

## Peer Review File

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**Reviewer A**

**Comment 1:** For tables 2, 3 and 4 the titles should be "comparison of" AND NOT "comparisons of".

Reply 1: Thank you for the comment. We have modified our text as advised.

**Comment 2:** Consider changing the title for Table 2 to "Comparison between QFT-GIT-negative and -positive patients with pleural TB".

Reply 2: Thank you for the comment. We have modified our text as advised.

**Comment 3:** For figure 3, you need to explain which figures are being plotted. Is it TB antigen-nil or TB antigen.

Reply 3: The figures are levels of TB antigen- nil, not TB antigen and we added it in the title of Y axis, "Quantitative levels of QFT-GIT test (TB antigen - nil, IU/mL)"

**Comment 4:** For line 155 change to "quantitative levels of QFT-GIT test (TB antigen - nil)"

Reply 4: Thank you for the comment. We have modified our text as advised.

## **Reviewer B**

Thank you for this interesting, relevant and well-written article.

I have a few important comments and questions for the authors:

**Comment 1: Line 60 – 62: Most regions in the world have not had access to the QFT GIT since 2018, as it was phased out by Qiagen and replaced with QFT Gold Plus. So the QFT GIT is no longer the most commonly used IGRA. Consider revising this sentence. In the methods section, please indicate if this study did include some patients who had QFT Gold Plus rather than QFT GIT or if your centre had access to GIT for the whole study period. This might also be mentioned as a limitation since we do not know if the QFT Gold Plus would perform the same.**

Reply 1: Thank you for your valuable comment. As our institution continued to use QFT GIT, we were unaware that it had been replaced by QFT Gold Plus worldwide. What the reviewer pointed out has been corrected in the text as follows. In addition, during this study, only 3 patients performed the QFT Gold Plus test in our medical center. As your comment we corrected our manuscript as follows.

### **Introduction Section Line 82-84**

In the current study, we aimed to investigate the clinical relevance of false-negative results of the QuantiFERON-TB Gold In-Tube (QFT-GIT; Cellestis Ltd, Carnegie, Australia) assay in the peripheral blood, ~~one of the most common commercial IGRA kits~~, and its associated risk factors in patients diagnosed with pleural TB.

### **Methods Section Line 94**

QFT-Gold plus, a new generation of QFT assay was used in three subjects out of 650 patients in our study.

### **Discussion section Line 307~**

We added below paragraph as you suggested.

“Lastly, few patients (3 out of 199 cases) were tested with QFT-Gold Plus instead of QFT-GIT. However, the two tests are known to have similar diagnostic efficacy (26), so it seems little effect on our final conclusion.”

26. Takeda K, Nagai H, Suzukawa M, et al. Comparison of QuantiFERON-TB Gold Plus, QuantiFERON-TB Gold In-Tube, and T-SPOT.TB among patients with tuberculosis. J Infect Chemother 2020;26:1205-12.

**Comment 2: Methods: include study design please.**

Reply 2: Thank you for your comment. As your comment we revised our manuscript as follows.

**Abstract, Line 37, Methods , and December 2020** were reviewed retrospectively

### **Methods section**

#### **Line 88~ Study design and population**

This observational retrospective study reviewed medical charts of 650 patients diagnosed with pleural TB by ICD 10 code A165 between January 2009 and December 2020 at a tertiary, referral hospital (1369 beds) in South Korea. The patients who underwent QFT-GIT in the peripheral blood and analysis of pleural fluid simultaneously before starting anti-TB medication were included. Patients aged <18 years were excluded.

**Comment 3: Methods and introduction: you need to specify somewhere that the QFT were performed on blood (as opposed to pleural fluid), for the reader's clarity**

Reply 3: We have stated that we had performed tests on blood samples for the readers to clearly understand in the whole manuscript, including Methods and Introduction

**Comment 4: Line 91-91: check if 'mitogen minus nil' is correct?**

Reply 4: We are sorry for the mistake and changed it to "TB antigen minus nil". The entire manuscript has been revised for the QFT-GIT inspection method as follows

#### **QuantiFERON TB Gold In-Tube (QFT-GIT) test**

IGRA was performed using the QFT-GIT tool before anti-TB treatment according to the manufacturer's instructions. The IGRA test was performed using the peripheral blood. The

