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Commentary

CD8 response measured by QuantiFERON-TB Gold Plus and tuberculosis disease status☆

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SUMMARY

Background: Clinical application of the CD8 response as measured by the newer interferon gamma release assay, QuantiFERON-TB Gold-Plus (QFT-Plus), remains to be investigated.
Method: We performed this prospective study and recruited active TB patients, contacts with latent tuberculosis infection (LTBI) and contacts without LTBI in two centres in northern Taiwan in 2017. Subjects were tested with both QuantiFERON-TB Gold In-Tube (QFT-GIT) and QFT-Plus. LTBI was defined by positive result by QFT-GIT and exclusion of active TB.
Results: A total of 336 participants (118 uninfected contacts, 105 LTBI, 113 active TB) were included. The concordance rate of QFT-GIT and QFT-Plus was high ($n = 300$, 89.3%). The kappa value was 0.811 among contacts and 0.708 among active TB. While TB1 and TB2 quantitative responses were not different between active TB and LTBI (TB1: 1.74 ± 2.73 IU/ml vs. 2.03 ± 2.28 IU/ml, $p = 0.403$; TB2: 2.21 ± 3.09 IU/ml vs. 2.15 ± 2.40 IU/ml, $p = 0.867$), CD8 response was higher in active TB than LTBI (0.47 ± 1.53 IU/ml vs. -0.06 ± 1.47 IU/ml, $p = 0.011$). Culture-confirmed TB had a higher CD8 response compared with LTBI (0.63 ± 1.74 IU/ml vs. -0.05 ± 1.47 IU/ml, $p = 0.004$).
Conclusions: This study demonstrated specific CD8 responses among uninfected contacts, LTBI as well as active TB.

Introduction

Tuberculosis (TB) remains an important global infectious disease and latent tuberculosis infection (LTBI) is the pre-clinical stage of TB infection now being considered an important target of TB elimination strategy.[1,2] Interferon-gamma release assay (IGRA) offered the advantage of reducing false positive results due to prior BCG vaccination compared with tuberculin skin test.[3,4] Currently, the QuantiFERON-TB Gold In-Tube assay (QFT-GIT; Cellestis/Qiagen, Carnegie, Australia) is widely used as a diagnostic tool for LTBI.[4] Its successor, QuantiFERON-TB Gold-Plus (QFT-Plus) (Qiagen) was launched in 2015 and FDA approved in the US in 2017. QFT-Plus contains two antigen tubes (TB1 and TB2) for the detection of adaptive immune responses in TB infection or in TB infected individuals. While TB1 tube was designed to measure interferon (IFN)-gamma response from CD4+ helper T lymphocytes, TB2 tube was designed to measure responses from both CD4+ and CD8+ cytotoxic T lymphocytes. The difference between TB1 and TB2 tube was thus assumed to be a surrogate of isolated CD8+ T cell response.[5]

As a new feature of the QFT-Plus assay, the TB2 antigen tube can characterize TB-specific CD8+ T cell responses of those tested. While studies on QFT-Plus have been published, most have focused on performance comparing QFT-GIT and QFT-Plus in various patient groups. Few, however, have investigated the role and clinical application of CD8 response by QFT-Plus. Previous studies have demonstrated a higher proportion of CD8 TB antigen-responsiveness in patients with active TB compared to latent infection.[6] The study by Barcellini et al., which was the first independent evaluation of QFT-Plus that describes the performance characteristic induced by TB1 and TB2 in active TB group, demonstrated that CD8+ T lymphocyte responses were higher in recent infection than remote infection and correlated to a higher intensity of TB exposure.[7] Furthermore, while numerous reports regarding TB biomarkers have been published, there is still a need

☆ **Take Home Message**: CD8 response measured by QFT-Plus was different and incremental among uninfected contacts, LTBI contacts and active TB. CD8 response was also higher among culture-confirmed active TB than histology-confirmed active TB.

