IL, USA) with figures generated by GraphPad Prism (v6.0; GraphPad Software Inc, CA, USA) and Sankey Diagram tool (https://sankeymatic.com/build/).

Results

Characteristics of participants

Over the 3-year period, 295 TB patients and 1051 contacts were enrolled (Fig. 1). Nearly all TB patients (97.9%) were NAAT-or culture-positive for MTB. The mean age of TB patients and contacts were similar (43.9 ± 12.6 vs 42.6 ± 17.3 years) but 10.6% of contacts were under 18 years old. Notably, 77% of contacts had exposure to index TB patients that were AFB smear-positive and 40% to those with AFB >1+ (Table 2, also see Supplementary Table S1 for detailed data). After excluding participants with indeterminate QFT-Plus results, 72% of TB patients (n = 204) and 35% of contacts (n = 361) were positive QFT-Plus ($p < 0.001$).

Clinical factors and QFT-Plus at baseline

In TB patients, TB2 IFN-γ response was significantly higher than TB1 ($p = 0.003$). In contacts, there were no significant differences between TB1 and TB2 responses. Both TB1 and TB2 results were higher in TB patients than in contacts ($p < 0.001$) (Fig. 2A). The CD8

response was higher in TB patients than in contacts ($p < 0.001$) (Table 2). In positive QFT-Plus population, CD8 response was also higher in TB subgroup than in contact subgroup (0.30 ± 1.44 vs -0.06 ± 0.75, $p = 0.001$) although both TB1 and TB2 IFN-γ response were not statistically different between the subgroups (Fig. 2A). The proportion of strong CD8 response was 15% in TB patients, which was higher than the 3% in all contacts ($p < 0.001$) and the 9% in the contacts with positive QFT-Plus (i.e., contacts with LTBI) ($p = 0.026$). In positive QFT-Plus population, a strong CD8 response was associated with a 2.66-odds for TB diagnosis versus LTBI (95% CI 1.62–4.34, $p < 0.001$).

Contacts of TB patients with AFB >1+ had higher TB1 and TB2 IFN-γ responses than contacts of TB patients with AFB ≤1+ ($p = 0.012$ and $p = 0.022$) and contacts of TB patients with AFB >1+ were more likely to have a positive QFT-Plus than contacts of index patients without (46.2% [146/316] vs 31.4% [154/491]) ($p < 0.001$). Moreover, contacts of TB patients with bilateral lung disease had higher TB2, but not TB1, IFN-γ response than contacts of TB patients without bilateral lung disease ($p = 0.046$) (Fig. 2B). Contacts of index patients with bilateral lung disease were also more likely to have strong CD8 responses than contacts of index patients without (4.7% [20/430] vs 2.1% [9/438]) ($p = 0.038$) (Fig. 2C).

Longitudinal QFT-Plus results among contacts

Contacts with follow-up QFT-Plus data ($n = 695$) were older than those without (43.6 ± 17.4 vs 40.7 ± 16.8 years, $p = 0.011$) but the male proportion was not significantly different (41% vs 46%, $p = 0.165$). Among contacts with initial high-positive QFT-Plus, 70% of contacts ($n = 89$) had persistent high-positive QFT-Plus results at 6 months and 92% ($n = 47$) were high-positive QFT-Plus at 12 months (Fig. 3A–B). Importantly, among contacts with initial QFT-Plus negative results, over 6% of contacts ($n = 20$) had stringent conversions by 6 months and 14% ($n = 21$) had stringent conversions by 12 months, with a total of 10% of them having QFT-Plus stringent conversion over 6–12 months ($n = 41/423$).

Factors associated with stringent conversion

Baseline TB1 and TB2 IFN-γ responses were higher in contacts with stringent conversion at month 6 and 12 than those without (all $p < 0.001$) while CD8 responses were not different in any subgroup (Table 3). The smear status and lung lesions of index TB patients were not associated with stringent conversion. In ROC curve analyses, the AUC for using baseline TB1 and TB2 IFN-γ responses to predict stringent conversion at month 6 were 0.793 (95% CI, 0.672–0.913) and 0.790 (95% CI, 0.686–0.895) with predictions slightly higher for conversion at 12 months with AUCs of 0.865 (95% CI, 0.789–0.940) and 0.854 (95% CI, 0.766–0.941). The optimized threshold values for prediction at month 6 was ≥0.03 IU/ml for TB1 and ≥0.06 IU/ml for TB2 with similar threshold values for month 12 follow ups (Table 3). The sensitivity and specificity of using TB1 ≥0.03 and TB2 ≥0.06 IU/ml to predict stringent conversion was 0.75 and 0.81 for 6 months and 0.78 to 0.85 for 12 months (see Supplementary Table S2).

In a logistic regression analysis (Table 4), contacts with TB1 ≥0.03 and TB2 ≥0.06 IU/ml at baseline had an increased risk of QFT-Plus stringent conversion at month 6 (adjusted OR

17.601 [5.520–56.121], p < 0.001) which was even higher at month 12 (adjusted OR 23.642 [6.752–82.782], p < 0.001). In a Kaplan–Meier curve analysis (Fig. 4), the cumulative probability of QFT-Plus stringent conversion during follow-up was exponentially higher in contacts with TB1 IFN-γ ≥0.03 plus TB2 ≥0.06 IU/ml (n = 94, 22.2%) than in the other two groups (both p = 0.001). While TB1 ≥0.03 IU/ml alone was associated with a 14.4-fold increased risk of stringent conversion (adjusted OR 14.389 [95% CI 6.345–32.627], p < 0.001) in contacts over a 6-12-month period, TB1 IFN-γ ≥0.03 plus TB2 ≥0.06 IU/ml was correlated with a 19.6-fold increased risk for stringent conversion after adjustment for age and sex (adjusted OR 19.565 [8.484–45.116], p < 0.001) (Table 4).