QFT-GIT result was defined as positive, indeterminate or negative. The tests were defined as positive if the IFN- $\gamma$  level of Nil was  $\leq 8.0$  IU/mL, and that of TB antigen minus Nil was  $\geq 0.35$  IU/mL and 25% of the Nil value. The negative result was if the IFN- $\gamma$  level of Nil was  $\leq 8.0$  IU/mL, that of mitogen minus Nil was  $\geq 0.5$  IU/mL, and that of TB antigen minus Nil was  $< 0.35$  IU/mL or 25% of the Nil value. An indeterminate result was defined when the IFN- $\gamma$  level of Nil was  $\leq 8.0$  IU/mL, that of TB antigen minus Nil was  $< 0.35$  IU/mL or  $< 25\%$  of Nil value, and mitogen minus Nil was  $< 0.5$  IU/mL or if the INF- $\gamma$  level of Nil was  $> 8.0$  IU/mL. In the current study, negative and indeterminate results of QFT-GIT were classified as a negative subgroup from a clinical point of view.

**Comment 5: Lines 102-107: Please indicate if all participants had all these tests? If not, then a simple ‘where done’ would demonstrate this.**

Reply 5: We checked microbiological tests according to each respiratory specimen as below. As you commented, we edited the sentence clearly because all the patients did not have all the tests.

**Line 102:** Sputum, bronchial washing fluid or pleural fluid were used for microbiological tests for M. tuberculosis such as AFB stain or culture, TB PCR.

Pleural fluid AFB: 196

Sputum AFB: 195

Pleural fluid AFB culture: 198

Pleural fluid PCR: 171

Sputum PCR: 187

Sputum AFB culture: 195

**Comment 6: Statistical analysis: did you consider adjusting for multiple testing effect?**

**Reply 6:** Yes, we are fully aware of the multiple testing effect and we know that it can cause bias. In most cases of multiple testing effect, it may occur in the part where t-test is performed multiple times for statistics that need to be analyzed at once, such as ANOVA. A review of the statistical methods used in our study found no common issues associated with multiple testing effects. Rather than inserting each variable into the multivariate analysis and observing the results, we included all the variables which was statistically significant by univariate analysis in the multivariate analysis and got the results of them.

We already addressed the context in the sentence, Line 143-145 “Multivariate analysis using multiple logistic regression was performed for statistically significant predictors in the univariate analysis to determine the risk factors associated with negative QFT-GIT results.”

**Comment 7: Line 208: please specify what type of TB the metaanalysis looks at? Pulmonary?**

Reply 7: Of the 17 papers included in this meta-analysis, five were studies on extrapulmonary TB and the rest were pulmonary tuberculosis. Therefore, the authors decided to leave the script unchanged as "patients with tuberculosis".

