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Authors

Slater, Madeline
Tran, Minh-Chi
Platt, Lauren
et al.

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In vitro immunomodulation for enhancing T cell–based diagnosis of *Mycobacterium tuberculosis* infection

Madeline Slater[a], **Minh-Chi Tran**[b], **Lauren Platt**[f], **Lien T. Luu**[c], **Ha T. Phan**[c], **Phuong T. Pham**[c], **Tam B. Do**[c], **Hanh T. Nguyen**[c], **Rajiv L. Gaur**[e], **Julie Parsonnet**[a], **Adithya Cattamanchi**[d], **Robert Luo**[e], **Payam Nahid**[d], and **Niaz Banaei**[a,e,f,*]

[a]Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

[b]University of California, San Francisco School of Medicine, San Francisco, CA, USA

[c]Hanoi Lung Hospital, Hanoi, Vietnam

[d]Division of Pulmonary and Critical Care Medicine, Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA, USA

[e]Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA

[f]Clinical Microbiology Laboratory, Stanford University Medical Center, Palo Alto, CA, USA

Abstract

Interferon-gamma release assays have limited sensitivity for detecting latent tuberculosis infection. In this study, we determine if the addition of immunomodulators to the QuantiFERON-TB Gold In-Tube (QFT-GIT) increased test sensitivity without compromising specificity. We prospectively compared QFT-GIT results with and without incubation with2immunomodulators (lipopolysaccharide [LPS] and polyinosine-polycytidylic acid [PolyIC]) in 2 cohorts–113 culture-confirmed tuberculosis (TB) subjects in Hanoi, Vietnam, and 226 documented QFT-GIT–negative, low TB risk health care workers undergoing annual TB screening at a US academic institution. Sensitivity ofthe tests in TB subjects was 84.1% with the standard QFT-GIT and 85.8% and 74.3% after incubation with LPS and PolyIC, respectively. Specificity in low TB risk health care workers was 100% with the standard QFT-GIT by design and 86.7% with LPS and 63.3% with PolyIC. Inconclusion, use ofthe 2 immunomodulators did not improve sensitivity of the QFT-GIT in TB patients and reduced specificity in low-risk health care workers.

Keywords

Tuberculosis; Interferon gamma release assays; Quantiferon; Pathogen associated molecular patterns

1. Introduction

Mycobacterium tuberculosis (Mtb) infects an estimated 2 billion people worldwide (World Health Organization Global Report, 2013). With tuberculosis (TB) eradication efforts

*Corresponding author. Tel.: +1-650-736-8052; fax: +1-650-725-5671.

primarily focused on active TB stalled (World Health Organization Global Report, 2013), it is increasingly evident that the large reservoir of individuals with latent TB infection (LTBI) needs accurate diagnosis and treatment to decrease global TB burden (Abu-Raddad et al., 2009; Blower et al., 1995; Hill et al., 2012).

Unfortunately, identifying individuals with LTBI relies on indirect methods suchas measuring the host immune response to Mtb antigens. For a century, the tuberculin skin test (TST) was the only available LTBI diagnostic. However, in the last decade, interferon-gamma (IFN-γ) release assays (IGRAs) were developed with resulting widespread use (Mazurek et al., 2010). IGRAs detect the sensitized T cell response in infected subjects through in vitro stimulation with Mtb antigens (Mazurek et al., 2010; QuanteFERON-TB Gold, nd). As compared to the TST, IGRAs have improved specificity in bacille calmette-guérin (BCG)-vaccinated individuals and individuals with nontuberculous mycobacterial infection. IGRAs also improve the logistics of LTBI diagnosis because, unlike the TST, IGRAs do not require trained readers or a return visit (Farhat et al., 2006; Pai et al., 2008). However, similar to the TST, IGRAs have an estimated sensitivity of approximately 80% (Diel et al., 2011; Mazurek et al., 2010; Pai et al., 2014). The sensitivity is further reduced in populations at highest risk for progression to active TB such as HIV-infected individuals, immunocompromised hosts, and children (Cattamanchi et al., 2011; Machingaidze et al., 2012; Sollai et al., 2014). Additionally, as data in serial testing emerge, poor reproducibility has been demonstrated (van Zyl-Smit et al., 2009; Ringshausen et al., 2012; Slater et al., 2013; Zwerling et al., 2013). The suboptimal sensitivity and reproducibility of the IGRAs highlight the critical need for an improved assay to diagnose LTBI, thereby promoting efforts to ultimately reduce the global TB burden.

