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# Serum level of IL-8 is associated with reversion of QuantiFERON-TB gold in-tube tests

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SUMMARY

*Background:* Frequent reversion has been commonly observed in serial QuantiFERON-TB Gold In-Tube (QFT) tests, which limited its accuracy in defining the status of *Mycobacterium tuberculosis* (MTB) infection. Serum cytokine profiles might provide additional information to clarify the infection status.

*Method:* Based on a population-based cohort study aiming to track MTB infection acquisition and disease development, serum profiles of 12 cytokines were determined by bead-based multiplex assays in parallel with QFT and tuberculin skin tests (TST) to explore potential relation between serum cytokines and MTB infection status.

*Results:* Totally, 309 subjects got QFT conversion in one year (2013–2014) and 46.92% (145/309) of them got reversion in 2015. The study subjects were classified into three groups according to their QFT and TST results in 2015 (QFT persistence positive, QFT-/TST + and QFT-/TST-). The serum levels of MCP-1 and IL-8 were significantly different among the three groups. Furthermore, level of IL-8 was dramatically lower in QFT-/TST- group as compared to the other two groups, and no significant difference was observed for QFT-/TST + group as comparing with persistent positive group.

*Conclusion:* Our results suggested that the decreased serum level of IL-8 might be potential biomarker to identify QFT reversion caused by infection clearance.



## Introduction

Interferon-gamma release assays (IGRAs) showed advantages for diagnosing *Mycobacterium tuberculosis* (MTB) infection, as compared to tuberculin skin test (TST), with improved specificity in BCG vaccinated individuals and convenience without repeat visits.[1] Two commercial IGRAs, QuantiFERONTB Gold In-Tube (QFT, Qiagen, USA) and T-SPOT.TB (Oxford Immunotec, Oxon, UK), have been recommended to replace TST in some high-income countries, such as United States.[2] However, frequent QFT conversion and reversion in serial tests have been observed in several studies.[3–6] Recently, MTB infection was suggested to be one continuous spectrum ranging from near active tuberculosis (TB) with obvious lesions to cleared infection with no or only minimal risk of developing disease.[7,8] Whether such conversion/reversion represents new infection/infection clearance, dynamic immunologic process or simply "wobble" around the cut-point due to test reproducibility is still unclear.[9] In addition, QFT is susceptible to modulation by various factors, such as tube order, blood volume, incubation duration or even manufacturing defects.[10] As a result, QFT conversion should be interpreted with caution to be used for targeting high-risk population for latent tuberculosis infection (LTBI) preventive treatment or for estimating vaccine efficacy.[11] In the same way, the protection effect of preventive treatment cannot be estimated by QFT reversion either.[12]

Increasing cut-off value or choosing an interval as positive criterion has been suggested to be a potential solution to improve the definition of QFT conversion.[13] Moreover, there were growing numbers of studies explored optimized biomarkers for diagnosis of LTBI and active disease. A better diagnostic performance of several cytokines other than IFN-$\gamma$ had been found to increase the detection rate among TB patients.[14–16] Thus, the expression profiles of cytokines/chemokines might provide new insights to define MTB infection status giving a result of QFT conversion or reversion. To date, there are few published data on the relation between serum level of cytokines and QFT results from serial tests. Based on a population-based cohort study, we assessed the expression profiles of 12 selected cytokines for individuals with QFT conversion and subsequent retest results.

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**Table 1**
Characteristics of the study participants according to QFT retested results.

| Variable | QFT persistent positive | QFT reversion | *p* for $\chi 2$ test |
|---|---|---|---|
| **Age** | | | |
| ≤45 years | 32 (19.51) | 43 (29.66) | 0.106 |
| 46–55 years | 42 (25.61) | 37 (25.52) | |
| 56–65 years | 43 (26.22) | 25 (17.24) | |
| ≥ 65 years | 47 (28.66) | 40 (27.59) | |
| Median (Q25–Q75) | 59 (48–66) | 52 (44–66) | 0.647 |
| **Gender** | | | |
| Female | 98 (59.76) | 74 (51.03) | 0.124 |
| Male | 66 (40.24) | 71 (48.97) | |
| **QFT in 2014 (UI/ml)** | | | |
| Median (Q25–Q75) | 0.70 (0.53–1.35) | 0.54 (0.40–0.82) | **<0.001** |

Abbreviations: QFT = QuantiFERON-TB Gold. Q25 = 25% quantile; Q75 = 75% quantile.

