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ORIGINAL ARTICLE

The effect of combining QuantiFERON-TB Gold In-Tube test with tuberculin skin test on the detection of active tuberculosis

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Background: Interferon-γ release assays (IGRAs) and tuberculin skin tests (TSTs) play an important role in the detection of tuberculosis (TB) infection. However, the interaction between these tests in detecting active pulmonary TB in adults has never been researched.

Methods: A matched case–control study was conducted in Taiwan from 1 March 2012 to 31 December 2013 by enrolling 150 confirmed TB cases and 852 matched controls dwelling at the same area (stratified by low, medium and high incidence).

Results: Compared with a negative QuantiFERON-TB Gold In-Tube (QFT-GIT) test and a negative tuberculin skin test (TST), the effect size for detecting active pulmonary TB was highest for both a positive QFT-GIT and positive TST (adjusted odds ratio [aOR] 8.77 [95% confidence interval {CI} 4.51 to 17.05]), but the detectability was substantially attenuated in the absence of either a positive TST (aOR 1.59 [95% CI 0.70 to 3.63]) or positive QFT-GIT (aOR 1.15 [95% CI 0.48 to 2.71]) after controlling for age and gender. The joint effect of detecting active TB was positively synergistic according to a large positive value of relative excess risk due to interaction (7.05 [95% CI 2.48 to 11.61]).

Conclusions: The administration of both the IGRA and TST for enhancing the detectability of active pulmonary TB in an area with a moderate prevalence of TB such as Taiwan is strongly suggested.

Keywords: IGRA, Matched case–control study, QFT-GIT, TST, Tuberculosis

Introduction

The tuberculin skin test (TST) measures a delayed-type hypersensitivity response to the purified protein derivative (PPD), which is a crude mixture of antigens, many of which are shared among *Mycobacterium tuberculosis*, *Mycobacterium bovis*, bacillus Calmette–Guérin (BCG) and several non-tuberculous mycobacteria (NTM).[1] Interferon-γ (IFN-γ) release assays (IGRAs) measure IFN-γ release from T cells in response to *M. tuberculosis*–specific antigens such as early secreted antigenic target-6 (ESAT-6), culture filtrate protein-10 (CFP-10) and tuberculosis (TB) 7.7 (Rv2654) are useful diagnostic tools.[2]

The TST for detecting latent TB infection (LTBI) has been widely used for decades. However, the lower sensitivity of the TST among the elderly was reported in previous studies, because of booster phenomenon,[3] and the results of TSTs were affected by BCG vaccine.[4] Although the effect of remote vaccination with BCG on a positive tuberculin response in adults ≥30 y of age is probably negligible,[5] the IGRA has emerged as an attractive alternative because it is unaffected by BCG and most NTM exposure and has no booster phenomenon. IGRAs also have dynamic characteristics over time during *M. tuberculosis* infection.[6,7] This new test appears to demonstrate better specificity than the TST and is highly correlated with the risk of exposure to TB.[8] However, the performance of IGRAs was different between high and low TB incidence scenarios in a previous report.[9] Relatively lower sensitivity in high-incidence countries was also reported.[10,11] TST can be used to screen children for LTBI[12] and

increase sensitivity for finding LTBI in patients at high risk of developing progression from LTBI to disease.[13] IGRAs are most useful in children >5 y of age for helping to diagnose TB disease and increasing test specificity in children who have received a BCG vaccine.[13]

While there were no gold standard criteria for diagnosing LTBIs when the TST and IGRA were compared, active TB was a widely accepted surrogate outcome for evaluation of the TST and IGRA. It is unclear about the joint effect of IGRA and TST responses on the risk for active TB. We therefore conducted a matched case–control study to investigate the effects of combining the QuantiFERON-TB Gold In-Tube (QFT-GIT) test with TST on the detection of active TB.

Methods

Changhua County is located in the middle Taiwan, with a population of around 1 300 000, 12.5% of whom are >65 y of age. Nearly 0.7% of the Changhua people reside in long-term care facilities. There are 26 townships in Changhua County, divided into 2 cities, 6 urban townships and 18 rural townships. The incidence of active TB in these townships ranges from 40.9 to 108.9 per 100 000, with an average incidence of 61.4 per 100 000 in year 2010.