Recent advancements in immunology offer insights into improving the sensitivity of existing IGRAs, such as enhancing the in vitro response to pathogenic antigens. The recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors–such as tolllike receptors (TLRs)–serves as an important trigger for the maturation of antigen presenting cells (APCs). The maturation of APCs is essential for the development of naïve T cells to effector cells (Honda et al., 2003; Schnare et al., 2001) and the initiation of adaptive immune responses (Hoebe et al., 2003; Hoshino et al., 2002). PAMPs therefore play a key role in effector T cell development and adaptive immunity. In addition, PAMPs are now recognized to have direct effects on effector T cells (Huang et al., 2009). The PAMP augmentation of host T cell responses suggests that PAMPs may serve to improve the performance of T cell–based diagnostic tests such as those used to detect LTBI. We found in preliminary work evaluating multiple different PAMPs that compared to the standard assay, the addition of 2 specific PAMPs–TLR agonists polyinosine-polycytidylic acid (PolyIC; TLR3) and lipopolysac-charide (LPS; TLR4)–resulted in a dose-dependent enhancement of IGRA response in blood from a small group of subjects with LTBI (range of 1-to 12-fold) but not from the uninfected controls (Gaur et al., 2012). Furthermore, the addition of PAMPs induced a response in IGRA-unresponsive individuals with known LTBI.

In this validation study, our objective was to conduct an expanded evaluation of the performance of an IGRA, the QuantiFERON TB Gold In-Tube (QFT-GIT; Qiagen/Cellestis, Valencia, CA, USA), for the diagnosis of Mtb infection by the novel techniqueof assay

immunomodulation by adding the PAMPs LPS or PolyIC. We hypothesized that compared to the standard QFT-GIT, the QFT-GIT with PAMP addition would bemore sensitive in active pulmonary TB patients after completing TB treatment (used as a proxy for LTBI to ensure true infection), while maintaining high specificity in a lowTB risk population. Additionally, in a pilot cohort of HIV-positive individuals with active pulmonary TB, we explored the test performance of the standard QFT-GIT as compared to the QFT-GIT with LPS or PolyIC addition.

2. Methods

2.1. Ethics statement

The Committee on Human Research at the University of California, San Francisco (UCSF), Institutional Review Board at Stanford University, and the Independent Ethics Committee at the Ministry of Health of Vietnam approved the study protocol. All subjects were >18 years of age and provided written informed consent.

2.2. Study population

We prospectively enrolled participants in 1 of 3 groups for this pilot diagnostic study (Fig. 1). Group 1, enrolled to assess assay sensitivity in infected immunocompetent individuals, consisted of patients with active pulmonary TB initially diagnosed by positive Mtb sputum culture or GeneXpert MTB/RIF Assay (Cepheid, Sunnyvale, CA, USA) who were 6–8 months into standard TB treatment and culture negative at the time of enrollment. We selected these patients to ensure definitive, microbiologically confirmed Mtb infection. Since there is no gold standard for diagnosing LTBI, using active TB as a proxy for LTBI has been established as the standard method of evaluating the accuracy of LTBI diagnostics (Mazurek et al., 2010). The timing of enrollment after 6– 8 months of treatment was implemented because of data showing CD4 to CD8 T cell ratios in LTBI more closely approximate those after treatment for active TB as compared to untreated active TB (Rodrigues et al., 2002). We excluded patients with histories of immunosuppressive conditions such as HIV, steroid use, or malignancy requiring chemotherapy. This study group was screened and enrolled using standardized criteria by the UCSF/Vietnam National TB Program research staff at the Hanoi Lung Hospital and at UCSF-affiliated district health centers in Hanoi from February 2012 to May 2013.