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for TB biomarkers that can identify disease status and those more likely to progress to active TB.[8,9] Though many immune cell specific biomarkers hold promise for more accurately differentiating LTBI from active TB,[10] the clinical application of CD8 responses of the commercially available QFT-Plus is compelling in the context of the spectrum of TB infection. We, therefore, initiated this study on contacts and active TB patients to compare the performance of the QFT-GIT and QFT-Plus and to investigate the clinical utilization of CD8 response measured by QFT-Plus for discrimination among different status of *Mycobacterium tuberculosis* (*M.tb*) infection and disease.

Materials and methods

Study design and duration

This prospective study was conducted in National Taiwan University Hospital and National Taiwan University Hospital Hsin-Chu Branch from Jan 2017 to December 2017 (NTUH REC: 201612143RINB).

Study population and blood sampling

Adult patients (age between 20 and 90 years) with culture- or histology-confirmed (based on caseating granulomatous inflammation) active TB were prospectively enrolled (**TB** group).[11] Close contacts of TB, defined as an exposure duration of 8 h or more in a single day or a cumulative duration of 40 h or more without wearing adequate personal protective equipment for airborne transmission precautions, were screened for LTBI by using the QuantiFERON-TB Gold In-Tube assay (QFT-GIT) and QFT-Plus (Qiagen). The contacts also received chest radiography, and mycobacteriologic study (acid-fast smear and mycobacterial culture) for 3 sputum samples if symptomatic, to exclude the possibility of active TB disease.[11] Those with negative QFT-GIT were designated as **Uninfected** contacts whereas those with positive QFT-GIT were considered as **LTBI** cases.

TB patients and contacts were excluded if having any of the following conditions: (1) human immunodeficiency virus infection; (2) bleeding tendency that increased the risk of blood sampling; (3) life expectancy of less than 6 months in TB patients or less than 2 years as a household contact. We also excluded participants with QFT-GIT or QFT-Plus indeterminate results. QFT-GIT and QFT-Plus were tested prior to initiation of anti-TB treatment.

QuantiFERON-TB Gold In-Tube (QFT-GIT) and QuantiFERON-TB Gold In-Tube Plus (QFT-Plus)

QFT-GIT and QFT-Plus were performed according to the manufacturer's instructions (Qiagen. QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert. 2015).

Data Collection and definition

Patients with 2-month sputum samples that remained culture-positive for *M.tb* were considered persistent culture positivity. CD8 response was defined as the difference between TB2 and TB1 tube (TB2 – TB1). A significant CD8 response was defined as difference between TB2 and TB1 more than 0.6 IU/ml.[7] This cut-off level was chosen according to previous study, in order to solve the issue of intrinsic variability and also for easier inter-study comparison.[7]

The type and proximity of contacts were divided into household, school (in the same classroom) and workplace (in the same office) contacts. Household contact was further divided into living with index in the same house but not in the same room and sleeping with index case in the same room.

Statistical analysis

Student's t-test and ANOVA were used for the comparison of TB1, TB2 and CD8 response among different groups of participants. For categorical variables, chi-square or fisher's exact test was used for comparison. Paired t-test was used to compare between CD8 response before and at two months after anti-TB treatment. All data analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC, USA).

Results

Demographic data

We recruited 336 participants during the study period and 8 participants were classified as indeterminate results (3 were due to high negative control (nil tube) value and 5 were due to low positive control (mitogen) value). Among indeterminate results, both tests were indeterminate. Three cases with indeterminate results were active TB and 5 were contacts. After excluding the 8 participants with indeterminate results, a total of 113 active TB patients, 105 LTBI contacts and 118 uninfected contacts, were included. All TB patients had active pulmonary TB and 10 of them had concomitant TB pleurisy. No other sites of extra-pulmonary TB were observed in this study. TB were culture-confirmed in 81 (71.7%) and histology-confirmed in the other 32 (28.3%). After anti-TB treatment, 43 culture-confirmed patients had follow-up data at 2nd month. Clinical characteristics of these 336 participants were summarized in Table 1. Briefly, the participants were middle-aged (median: 41-year-old) and there was a male preponderance ($n = 176$, 56.1%). Among contacts, the vast majority of their index cases were smear positive (94.2%) and 26.9% had cavitation on CXR. Among active TB patients, 25 patients (22.1%) had smear-positive disease. The most common exposure type was classmates studying in the same classroom (42.2%).