Discussion

In this study, we completed serial QFT-Plus monitoring of contacts of TB patients and found that 10% of participants with QFT-Plus negative results at baseline had converted to high-positive results (e.g., stringent conversion) by 6–12 months after presumed exposure. A baseline IFN-γ response of TB1 ≥0.03 and TB2 ≥0.06 IU/ml, while still negative by the manufacturer's instructions,[8] was highly predictive of the stringent conversion with a sensitivity of 81% and specificity of 85% and a 19.6-fold risk of conversion over 6–12 months.

Our findings are consistent with previous longitudinal studies. A cohort study in India documented a 12% annual incidence of stringent QFT-GIT conversion (IFN-γ change from <0.35 to ≥0.70 IU/ml),[17] and another recent cohort study in healthy adolescents in South Africa found that 20% of QFT-GIT negative persons experienced stringent conversions (IFN-γ change from <0.2 at baseline to >0.7 IU/ml) at 12 months.[18] Importantly, stringent conversions were associated with 10-fold increase in incidence of TB disease over a 2-year period,[18] suggesting a very real impact of IGRA conversion on future risk of TB disease. To our knowledge, the present study is the first that uses QFT-Plus to monitor the IFN-γ response "journey" in TB contacts. The real-world clinical relevance and additional contribution of our findings suggests the global health community is missing an opportunity to treat a small, clearly identifiable, high-risk subpopulation of baseline QFT-Plus negative contacts of TB patients. Of note, baseline TB1 IFN-γ response ≥0.03 IU/ml (alone) was associated with a 14-fold increased risk of stringent conversion, suggesting a significant additive value of TB2 ≥0.06 IU/ml in the predictive model. Our findings highlight the potential synergistic role of CD4 and CD8 IFN-γ response in TB contacts undergoing active transition from LTBI to a stage at higher risk of TB disease. However, the TB1 and TB2 values indicated are very low, which may differ over different laboratories. Thus, its clinical values should be confirmed in other larger and different settings.

Our focus on the CD8 response in the QFT-Plus results also showed that while over 15% of our TB patients had strong CD8 responses (TB2-TB1 >0.6 IU/ml) in their QFT-Plus results, 9% of the contacts diagnosed as LTBI did as well, which is in line with other reports.[9] Interestingly, among participants with positive QFT-Plus results, strong CD8 response yielded a 2.7-fold odds of differentiating TB disease from LTBI, suggesting a moderately added value of the TB2 tube since our data showed that TB1 response itself was not different between the two subgroups. Although CD8 IFN-γ response at baseline,

which should be very low by definition in QFT-Plus negative condition, was not predictive of QFT-Plus stringent conversion, other prognostic values of CD8 IFN-γ response deserves further investigation in various clinical settings.

Moreover, our study found that bilateral lung disease of index TB patients was not only marginally correlated in strong CD8 response in TB patients but also significantly associated with strong CD8 response in contacts. Our findings suggest that lung inflammatory status of index TB patients was associated with CD8 IFN-γ response in their contacts. A clinical study revealed that a modern MTB lineage was associated with severe lung damage and increased level of TNF-α but not related to smear status of TB patients.[20] Another contact investigation study reported that certain MTB strains in TB patients may induce different IFN-γ response in their contacts.[12] Thus, it is possible that infection caused by certain virulent MTB lineages may result in more severe lung inflammation leading to bilateral lung disease in TB patients and induce strong CD8 responses in TB patients as well as elevated CD8 responses in their contacts. Our study joins this growing body of literature, but this hypothesis warrants further MTB lineage infection modeling to support possible associations.

We also found that smear status of index TB patients was associated with higher TB1 and TB2 IFN-γ levels, but not CD8 responses, in their contacts. Two recent mouse models have reported that CD4+ T cells is more important than CD8+ T cells to control the bacterial load in the early phase of MTB infection,[21] and that in immune deficient mice infected by *Mycobacterium bovis* Bacillus Calmette-Guérin, CD4+ T cells transferring to mice reduced bacterial burden in the lung but CD8+ T cells transferring did not.[22] Thus, we hypothesize that CD4 immune response may be more sensitive to high bacterial burden upon MTB exposure, which may in turn result in correspondingly high CD4 generated TB1 and TB2 IFN-γ response in contacts. Our findings further suggest smear status of index case may be preferentially associated with CD4 IFN-γ response in contacts.

The present study has some limitations. First, due to the COVID-19 pandemic, some of the missing data was not recoverable and identification of incident TB in contacts on a regular basis was not always possible. There is a need for additional study to investigate the association between QFT-Plus response at baseline and incident TB during follow-up in contacts with untreated LTBI. Second, we were unable to confirm if contacts had community exposure to other TB patients during follow-up, and this is especially relevant given the study location known ongoing local TB transmission. Third, we did not analyze MTB lineage isolated from the index case, nor the environment of the household and exposure period between index TB patients and contacts.[6,10,12] Finally, the impact of HIV on QFT-Plus conversion could not be assessed because there was few cases of HIV in the cohort.[3]