Group 2, enrolled to assess assay specificity, consisted of Stanford University Medical Center (SUMC) health care workers (HCWs) undergoing annual QFT-GIT screening from July 2011 to April 2012, all of whom had a negative QFT-GIT prior to enrollment and were US born. We excluded HCWs with any signs or symptoms of active pulmonary tuberculosis (fever, weight loss, night sweats, and persistent cough) in the preceding2 months; historyof active TB or LTBI; presenceof immunosuppressive conditions such as HIV, steroid use, or malignancy requiring chemotherapy; residency outside the United States for >6 months in the last 2 years; history of receiving BCG vaccine; or history of providing care or being in close contact with a person diagnosed with TB. Subjects with a positive standard QFT-GIT at the time of enrollment were excluded from the analysis.

Group 3, enrolled to collect preliminary data on assay sensitivity in HIV/TB coinfected individuals, included patients with positive HIV testing and active pulmonary TB by MTB culture or GeneXpert MTB/RIF assay prior to initiating TB therapy. This study group was screened and enrolled at the Hanoi Lung Hospital and affiliated district health centers.

2.3. Data collection

We administered a standardized questionnaire to all participants. For the participants in Hanoi, Vietnam, we reviewed hospital records to collect clinical information, including confirmation of HIV testing, sputum culture, and GeneXpert results. A blood sample was collected for the QFT-GIT assays. In group 3, HIV-positive patients with suspected TB were enrolled. Only data from GeneXpert-positive or GeneXpert-negative/culture-positive patients were included in the analysis.

2.4. QFT-GIT testing

A 1-time peripheral blood sample (8 mL) was collected for the QFT-GIT assays into a heparin-containing 10-mL Kendall Monoject Green Stopper glass tube (catalog no. 320751). After mixing, 1-mL aliquots of blood were transferred to a standard set of QFT-GIT tubes; into a TB antigen tube and a nil tube with the addition of 4 μL of LPS (0.25 ng/mL); and into a TB antigen tube and a nil tube with the addition of 4 μL/mL of PolyIC (40 μg/mL). The concentration of each agonist was chosen from a dose–response curve performed in the nil tube in preliminary work (Gaur et al., 2012). LPS and PolyIC aliquots (InvivoGen, San Diego, CA, USA) were stored at −70 °C and thawed to room temperature prior to use. The tubes were shaken according to the package insert and incubated for 20–24 hours. Plasma extraction and IFN-γ enzyme linked immunosorbent assay (ELISA) were performed manually according to the QFT-GIT package insert (QuanteFERON-TB Gold, nd). The IFN-γ concentrations in the 7 plasma samples were measured in the same ELISA run. All remaining plasma was stored at −70 °C.

QuantiFERON®-TB Gold IT Analysis software (version 2.17 or later) was used to analyze raw data and calculate quantitative results. The IFN-γ concentration in the single mitogen tube was used to interpret the standard QFT-GIT and assays when TB antigen tubes and nil tubes were augmented with LPS or PolyIC.