Study design

A matched case–control study was conducted in Changhua County from 1 March 2012 to 31 December 2013. TB cases (active TB; the case group) were confirmed by *M. tuberculosis* isolates, pathologic reports or physicians' clinical diagnosis on the basis of symptoms, physical findings and radiologic evidence without laboratory confirmation. The definition of TB contacts (the control group) was pursuant to the guideline of the Taiwan Centers for Disease Control: a shared airspace >8 h/d or >40 h exposure with TB cases.[14] For each TB case, four to seven controls were invited from reported TB contacts living in the same household or sharing airspaces with active TB cases in the 26 townships in Changhua County matched by the living location of the same TB incidence area, which was classified into three categories: high incidence area (>79.7/100 000 per year), intermediate incidence area (62.6–79/100 000 per year) and low incidence area (<62.6/100 000 per year).

A total of 215 suspected TB cases and 954 controls (TB contacts) were recruited consecutively. Written informed consent with signature was obtained from each of the participants before enrolment in our study. Suspected TB cases were ascertained and referred to have a confirmatory diagnosis during the study period. By the end of the study (31 December 2013), the status of active TB cases was ascertained for the controls by comparison with the TB registry. None of the selected controls turned into active TB cases within the 2-y follow-up period. All participants were administered questionnaires, TSTs and QFT-GITs during the study. Of those suspected TB cases and contacts participating in the study, 151 TB cases and 858 controls (TB contacts) met the study criteria. Finally, 150 TB cases and 852 TB contacts were enrolled in this case–control study after one TB case and six TB contacts were excluded because the results of the TST and QFT-GIT were not available. Among TB cases, 93 were confirmed by positive sputum culture, 4 by positive pleural effusion culture, 14 by pathologic reports and 39 by clinical criteria. There were two patients with extrapulmonary TB and the others were pulmonary TB.

The questionnaires were designed to collect sociodemographic information, clinical history of TB and factors relevant to the disease, including residence, travel history, contact with TB cases, smoking, alcohol drinking, betel nuts chewing, medical prescription and comorbidity (such as dementia, cerebrovascular disease, chronic lung disease, chronic liver disease, chronic kidney disease, hypertension, diabetes and malignant tumour).

Specimen collection and laboratory methods

In addition to the confirmatory diagnosis of all active TB cases as indicated above, TSTs that contained 2 tuberculin units of PPD of the RT23 strain were performed immediately and an induration ≥10 mm was considered positive. The IFN-γ assay was performed in two stages according to the manufacturer's instructions. A cut-off value of 0.35 IU/mL was defined as a positive response.[15,16]

All isolated *M. tuberculosis* was sent to the National Reference Laboratory of Mycobacteriology for further confirmation. The interpretation criteria approved by US Food and Drug Administration in 2007 for QFT-GIT were followed.[17]

Statistical analysis

We estimated univariate odds ratios (ORs) and 95% confidence intervals (CIs) using a conditional logistic regression model. A stepwise selection procedure (p-value to enter <0.1, p-value to remove >0.05) using Akaike information criterion (AIC) was applied to determine both the covariates included and the interaction effect between covariates to attain the parsimonious model for the multivariate model. The process of building the multivariate model is included in Appendix A. The smaller the AIC value, the better the model is indicated. We measured the interaction between the TST and QFT-GIT by using the synergistic index (S_{OR}) and its 95% CI.[18,19] If $S_{OR}>1$, there is a positive interaction and if $S_{OR}<1$, the interaction is said to be negative between two factors.

Results

The comparisons of characteristics were made across confirmed TB cases, after excluding those cases not meeting inclusion criteria, and controls and are listed in Table 1. The proportion was identical between TSTs and QFT-GITs with a consistent positive finding (80.4% vs 83.1%; κ=0.345 [range 0.153–0.537]) among TB cases. However, the percentage of positive TSTs (54.2%) was higher than positive QFT-GITs (39.6%) among the control group (κ=0.089 [range 0.023–0.155]).