In conclusion, the combined baseline TB1 and TB2 IFN-γ response was highly predictive of a QFT-Plus stringent conversion during the 6–12 months of follow-up in TB contacts. Moreover, increased CD4 and CD8 responses in contacts were associated with smear status and bilateral lung disease of index TB patients, respectively. Furthermore, among positive QFT-Plus population, the CD8 IFN-γ response, but not TB1 or TB2 response, was higher in TB patients compared with contacts with LTBI, suggesting additional diagnostic value

of CD8 response. Further studies are warranted to assess the potential impact of setting thresholds for close monitoring based on baseline CD4 and CD8 IFN-γ responses from the QFT-Plus assay among contacts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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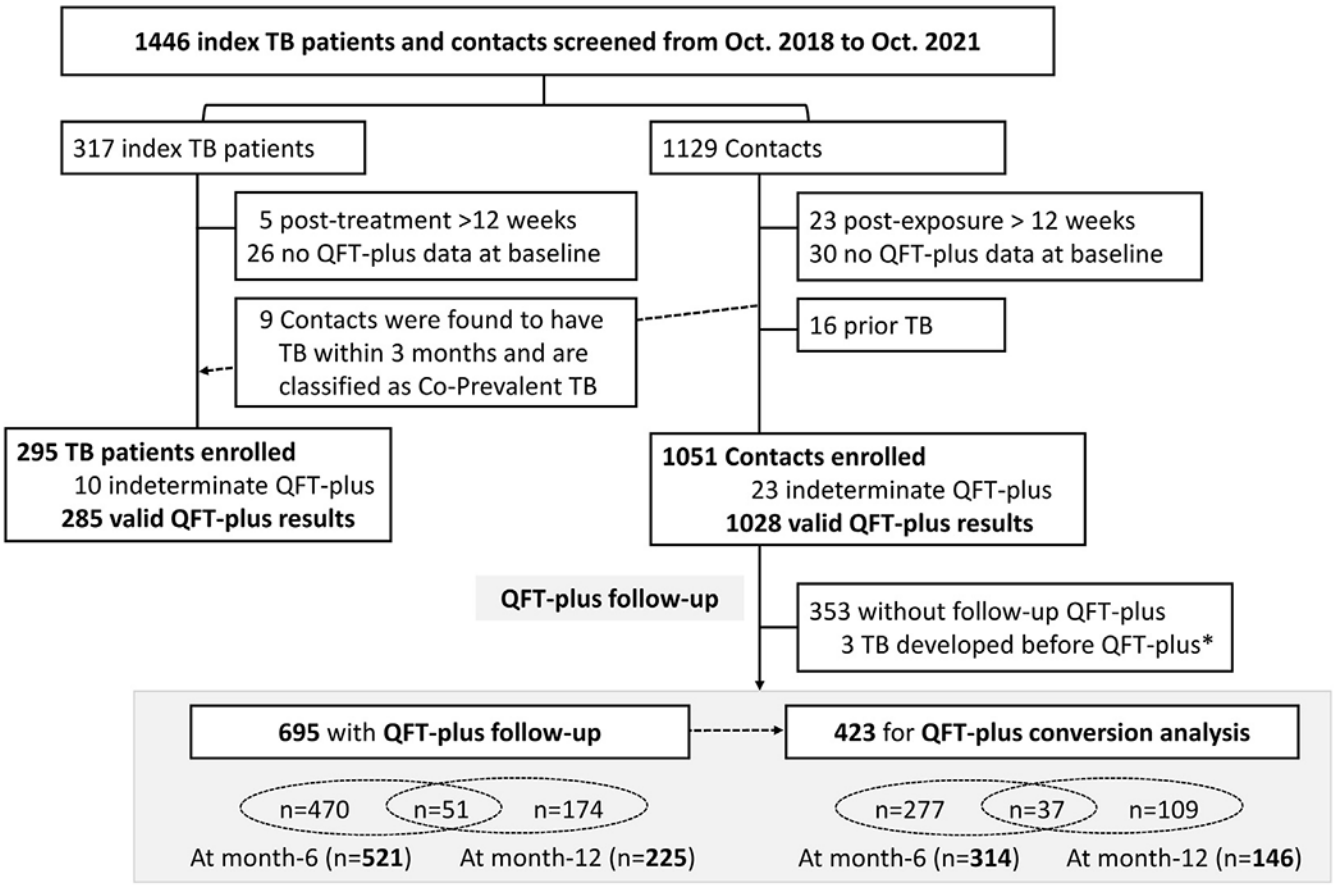

Figure 1.

Flow chart of enrollment. Follow-up QFT-Plus data were available for 695 contacts: 521 tested at 6 months (median 6.5 months [IQR 6.0–7.5]) and 225 at 12 months (median 13.2 months [11.8–14.7]), including 51 at both time points. *During follow-up, 16 contacts were known to have microbiologic confirmed TB after the initial 3 months: 5 with lost to follow-up for QFT-Plus, 8 with follow-up QFT-Plus and TB development later, and 3 with TB developed before QFT-Plus. The last 3 ones were excluded from analysis.