Author, year	Nationality	Study design	Sample size	Age, years median (range, IQR or $\pm$ SD)	Male (%)	HIV (%)	EPTB (%)	History of TB (%)	IGRA	True-positive (%)	False-negative (%)
Kim 2018	South Korea	retrospective	163	55 (65 < 35%)	85 (52.1)	1 (0.6)	163 (100)	18 (11.0)	QFT-GIT	69.9	28.8
Yang 2018	China	retrospective	2,425	43.6 $\pm$ 18.5	1,561 (64)	0	143 (5.9)	nd	T-SPOT	75.1	24.9
Nugyen 2018	USA	retrospective	1,487	47 (IQR: 30–61)	942 (63.3)	90 (13.2)	196 (13.2)	32 (2.2)	875 (65.4) in QFT-GIT 463 (34.6) in T-SPOT	87.7 in total nd in QFT-GIT nd in T-SPOT	12.3 in total 12.2 in QFT-GIT 16.4 in T-SPOT
Di 2018	China	retrospective	98	nd (<30 21.4%, 30–60 50.0%, 60 < 28.6%)	55 (56.1)	nd	69 (70.4)	0	T-SPOT	83.7	16.3
Lian 2017	China	retrospective	556	44.2 (range 0.75–85)	333 (59.9)	2 (0.4)	358 (64.4)	nd	T-SPOT	86.2	13.8
Kown 2015	South Korea	retrospective	1,264	50.3 (IQR: 35–69)	718 (56.8)	0	158 (12.5)	165 (13.1)	QFT-GIT	85.6	14.4
Choi 2015	USA	retrospective	300	48.1 $\pm$ 22.1	195 (65.0)	18 (6)	52 (17.3)	nd	QFT-GIT QFT-2G	70.3	29.7
Visser 2015	Europe	retrospective	664	41 (IQR: 30–53) in QFT 41 (IQR: 28–56) in T-SPOT	nd	nd	nd	nd	QFT-GIT T-SPOT	66.6 in total nd in QFT-GIT nd in T-SPOT nd in both IGRA	33.2 in total 22.7 in QFT-GIT 4.8 in T-SPOT 1.0 in both IGRA
Pan 2014	China	prospective	774	45 (range 11–91)	465 (60.1)	0	244 (31.5)	nd	T-SPOT	89.9 in total 91.3 in PTB 86.9 in EPTB	10.1 in total 8.7 in PTB 13.1 in EPTB
Lee 2013	South Korea	prospective	128	65 < 21.1%	53 (41.4)	5 (3.9)	84 (66)	13 (10.2)	T-SPOT	82.8	17
Joen 2013	South Korea	retrospective	168	54.8 $\pm$ 20.1	102 (60.7)	0	10 (5.9)	3 (1.8)	QFT-GIT	76.8	23.2
Kim 2013	South Korea	retrospective	44	64 $\pm$ 19.0	17 (39)	2 (4.5)	nd	nd	QFT-GIT	68.2	16
Aabye 2012	Denmark Tanzania	retrospective	34 172	50 (range 23–76) 32 (range 15–84)	24 (70.5) 64 (37.2)	4 (8) 75 (43.6)	8 (23.1) nd	0	QFT-GIT	64.7 71.5	11.3 3.8
Hang 2011	Viet Nam	prospective	504	38.8 (IQR: 29.2–50.8)	399 (79.2)	44 (8.7)	0	nd	QFT-GIT	92.3	4.8
Kim 2011	South Korea	retrospective	362	49 (IQR: 16–94)	197 (54.4)	0	0	55 (15.2)	QFT-GIT	85.9	14.1
Komiya 2010	Japan	retrospective	215	67 (IQR: 50–79)	156 (73)	0	0	nd	QFT-G ELISPOT	74 93	23 7.4
Raby 2008	Zambia	retrospective	112	31 (IQR: 25–36)	71 (63)	59 (52.7)	nd	20 (18)	QFT-GIT	74	12

**Table 1.** Characteristics of the studies included in this systematic review. EPTB, extrapulmonary TB; nd, not described.

**Comment 8: Line 210-213: There was a significantly lower serum lymphocyte count in the false negative group, which may be mentioned, though it did not come up in the univariate and multivariate analysis.**

Reply 8: Thank you for the valuable comment. As the reviewer noted, the serum lymphocyte count was lower in false-negative group in our study, although not significant. However, the serum lymphocyte percentage was significantly lower in false-negative group. So, we revised manuscript as follows.

**Line 241~243:** “Although serum lymphocyte count and age were not statistically significant in our study, we also observed that the QTF-GIT-negative group tended to have lower serum lymphocyte count and be older than the QTF-GIT-positive group.”

**Comment 9:** I would like more detail on what kind of pneumoconiosis were present in the patients – silicosis? CWP? Asbestos? The explanation in the discussion suggests it is all silica-related, but pneumoconiosis is a broad category of disease.

Reply 9: Of the 8 pneumoconiosis patients enrolled in the study, 6 had CWP and 2 had silicosis. Although we do not think that the pathophysiological mechanism is different, there were only studies on the pathogenesis of tuberculosis caused by silicosis rather than tuberculosis related to CWP, so we cited the studies with tuberculosis. We also searched for pathogenesis of TB in CWP and revised manuscript as follows.

**Line 256:** Of 8 pneumoconiosis patients enrolled in our study, 6 patients had coal workers pneumoconiosis and 2 patients had silicosis. Pneumoconiosis, especially silicosis is well known to be vulnerable to pulmonary TB because silica particles in the lungs induce macrophage dysfunction and decrease cell-mediated immunity (18,19). Also, coal workers pneumoconiosis is known to be vulnerable to pulmonary tuberculosis because coal dust is not a pure substance and usually contains 1-2% free silica (20).

**Comment 10: Line 247:** could the hypoproteinaemia also be related to the end stage renal disease patients? Could this be a confounder, or rather could these two significant results be pointing toward the same thing?