When the conditional logistic regression model was applied to data on TB cases and controls, both the TST and QFT-GIT were found to be independent predictors for detecting TB with adjustment for age and sex (see Table 2). The risk for developing TB increased with advancing age, ranging from 2-fold for the 35–64 y age group to 13-fold for the those ≥65 y. Males were

Table 1. Characteristics of confirmed TB cases, excluded TB cases and controls in Changhua County, Taiwan

Characteristics	Excluded TB cases (n=62)	Confirmed TB cases (n=150)	Controls (n=852)
Location, n (%)			
High-incidence area	22 (35.5)	60 (40.0)	275 (32.3)
Medium-incidence area	22 (35.5)	48 (32.0)	263 (30.9)
Low-incidence area	18 (29.0)	42 (28.0)	314 (36.9)
Age (y)			
Mean (SD)	62.5 (13.0)	64.7 (15.7)	47.9 (16.1)
Age group (y)[a], n (%)			
<35	1 (1.6)	11 (7.3)	223 (26.2)
35–65	33 (53.2)	55 (36.7)	503 (59.0)
≥65	28 (45.2)	84 (56.0)	126 (14.8)
Gender, n (%)			
Male	28 (45.2)	105 (70.0)	380 (44.6)
Female	34 (54.8)	45 (30.0)	472 (55.4)
TST[b]			
Mean (SD)	8.5 (7.0)	14.2 (7.3)	10.3 (6.7)
TST[b], n (%)			
<10 mm	36 (62.1)	29 (19.6)	390 (45.8)
10–14 mm	10 (17.2)	38 (25.7)	218 (25.6)
≥15 mm	12 (20.7)	81 (54.7)	243 (28.6)
QFT-GIT[c], n (%)			
Negative	39 (63.9)	24 (16.2)	484 (57.4)
Positive (≥0.35 IU/ml)	22 (36.1)	123 (83.1)	334 (39.6)
Indeterminate	0 (0.0)	1 (0.7)	26 (3.1)
QFT-GIT, n (%)			
<0 IU/ml	7 (11.5)	2 (1.4)	123 (14.6)
0–0.01 IU/ml	4 (6.6)	4 (2.7)	38 (4.5)
0.01–0.35 IU/ml	28 (45.9)	18 (12.2)	305 (36.1)
≥0.35 IU/ml	22 (36.1)	123 (83.1)	352 (41.7)
Indeterminate	0 (0.0)	1 (0.7)	26 (3.0)

[a] Five TB cases with age <30 y and 131 TB contacts with age <30 y.
[b] The results of the TST were missed in two TB cases and one TB contact.
[c] The results of the QFT-GIT were missed in two TB cases and eight TB contacts.

three times more likely to have TB than females. The OR for the association between the detection of TB and different cut-offs of the TST was increased from 2 given a 5-mm cut-off to 3 given a 15-mm cut-off. A QFT-GIT >0.35 IU/ml was approximately seven times more likely to detect TB than one <0.35 IU/ml. Similar findings were noted for the QFT-GIT with the refined classification, with the order of ORs being 5.43 (95% CI 1.16 to 25.33) for 0–0.01 IU/ml, 2.97 (95% CI 0.86 to 10.25) for 0.01–0.35 IU/ml and 17.33 (95% CI 5.43 to 55.34) for ≥0.35 IU/ml.

The estimated adjusted odds ratios (aORs) in the multivariable logistic regression model are shown in Table 3. The aOR of a positive QFT-GIT with adjustment for age, gender and TST was 4.28 (95% CI 2.69 to 6.82). By using the stepwise selection procedure based on comparisons of AIC values across a series of candidate models, the model was the most parsimonious one (see Table 4) when considering the main effect of age, sex, TST and QFT-GIT as well as the interaction term between TST and QFT-GIT. Given the significant findings of interaction between the TST and QFT-GIT, each stratum-specific aOR in combination with the TST and IGRA are listed in Table 3 (right bottom). Compared with the participants with a negative TST and negative QFT-GIT, the aOR for participants with a positive QFT-GIT and positive TST was 8.77 (95% CI 4.51 to 17.05), but the corresponding figures decreased to 1.59 (95% CI 0.70 to 3.63) for those with a positive QFT-GIT and negative TST and 1.15 (95% CI 0.48 to 2.71) for those with a negative QFT-GIT and positive TST. The estimated S_{OR} was 7.05 (95% CI 2.48 to 11.61), indicating a positive interaction. Similar results were obtained from the same model in diagnosing culture-negative TB cases (Appendix Table B).