## Materials and methods

### *Study population*

Serum samples used in the present study were collected from a population-based, multicenter prospective study addressing in MTB infection and active disease development conducted in rural China between 2013 and 2015.[17,18] In the baseline survey in 2013, 21,022 rural residents aged 5 years older from 4 study sites underwent both QFT and TST tests. One year later, those with either TST negative (induration < 10 mm) or QFT negative (IFN-$\gamma$ < 0.35 IU/mL) were retested. Among them, 390 participants were identified with QFT conversion (IFN-$\gamma \geq 0.35$ IU/ml). Subsequently, 330 (84.6%) converters in 2014 completed the second QFT retest in 2015, and TST was offered only to those QFT reversed. Finally, a total of 309 QFT converters that should have both serum samples in 2014 and in 2015 were included in this study.

The ethics committees of the Institute of Pathogen Biology and the Chinese Academy of Medical Sciences approved the study.

### *QFT and TST measurements*

QFT was performed as recommended by the manufacturer using a cut-off value of ⩾0.35 IU/mL. TST was done immediately after QFT blood collection using the Mantoux method by injecting 0.1 mL of 5 tuberculin units purified protein derivative (Xiangrui, Beijing, China) intradermaly, into the left forearm as a preference.[19] TST positive was defined by an induration ≥ 10 mm. QFT conversion was defined as baseline IFN-$\gamma$ < 0.35 IU/ml in 2013 and ⩾ 0.35 IU/ml in the first yearly retest in 2014. QFT reversion was defined as IFN-$\gamma$ ⩾ 0.35 IU/ml in 2014 and < 0.35 IU/ml in the second yearly retest in 2015. QFT persistent positivity was defined as IFN-$\gamma$ ⩾ 0.35 IU/ml in both retests in 2014 and in 2015.

### *Cytokines measurements*

Twelve cytokines (interleukin (IL)-1$\beta$, IL-1$\alpha$, IL-2, IL-6, IL-8/CXCL8, IL-10, IL12p40, IL17A, IP-10/CXCL10, macrophage chemoattractant protein 1 (MCP-1)/CCL2, tumor necrosis factor (TNF), IFN-$\gamma$) were selected for examination due to potential relation to MTB infection and disease development with evidence of publication (Supplementary Table 1). Levels of the selected cytokines were determined for each sample (25 µl) by magnetic bead suspension array using the Bio-Plex Pro Human Cytokine panels (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The results were analyzed on a Multiplex Analyzer using Bio-Plex Manager 6.0 (Bio-Rad Laboratories). For cytokines with > 50% of the samples below the lower detection level (LDL) of the assay will be excluded from further statistical analysis. For cytokines with occasional values (< 25%) below the LDL were assigned an averaged value between 0 and the lowest detectable level in each assay plate.

**Table 2**
Incidence of reversion was inversely associated with the quantitative value of QFT.

| Primary IFN-$\gamma$ value in 2014 (IU/ml) | Incidence of reversion (%) | *p* value for $\chi 2$ test |
|---|---|---|
| 0.35–0.44 | 52/78 (66.67%) | **<0.001** |
| 0.45–0.63 | 36/77 (46.75%) | |
| 0.64–1.04 | 30/77 (38.96%) | |
| >1.04 | 27/77 (35.06%) | |

### *Statistical analysis*

Statistical analyses were performed using SAS 9.4 version (SAS institute, Cary, NC) and GraphPad Prism 5. The Chi-square ($\chi^2$) test were used to compare the distribution of categorical variables between various groups. The level of cytokines were presented with median (Q25-Q75) and compared with Kruskal-Wallis test. If significant difference were found among groups ($p < 0.05$), multiple comparisons were conducted using the Dwass-Steel-Critchlow-Fligner (DSCF) test. Wilcoxon signed rank test was used to evaluate changes of cytokines levels for the same person in different time points. In addition, sensitivity analyses were conducted using a more strict alternative definition of "conversion": IFN-$\gamma$ < 0.35 IU/ml in 2013 baseline test and ⩾ 0.70 IU/ml in 2014 retest. QFT persistent positivity was defined as IFN-$\gamma$ ⩾ 0.70 IU/ml in both retests in 2014 and in 2015.