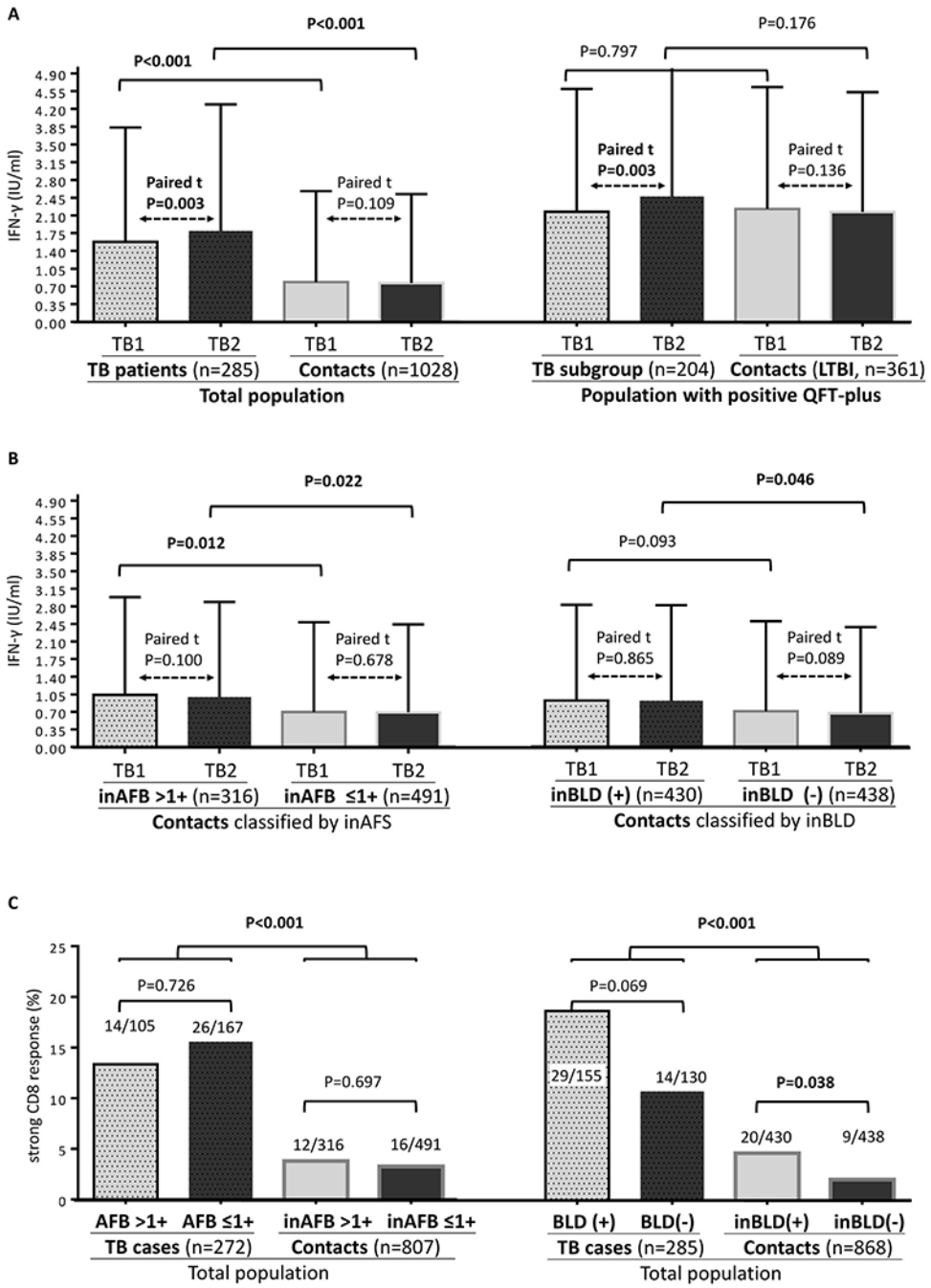

Figure 2.

(A) QuantiFERON Gold Plus (QFT-Plus) TB Antigen Tube 1-nil (TB1) and TB Antigen Tube 2-nil (TB2) interferon gamma (IFN-γ) responses in TB patients and contacts as well as IFN-γ responses in positive QFT-Plus TB patients and positive QFT-Plus contacts (contacts with latent TB infection). (B) TB1 and TB2 IFN-γ responses in contacts subgroups classified by index factors were compared. The factors were sputum acid-fast bacilli (AFB) smear grade of index case (inAFB) and bilateral lung disease (BLD) of index TB patients (inBLD). Of note, inAFB >1+ was correlated with initial positive QFT-Plus (odds ratio [OR]

1.879 [95% confidence interval 1.403–2.517], $p < 0.001$) and high-positive QFT-Plus in contacts (OR 1.674 [1.229–2.281], $p = 0.001$) but it was not associated with strong CD8 responses in contacts. (C) The proportions of strong CD8 response (TB2-TB1 IFN-γ ≥0.60 IU/ml) were showed in TB patients classified by related AFB and BLD as well as in contacts by inAFS and inBLD. Notably, inBLD was associated with strong CD8 response in contacts (OR 2.325 [1.047–5.166], $p = 0.038$) but was not correlated with positive QFT-Plus. In TB patients, the presence of BLD, but not AFB status, was marginally associated with strong CD8 response.