The QFT-GIT was considered positive if the IFN-γ concentration in the nil tube (Nil) was <8.0 IU/mL, and the concentration in the TB antigen tube minus Nil (TB response) was ≥0.35 IU/mL and ≥25% of Nil; negative if Nil was <8.0 IU/mL, the TB response was <0.35 IU/mL or <25% of Nil, and the concentration in the mitogen tube minus Nil (Mitogen response) was <0.5 IU/mL; or indeterminate if either Nil was >8.0 IU/mL, or the mitogen response was <0.5 IU/mL and TB response was <0.35 IU/mL or <25% of Nil (as described by manufacturer). The same criteria were used to interpret assays modulated with LPS and PolyIC. The effect of modulation was quantitatively assessed by comparing median TB response of standard QFT-GIT with the TB response after the addition of LPS or PolyIC. Per the package insert, the software reports all concentrations >10 IU/mL as “>10” as such values fall beyond the validated linear range of the ELISA (QuanteFERON-TB Gold, nd). When the IFN-γ concentration in the TB antigen tube was >10 IU/mL, the TB antigen and

nil were diluted by 10-fold serially with endotoxin-free phosphate-buffered saline (PBS) and reprocessed. All testing was performed in-country at both sites.

2.5. Statistical methods

Sensitivity (defined as the percent positive in groups 1 and 3) and specificity (defined as the percent negative in group 2) with 95% confidence intervals were calculated. In order to avoid inflating test sensitivity, indeterminate results were included in the analyses and classified as indeterminate. We used the z-test of proportions, the Student's t test, and the Wilcoxon signed rank sum test for comparison of categorical variables, normally distributed continuous variables, and nonnormally distributed continuous variables, respectively. All P values are based on 2-tailed comparisons with statistical significance set at $P < 0.05$. Statistical analyses were performed using Stata 11.1 (StataCorp, College Station, TX, USA).

3. Results

3.1. Study population

Sensitivity was assessed in a cohort with treated active TB, and 113 subjects (74.3% male, mean age 45.2 years) were included in the analysis. Specificity was assessed in a cohort of HCWs with low TB risk and documented negative QFT-GIT testing; 226 subjects (29.7% male, mean age 43.0 years) were included in the analysis (Fig. 1). Multiple patient characteristics were significantly different between the 2 groups, most notably body mass index (BMI) and the prevalence of diabetes, underlying lung disease, and smoking (Table 1). Additionally, exploratory sensitivity data were collected in 10 HIV-positive individuals newly diagnosed with culture or GeneXpert-confirmed TB (70% male, mean age 36.1 years).

3.2. Sensitivity

Sensitivity was not significantly improved with the addition of either PAMP to the QFT-GIT assay (Table 2). Sensitivity in the cohort with treated active TB was 84.1% using the standard QFT-GIT. Comparatively, the sensitivity of the QFT-GIT + LPS was 85.8% ($P = 0.71$), and the sensitivity of the QFT-GIT + PolyIC was reduced to 74.3%. Of the 18 subjects testing negative with the standard QFT-GIT, only 3 tested positive with QFT-GIT + LPS. Conversely, 1 subject testing positive with the standard QFT-GIT tested negative with QFT-GIT + LPS. There were no indeterminate results in the standard QFT-GIT or the QFT-GIT + LPS; 17 indeterminate results (15.0%) occurred with the QFT-GIT + PolyIC, all secondary to a nil value of >8.0 IU/mL.

3.3. Specificity

Specificity was assessed in the cohort of low TB risk HCWs. Specificity declined with the addition of PAMPs to the QFT-GIT assay. Of the initial 240 HCWs recruited, 14 were positive by the standard QFT-GIT (5.8%). These individuals, who likely had false-positive results, were excluded from the analysis, resulting in a specificity artificially set at 100% with the standard QFT-GIT. Comparatively, the specificity decreased to 86.7% and 63.3% with QFT-GIT + LPS and QFT-GIT + PolyIC, respectively. There were no indeterminate results with the standard QFT-GIT given the inclusion of only negative standard QFT-GIT

results. With QFT-GIT + LPS, 4 indeterminate results (1.8%) occurred; 48 indeterminate results (21.2%) occurred with the QFT-GIT + PolyIC, all secondary to a Nil value of >8.0 IU/mL.

