Title

Multi-cytokine detection improves latent tuberculosis diagnosis in healthcare workers

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ABSTRACT

In a low incidence setting, healthcare workers (HCW) are at higher risk of tuberculosis than the general population. The suboptimal sensitivity of QuantiFERON®-TB Gold In-Tube (QFT) remains a critical issue when identifying occupational latent tuberculosis infection (LTBI) in HCW. The aim of this study is to identify additional biomarkers in order to overcome the limits of interferon-gamma release assays (IGRAs) and improve the performance of LTBI diagnosis within this population.

Seventy Bacille Calmette Guerin-vaccinated HCW regularly exposed to Mycobacterium tuberculosis were grouped according to QFT results in a LTBI-positive group (positive QFT, n=8), a LTBI-negative group (normal QFT and negative TST, n=21) and an undetermined group (sub-positive QFT and/or positive TST, n=41). Secretions of 22 cytokines were quantified using a multiparameter-based immunoassay in response to QFT-specific stimulation.

As a result, thresholds discriminating LTBI-positive from LTBI-negative HCW were established when comparing areas under the receiver operating characteristic curves for IL-2, IL-15, IP-10 and MIG that are biomarkers differentially secreted between the two groups. Combining IL-15 and MIG, they provided a sensitivity of 100% and a specificity of 94.1% in distinguishing LTBI-positive from LTBI-negative HCW. When using IL-15 and MIG among the undetermined group, 6/45 HCW could be classified in the LTBI-positive group.

The use of additional biomarkers after IGRA screening could improve the diagnostic performance of LTBI. Performances of these biomarkers and their use in combination with TST and/or QFT, as well as the cost-effectiveness of such a diagnostic strategy, should be evaluated in further larger clinical trials.
INTRODUCTION

In countries with a low incidence of tuberculosis (TB), healthcare workers (HCW) are at higher risk of TB than the general population (12, 15, 28). In this context, TB remains a significant occupational health problem. HCW working in a high-risk TB environment have long been screened periodically for latent TB infection (LTBI) using chest X-rays and tuberculin skin test (TST). The TST has an estimated sensitivity of 78% (17) but its specificity is poor, mainly due to the cross-reactive immune responses to Bacille Calmette-Guérin (BCG) in vaccinated population such as HCW. Recently, interferon-gamma release assays (IGRA), which are based on T-cell measurements of anti-mycobacterial immunity, have emerged as an alternative to TST when diagnosing LTBI. IGRA are not confounded by cross-reactivity because peptides used for T-cell stimulation are expressed by Mycobacterium tuberculosis and not by the BCG strains nor by most of the non-tuberculous mycobacteria (5). Furthermore, unlike TST, IGRA can be repeated without a sensitization risk or boosting effect making them attractive tests for TB control programs, particularly in an occupational setting. The use of IGRA for HCW screening instead of TST is recommended in the USA (2). In France, the current policy on TB infection control indicates that IGRA can be used for the LTBI pre-employment screening of HCW as a replacement for TST (1).

The QuantiFERON® test, manufactured by Cellestis, is one of these IGRA tests quantifying the IFN-γ secreted in response to Mycobacterium tuberculosis-specific RD1 antigens derived from early secretory antigenic target 6 kDa (ESAT-6), culture filtrate protein 10 kDa (CFP-10) and TB7.7. The third generation of this assay named QFT-TB Gold In-Tube (QFT) has a specificity close to 100% when diagnosing active tuberculosis (9, 24). However, the sensitivity of this test is suboptimal, between...
55 and 94%, when a majority of patients with active tuberculosis were enrolled (9, 17, 24). The sensitivity of LTBI diagnostic tests may be significantly improved by selecting adequate secreted cytokines, beyond IFN-\(\gamma\). For example, IFN-\(\gamma\)-inducible protein 10 kDa (IP-10) or interleukin (IL)-2 may be adjunct biomarkers to IFN-\(\gamma\) for \textit{in vitro} testing of LTBI (6, 21, 22, 27).