Concordance rate of QFT-GIT and QFT-Plus

Comparing QFT-GIT and QFT-Plus results among the 223 contacts, 202 (90.6%) were concordant and kappa statistic was 0.811. The positive rate of QFT-GIT and QFT-Plus was 47.1% ($n = 105$) and 45.7% ($n = 102$). In 9 contacts, the QFT-GIT was negative while QFT-Plus was positive. Among them, four had a QFT-GIT response between 0.20 and 0.35 IU/ml. Another three had a CD8 response >0.6 IU/ml. In 12 contacts, the QFT-GIT was positive while the QFT-Plus was negative. Among them, four had a QFT-Plus response between 0.20 and 0.35 IU/ml while none had a CD8 response >0.6 IU/ml. The characteristics of the 21 contacts and their index cases with discrepant QFT results were summarized in Table 2.

For 113 active TB patients, the positive rate of QFT-GIT and QFT-Plus was 63.7% ($n = 72$) and 66.4% ($n = 75$), respectively. Results were concordant in 98 (86.7%) patients (32 were both negative and 66 were both positive) and kappa statistic was 0.708. In culture-confirmed patients, the positive rate of QFT-GIT and QFT-Plus was 74.1% ($n = 60$) and 79% ($n = 64$) while in histology-confirmed patients, the positive rate of QFT-GIT and QFT-Plus was 37.5% ($n = 12$) and 34.4% ($n = 11$). Six patients were positive by QFT-GIT but negative by QFT-Plus; all had a QFT-GIT response between 0.35 and 0.70 IU/ml. None of the six patients had a CD8 response >0.6 IU/ml. Nine patients were negative by QFT-GIT but positive by QFT-Plus. Among them, six had a QFT-GIT response between 0.20 and 0.35 IU/ml, and two more at 0.19 IU/ml. Six had systemic comorbidities, including diabetes mellitus in three patients, cancer in one, concomitant DM and end-stage renal disease (ESRD) in one, and concomitant DM and cancer in one. Participants who were QFT-Plus positive but QFT-GIT negative were more likely to have

Table 1
Demographic data of uninfected contacts, LTBI contacts (LTBI diagnosed by QFT-GIT), and active TB patients.

	All ($n=336$)	Contacts ($n=223$)	Uninfected ($n=118$)	LTBI ($n=105$)	P (LTBI vs. Uninfected)	TB ($n=113$)	P (TB vs. Contacts)	Cul+ ($n=81$)	His+ ($n=32$)	P (Cul+ vs. His+)	P (Cul+ vs. LTBI)
Age	43.3 ± 19.0	37.2 ± 17.4	31.3 ± 14.1	43.7 ± 18.4	<0.001	55.5 ± 16.1	<0.001	56.2 ± 16.3	53.6 ± 15.6	0.438	<0.001
BMI	22.8 ± 3.54	23.3 ± 3.54	23.2 ± 3.49	23.3 ± 3.61	0.766	22.0 ± 3.39	0.002	22.0 ± 3.29	22.0 ± 3.69	0.998	0.01
Men	176 (56.1)	112 (54.1)	69 (58.5)	49 (46.7)	0.078	64 (59.8)	0.116	55 (67.9)	15 (46.9)	0.038	0.004
Comorbidity											
DM	29 (8.6)	4 (1.8)	1 (0.9)	3 (2.9)	0.344	25 (21.1)	<0.001	21 (25.9)	4 (12.5)	0.140	<0.001
ESRD	2 (0.6)	0	0	0	NA	2 (1.8)	0.113	2(2.5)	0	1.000	0.190
Cancer	18 (5.4)	0	0	0	NA	18 (15.9)	<0.001	10 (12.4)	8 (25)	0.100	<0.001
Smoking	64 (19.0)	35 (15.7)	9 (7.6)	26 (24.8)	<0.001	29 (25.7)	0.028	21 (25.9)	8 (25)	0.919	0.856
Index Case											
Sm+	NA	210 (94.2)	108 (91.5)	102 (97.1)	0.074	NA	NA	NA	NA	NA	NA
Cavitation	NA	60 (26.9)	21 (17.8)	39 (37.1)	0.001	NA	NA	NA	NA	NA	NA
Exposure											
HHs, same house	NA	28 (12.6)	12 (10.2)	16 (15.2)	0.254	NA	NA	NA	NA	NA	NA
HHs, same room	NA	17 (7.6)	6 (5.1)	11 (10.5)	0.130	NA	NA	NA	NA	NA	NA
Same classroom	NA	94 (42.2)	69 (58.5)	25 (23.8)	<0.001	NA	NA	NA	NA	NA	NA
Same office	NA	37 (16.6)	13 (11.2)	24 (22.9)	0.018	NA	NA	NA	NA	NA	NA
Other	NA	47 (21.1)	18 (15.3)	29 (27.6)	0.024	NA	NA	NA	NA	NA	NA
TB1-Nil (IU/ml)	1.23 ± 2.22	0.97 ± 1.86	0.03 ± 0.23	2.03 ± 2.28	<0.001	1.74 ± 2.73	0.008	1.77 ± 2.50	1.67 ± 3.30	0.868	0.461
TB2-Nil (IU/ml)	1.43 ± 2.46	1.04 ± 1.96	0.05 ± 0.03	2.15 ± 2.40	<0.001	2.21 ± 3.09	<0.001	2.39 ± 2.99	1.73 ± 3.32	0.311	0.542
TB2-TB1 (IU/ml)	0.15 ± 1.24	−0.02 ± 1.03	0.02 ± 0.01	−0.05 ± 1.47	0.575	0.47 ± 1.53	0.003	0.63 ± 1.74	0.07 ± 0.67	0.016	0.004
CD8>0.6	42 (12.5)	18 (8.1)	4 (3.4)	14 (13.3)	0.012	24 (21.2)	0.001	21 (25.9)	3 (9.4)	0.073	0.029