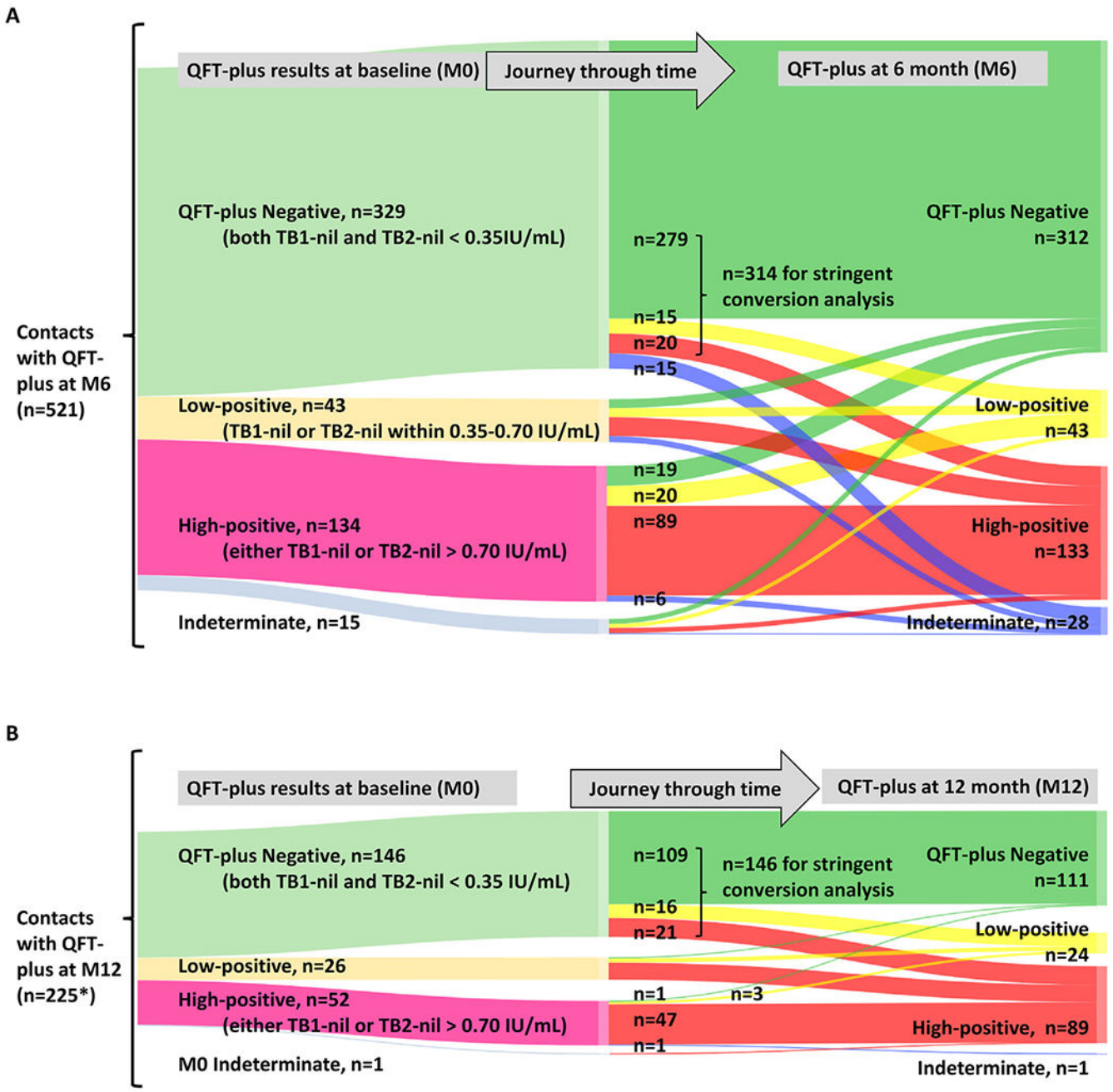

Figure 3.

Journey of QFT-Plus status of contacts from baseline (M0) to follow-up at months 6 (M6) (A) and months 12 (M12) (B). *Among contacts with follow-up QFT-Plus at both 6 and 12 months, 37 were initially QFT-negative: 31 (83.7%) of them were always QFT-Plus negative at both time points, 5 (13.5%) moved from persistent negative at months 6 to high-positive at months 12 (QFT-Plus stringent conversion), and 1 (2.7%) from stringent conversion status at month 6 to negative reversion at month 12.