3.4. Quantitative results

The quantitative results of the 3 assays were compared to evaluate for boosting of the TB response (Table 3; Figs. 2 and 3). In the treated active TB cohort, the median TB response was significantly higher with QFT-GIT + LPS (2.31 IU/mL) and QFT-GIT + PolyIC (11.74 IU/mL with indeterminates included; 5.72 with indeterminates excluded) as compared to the standard QFT-GIT (2.0 IU/mL; $P < 0.001$). The medianTB response in individuals with negative results with the standard QFT-GIT (n = 18) was 0.12 IU/mL as compared to a median of 0.19 IU/mL with the QFT-GIT + LPS and 0.14 IU/mL with QFT-GIT + PolyIC. In the low TB risk HCWs, the median TB response was significantly higher with QFT-GIT + LPS (0.03 IU/mL) but not with QFT-GIT + PolyIC (0 IU/mL) as compared to the standard QFT-GIT (0.01 IU/mL; $P < 0.001$ and 0.31, respectively).

3.5. TB/HIV coinfected cohort

In the TB/HIV coinfected patients tested (n = 10) to collect exploratory data on the sensitivity of the addition of PAMPs to the QFT-GIT in an immunocompromised population, the sensitivity of the standard QFT-GIT was 60%, equivalent to the sensitivity with the QFT-GIT + LPS. One additional subject had a positive result with the QFT-GIT + PolyIC resulting in a sensitivity of 70% (Table 2). One subject had an indeterminate result on all 3 assays secondary to a low mitogen response. The median TB response was equivalent with the standard QFT-GIT and QFT-GIT + LPS assays at 1.5 IU/mL and increased to 2.0 IU/mL with QFT-GIT + PolyIC (Table 3).

4. Discussion

We found that the novel techniqueof invitro immunomodulation of the QFT-GIT assay with LPS or PolyIC did not improve sensitivity over the standard QFT-GIT using identical interpretation criteria in immuno-competent subjects in treated active TB. The specificity declined in HCWs at low TB-risk with the QFT-GIT with PAMP addition. The high proportion of indeterminate results with the QFT-GIT + PolyIC due to high boosting of IFN-γ in the nil tube negates the usefulness of the PolyIC assay. Although a significant difference was found in the quantitative TB response between the standard QFT-GIT and QFT-GIT + LPS, the absolute difference is unlikely clinically significant because the difference falls within the within-subject variability measurements reported for the QFT-GIT assay (Metcalfe et al., 2013; van Zyl-Smit et al., 2009; Whitworth et al., 2012). Given the small absolute difference in median TB response, particularly with the negative standard QFT-GIT results in the treated TB cohort, there was no utility in changing the cutoff in the assay with PAMPadditiontomaintainanimproved sensitivity without sacrificing specificity.

In a pilot cohort of HIV-positive individuals with active TB, we found no improvementinsensitivityofthe QFT-GITassaywithLPSorPolyIC as compared to the standard QFT-GIT. Only 10 HIV/TB coinfected subjects were evaluated. Because of the poor

sensitivity of the QFT-GIT in HIV-positive individuals as well as the increased risk of progression to TB (Cattamanchi et al., 2011), more studies of PAMP addition to the QFT-GIT could be warranted in an HIV/TB coinfected cohort. However, preliminary studies to identify optimal PAMPs would first need to be repeated in this population since our preliminary studies were performed in healthy HCW (Gaur et al., 2012).