The aim of this study was to identify additional biomarkers that can be used with QFT to improve the diagnostic of LTBI among HCW exposed to TB.
MATERIAL AND METHODS

Data collection and participants

This study was carried out within a large multicenter study named ‘QuantiFERON for
detection of latent tuberculosis in healthcare workers’ (QUANTIPS), which assessed
the cost-effectiveness of QFT vs. TST to detect latent tuberculosis among exposed
HCW. The participants were adults from French university hospitals, working in
medical units with a high risk of Mycobacterium tuberculosis exposure. In the present
sub-study, the population consisted of 70 BCG-vaccinated healthcare workers from
the Respiratory Diseases and the Infectious Diseases Departments of CHRU
Montpellier in France. None of the workers enrolled were infected with human
immunodeficiency virus (HIV), on anti-TB treatment and/or had a clinical examination
suggesting an active disease. At the baseline, a TST was done using the Mantoux
technique unless a previous test was positive before enrolment. All TST results were
checked between 48 to 72 hours later and were considered positive when the
induration area was ≥10 mm. Inform consents were obtained from all participants.
This study was registered under the identifier NCT00797836 and was approved by
the Institutional Review Board of Assistance Publique – Hôpitaux de Paris.

Study design

The study was conducted in two steps. During the first step, participants were
classified in three groups according to their TB screening: i) the LTBI-positive group
was composed of HCW positive for QFT using the IFN-γ cut-off of 0.35 IU/ml,
independently of the TST; ii) the LTBI-negative group was composed of HCW with a
normal value of QFT (under 0.1 IU/ml) and a negative TST result (<10 mm); iii) the
undetermined group was composed of HCW having a sub-positive QFT result (between 0.1 and 0.35 IU/ml) and/or a positive TST (≥10 mm) (Fig. 1).

Secondly, the concentrations of additional cytokines secreted were measured in responses to RD1 stimulation during the QFT assays. Cytokines with a concentration that discriminates between the LTBI-positive and the LTBI-negative group were selected using receiver operating characteristic (ROC) curves. The selected cytokines were then used with their positive cut-off values to identify HCW from the undetermined group suspected to have LTBI.

**The QuantiFERON®-TB Gold In-Tube assay**

The whole blood stimulation and quantification of IFN-γ production were performed according to manufacturer’s instructions (Cellestis, Darmstadt, Germany). After the 24 hours incubation, plasma was collected and IFN-γ concentration was measured. The optical density (OD) was read using a 450 nm filter and an ELISA plate reader. Positivity for QFT was first defined on the basis of the IFN-γ threshold recommended by the manufacturer (>0.35 IU/ml). Because using an uncertainty zone around the cut-off could improve the LTBI diagnostic in healthcare workers (16, 19), we considered HCW with IFN-γ levels between 0.1 and 0.35 IU/ml as having a sub-positive QFT result, suggesting a possible LTBI in this population exposed to TB. Remaining plasma samples were stored at -80°C until quantification of cytokines was carried out.

**Cytokine profile in cell-free culture supernatant of QFT**

After 24 hours of stimulation by antigens from the QFT assay, cytokine secretion was quantitated in cell-free culture supernatants of 69/70 HCW by a microbeads-based
multiplex method (Cytokine human panel, Invitrogen, Villebon sur Yvette, France) and a Luminex 100 apparatus (Luminex, Oosterhout, The Netherlands) according to manufacturer’s instructions. Detection sensitivities of these cytokines range between 3 and 30 pg/ml. Data were acquired using 7800-15200 double discriminator gate settings. Standard curves were established to determine cytokine and concentrations and a minimum of 100 microspheres per analyte were used. Concentration values above the superior point of the curve were given an arbitrary value equal to the superior point of the standard curve for each biomarker.

**Statistical analysis**

The median cytokine levels (interquartile range, IQR) were compared between the LTBI-positive and LTBI-negative group using the non-parametric Mann-Whitney U test. The individual alpha errors for multiple comparisons were corrected by adjusting the P values using the Holm technique (adjusted P < 0.05 was considered significant). Then, ROC curves of the selected biomarkers were constructed by plotting the true positive samples (sensitivity) against the false positive samples (1-specificity) for each possible cut-off point. Areas under the curve (AUC) were calculated along with their 95% confidence intervals using a non-parametric approach. Cut-offs for antigen-specific IL-2, IL-15, IP-10 and MIG were estimated at various sensitivities and specificities. To avoid false positive results, a cut-off value was retained corresponding to the maximum Youden's index (YI) defined as sensitivity + specificity - 1. Selected cytokines with their optimal cut-off were applied to the undetermined group to detect additional LTBI.