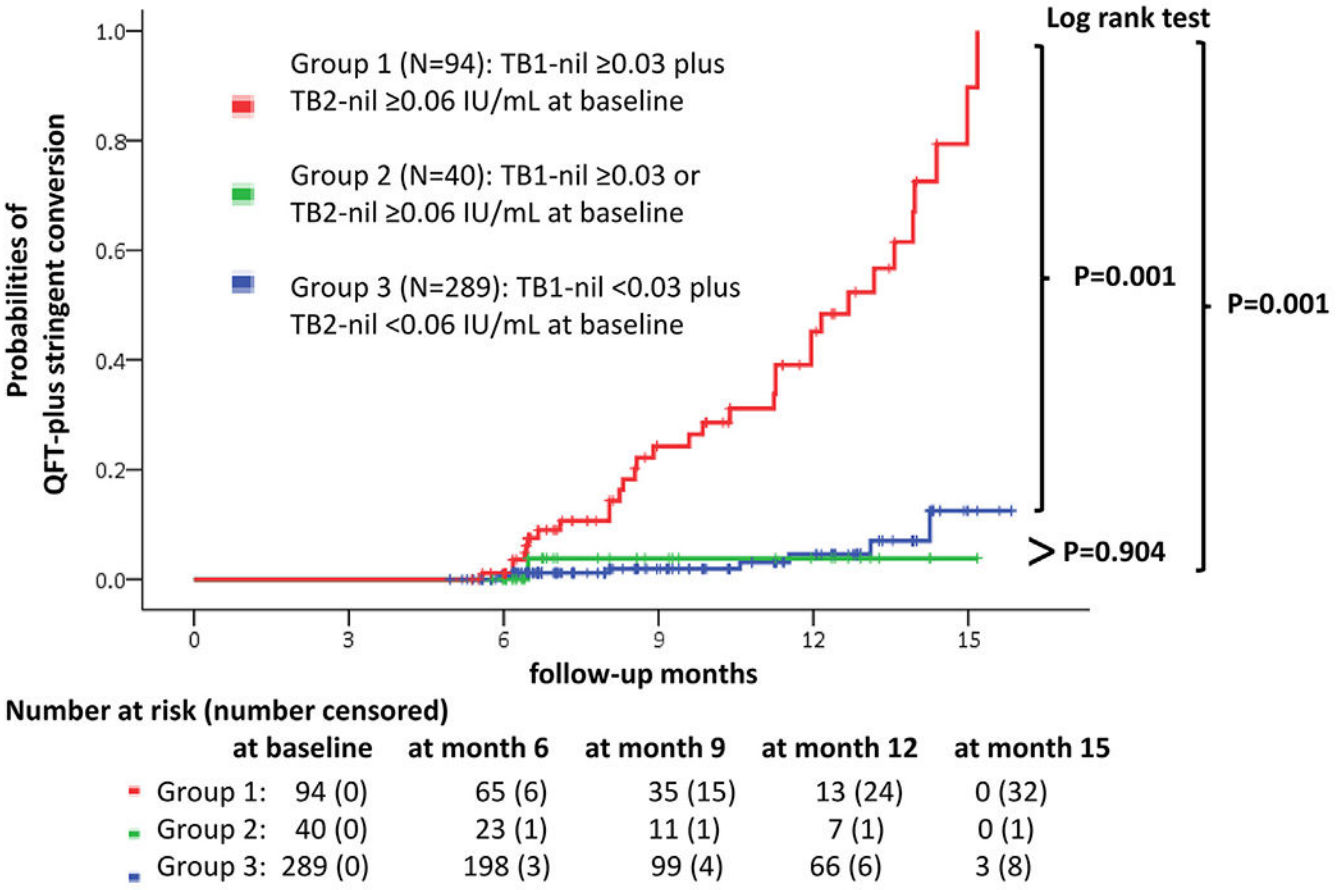

Figure 4.

Kaplan–Meier survival curve showing the probability of QFT-Plus stringent conversion in contacts stratified by baseline TB1 and TB2 interferon gamma responses (n = 423).

Table 1

Classification and definition of QuantiFERON-TB Gold Plus (QFT-Plus) results in this study.

Classification	Definition
TB1 IFN-γ response	Interferon gamma (IFN-γ) level in TB1 antigen tube minus that in nil tube (TB1-nil)
TB2 IFN-γ response	IFN-γ level in TB2 antigen tube minus that in nil tube (TB2-nil)
QFT-Plus negative	Both TB1-nil and TB2-nil IFN-γ levels <0.35 IU/ml or <25% of nil value[a]
Positive QFT-Plus	Either TB1-nil or TB2-nil IFN-γ level >0.35 IU/mL and>25% of nil value[b]
Low-positive QFT-Plus	Highest level of TB1-nil and TB2-nil IFN-γ between 0.35 and 0.69 IU/ml[b]
High-positive QFT-Plus	Highest level of either TB1-nil or TB2-nil IFN-γ ≥0.70 IU/ml[b]
CD8 IFN-γ response	TB2 IFN-γ response minus TB1 (TB2-TB1)
Strong CD8 response	TB2-TB1 IFN-γ level >0.6 IU/ml
QFT-Plus stringent conversion	On follow-up, change from QFT-Plus negative to high-positive QFT-Plus
Indeterminate QFT-Plus	1) IFN-γ level in nil tube >8.0 IU/ml or 2) IFN-γ level in mitogen tube <0.5 IU/ml when TB1-nil and TB2-nil IFN-γ levels were <0.35 IU/ml or was <25% of nil value

[a]Along with interferon gamma level in mitogen tube ≥0.5 IU/ml and that in nil tube ≤8.0 IU/ml.

[b]Along with interferon gamma level in nil tube ≤8.0 IU/ml.

Table 2

Demographic data of patients with pulmonary tuberculosis (TB) and TB contacts (n = 1346).

Variables	TB patients (n = 295)	Contacts (n = 1051)	Contacts subgroups by baseline QFT-Plus results		
			Positive (n = 361)	Negative (n = 667)	IDT (n = 23)
Age, years (n = 1337)[a]	43.9 ± 12.6	42.6 ± 17.3	43.4 ± 17.9	42.3 ± 16.8	37.6 ± 21.2
Male sex (n = 1340)	224 (76%)	447 (43%) ***	153 (43%)	285 (43%)	9 (39%)
Ever smoker (n = 1249)	179 (63%)	217 (22%) ***	71 (22%)	141 (23%)	5 (24%)
HIV positive (1142)	12 (4%)	8 (1%) **	3 (1%)	5 (1%)	0
Diabetes mellitus (1136)	11 (4%)	24 (3%)	8 (3%)	16 (3%)	0
Clinical presentation of TB patients					
Smear positive (n = 280)	210 (75%)				
Smear grade >1+ (n = 280)	108 (39%)				
Bilateral lung disease (n = 295)	159 (54%)				
Cavitary lung lesion (n = 295)	90 (31%)				
Characteristics of index TB patients					
Smear positive (n = 827)		640 (77%)	242 (81%)	381 (75%)	17 (85%)
Smear grade >1+ (n = 827)		328 (40%)	146 (49%)	170 (34%) ***	12 (60%)
Bilateral lung disease (BLD, n = 891)		442 (50%)	169 (54%)	261 (47%)	12 (52%)
Cavitary lung lesion (CLD, n = 891)		280 (31%)	102 (32%)	173 (31%)	5 (22%)
BLD and/or CLD (n = 891)		500 (56%)	189 (60%)	299 (54%)	12 (52%)
QFT-Plus results at M0 (n = 1313) [b]	**(n = 285)**	**(n = 1028)**	**(n = 361)**	**(n = 667)**	
Positive QFT-Plus	204 (72%)	361 (35%) ***	361 (100%)	0 ***	
High-positive QFT-Plus	153 (54%)	275 (27%) ***	275 (76%)	0 ***	
TB1-nil INF-γ (IU/ml)	1.58 ± 2.25	0.79 ± 1.79 ***	2.23 ± 2.40	0.00 ± 0.30 ***	
TB2-nil INF-γ (IU/ml)	1.80 ± 2.49	0.76 ± 1.76 ***	2.17 ± 2.36	0.00 ± 0.31 ***	
CD8 IFN-γ response (IU/ml)	0.22 ± 1.23	−0.02 ± 0.45 **	−0.06 ± 0.75	−0.00 ± 0.07 **	
Strong CD8 response	43 (15%)	33 (3%) ***	33 (9%)	0 ***	
QFT-Plus results at M6 (n = 493) [c]		**(n = 493)**	**(n = 165)**	**(n = 314)**	**(n = 14)**
Positive QFT-Plus		182 (37%)	137 (83%)	35 (11%) ***	9 (64%)