The negative results from our study contrast with our preliminary data that suggest that PAMPs are a useful strategy to address the suboptimal sensitivity of the QFT-GIT. Gaur et al. (2012) found that the TB responses significantly increased with the addition of LPS and PolyIC to the QFT-GIT in individuals with LTBI–defined by a positive TST and QFT-GIT and at least 1 risk factor for TB–but were unaffected in uninfected individuals. We postulate that the most likely cause of the discrepant results in sensitivity in this study relate to differences in immune responses in LTBI as compared to recently treated TB, impacting the results of the QFT-GIT, a functional immunodiagnostic assay. Recent literature describes newly discovered differences in T cell profiles between latent and active disease (Nikolova et al., 2013; Perreau et al., 2013; Rozot et al., 2013). However, because there is no gold standard for LTBI diagnosis, sensitivity analyses of LTBI diagnostics are problematic typically resulting in reliance on the proxy of active TB infection–the approach that we adopted in this study (Mazurek et al., 2010). To mitigate differences in immune response during active disease, we sampled patients nearing the end of treatment. Limited data suggest that these patients have similar CD4/CD8 T cell responses to those with LTBI (Rodrigues et al., 2002). However, given the paucity of data, we acknowledge the limitation of the study group selection as potentially not being reflective of an LTBI group. Therefore, further investigation into the enhancement of the QFT-GIT assay with the addition of LPS (PolyIC proved to be too problematic in negative controls) should be conducted in a population of individuals likely to have LTBI such as in individuals in contact investigations who are followed for progression to active TB.

Multiple additional potential causes of the unexpectedly negative results exist relating to host immune response. Results could be affected by genetic polymorphisms influencingTcell immune response and IFN-γ production impacting both the standard QFT-GIT and the QFT-GIT with PAMPs. A study also conducted in Hanoi, Vietnam, showed an association between HLA type and negative QFT-GIT results (Hang et al., 2011). Our sensitivity study, conducted in a high TB incidence setting to ensure sample size attainment, included only Vietnamese subjects as compared to the preliminary studies conducted in individuals with LTBI with diverse ethnic backgrounds. The attenuation of the QFT-GIT responses with the addition of PAMPs could result from high levels of endogenous PAMPs in the Vietnamese cohort secondary to the impact of diet on microbiome-derived PAMPs (Pai et al., 2014).

Problems with the QFT-GIT assay could also be the source of discordant results between the preliminary and current studies. Contamination of the QFT-GIT tubes with skin and environmental microorganisms has been shown to cause false-positive QFT-GIT results (Gaur and Banaei, 2014). However, multiple quality assurance measures were taken at both sites without any evidence of invalid results. Although the PAMPs used and the concentrations chosen were supported by the preliminary results (Gaur et al., 2012), it is

possible that an alternate PAMP or an alternate concentration would have more promising results. The inherent poor reproducibility of the QFT-GIT (Honda et al., 2003; Ringshausen et al., 2012; Slater et al., 2013; van Zyl-Smit et al., 2009; Zwerling et al., 2013) is a limitation of our results; however, the methods of performing the assays on blood from a single draw were as standardized as possible to limit the variability. The impact of blood collection in a green top tube with subsequent endotoxin-free PBS dilution is also unknown. Lastly, our method of serial dilution in the small number of samples with IFN-γ concentrations in the TB antigen tube of >10 IU/mL is not validated by the manufacturer.

This proof-of-principle study had limitations inherentto the QFT-GIT assay and TB epidemiology. The study design and study sites were chosen becauseofthe lack of gold standard for LTBI and to allow for efficient subject recruitment. We enrolled subjects with active TB as a proxy for LTBI from a high TB incidence country to assess sensitivity and subjects with low TB risk from a low TB incidence country to assess specificity. The differences in study sites and patient populations lead to many possible confounders. However, our aim was to diversify patient populations if results were promising.

In conclusion,wewere unableto show enhancementinthe accuracy of the QFT-GIT with the addition of PAMPs in an immunocompetent population with treated active TB. Given the suboptimal accuracy of LTBI diagnostics, further testing of the QFT-GIT with the addition of PAMPs could be warranted in a population with LTBI.