Note. Cul+, culture-confirmed TB; DM, diabetes mellitus; ESRD, end-stage renal disease; HHs, households; His+, histology-confirmed TB; LTBI, latent tuberculosis infection; NA, not available; SD, standard deviation; Sm, smear; TB, tuberculosis.
Data are number (%) or mean ± standard deviation unless otherwise mentioned.

diabetes than other participants (5/9 (55.6%) vs. 20/104 (18.4%), $p=0.024$). Among all 15 patients with discrepant QFT results, all were culture-confirmed while only one QFT-GIT positive but QFT-Plus negative patient was histology-confirmed. Characteristics of active TB with discrepant QFT-GIT and QFT-Plus results were summarized in Table 3. Also, the distribution of the results of QFT-GIT / TB1 – Nil / TB2 – Nil was described in Appendix Table 1. Briefly, 24 (7%), 17 (5%), 25 (7%) and 37 (11%), 34 (10%), 30 (9%) participants had values between 0.2 and 0.35 IU/ml and 0.35 and 0.7 IU/ml for QFT-GIT / TB1 – Nil / TB2 – Nil, respectively.

QFT-Plus CD8 response in uninfected contacts, LTBI contacts and active TB patients

The results of CD8 response, TB1 and TB2 response among active TB patients, LTBI and uninfected contacts were described in Table 1. TB1 response was higher in LTBI (2.03 ± 2.28 IU/ml) and active TB (1.74 ± 2.73 IU/ml) compared with uninfected contacts (0.03 ± 0.23 IU/ml) (both p<0.001). TB2 response was also higher in LTBI (2.15 ± 2.40 IU/ml) and active TB (2.21 ± 3.09 IU/ml) compared with uninfected contacts (0.05 ± 0.03 IU/ml) (both $p<0.001$). While TB1 and TB2 response were not different between active TB and LTBI ($p=0.403$ and $p=0.867$, respectively), quantitative CD8 responses were higher in active TB than LTBI (active TB vs. LTBI: 0.47 ± 1.53 IU/ml vs. −0.05 ± 1.47 IU/ml, $p=0.011$). Among active TB patients, the CD8 response was higher among culture-confirmed patients ($n=81$, 0.63 ± 1.74 IU/ml,) compared to histology-confirmed patients ($n=32$, 0.07 ± 0.67 IU/ml, $p=0.016$) and LTBI contacts (0.05 ± 1.47 IU/ml, $p=0.004$). Significant CD8 response (CD8>0.6 IU/ml) among culture-confirmed patients (21/81, 25.9%) was more common than in histology-confirmed patients (3/32, 9.4%, $p=0.073$) and in LTBI contacts (14/105, 13.3%, $p=0.029$). Also, though statistically insignificant, we also observed higher TB2 and CD8 response in smear-positive TB compared with smear-negative TB (TB2: 2.40 ± 3.25 vs. 2.15 ± 3.06 IU/ml, $p=0.731$; CD8: 0.64 ± 2.28 vs. 0.42 ± 1.26 IU/ml, $p=0.653$).