## Results

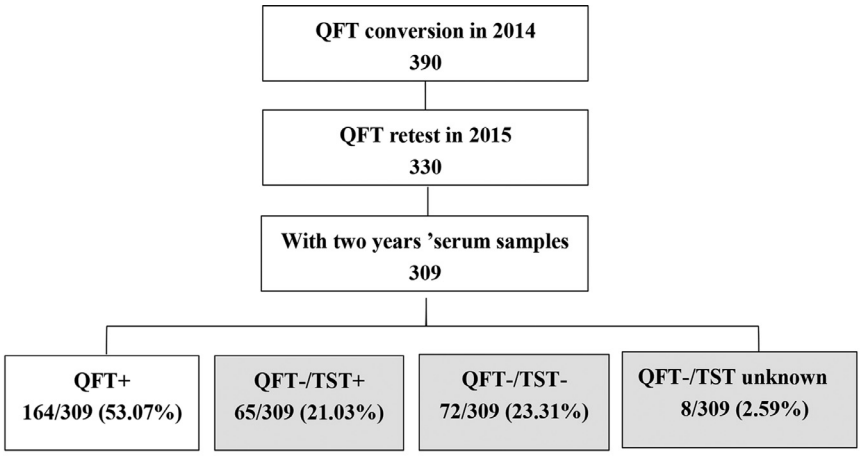
### *Characteristics of the study participants and their QFT/TST results*

A total of 390 QFT converters were identified in 2014, 330 of them completed the retest in 2015 and 309 with serum samples for both retests were included in the present study. Table 1 shows characteristics of the study participants according to their QFT results in 2015 (persistent positive vs. reversed). No significantly differences were found for two groups with respect of age and sex. Quantitative results of QFT in 2014 were remarkably lower in QFT reversion group (median = 0.54UL/ml) as compared with QFT persistent positive group (median = 0.70UL/ml). In 2015, 46.92% (145/309) of the new convertors got reversed. The incidence of reversion was inversely associated with the quartile value of QFT quantitative results in 2014 ($p < 0.001$) as shown in Table 2.

TST was retested for QFT reversions and the results were showed in Fig. 1A. Apart from 8 participants without TST results, 72 participants with TST negative results and 65 participants with TST positive results. The participants were divided into three groups according to their QFT/TST status (QFT persistence group, QFT-/TST + group and QFT-/TST- group). Comparison of QFT quantitative results were further conducted among three groups. As Fig. 1B shows, although dichotomous results were positive, the median level of IFN-$\gamma$ in 2014 in QFT persistence group was higher than the other two groups. In addition, for participants got reversed, the median level of IFN-$\gamma$ in QFT-/TST + group was significantly higher than QFT-/TST- group in 2015, no statistical difference were found for them in 2014.

### *Serum levels of selected cytokines in different time-points between subgroups*

Twelve cytokines were detected using Luminex technology. Six of them (IL-1$\alpha$, IL-2, IL17A, TNF, IFN-$\gamma$, IL12p40) were below the LDL, thus no further data analysis was conducted. For the rest six

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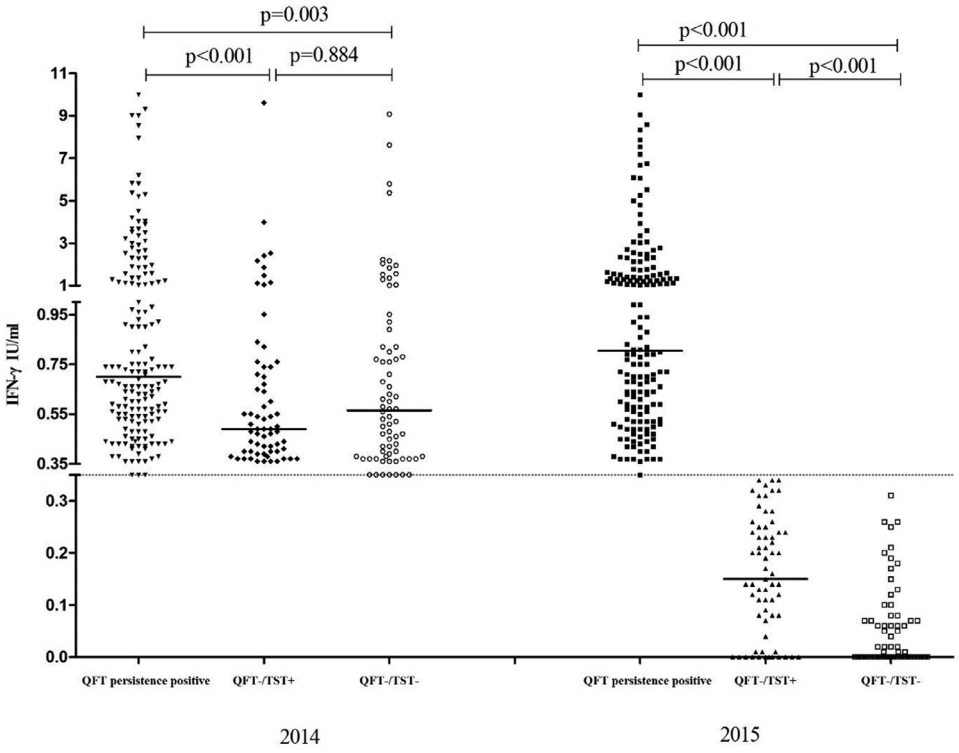