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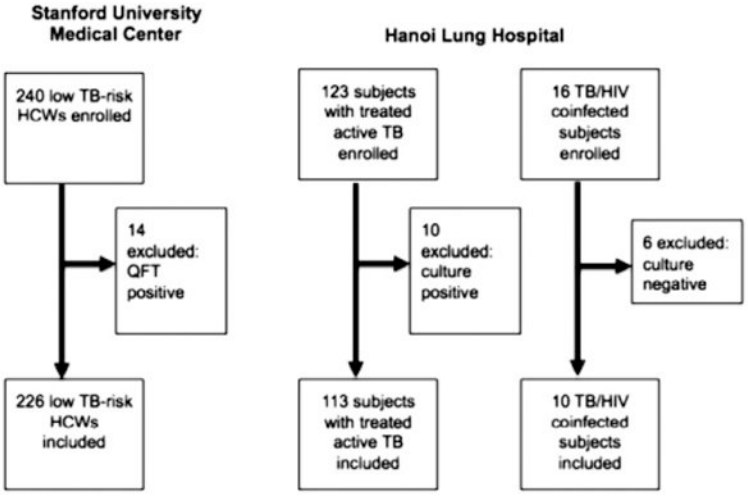

Fig. 1.
Flow chart of subject inclusion.

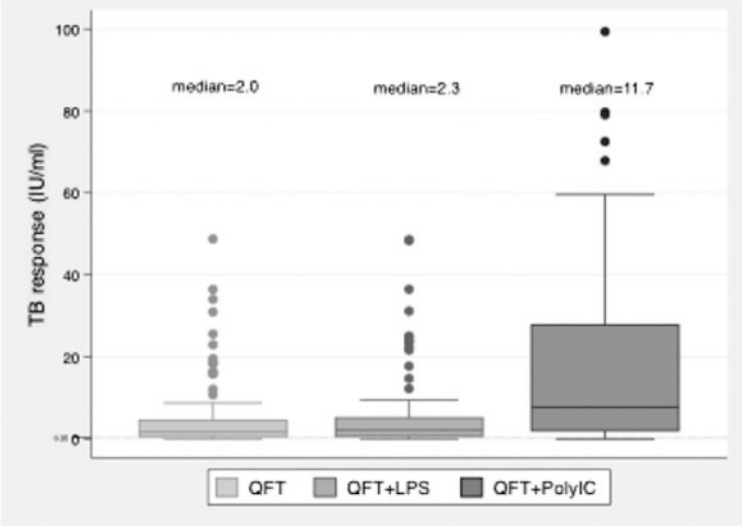

Fig. 2.

Quantitative results of the standard QFT and QFT with LPS or PolyIC in the cohort with treated TB. Boxes show interquartile ranges, and the lower whisker represents data within 1.5 times the IQR of the lower quartile, and the upper whisker represents data within 1.5 times the IQR of the upper quartile. Dots represent outliers above 1.5 times the IQR of the upper quartile. The reference line at TB response of 0.35 IU/mL is the QFT cutoff. QFT = QuantiFERON-TB Gold In Tube; TB response = TB antigen minus nil; IQR = interquartile range.

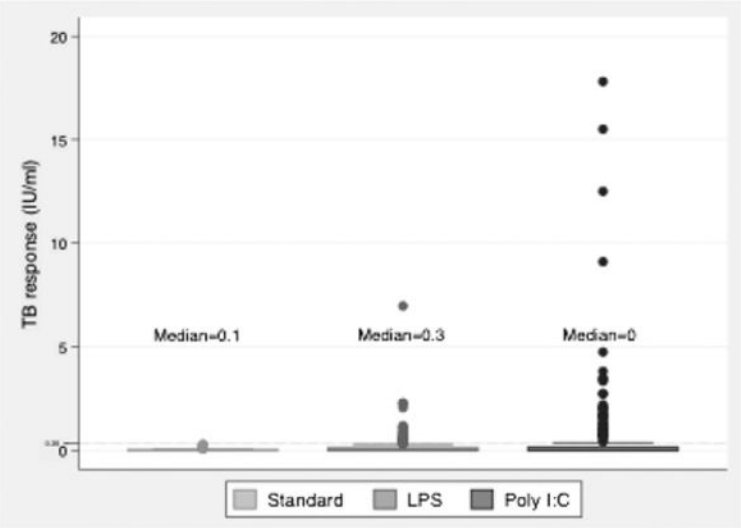

Fig. 3.