Among contacts, the CD8 response was 0.35 ± 0.52 IU/ml among those sleeping with index case in the same room ($n=17$), 0.19 ± 1.08 IU/ml among those living with index case in the same house but not in the same room ($n=28$), −0.09 ± 1.17 IU/ml among those studying with index case in the same classroom ($n=94$) and −0.06 ± 0.86 IU/ml among those working with index case in the same office ($n=37$). The CD8 response was higher in contacts living with index case in the same room compared with others (0.35 ± 0.52 IU/ml vs. −0.05 ± 1.05 IU/ml, $p=0.010$). The positive rate of QFT-GIT and QFT-Plus was highest among contacts working the same office (24/37, 64.9% and 25/37, 67.6%) and contacts living with index case in the same room (11/17, 64.7% and 10/17, 58.8%). The results of QFT response among contacts were summarized in Appendix Table 2.

CD8 response before and at two months after anti-TB treatment

The results of CD8 response before and at two months after anti-TB treatment among different groups of patients are summarized in Table 4. No statistical significance was detected between before and at two months after anti-TB treatment among different groups of patients. Quantitative decline of CD8 response was observed among those with smear-positive TB who had culture conversion while no quantitative change in CD8 responses was observed in the smear-negative TB cohort. In contrast, the CD8 response among patients with persistent second-month culture positivity increased after two months of treatment.

Table 2
Clinical characteristics of contacts with discordant QFT-GIT and QFT-Plus.

QFT-GIT	QFT-Plus TB1	QFT-Plus TB2	TB2 – TB1	Index Cavitation	Relationship	Exposure
QFT-GIT (−) QFT-Plus (+)						
Neg (−0.14)	Pos (0.74)	Pos (1.51)	0.77	No	colleague	same dormitory
Neg (0.01)	Neg (0.05)	Pos (1.04)	0.99	No	classmate	same classroom
Neg (0.02)	Pos (0.53)	Pos (1.38)	0.85	Yes	colleague	same dormitory
Neg (0.02)	Pos (1.30)	Neg (0.04)	−1.26	No	classmate	same classroom
Neg (0.16)	Neg (0.19)	Pos (0.42)	0.23	Yes	colleague	same office
Neg (0.26)	Neg (0.24)	Pos (0.54)	0.30	No	husband	same room
Neg (0.29)	Neg (0.16)	Pos (0.40)	0.24	No	classmate	same classroom
Neg (0.29)	Pos (0.71)	Neg (0.23)	−0.48	No	daughter	same house
Neg (0.32)	Pos (0.43)	Neg (0.27)	−0.16	Yes	colleague	same office
QFT-GIT (+) QFT-Plus (−)						
Pos (1.33)	Neg (0.16)	Neg (0.34)	0.18	Yes	wife	same room
Pos (1.08)	Neg (0.19)	Neg (0.34)	0.15	No	son	same house
Pos (0.99)	Neg (0.08)	Neg (0.16)	0.08	Yes	classmate	same classroom
Pos (0.94)	Neg (0.00)	Neg (0.02)	0.02	No	classmate	same classroom
Pos (0.86)	Neg (0.11)	Neg (0.09)	−0.02	Yes	sister	different house
Pos (0.80)	Neg (0.13)	Neg (0.22)	0.09	No	son	same house
Pos (0.68)	Neg (0.09)	Neg (0.17)	0.08	No	husband	same room
Pos (0.47)	Neg (−0.44)	Neg (−0.49)	−0.05	No	father	same house
Pos (0.37)	Neg (0.13)	Neg (0.04)	−0.09	No	classmate	same classroom
Pos (0.36)	Neg (0.09)	Neg (0.12)	0.03	Yes	classmate	same classroom
Pos (0.36)	Neg (0.14)	Neg (0.24)	0.10	No	classmate	same classroom
Pos (0.36)	Neg (0.14)	Neg (0.09)	−0.05	Yes	classmate	same classroom

Note. Neg, negative; Pos, positive.
Values in brackets are QFT response, calculated by subtracting interferon-gamma level (IU/ml) of the negative control tube from that of corresponding antigen tube.