B

**Fig. 1.** Qualitative and quantitative results of QFT in serial tests. A total of 12,716 participants with baseline QFT negative results in 2013 were retested in 2014 and 390 of them got conversion, 330 converters completed the second yearly retest in 2015 and 309 of them whose serum samples available were included in the present study. In 2015, 46.92% (145/309) converters got reversed (Fig. 1A) and were tested by TST subsequently. For QFT test, the median level of IFN-$\gamma$ in 2015 in QFT-/TST + group was 0.15UI/ml, which was significantly higher than the level in QFT-/TST- group ($p < 0.001$). However, no difference was found for the median level of IFN-$\gamma$ in 2014 between these two groups (Fig. 1B). Horizontal line indicates median levels of IFN-$\gamma$.

cytokines (IL-1$\beta$, IP-10, IL-6, IL-8, IL-10, MCP-1), occasional values below the LDL were assigned an averaged value between 0 and the lowest detectable level in each assay plate.

As shown in Table 3, the levels of the cytokines were presented by median (Q25-Q75). No significant difference was found for any cytokine among the three groups in 2014. While in 2015, two cytokines (IL-8 and MCP-1) were discriminatively expressed among the three groups ($p = 0.011$ for IL-8 and $p = 0.017$ for MCP-1).

As showed in Fig. 2A, multiple comparisons were further conducted among the three groups with respect of the level of IL-8 and MCP-1. The serum levels of MCP-1 and IL-8 (Median ± IQR, 4.20 ± 6.25 pg/ml for IL-8, 32.39 ± 65.83 pg/ml for MCP-1) were dramatically lower in QFT-/TST- group than in QFT persistent positive group (Median ± IQR, 6.81 ± 10.58 pg/ml for IL-8, 61.70 ± 86.87 pg/ml for MCP-1). No such difference was found for QFT-/TST + group (Median ± IQR, 6.67 ± 10.76 pg/ml for IL-8, 40.63 ± 69.59 pg/ml for MCP-1) as comparing with QFT persistent positive group. In addition, the results were not changed for IL-8 when using strict definition with IFN-$\gamma > 0.70$ IU/ml as definition of conversion. (Fig. 2B).

**Table 3**
The serum levels of the selected cytokines in different subgroups.

| Cytokines | QFT persistence positive | QFT-/TST+ | QFT-/TST- | *p* for *Kruskal-Wallis* test |
|---|---|---|---|---|
| | *>Median value (Q25-Q75) in 2014* | | | |
| *IL-1β* | 0.42 (0.07–0.91) | 0.42 (0.08–1.47) | 0.57 (0.21–1.41) | 0.116 |
| *IL-6* | 2.27 (0.94–4.76) | 2.29 (0.92–5.84) | 2.78 (0.64–6.39) | 0.659 |
| *IL-8* | 4.56 (1.92–10.90) | 4.64 (2.62–11.34) | 5.53 (2.17–9.35) | 0.929 |
| *IL-10* | 2.08 (0.52–4.26) | 1.99 (0.48–5.01) | 2.76 (0.64–5.73) | 0.235 |
| *MCP-1* | 33.39 (4.16–61.33) | 33.67 (4.24–70.13) | 38.81 (6.92–71.60) | 0.584 |
| *IP-10* | 402.70 (293.92–588.64) | 449.66 (334.59–585.64) | 439.52 (351.43–626.04) | 0.211 |
| | *Median value (Q25-Q75) in 2015* | | | |
| *IL-1β* | 0.82 (0.24–2.16) | 1.16 (0.08–1.95) | 0.65 (0.07–2.80) | 0.711 |
| *IL-6* | 3.14 (1.16–8.06) | 2.75 (0.90–7.71) | 2.29 (0.99–8.37) | 0.603 |
| *IL-8* | 6.81 (2.81–13.39) | 6.67 (2.62–13.38) | 4.20 (1.84–8.09) | *0.011* |
| *IL-10* | 2.59 (0.47–6.71) | 2.54 (0.39–4.93) | 2.24 (0.33–9.96) | 0.684 |
| *MCP-1* | 61.70 (23.54–110.41) | 40.63 (13.99–83.58) | 32.39 (13.50–79.33) | *0.017* |
| *IP-10* | 591.08 (417.43–912.27) | 529.99 (345.23–767.70) | 519.05 (368.80–864.46) | 0.224 |