Reply 10: When the serum protein level was analyzed by dividing the group into groups with and without ESRD, the group with ESRD had significantly lower serum protein. However, there was no significant difference in hypoproteinemia between the two groups.

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	Total (N=199)	None ESRD group (N=184)	ESRD group (N=15)	<i>P</i> value
Serum protein	6.7 ± 0.8	6.7 ± 0.8	6.2 ± 0.4	< 0.001
Hypoproteinaemia	36 (18.1%)	32 (17.4%)	4 (26.7%)	0.482

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We also analyzed only patients without ESRD, excluding ESRD patients, to determine whether ESRD acted as a confounding factor. However, even when only patients without ESRD were analyzed separately, plasma protein was significantly lower in the false-negative group, and there were significantly more hypoproteinemia patients in the false-negative group.

<b>When patients with ESRD were excluded from the analysis.</b>	Total (N=184)	QFT-GIT-negative group (N=30)	QFT-GIT-positive group (N=154)	<i>P</i> value
Serum protein	6.7 ± 0.8	6.4 ± 1.0	6.8 ± 0.7	0.029
Hypoproteinaemia	32 (17.4%)	13 (43.3%)	19 (12.3%)	< 0.001

In addition, in the case of multivariate regression analysis presented in this text, ESRD and hypoproteinemia were considered and corrected as confounding factors. Therefore, the authors agree with the reviewer's opinion, and think that the two factors, ESRD and hypoproteinemia, have a certain relationship and affected the false-negative result.

**Comment 11: Line 259 onwards: Please comment on if any of those with poor outcomes were classified as ‘probable TB’, to rule out the possibility of misdiagnosis as a reason for both negative IGRA and poor outcome after TB treatment?**

Reply 11: As defined in the method, we divided all patients diagnosed with pleural tuberculosis into strictly confirmed and probable groups when collecting data. Probable pleural TB was defined as lymphocyte dominant exudate in the pleural fluid based on Light’s criteria, 79 with ADA >40 IU/L, and clinical improvement after anti-TB medication.

To alleviate the reviewer's concerns, both the probable TB group and confirmed TB group were analyzed separately by dividing the results into QFT-GIT-negative and -positive groups. There was no significant difference in treatment results between the two groups.

<b>Only the probable TB group was analyzed separately.</b>	Total (N=80)	QFT-GIT-negative group (N=17)	QFT-GIT-positive group (N=63)	<i>P</i> value
Favorable outcome	68 (85.0%)	12 (70.6%)	56 (88.9%)	0.118
Unfavorable outcome	12 (15.0%)	5 (29.4%)	7 (11.1%)	0.118
Discontinuation of anti-TB medication	5 (6.2%)	3 (17.6%)	2 (3.2%)	0.062
Follow-up loss	3 (3.8%)	0 (0.0%)	3 (4.8%)	1.000

Death	4 (5.0%)	2 (11.8%)	9 (7.6%)	0.197
- TB-related death	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
- Not TB-related death	4 (5.0%)	2 (11.8%)	2 (3.2%)	0.197

Only the confirmed TB group was analyzed separately.	Total (N=119)	QFT-GIT-negative group (N=17)	QFT-GIT-positive group (N=63)	<i>P</i> value
Favorable outcome	95 (79.8%)	12 (63.2%)	83 (83.0%)	0.063
Unfavorable outcome	24 (20.2%)	7 (36.8%)	17 (17.0%)	0.063
Discontinuation of anti-TB medication	1 (0.8%)	0 (0.0%)	1 (1.0%)	1.000
Follow-up loss	14 (11.8%)	3 (15.8%)	11 (11.0%)	0.696
Death	9 (7.6%)	4 (21.1%)	5 (5.0%)	0.035
- TB-related death	4 (3.4%)	1 (5.3%)	3 (3.0%)	0.506
- Not TB-related death	5 (4.2%)	3 (15.8%)	2 (2.0%)	0.028

**Comment 12: Line 54: I suggest you leave out the cutoff of ‘42’ as an example here, as cutoff values vary in different prevalence regions.**

Reply 12: We agreed with your opinion, so we modified it as follows.

**Line 76:** When there is a clinical suspicion of TB pleurisy but with borderline results in analysis of pleural effusion, such as mild elevated adenosine deaminase (ADA) and no microbiological evidence, a positive IGRA result in the blood could help clinician support a diagnosis of TB pleurisy.