Table 3
Clinical characteristics of active tuberculosis (TB) patients with discordant QFT-GIT and QFT-Plus.

QFT-GIT	QFT-Plus TB1	QFT-Plus TB2	TB2 – TB1	Sex	Age	Comorbidity	Acid-fast stain	Diagnosis
QFT-GIT (-) QFT-Plus (+)								
Neg (−0.01)	Neg (0.03)	Pos (0.49)	0.46	Male	51	DM, ESRD	Neg	Culture
Neg (0.19)	Neg (0.21)	Pos (0.73)	0.52	Female	70	Nil	1+	Culture
Neg (0.19)	Neg (0.14)	Pos (0.53)	0.39	Male	77	DM	Neg	Culture
Neg (0.22)	Pos (0.72)	Neg (1.45)	0.73	Male	64	Nil	Neg	Culture
Neg (0.23)	Pos (0.36)	Pos (0.73)	0.37	Male	81	DM	2+	Culture
Neg (0.24)	Pos (0.55)	Pos (0.86)	0.31	Female	29	Nil	4+	Culture
Neg (0.24)	Pos (0.45)	Pos (0.46)	0.01	Male	56	DM, cancer	Neg	Culture
Neg (0.24)	Pos (0.35)	Pos (1.48)	1.13	Male	62	Cancer	Neg	Culture
Neg (0.31)	Pos (1.05)	Pos (0.91)	−0.14	Female	57	DM	1+	Culture
QFT-GIT (+) QFT-Plus (−)								
Pos (0.60)	Neg (0.07)	Neg (0.06)	−0.01	Male	67	Nil	Neg	Culture
Pos (0.47)	Neg (0.14)	Neg (0.16)	0.02	Female	29	Nil	Neg	Culture
Pos (0.43)	Neg (0.15)	Neg (0.14)	−0.01	Male	60	DM	Neg	Histology
Pos (0.40)	Neg (0.13)	Neg (0.20)	0.07	Male	60	Nil	Neg	Culture
Pos (0.39)	Neg (0.11)	Neg (0.12)	0.01	Male	79	Nil	Neg	Culture
Pos (0.35)	Neg (0.08)	Neg (0.06)	−0.02	Male	81	Cancer	1+	Culture

Note. DM, diabetes mellitus; ESRD, end-stage renal disease; Neg, negative; Pos, positive.
Values in brackets are QFT response, calculated by subtracting interferon-gamma level (IU/ml) of the negative control tube from that of corresponding antigen tube.

Table 4
CD8 response (IU/ml) before treatment and at 2nd month after treatment among culture-confirmed active TB patients.

	Before Tx	After 2-months Tx	P value
All ($n=43$)	0.63 ± 1.80	0.42 ± 1.32	0.371
2nd month culture conversion ($n=38$)	0.66 ± 1.89	0.41 ± 1.33	0.331
2nd month culture persistence ($n=5$)	0.39 ± 0.93	0.53 ± 1.40	0.560
Initial smear positive ($n=14$)	1.38 ± 2.83	0.84 ± 2.24	0.379
Initial smear negative ($n=29$)	0.27 ± 0.87	0.22 ± 0.38	0.817

Diagnosis of LTBI by QFT-GIT and quantitative differences from active TB

When diagnosing LTBI using QFT-Plus, the results were similar compared with the results made by QFT-GIT (Appendix Table 3). CD8 responses were higher in culture-confirmed TB (0.63 ± 1.74 IU/ml) than histology-confirmed TB (0.07 ± 0.67 IU/ml, $p=0.016$) and LTBI (-0.05 ± 1.51 IU/ml, $p=0.005$). TB1 ($p=0.270$) and TB2 ($p=0.905$) responses were not significantly different between active TB and LTBI.