Abbreviations: QFT = QuantiFERON-TB Gold. Q25 = 25% quantile; Q75 = 75% quantile; TST = tuberculin skin test.

**Table 4**
The median level of the selected cytokines classified by the quartile value of 2014 QFT quantitative results among QFT reversions.

| Cytokines | 0.35 IU/ml ≤ IFN-$\gamma$ ≤ 0.44 IU/ml | 0.45IU/ml ≤ IFN-$\gamma$ ≤ 0.63IU/ml | 0.64IU/ml ≤ IFN-$\gamma$ ≤ 1.04IU/ml | IFN-$\gamma$ > 1.04IU/ml | *p* for *Kruskal-Wallis* test |
|---|---|---|---|---|---|
| | *Median value (Q25-Q75) in 2014* | | | | |
| *IL-1β* | 0.48 (0.15–0.1.36) | 0.51 (0.17–1.62) | 0.49 (0.19–1.11) | 0.70 (0.21–1.58) | 0.982 |
| *IL-6* | 2.04 (0.40–5.98) | 2.94 (0.72–6.06) | 2.32 (1.31–6.23) | 1.75 (0.26–6.58) | 0.839 |
| *IL-8* | 4.45 (2.13–8.99) | 5.77 (2.38–10.57) | 4.91 (2.81–9.50) | 5.58 (2.01–13.25) | 0.812 |
| *IL-10* | 2.78 (0.45–6.45) | 2.33 (0.50–4.40) | 2.21 (0.64–7.39) | 2.46 (1.56–8.05) | 0.643 |
| *MCP-1* | 33.55 (1.55–75.22) | 34.51 (15.18–59.16) | 45.69 (4.48–74.39) | 39.60 (19.94-68.71) | 0.771 |
| *IP-10* | 460.68 (339.19–626.27) | 411.05 (333.32–598.02) | 447.45 (367.05–559.76) | 405.22 (303.17–530.69) | 0.735 |
| | *Median value (Q25-Q75) in 2015* | | | | |
| *IL-1β* | 0.87 (0.19–2.13) | 0.43 (0.11–1.76) | 1.07 (0.07–1.98) | 0.38 (0.07–2.65) | 0.772 |
| *IL-6* | 4.16 (1.12–7.31) | 2.16 (0.83–7.52) | 2.23 (0.82–7.31) | 1.85 (0.57–8.93) | 0.820 |
| *IL-8* | *5.62 (2.22–11.84)* | *5.52 (2.45–9.67)* | *3.94 (1.83–7.56)* | *3.84 (1.78–6.73)* | 0.617 |
| *IL-10* | 2.81 (0.34–10.53) | 0.62 (0.33–4.97) | 1.89 (0.41–4.29) | 1.77 (0.18–8.71) | 0.477 |
| *MCP-1* | 42.57 (14.03–78.01) | 29.64 (13.50–52.72) | 36.83 (12.54–90.35) | 34.02 (1.74–95.09) | 0.780 |
| *IP-10* | 517.21 (347.06–903.35) | 568.23 (362.74–741.87) | 492.50 (339.17–675.82) | 552.98 (336.71–817.53) | 0.800 |

Abbreviations: IFN = interferon. QFT = QuantiFERON-TB Gold. Q25 = 25% quantile; Q75 = 75% quantile.