Finally, the non-parametric Spearman’s rank correlation coefficient was used to evaluate associations between secretions of selected cytokines.
RESULTS

Study participants

Clinical characteristics of HCW are shown in Table 1. Forty-one out of the 70 participants (59%) had a TST superior to the positive threshold established at 10 mm (median = 17 mm, IQR 14-20) whereas 29 participants (41%) displayed a negative TST. The participants were classified into three groups based on QFT and TST values. Eight HCW (11%) were positive for QFT using the manufacturer cut-off and were considered as having LTBI. In contrast, 21/70 HCW (30%) had a negative testing with QFT <0.1 IU/ml and TST <10 mm. Thus, a majority of HCW (59%) included was classified in an undetermined group because of a sub-positive IFN-γ secretion (between 0.1 and 0.35 IU/ml) and/or a TST ≥10 mm (Fig. 1).

Identification of four additional biomarkers discriminating LTBI-positive from LTBI-negative HCW

Besides IFN-γ, 22 cytokines were analyzed after overnight T-cell specific stimulation by RD1 peptides contained in QFT tubes. IL-2, IL-15, IP-10 and MIG were secreted at higher concentration in the LTBI-positive group than in the LTBI-negative group of HCW after QFT-specific stimulation (P <0.05) (Table 2).

Performances of IL-2, IL-15, IP-10 or MIG testing in HCW positive or negative for LTBI

Using ROC curves, the performance of IL-2, IL-15, IP-10 and MIG testing was evaluated to discriminate LTBI-positive from LTBI-negative HCW (Fig. 2). The most discriminating cytokines were IL-2 (threshold: 66 pg/ml, AUC = 1, 95%CI 1-1), IL-15 (threshold: 200 pg/ml, AUC = 0.88, 95%CI 0.68-0.96), IP-10 (threshold: 1259 pg/ml,
AUC = 0.93, 95%CI 0.75-0.98) and MIG (threshold: 392 pg/ml, AUC = 0.93, 95%CI 0.67-0.99). These four cytokines detect all HCW from the LTBI-positive group.

Regarding the non-TB group, 7 HCW were misclassified for IL-15, 3 for IP-10 and MIG and none for IL-2 suggesting that increasing the sensitivity up to 100% will lead to a concomitant reduction of the specificity.

**LTBI detection using IL-2, IL-15, IP-10 or MIG in HCW undetermined for tuberculosis infection.**

We investigated TB infection in HCW from the undetermined group using the cytokines previously selected (Fig. 3a). Fifteen HCW out of 41 were positive when assessed for IL-15, 13 for IP-10 and 6 for MIG and IL-2 testing (Fig. 3a).

There was a positive correlation between IL-15 and IP-10 secretion (r = 0.57, P < 0.001), IL-15 and MIG (r = 0.69, P < 0.001), as well as between IP-10 and MIG (r = 0.78, P < 0.001) (Table 3). IL-2 secretion was also correlated to IL-15 (r = 0.55, P < 0.001), IP-10 (r = 0.39, P = 0.001) and MIG (r = 0.49, P < 0.001) (Table 3).

**Improving specificity by using combination of biomarkers.**

IL-2 appeared as a perfect marker in our sample, with a specificity and a sensitivity of 100%. Although IL-2 is certainly a marker of interest (6), such a perfect diagnostic performance could be a pure luck; its true value of sensitivity is between 63% and 100%, and its true value of specificity lies between 80% and 100%. As a result, we decided to consider IL-2 by itself for further analyses. We also investigated in parallel whether an approach combining the other biomarkers could increase the specificity of the LTBI diagnostic test, which was 58.8%, 82.4% and 88.2% by using single IL-15, IP-10 or MIG (Fig. 3b). The sensitivity was still 100% and the specificity had
increased up to 94.1% when HCW had both IL-15 ≥ 200 pg/ml and MIG ≥ 392 pg/ml.

In contrast, MIG/IP-10 (sensitivity = 100%, specificity = 88.2%), IL-15/IP-10 (sensitivity = 100%, specificity = 88.2%) and IL-15/IP-10/MIG combinations (sensitivity = 100%, specificity = 94.1%) did not provide better results than MIG/IL-15 combination (sensitivity = 100%, specificity = 94.1%), which is the greatest combination for discriminating LTBI-positive from LTBI-negative subjects.