Discussion

By measuring CD8 response (TB2 – TB1) using QFT-Plus, we found that CD8 response was higher in the active TB patients compared to LTBI and uninfected contacts. CD8 response was also

higher in culture-confirmed TB than histology-confirmed TB. While QFT-Plus TB1 and TB2 antigen tube responses were higher in active TB than uninfected contacts, the inter-test differences in TB1 and TB2 response were similar between active TB and LTBI participants. While the addition of TB2 tube based in qualitative interpretation of positive or negative values did not seem to enhance clinical utility, CD8 response as measured by TB2 minus TB1, holds promise as a potential biomarker for TB disease status and exposure intensity.

Differentiating between active TB and LTBI is difficult and even controversial. Previous studies using positron emission and computed tomography and *M.tb* mRNA detection hinted that TB infection is a disease with continual spectrum, highlighting the importance of immune response and control.[12,13] Given the heterogeneity and complexity of TB disease status, the search of biomarkers that can differentiate disease status and risk of progression are needed.[9,14] Previous studies have primarily focused on CD4 T cell as potential biomarker while CD8 T cell remained less emphasized.[10,15] The novelty of our study, therefore, lies in discovering potential use of CD8 response. As a result, our study found that the CD8 response measured by QFT-Plus was different between LTBI and active TB as well as between culture-confirmed TB and histology-confirmed TB, while the TB1 (CD4) response was not different.

CD8 T cells play an important role in the adaptive immune response to TB. CD8 T cells possess cytolytic functions to kill *M.tb*-infected cells and can also kill *M.tb* directly by producing granulysin.[16] CD8 T cells can also produce cytokines that are known to play critical functions during *M.tb* infection, such as IL-2, IFN-gamma, and TNF.[17] Theoretically, the addition of CD8 T cell response was expected to increase the sensitivity of detecting LTBI with QFT-Plus. In our study, however, only six (2.7% of all contacts) cases were QFT-GIT negative but QFT-Plus positive according to positivity of TB2 tube results. The incremental gain of TB2 tube, therefore, was limited in contact evaluation.

There still remains controversies and pitfalls for utilizing and studying QFT-GIT, QFT-Plus and CD8 response. First, CD8 response can also be detectable in response to TB1 peptides stimulation.[18] Second, the 0.6 IU/ml cut-off value for CD8 was used in our study, mainly in order to facilitate comparison between previous studies.[7,19] An optimal cut-off value for a significant CD8 response, however, has not been established. Also, previous studies have emphasized that IGRA results falling in the uncertain range should be carefully monitored in contact screening to avoid false-negative results.[20,21] Indeed, around 7% of our participants had interferon-gamma results between 0.2 and 0.35 IU/ml, which warrants judicious follow-up.

Since the launch of QFT-Plus in 2015, several studies have been reported and examined the clinical utility of QFT-Plus. The first study evaluated 119 contacts which only 12 (10.1%) discordant results (12 QFT-GIT (-) but QFT-Plus (+) contacts) were observed, along with a better association to exposure gradient by QFT-Plus.[7] This study also found a higher, though insignificant, TB2 – TB1 response in smear-positive versus smear-negative active TB patients.[7] In another study, selective response to TB2 was found to be associated with active TB and severe TB disease.[22] A more recent multicentre verification study also showed that subjects considered to be recently exposed to TB had significantly more often a true difference between TB2 – TB1.[19] The same study also reported high concordant rate of 94.6% (121/128) between QFT-GIT and QFT-Plus.[22] Our study also included 223 contacts and the concordance rate was 90.6% with a high Kappa value of 0.811.