When QFT reversions were classified into four groups according to the quartile value of QFT quantitative results in 2014 (0.35 IU/ml ≤ IFN-$\gamma$ ≤ 0.44 IU/ml, 0.45 IU/ml ≤ IFN-$\gamma$ ≤ 0.63 IU/ml, 0.64 IU/ml ≤ IFN-$\gamma$ ≤ 1.04 IU/ml, IFN-$\gamma$ > 1.04 IU/ml). The median levels of the selected cytokines among the four groups in both 2014 and 2015 were showed in Table 4. The concentrations of IL-8 were inversely expressed with the increasing of initial QFT results in 2014 although no significantly difference were found.

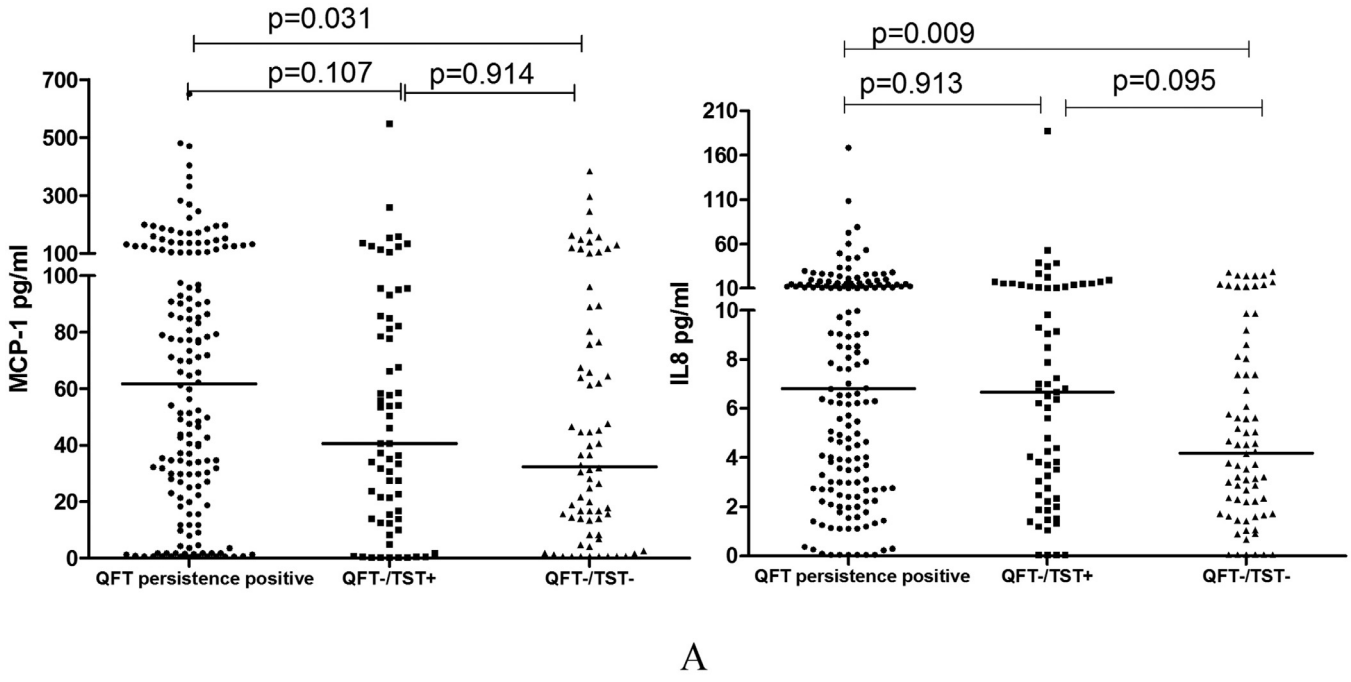
## Discussion

The present study reported serum expression profiles of six selected cytokines along with QFT serial tests based on a population-based prospective study. Nearly a half of QFT convertors (46.92%, 145/309) got reversed in one year time. Serum levels of MCP-1 and IL-8 were found to be significantly associated with QFT reversion, and the decreasing serum IL-8 might potentially predict infection clearance indicated by negative results for both TST and QFT.