Using IL-15 and MIG cut-offs, 6 of the undetermined HCW could have LTBI and defined as ‘second look LTBI diagnosis’ (Fig. 4).
DISCUSSION

Currently, QFT assay stands as the most specific immunoassay dedicated to LTBI screening. However, the IGRA's sensitivity to detect LTBI is questionable, as these tests were mainly evaluated against active TB defined by a positive microbiological test (either the microscopic detection of acid-fast bacilli in sputum or sputum culture).

Recently, a 75% sensitivity and a 37% specificity have been reported in smear-negative subjects using the QFT assay, suggesting the IGRA's performance could be worse among smear-negative patients than among smear-positive subjects even in high-burden country (14). This insufficient sensitivity of the QFT assay cannot simply be overcome by reducing the positive cut-off since it would have an impact on the specificity.

The combination of TST and QFT could be an alternative in improving the LTBI diagnostic performance (3, 10, 18, 25). However, TST itself has a poor sensitivity evaluated at 80% (17) and a poor specificity among individuals vaccinated with BCG, which is the case of most HCWs exposed to TB. Our results confirm that this test is unlikely to be useful in this context unless it is used in combination with IGRA. In particular, 3/8 HCW with a positive QFT have a negative TST. This is in line with a recent review indicating that use of QFT, rather than TST, is currently recommended for contact tracing and healthcare workers as screening test for LTBI in low incidence countries and in BCG-vaccinated individuals (8). In addition, for HCW, QFT screening only is more cost-effective than TST screening (7, 13).

Our approach to improve the diagnosis of LTBI consisted of increasing the performance of the current IGRA based on the detection of additional cytokines to...
IFN-γ. The HCW undetermined for LTBI, i.e. with a possible diagnosis of LTBI, had a TST ≥10 mm and/or a QFT value (0.1-0.35 IU/ml) above the range generally observed in healthy controls suggesting that effector memory T-cells directed against RD1 antigens may be present in this exposed subjects. This group of HCW is of interest because a considerable within-subject variability in measured IFN-γ response during QFT has been revealed with serial testing (16, 19). Therefore, using an uncertainty zone around the cut-off of QFT, such as 0.1 to 0.35 IU/ml, could improve the discrimination between healthy HCW and LTBI-positive HCW with a sub-positive QFT response. Along with IFN-γ, IL-2, IL-15, MIG and IP-10 could be useful biomarkers to differentiate subjects with or without LTBI. Our results suggest that up to 15 QFT-negative individuals have a positive response to these cytokines and may have LTBI, which is consistent with the 'expected' 20% false-negative tests (i.e. 14/70 people) given the 80% sensitivity of QFT. Notably, this result was obtained without substantially weakening the specificity. Instead of serial testing, we propose to use a combination of additional biomarkers, such as IL-15 and MIG, as a second line diagnostic tool before offering preventive chemotherapy to HCW with borderline negative results in a low incidence country.

Alike IFN-γ, IL-2, MIG, IP-10 and IL-15 are involved in the T helper type 1 (Th1) cellular immune response, which is pivotal in the clearance of Mycobacterium tuberculosis. Our results are coherent with recent findings indicating that the measurement of T-cells directed against TB and secreting IP-10 may be useful for TB diagnosis (20-22, 27). In addition, MIG is expressed by IFN-γ-stimulated cells in TB (4) and was detected in the bronchial epithelium, which concurs to the recruitment of activated T-cells during tuberculosis (23). IL-15 shares many biological properties
with IL-2 despite having no sequence homology (26). Unlike IP-10, MIG and IL-15 have not yet been well documented in the case of latent TB diagnosis. To our knowledge, only one recent report suggested that IL-15 may be a reliable cytokine to detect TB (11). Testing several parameters may limit false negative results due to inter-individual variations in the immune response during TB infection.