Quantitative results of the standard QFT and QFT with LPS or PolyIC in the uninfected cohort. Boxes show interquartile ranges, and the lower whisker represents data within 1.5 times the IQR of the lower quartile, and the upper whisker represents data within 1.5 times the IQR of the upper quartile. Dots represent outliers above 1.5 times the IQR of the upper quartile. The reference line at TB response of 0.35 IU/mL is the QFT cutoff. QFT = QuantiFERON-TB Gold In Tube; TB response = TB antigen minus nil.

Table 1

Demographic characteristics of the study population.

	Uninfected	Treated active TB	HIV/TB coinfected	P value[a]
	n = 226	n = 113	n = 10	
Age mean (±SD)	43.0 (13.6)	45.2 (19.4)	36.1 (11.1)	0.22
BMI, mean ± SD	26.1 ± 4.9	19.2 ± 2.6	15.9 ± 2.2	<0.001
Gender (% male)	29.7	74.3	70.0	<0.001
Diabetes (%)	2.2	13.3	0	<0.001
Lung disease (%)	3.5	39.8	10.0	<0.001
Dialysis (%)	0	1.8	0	0.04
Cancer (%)	0.4	0.9	0	0.62
Liver disease (%)	0	6.2	20.0	0.01
Smoking (%)	2.2	44.6	40.0	<0.001

[a] *P* values compare results from the uninfected and treated active TB cohorts and were calculated using the Student's t test for continuous variables and the z-test of proportions for categorical variables.

Table 2

Qualitative QFT results using the manufacturer cutoff of 0.35 IU/mL.

	Uninfected, n = 226			Treated active TB, n = 113			HIV/TB coinfected, n = 10		
	QFT[a]	QFT + LPS	QFT + PolyIC	QFT	QFT + LPS	QFT + PolyIC	QFT	QFT + LPS	QFT + PolyIC
Positive result (n)	0	26	35	95	97	84	6	6	7
Negative result (n)	226	196	143	18	16	12	3	3	2
Indeterminate result (n)[b]	0	4	48	0	0	17	1	1	1
Sensitivity, % (95% CI)				84.1 (76.0–89.9)	85.8 (78.0–91.2)	74.3 (65.4–81.6)	60.0 (24.3–87.5)	60.0 (24.3–87.5)	70.0 (31.0–92.4)
Specificity, % (95% CI)	100.0	86.7 (81.6–90.6)	63.3 (5.7–69.3)						

CI = confidence interval.

[a]Artificially created specificity–14 subjects (5.8%) with positive results with the standard assay were excluded.

[b]All indeterminate results in the uninfected and treated active TB cohorts were secondary to high nil (>8.0 IU/mL); the indeterminate result in the HIV/TB coinfected cohort was secondary to low mitogen (TB antigen-nil <0.35 IU/mL and mitogen-nil <0.5).

Table 3

Quantitative QFT results.

Median TB response, IU/mL (IQR)					
	QFT	**QFT + LPS**	**P value[a]**	**QFT + PolyIC**	**P value[a]**
Uninfected	0.1 (0–0.3)	0.3 (0–0.1)	<0.001	0 (0–0.2)	0.31
Treated active TB	2.0 (0.6–5.9)	2.3 (0.7–6.4)	<0.001	11.7 (2.4–32.4)	<0.001
HIV/TB coinfected	1.5 (0.1–4.5)	1.5 (0.1–4.8)	0.3	2.0 (0.2–5.1)	0.01

TB response = TB antigen-nil; IQR = interquartile range.

[a]P values compare PAMP's median TB response to the standard QFT median TB response by the Wilcoxon signed rank sum test.