Discussion

Very few studies have been conducted to investigate the role of combining an IGRA with a TST in detecting TB. To the best of our

Table 2. Univariate analysis for demographic features associated with the risk of TB and both TST and IGRA tests in the detection of TB

Characteristics	OR (95% CI)
Age group (y)	
≥65	13.03 (6.70 to 25.34)
35–64	2.19 (1.12 to 4.25)
<35	Reference
Gender	
Male	2.92 (2.01 to 4.25)
Female	Reference
TST	
≥5 mm	2.00 (1.26 to 3.17)
<5 mm	Reference
TST	
≥10 mm	3.43 (2.24 to 5.27)
<10 mm	Reference
TST	
≥15 mm	2.98 (2.08 to 4.25)
<15 mm	Reference
QFT-GIT	
≥0.35 IU/ml	6.76 (4.35 to 10.50)
<0.35 IU/ml	Reference
QFT-GIT	
<0 IU/ml	Reference
0–0.01 IU/ml	5.43 (1.16 to 25.33)
0.01–0.35 IU/ml	2.97 (0.86 to 10.25)
≥0.35 IU/ml	17.33 (5.43 to 55.34)

Table 3. Multivariable analysis for independent role of the IGRA or TST alone and interaction between the IGRA and TST in detecting TB with adjustment for age and gender

Characteristics	IGRA or TST alone, OR (95% CI)	Interaction between IGRA and TST, OR (95% CI)
Age group (y)		
≥65	11.10 (5.40 to 22.80)	11.07 (5.36 to 22.85)
35–64	1.76 (0.88 to 3.52)	1.81 (0.90 to 3.65)
<35	Reference	Reference
Gender		
Male	2.90 (1.88 to 4.49)	2.97 (1.91 to 4.62)
Female	Reference	Reference
TST		
≥10 mm	3.47 (2.11 to 5.71)	—
<10 mm	Reference	—
QFT-GIT		
≥0.35 IU/ml	4.28 (2.69 to 6.82)	—
<0.35 IU/ml	Reference	—
TST^+ and $QFT\text{-}GIT^+$	—	8.77 (4.51 to 17.05)
TST^+ and $QFT\text{-}GIT^-$	—	1.15 (0.48 to 2.71)
TST^- and $QFT\text{-}GIT^+$	—	1.59 (0.70 to 3.63)
TST^- and $QFT\text{-}GIT^-$	—	Reference

TST^+: ≥10 mm; TST^-: <10 mm; $QFT\text{-}GIT^+$: ≥0.35 IU/ml; $QFT\text{-}GIT^-$: <0.35 IU/ml.

knowledge, this is the first study focused on the interaction between the TST and IGRA in the detection of active TB in adults ≥20 y of age. The unique finding is that the effect of the IGRA on the risk of TB was notably found among positive TST subjects but not among negative TST subjects, which is in contrast to previous studies showing a correlation between TB infection and positive QFT-GIT results.[9]

The gold standard for diagnosis of active TB is bacteriological diagnostic tests. However, they have limitations because the smear microscopy test is less sensitive and the culture test is time consuming. Nucleic acid amplification tests are useful for rapid diagnosis of TB but have only a moderate sensitivity (around 50–80%) in smear-negative pulmonary TB, which comprised more than half of all the pulmonary TB cases.[20] Hence rapid detection of individuals with TB is still difficult and new diagnostic tools are urgently needed. Although the QFT-GIT was developed for diagnosis of LTBI,[21] it plays a potential role in the evaluation of suspected active TB.[22] A recent published systematic review and meta-analysis revealed a pooled sensitivity of 80% (95% CI 75 to 84) for QFT-GIT among TB cases (confirmed and non-confirmed by culture), similar to the result of our study. However, the IGRA cannot distinguish active TB from latent infection, which impairs its specificity and utility for TB diagnosis, especially in TB-endemic countries, where most of the people are believed to be latently infected with *M. tuberculosis*.[22,23]

Several studies have suggested that the IGRA may be used as an adjunct test to rule out pulmonary TB when combined with the TST.[24,25] Similarly, as demonstrated in our study, the IGRA plays an independent role in the detection of active TB and a positive IGRA with a positive TST was more likely to detect active TB. Note that the predictive values of the combination of the TST and IGRA are highly influenced by the prior probability of active TB infection in different clinical settings.[25] Moreover, TB cases in an early disease state with low mycobacterial burden (e.g., culture-negative TB; Appendix B) and those in a more progressive state could differ in their immune response to TB so as to present different test results.[26] Further investigations are needed to assess their diagnostic utility for various disease states of TB by a risk-stratified approach.