Variables	TB patients (n = 295)	Contacts (n = 1051)	Contacts subgroups by baseline QFT-Plus results		
			Positive (n = 361)	Negative (n = 667)	IDT (n = 23)
High-positive QFT-Plus		134 (27%)	108 (65%)	20 (6%) ***	5 (36%)
TB1-nil INF-γ (IU/ml)		0.77 ± 1.89	2.12 ± 2.71	0.16 ± 0.64 ***	0.94 ± 1.08
TB2-nil INF-γ (IU/ml)		0.77 ± 1.78	1.93 ± 2.59	0.16 ± 0.60 ***	0.91 ± 1.13
CD8 IFN-γ response (IU/ml)		−0.06 ± 0.52	−0.19 ± 0.83	0.00 ± 0.23 *	−0.03 ± 0.29
Definite QFT-Plus results at M12 (n = 224) [d]		**(n = 224)**	**(n = 77)**	**(n = 146)**	**(n = 1)**
Positive QFT-Plus		105 (47%)	72 (94%)	31 (21%) ***	1 (100%)
High-positive QFT-Plus		90 (40%)	67 (87%)	21 (14%) ***	1 (100%)
TB1-nil INF-γ (IU/ml)		1.65 ± 2.83	4.13 ± 3.27	0.35 ± 1.33 ***	1.72
TB2-nil INF-γ (IU/ml)		1.64 ± 2.80	4.20 ± 3.28	0.30 ± 1.11 ***	1.54
CD8 IFN-γ response (IU/ml)		−0.01 ± 1.03	0.06 ± 1.63	−0.04 ± 0.47	−0.18

[a] For detailed information on missing data, please refer to Supplementary Table S1.

[b] Ten TB patients and 23 contacts with indeterminate QFT-Plus results at baseline (M0) were excluded from analysis.

[c] Among 521 contacts with follow-up QFT-Plus test at month 6 (M6), 28 with indeterminate results were excluded from analysis.

[d] Among 225 contacts with follow-up QFT-Plus test at month 12 (M12), only one with indeterminate result was excluded from analysis.

Continuous and categorical data are expressed as mean ± standard deviation (SD) and number (%).

IDT indicates indeterminate; QFT-Plus, QuantiFERON-TB Gold Plus; TB1-nil, the difference of Interferon-gamma (IFN-γ) between tube with TB1 antigen and without stimulation (nil); TB2-nil, the difference of IFN-γ between tube with TB2 antigen and without stimulation (nil); TB2-TB1, the difference of IFN-γ between tube with TB1-antigen and tube with TB2-antigen stimulation.

* indicates $p < 0.05$,

** $p < 0.01$ and

*** $p < 0.001$ for comparisons between index TB patients and contacts and between contacts with positive QFT-Plus and with QFT-Plus negative results.

Table 3

Characteristics of initially QFT-Plus negative contacts with stringent conversion (SC) and without SC during 6- and 12-month follow-up.