In current study, the incidence of reversion was inversely associated with the quantitative value of QFT at the time point of getting conversion. It was consistent with previous reports that the likelihood of reversion increased in subjects with initial test results close to the diagnostic threshold, generally within a borderline zone between 0.2 to 0.7 IU/ml.[20,21] Although, the phenomena were common in various populations, the mechanism of reversion has not been fully understood. When individuals with QFT reversion were sub-grouped with respect to the results of TST, nearly a half of the QFT reversions were TST positive, thus, we tend to consider QFT reversion in discordant group in 2015 might be mainly caused by dynamic immunologic process. On the other hand, the concordant group (QFT-/TST-) might reflect a non-infection status. One of the possible explanations was natural clearing of MTB infection. A study addressing the kinetics of MTB–specific Th1-type T-cell responses after MTB infection showed the disappearance of MTB-specific Th1-type T cell responses in untreated TB contacts with TB infection, suggesting that early infection may be eradicated in a minority of individuals.[22] Another possible explanation was the initial conversion in 2014 might be false-positive. Misclassification was inevitable with such subgrouping in the condition of lacking gold standard selection method.

As the likelihood of “wobble” caused by non-perfect test reproducibility and dynamic host immunity increased in subjects with initial test results close to the cut-point, the possibility of “true” reversion caused by infection clearance should be elevated with the increasing of initial QFT result. As shown in Table 4, the lowest level of IL-8 in 2015 was observed in the group with initial IFN-$\gamma$ > 1.04 IU/ml which might reflect attenuated inflammatory response to MTB infection. As a member of the CXC chemokine family, IL-8 serves as a chemoattractant for neutrophils, T-cells, and monocytes and controls trafficking of these cells to the sites of infection.[23] Enhanced IL-8 release and gene expression in macrophages or monocytes has been shown after exposure to MTB and its components.[24,25] IL-8 gene polymorphism was also found to be associated with susceptibility to TB.[26] Based on above evidences, decreased IL8 level might reflect status of non-TB-infection or reduced bacterial replication activity and further suggested the potential role of IL8 on predicting MTB infection clearance.

Normally, protection rate is used to evaluate the efficacy of LTBI treatment. However, it needs a long observation period to get the incidence results and is not suitable for practice without

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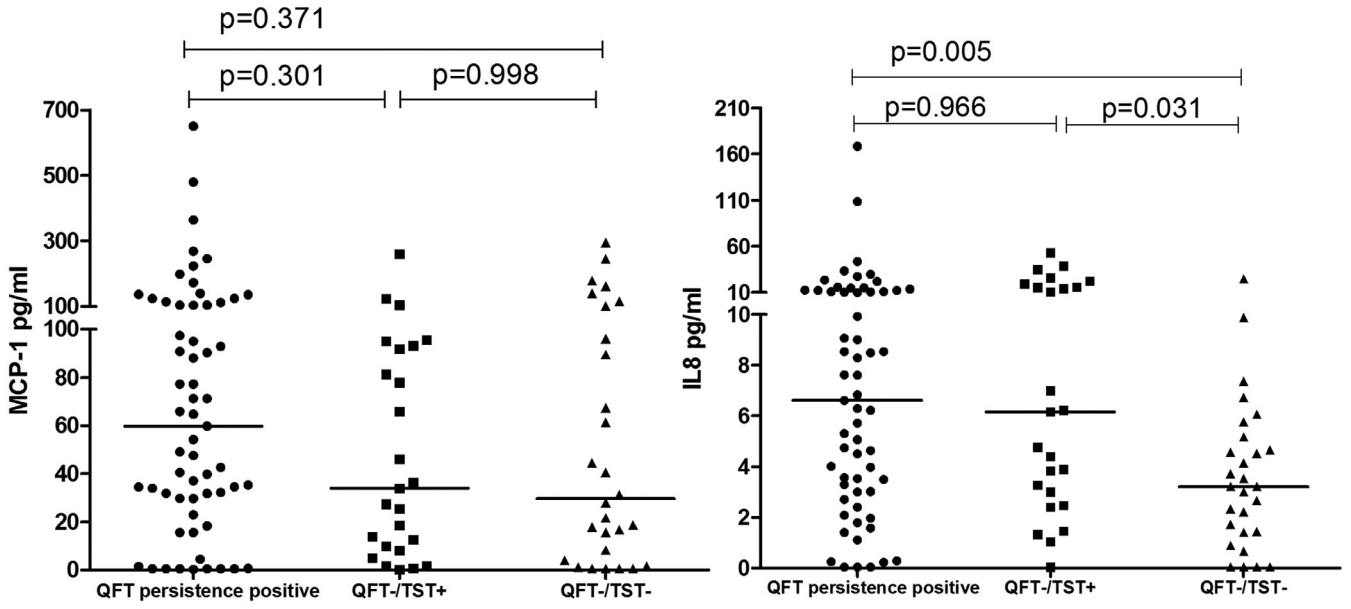