Our study revealed 12 contacts which were QFT-GIT positive but QFT-Plus negative. This finding poses a potential serious drawback for clinical utility of QFT-Plus. The removal of original TB-7.7 from QFT-Plus may have contributed to the negative results in the QFT-Plus among QFT-GIT positive patients (www.quantiferon.com/irm/content/package-inserts.aspx). TB-7.7 was highly specific for TB and the absence of TB-7.7 in QFT-Plus may lead to less release of IFN-gamma.[23] Another possible explanation may be all of these positive QFT-GIT responses were close to the cut-off value – 0.35 IU/ml.[24] An unexpected finding of our study was the significant association of diabetes with discordant QFT-GIT negative/QFT-Plus positive results among patients with active TB. Prior studies have noted that diabetes was associated with immunosuppression and lower IFN-gamma release, thus leading to negative IGRA results.[25,26] The findings suggest that QFT-Plus may be more sensitive among immunocompromised hosts but warrants further researches.[27,28]

Compared to prior studies on QFT-Plus sensitivity in patients with active culture-proven TB, sensitivity in our study was considerably lower. A positive rate of 99% (98 cases) was observed in a study enrolling 99 active TB patients with a median age of 42 (range: 29 – 55).[29] In our study (median age: 59; range: 21–86), QFT-Plus sensitivity was lower (64/81, 79%) among culture-confirmed active TB patients, though still slightly higher than QFT-GIT (60/81, 74.1%). The sensitivity was also much lower in histology-confirmed active TB patients. The low smear positivity rate (22.1%) and high proportion of histology-confirmed TB (28.3%) in our study may indicate lower bacteria load in our elderly patient group and this may have contributed to the lower positive rate by QFT-Plus. Our study suggested that utilization of QFT-Plus in the setting of active TB should be cautious especially among elderly patients with lower disease severity and less bacteria load.

Also, another advantage of adding TB2 tube was hypothesized to be that CD8 response represented more recent TB infection.[7] Previous studies have suggested that proximity of contact and more recent infection were associated with positive CD8 response.[7,19] In our study, the CD8 response was higher among those living with the index case in the same room while studying in the same classroom (school contacts) may be a less intense type of exposure compared with household contacts.[30] Interesting, while TB1 and TB2 response were similar in household contacts and colleagues working in the same office, CD8 response appeared to be higher in household contacts than colleagues in the same office.

In a previous study by Kamada, the CD8 response has been proposed to be a potential treatment monitoring tool in active TB.[5] The CD8 response has been reported to significantly decline during treatment, however the decline in CD4 and CD8 differed in that CD4 decline was only significant after the first 3 months of treatment whereas CD8 response decline was significant when measured after 6 months of treatment.[5] In our study, CD8 responses were not different before TB treatment and at 2 months after TB treatment. Interestingly, among those with 2-month culture persistence, CD8 level increased paradoxically after 2 months of anti-TB treatment, a finding that corresponds to bacterial burden. As the number of these individuals were small in our study, larger cohorts with 2-month culture persistence is needed to validate our findings.

Our study also has limitations. First, we were unable to find an optimal cut-off point of CD8 level to help differentiate between LTBI and active TB. This may limit the clinical use of QFT-Plus CD8 response. We acknowledge that currently CD8 response cannot be used to diagnose LTBI or active TB. We, however, would like to emphasize CD8 response as future direction for TB biomarkers. Second, the active TB patients recruited in our study tended to have lower bacteria loads and less severe disease. Whether our findings could extrapolate to patients with higher bacteria loads and more severe disease status remained to be studied. Third, we do not have information as to how testing with QFT-Plus or any difference between CD8 response improves prediction of progression towards active TB. To achieve this goal, a substantial number of TB contacts need to be recruited with long-term follow-up and this

should be the direction for future studies to enhance clinical utility of QFT-Plus.

In conclusion, our study compares QFT-Plus to its prior generation and analyzes the utility of the CD8 response in differentiating the spectrum of TB infection to disease among uninfected contacts, contacts with LTBI and those with active TB. Although there was no difference in positive rates among contacts with LTBI and sensitivity in active TB, we conclude that additional information from the differential CD8 response in contacts by extent of exposure and active TB status is a clinical advance.

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Author Contributions

M.R.L., J.T.W. and J.Y.W. conceived and designed the study; C.H.C., L.Y.C., C.Y.C. and H.Y.S. conducted the study and coordinated the laboratory work; M.R.L. and J.Y.W. conducted the data analysis. M.R.L. and J.Y.W. wrote the first draft of the manuscript. All authors reviewed the final manuscript.

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Potential conflicts of interest

The authors have no conflict of interest to disclose.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.01.007.

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