Variables	QFT-Plus at month-6 (n = 314)[a]		P value	QFT-Plus at month-12 (n = 146)[b]		P value
	With SC (20)	Without SC (n = 294)		With SC (n = 21)	Without SC (n = 125)	
Age, years	40.1 ± 14.0	43.3 ± 17.3	0.411	44.4 ± 11.9	44.2 ± 16.6	0.944
Male sex	8 (40%)	114 (39%)	1.000	4 (19%)	63 (50%)	0.009
Ever smoker	7 (35%)	51 (17%)	0.060	5 (24%)	33 (26%)	0.801
HIV positive	0	3 (1%)	1.000	0	1 (1%)	1.000
Diabetes mellitus	0	10 (3%)	0.654	1 (5%)	2 (2%)	0.352
Index case clinical data						
Bilateral lung disease	4 (20%)	115 (39%)	0.080	10 (48%)	58 (46%)	0.780
Cavitary lung lesion	6 (30%)	75 (26%)	0.786	7 (33%)	27 (22%)	0.227
Smear grade >1+	8 (40%)	67 (23%)	0.168	4 (19%)	29 (23%)	0.565
QFT-Plus results at baseline (M0)						
TB1-nil INF-γ (IU/ml)	0.14 ± 0.13	−0.02 ± 0.43	<0.001	0.11 ± 0.07	0.00 ± 0.10	<0.001
TB2-nil INF-γ (IU/ml)	0.12 ± 0.11	−0.02 ± 0.42	<0.001	0.12 ± 0.09	−0.01 ± 0.10	<0.001
TB1-nil IFN-γ ≥0.03 IU/ml	16 (80%)	72 (24%)	<0.001	17 (81%)	26 (21%)	<0.001
TB2-nil IFN-γ ≥0.06 IU/ml	15 (75%)	59 (20%)	<0.001	17 (81%)	23 (18%)	<0.001
CD8 IFN-γ response (IU/ml)	−0.01 ± 0.07	0.00 ± 0.07	0.302	0.01 ± 0.06	−0.01 ± 0.07	0.440
M0 QFT-Plus stratification			<0.001			<0.001
TB1-nil <0.03 and TB2-nil <0.06 IU/ml	4 (20%)	212 (72%)		4 (19%)	95 (76%)	
TB1-nil ≥0.03 or TB2-nil ≥0.06 IU/ml	1 (5%)	33 (11%)		0	11 (9%)	
TB1-nil ≥0.03 and TB2-nil ≥0.06 IU/ml	15 (75%)	49 (17%)		17 (81%)	19 (15%)	

[a] Among 329 initially QFT-Plus negative contacts with QFT-Plus testing at months 6, 314 contacts were included for analysis of stringent conversion after excluding 15 with indeterminate QFT-Plus results at months 6. Out of the 314 participants, there were missing data for smoking status, diabetes mellitus, radiographic findings, and sputum smear results in 21, 28, 49, and 66 subjects, respectively.

[b] There were missing data for diabetes mellitus, radiographic findings, and sputum smear results in 2, 36 and 51 subjects, respectively. Continuous and categorical data are expressed as mean ± standard deviation (SD) or median with interquartile range [IQR] and number (%).

QFT-Plus indicates QuantiFERON-TB Gold Plus; TB1-nil, the difference of Interferon-gamma (IFN-γ) between tube with TB1 antigen and without stimulation (nil); TB2-nil, the difference of IFN-γ between tube with TB2 antigen and without stimulation (nil); TB2-TB1, the difference of IFN-γ between tube with TB1 antigen and tube with TB2 antigen stimulation.

Table 4

Analysis for factors associated with stringent conversion (SC) in initially QFT-negative contacts.

Variables	Crude OR (95% CI)	P value	Age- & Sex-Adjusted OR (95% CI)	P value
1) M6 SC as the outcome (n = 314)				
M0 TB1-nil IFN-γ ≥0.03 IU/ml	12.333 (3.994–38.082)	<0.001	13.603 (4.333–42.708)	<0.001
M0 TB2-nil IFN-γ ≥0.06 IU/ml	11.949 (4.175–34.201)	<0.001	12.620 (4.374–36.409)	<0.001
M0 QFT-Plus stratification				
TB1-nil <0.03 and TB2-nil <0.06 IU/ml	1.0 (Reference)		1.0 (Reference)	
TB1-nil ≥0.03 or TB2-nil ≥0.06 IU/ml	1.606 (0.174–14.814)	0.676	1.716 (0.185–15.932)	0.635
TB1-nil ≥0.03 and TB2-nil ≥0.06 IU/ml	16.224 (5.159–51.028)	<0.001	17.601 (5.520–56.121)	<0.001
2) M12 SC as the outcome (n = 147)				
M0 TB1-nil IFN-γ ≥0.03 IU/ml	16.183 (5.014–52.229)	<0.001	19.148 (5.594–65.549)	<0.001
M0 TB2-nil IFN-γ ≥0.06 IU/ml	18.848 (5.795–61.302)	<0.001	20.126 (5.920–68.414)	<0.001
M0 QFT-Plus stratification				
TB1-nil <0.03 and TB2-nil <0.06 IU/ml	1.0 (Reference)		1.0 (Reference)	
TB1-nil ≥0.03 or TB2-nil ≥0.06 IU/ml	–		–	
TB1-nil ≥0.03 and TB2-nil ≥0.06 IU/ml	21.250 (6.430–70.228)	<0.001	23.642 (6.752–82.782)	<0.001
3) M6 or M12 developing SC (n = 423)				
M0 TB1-nil IFN-γ ≥0.03 IU/ml	13.580 (6.053–30.466)	<0.001	14.389 (6.345–32.627)	<0.001
M0 TB2-nil IFN-γ ≥0.06 IU/ml	14.799 (6.771–32.342)	<0.001	16.063 (7.265–35.518)	<0.001
M0 QFT-Plus stratification				
TB1-nil <0.03 and TB2-nil <0.06 IU/ml	1.0 (Reference)		1.0 (Reference)	
TB1-nil ≥0.03 or TB2-nil ≥0.06 IU/ml	0.901 (0.110–7.397)	0.922	0.956 (0.116–7.889)	0.967
TB1-nil ≥0.03 and TB2-nil ≥0.06 IU/ml	18.129 (7.968–41.249)	<0.001	19.565 (8.484–45.116)	<0.001

TB1-nil ≥0.03 and TB2-nil ≥0.06

TB1-nil, the difference of Interferon-gamma (IFN-γ) between tube with TB1 antigen and without stimulation (nil); TB2-nil, the difference of IFN-γ between tube with TB2 antigen and without stimulation.