In conclusion, adding at least MIG and IL-15 along with IFN-γ could markedly improve the performance of IGRA when detecting LTBI. These data should be confirmed in larger studies. Among HCW, a QFT assay could be first used to screen LTBI-positive individuals. Then, the QFT supernatants of patients i) with IFN-γ response between 0.1 and 0.35 IU/ml, or ii) with a QFT under 0.1 IU/ml but with a TST superior to 10 mm, should be tested for additional cytokines to detect supplemental LTBI-positive cases. Further studies, including cost-effectiveness analyses, are needed to provide evidence for the reliability of such a diagnostic strategy of LTBI in HCW from a low incidence country.
ACKNOWLEDGEMENTS

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Conflicts of interest: nothing to declare.
REFERENCES


Table 1. Clinical characteristics of the 70 healthcare workers including in the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Age [IQR] in years</strong></td>
<td>44 [36-50]</td>
</tr>
<tr>
<td><strong>Female gender</strong></td>
<td>59/70 (84.3%)</td>
</tr>
<tr>
<td><strong>Working groups</strong></td>
<td></td>
</tr>
<tr>
<td>Doctors</td>
<td>8/70 (11%)</td>
</tr>
<tr>
<td>Nurses</td>
<td>28/70 (40%)</td>
</tr>
<tr>
<td>Auxiliary nurses</td>
<td>24/70 (34%)</td>
</tr>
<tr>
<td>Paramedical staff</td>
<td>1/70 (1%)</td>
</tr>
<tr>
<td>Other hospital workers</td>
<td>9/70 (13%)</td>
</tr>
<tr>
<td><strong>Hospital departments</strong></td>
<td></td>
</tr>
<tr>
<td>Tropical and Infectious Diseases</td>
<td>45/70 (64%)</td>
</tr>
<tr>
<td>Pneumology</td>
<td>25/70 (36%)</td>
</tr>
<tr>
<td><strong>QFT(^b) results</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>62/70 (89%)</td>
</tr>
<tr>
<td>Positive</td>
<td>8/70 (11%)</td>
</tr>
<tr>
<td><strong>TST(^c) in mm</strong></td>
<td></td>
</tr>
<tr>
<td>Negative (&lt;10)</td>
<td>29/70 (41%)</td>
</tr>
<tr>
<td>Positive (≥10) [median – IQR]</td>
<td>41/70 (59%) [17 – 14-20]</td>
</tr>
<tr>
<td><strong>Abnormal Chest X-ray</strong></td>
<td>4/70 (6%)</td>
</tr>
</tbody>
</table>

\(^a\)IQR, interquartile range (25\(^{th}\) – 75\(^{th}\) percentiles)

\(^b\)QFT, quantiFERON® TB-Gold In-Tube

\(^c\)TST, tuberculin skin test
Table 2. Concentrations of cytokines secreted by peripheral blood mononuclear cells from healthcare workers according to QuantiFERON®-TB Gold In-Tube results.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Latent TB(^a) HCW(^b) (n=8) Median (IQR(^d))</th>
<th>LTBI-negative HCW (n=17) Median (IQR)</th>
<th>(P) values(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1RA</td>
<td>8491 (6966-9885)</td>
<td>5837 (5528-6869)</td>
<td>0.140</td>
</tr>
<tr>
<td>IL-2</td>
<td>87 (69-140)</td>
<td>10 (9-12)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>IL-2R</td>
<td>1751 (1696-1817)</td>
<td>1714 (1678-1751)</td>
<td>0.242</td>
</tr>
<tr>
<td>IL-4</td>
<td>271 (267-282)</td>
<td>263 (261-269)</td>
<td>0.126</td>
</tr>
<tr>
<td>IL-5</td>
<td>15 (14-23)</td>
<td>13 (13-15)</td>
<td>0.323</td>
</tr>
<tr>
<td>IL-6</td>
<td>656 (430-800)</td>
<td>546 (335-937)</td>
<td>0.852</td>
</tr>
<tr>
<td>IL-7</td>
<td>133 (133-134)</td>
<td>133 (131-133)</td>
<td>0.435</td>
</tr>
<tr>
<td>IL-10</td>
<td>8 (6-15)</td>
<td>7 (6-10)</td>
<td>0.448</td>
</tr>
<tr>
<td>IL-12p40/70</td>
<td>848 (783-894)</td>
<td>857 (823-892)</td>
<td>0.764</td>
</tr>
<tr>
<td>IL-13</td>
<td>31 (24-56)</td>
<td>28 (22-31)</td>
<td>0.263</td>
</tr>
<tr>
<td>IL-15</td>
<td>205 (200-211)</td>
<td>50 (39-200)</td>
<td>(0.021)</td>
</tr>
<tr>
<td>IL-17</td>
<td>208 (206-208)</td>
<td>208 (208-208)</td>
<td>0.545</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>167 (151-195)</td>
<td>161 (152-220)</td>
<td>0.815</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>262 (210-266)</td>
<td>261 (54-263)</td>
<td>0.300</td>
</tr>
<tr>
<td>MIP-1(\alpha)</td>
<td>2436 (1607-2728)</td>
<td>2193 (1078-4506)</td>
<td>0.966</td>
</tr>
<tr>
<td>MIP-1(\beta)</td>
<td>4037 (2286-4869)</td>
<td>1658 (768-1796)</td>
<td>0.061</td>
</tr>
<tr>
<td>IP-10</td>
<td>3903 (3004-5080)</td>
<td>225 (76-668)</td>
<td>(0.004)</td>
</tr>
<tr>
<td>MIG</td>
<td>515 (419-684)</td>
<td>358 (358-361)</td>
<td>(0.003)</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>117 (106-130)</td>
<td>106 (89-145)</td>
<td>0.618</td>
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</tr>
<tr>
<td>Rantes</td>
<td>16760 (15483-16760)</td>
<td>13402 (11651-16760)</td>
<td>0.216</td>
</tr>
<tr>
<td>MCP-1</td>
<td>15197 (5395-22172)</td>
<td>5449 (5131-7138)</td>
<td>0.107</td>
</tr>
<tr>
<td>IFN-α</td>
<td>109 (108-110)</td>
<td>108 (108-109)</td>
<td>0.181</td>
</tr>
</tbody>
</table>