Using active TB as a surrogate reference standard for LTBI, this study revealed the combination of the IGRA and TST may also be helpful for detecting LTBI and predicting its progression to active TB. While many cross-sectional studies have evaluated the performance of the IGRA and TST as two independent tests for LTBI, concordance and discordance between the two tests was unexplained or only explained by test characteristics (i.e., sensitivity and specificity).[27] Previous longitudinal cohort studies assessed the predictive ability of the IGRA and TST for active TB. They reported the result as a weak to moderate association between positive results and occurrence of TB, with the relative rates of positive TST/positive IGRA vs negative TST/negative IGRA at around 2–4.[28] In current study we found a strong synergistic

Table 4. Akaike information criterion values of models

Models	Number of parameters	AIC
Main effect		
TST, age group, sex	5	639.292
QFT-GIT, age group, sex	5	618.042
TST, QFT-GIT, age group, sex	6	587.264
Including interaction term		
QFT-GIT, age group, sex, (age group*QFT-GIT)	7	619.768
TST[a], QFT-GIT, age group, sex, (TST*QFT-GIT)	7	582.337
TST, QFT-GIT, age group, sex, (age group*TST)	8	585.891
TST, QFT-GIT, age group, sex, (age group* QFT-GIT)	8	591.183
TST, QFT-GIT, age group, sex, (age group*TST), (TST*QFT-GIT)	9	585.891
TST, QFT-GIT, age group, sex, (age group*TST), (age group*QFT-GIT)	10	594.216

Asterisk (*) refers to the interaction effect between variables.
[a] Refers to the parsimonious multivariate model.

effect between the TST and IGRA (aOR 8.77). This prominent effect is mainly due to the comparable history of TB exposure between active TB cases and matched contact controls established by the matching process using the background TB incidence as the criterion, which provides better contrast for elucidation of the effect of the TST and IGRA.

A positive TST may indicate recent infection or remote infection, because after infection with TB, positive results often remain lifelong until old age.[29] In contrast, the IGRA has a dose–response relationship with TB exposure, but it wanes quickly.[30] Previous studies have shown the IGRA response in the discordant pairs of negative TST/positive IGRA may reverse spontaneously to negativity.[30] On the other hand, positive TST/negative IGRA was attributable to remote infection or lower sensitivity of the IGRA compared with the TST, especially in those people with a minimal risk of infection.[31] The findings of this study support these previous reports that the two tests identified different populations with distinct immunologic processes.[25] Apparent interaction between the TST and IGRA, as revealed in our analysis, strongly suggests that the TST and IGRA must be used simultaneously in targeted testing for LTBI, particularly for those high-risk groups with recent exposure to TB, such as in contacts tracing and outbreak investigation.

The positive interaction between the QFT-GIT and TST in detecting active pulmonary TB noted in this study may result from immune responses that evolved during infection with *M. tuberculosis*. Latent infection may denote a stable state or preclinical stage of active TB.[7] A PPD-stimulated response is the same as the above conditions or active TB. However, higher responses of the IGRA have been shown in patients with active TB compared with those with LTBI.[32,33] The estimated S_{OR} is based on a multivariate conditional logistic regression controlling for age and sex with a matched incidence rank between TB cases and their contacts, which demonstrated the positive synergistic effect of the TST and QFT-GIT on active TB.

There are several limitations in this study. It is possible to have false-negative TST results, which is more common in the elderly without two-step testing to measure tuberculin reactivity.[34] False-positive TST results are not uncommon, due to an atypical mycobacteria infection or following BCG vaccination, although the effect of remote vaccination with BCG on positive tuberculin response in adults >30 y of age is probably negligible.[5] The subjects with an indeterminate QFT-GIT were excluded from the analysis for risk factors of TB. Furthermore, misclassification bias in controls may happen, as some TB cases were classified as controls as a result of the 2-y follow-up period. However, the bias toward the null is common because of underestimation of the risk. In addition, the multivariate model was built by comparing the AIC values of a series of nested models to determine the covariates and interaction terms on the TST and QFT-GIT included in the model. This approach compares the models for loss of information and tries to select the best model based on simplicity and goodness of fit rather than the perfect model. Our result is thus limited to assessing the relative effect of the TST and QFT-GIT on the risk of active TB rather than predicting risk based on the proposed multivariate model.