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**Fig. 2.** Distribution of the serum levels of MCP-1 and IL-8 in sub-grouped participants**.** According to QFT/TST results in 2015, the participants were divided into three groups as with QFT persistent positive, with QFT-/TST+, and with QFT-/TST-. Serum levels of MCP-1 and IL-8 were compared among the groups by Kruskal–Wallis test and multiple comparisons were conducted by the DSCF test. Horizontal line indicates the median value of the two cytokines. Two definitions of QFT conversion were used: (1) IFN-$\gamma$ < 0.35 IU/ml in baseline test in 2013 and ≥ 0.35 IU/ml in retest in 2014 (Fig 2A) and (2) IFN-$\gamma$ < 0.35 IU/ml in baseline test in 2013 and ≥ 0.70 IU/ml in retest in 2014 (Fig 2B).

untreated control. Therefore, it is warranted to explore biomarkers to evaluate the performance of LTBI treatment. QFT reversion has been frequently studied to predict LTBI treatment success.[27–29] However, the findings were inconsistent. Our new published meta-analysis showed that similar reversion rates were observed among participants without preventive treatment as compared with those completed prophylactic therapy. Hence, QFT reversion might be occurred in many cases but not solely specifically caused by prophylactic treatment.[12] By far, numerous studies have been engaged in identifying other potential diagnostic or prognosis biomarkers for LTBI management. However, very few well-qualified or validated ones have been developed for application in practice. The advent of simple and rapid bead-based multiplex assays has allowed for quantification of multiple cytokines and chemokines as alternative immunodiagnostic markers to IFN-$\gamma$.[30] A study conducted by M. Ruhwald and colleagues evaluated the potential of using antigen specific IP-10 and MCP-2 expression for in vitro diagnosis of MTB infection. By combining IP-10 and IFN-$\gamma$ tests,

the detection rate increased to 90% among TB patients.[14] Another study conducted in LTBI patients aiming to determine changes in MTB-specific antigen-induced cytokine biomarkers in patients receiving therapy for latent TB. A statistically significant decline in IL-1ra responses were observed over therapy at 6 and 9 months with all 3 stimulants (CFP-10, ESAT-6, PPD) which prompted the potential of Mycobacteria-specific cytokine responses as correlates of treatment response in LTBI.[31] In current study, the decreased level of IL-8 might provide its potential as prognosis biomarkers for LTBI treatment in the future.

When interpreting our results, several limitations should be kept in mind. First, although we used the same Luminex kit lot to test serum samples of different time points and detected them in the same time, the influence of preservation time on sample quality couldn't be completely excluded as the median levels of all six cytokines elevated from 2014 to 2015 significantly for QFT persistence groups. Nevertheless, for QFT-/TST- group, no such trend was observed and the concentrations of several cytokines even down-regulated (Supplementary Table 2). Therefore, the possibility of the depressed levels of cytokines reflecting the attenuated anti-TB immune responses couldn't excluded as well. Further studies with larger sample size are needed for verification. Second, even lacking golden standard to define MTB infection, grouping QFT reversion by TST results could not absolutely reflect the real infection status. Thus, misclassification bias could not be excluded. Third, as with other studies reported,[32,33] the expression of IP-10 were hundreds fold higher than other cytokines which suggested the expression level of cytokines itself would influence the sensitivity of the test result. In current study, serum cytokine testing was failed for 6 selected cytokines because of under detection level. Thus, largely different expression levels of the selected cytokines might influence the accuracy of the relation estimation in various study population.

In conclusion, frequently occurred reversion in TB infection retests might be mainly explained by the technical and host immunological factors, but reversion caused by infection clearance could not completely excluded. Decreased serum IL-8 might be a potential marker to reflect such reversion due to infection clearance indicated by negative result of TST as well. It provided a clue for exploring prognosis biomarkers for LTBI treatment, but our findings need further verification supported by more solid evidence of infection clearance.

## Conflict of interest

We declare no competing interests.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2018.08.010.

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