In conclusion, we demonstrate in an area with intermediate prevalence of TB (i.e., Taiwan) that combined IGRA and TST tests enhance the detectability of active pulmonary TB, which implies the IGRA can be used as an element in the diagnosis of TB in combination with the TST and clinical, biological and radiological assessments.

Authors' contributions: Study concept and design: Y-PY and H-HC. Analysis, acquisition and interpretation of data: C-CL, C-YH and Y-CH. Drafting of the manuscript: C-CL, C-YH, Y-PY and H-HChen. Critical revision of the manuscript for important intellectual content: Y-PY and H-HC. Statistical analysis: C-CL, C-YH. Administrative, techinical, or material support: Y-CH. C-CL and C-YH contributed equally to this study. Y-PY and H-HC had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix

A. Process for building the multivariate model

The covariates, including the effect of the TST, effect of the QFT-GIT, age group and sex, were considered in the multivariate model. The optimal model was selected by using a series of comparisons as follows based on the AIC values listed in Table 4 of the main text.

1. Determine significant covariates included in the model

We first compared the model with only the main effect of the TST and QFT-GIT adjusting for age and sex by using the AIC (639.292 and 618.042), which demonstrated a significantly lower AIC for the model with the QFT-GIT, age and sex (AIC 618.042). We then included the TST in this model, which resulted in a further significant reduction in the AIC, with the difference of 30.78 with 1 degree of freedom (AIC 618.042 to 587.264).

2. Determine the structure of interaction

2.1 Interaction between age group and the TST and QFT-GIT

Due to previous evidence on the effect of age group on TST positivity,[5] we further explored the interaction between age group and the two tests. Based on the model including TST, QFT-GIT, age and sex, models with an interaction between age and TST and QFT-GIT were scrutinized. All three of the age-based interaction models ([age group*TST], [age group*QFT-GIT] and [age group*TST] and [age group*QFT-GIT]) were not significant compared with the model without the interaction term (AIC 587.264).

The comparison between the model with covariates of TST, QFT-GIT, age and QFT-GIT*age (AIC 591.183) and that with covariates of QFT-GIT, age and QFT-GIT*age (AIC 619.768) demonstrated the significant effect of the TST, which confirmed the significant role of the TST.

2.2 Interaction between the TST and QFT-GIT

Compared with the model with covariates of TST, QFT-GIT, age and sex (AIC 587.264), the model that contained the interaction between TST and QFT-GIT (AIC 582.337) demonstrated the significant role of including this interaction term. The further inclusion of the interaction term between age and TST in the model was not significant (AIC 585.891). We thus selected the model with TST, QFT-GIT, age, sex and the interaction between TST and QFT-GIT as our parsimonious multivariate model.

B. Multivariable analysis for culture-negative TB

There were 53 cases with negative culture results for TB among the case group. Estimated results based on the model with interaction (shown in Table 4) are listed in Table B while considering the main effect of age, sex, TST and QFT-GIT as well as the interaction term between TST and QFT-GIT.

Table B. Multivariable analysis for the interaction between IGRA and TST in detecting culture-negative TB with adjustment for age and gender

Characteristics	Interaction between IGRA and TST, OR (95% CI)
Age	
≥65	5.49 (2.06 to 14.67)
35–64	1.90 (0.77 to 4.73)
<35	Reference
Gender	
Male	2.29 (1.25 to 4.17)
Female	Reference
TST^+ and $QFT\text{-}GIT^+$	3.29 (1.49 to 7.28)
TST^+ and $QFT\text{-}GIT^-$	1.14 (0.44 to 2.92)
TST^- and $QFT\text{-}GIT^+$	0.77 (0.25 to 2.40)
TST^- and $QFT\text{-}GIT^-$	Reference

TST^+: ≥10 mm; TST^-: <10 mm; $QFT\text{-}GIT^+$: ≥0.35 IU/ml; $QFT\text{-}GIT^-$: <0.35 IU/ml.