\(^a\) TB, tuberculosis  
\(^b\) HCW, healthcare workers  
\(^c\) Mann-Whitney test comparing the two groups, with \( P < 0.05 \) statistically significant  
\(^d\) IQR, interquartile range
Table 3. Spearman correlation coefficient between two-by-two combinations of IL-2, IL-15, IP-10 and MIG secretion levels after QuantiFERON®-TB Gold In-Tube in 69 healthcare workers.

<table>
<thead>
<tr>
<th>Spearman coefficients (P values&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>IL-2</th>
<th>IL-15</th>
<th>IP-10&lt;sup&gt;b&lt;/sup&gt;</th>
<th>MIG&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-2</strong></td>
<td>0.55 (&lt; 0.001)</td>
<td>0.39 (0.001)</td>
<td>0.49 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>IL-15</strong></td>
<td>0.55 (&lt; 0.001)</td>
<td>0.57 (&lt; 0.001)</td>
<td>0.69 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>IP-10</strong></td>
<td>0.39 (0.001)</td>
<td>0.57 (&lt; 0.001)</td>
<td>0.78 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>MIG</strong></td>
<td>0.49 (&lt; 0.001)</td>
<td>0.69 (&lt; 0.001)</td>
<td>0.78 (&lt; 0.001)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mann-Whitney test, with P < 0.05 statistically significant

<sup>b</sup>IP-10, IFN-γ induced-protein 10

<sup>c</sup>MIG, Monokine induced by IFN-γ
Figure 1: The QuantiFERON-TB Gold In-Tube (QFT) and tuberculin skin test (TST) results of the 70 healthcare workers (HCW).
QFT results were obtained after infection with Mycobacterium avium intracellulare (MAC) or the bacillus Calmette-Guérin (BCG) vaccine when the results were negative. Participants were divided into three groups based on QFT and TST results. The positive cut-off value of QFT is 0.35 IU/mL and TST is 15 mm while the dotted line represents the threshold value of both positive QFT response (at 0.35 IU/mL) and TST (at 15 mm).
Figure 2

Data regarding additional biomarkers for the diagnosis of Helicobacter pylori (HP) in ulcerative colitis (UC). The accuracy of the tests was evaluated using the area under the receiver operating characteristic curve (AUC). The figure shows the AUC values for various biomarkers (IL-2, IL-15, IFN-γ, and IL-18) with statistical significance compared to the control group. The AUC values were calculated for each biomarker to differentiate between UC patients and healthy controls.
The positivity to additional biomarkers for the diagnosis of latent tuberculosis infection (LTBI) in 69 healthcare workers (HCW) after RD1 stimulation. Cytokine secretion is shown as a heat map, with each row representing the cytokine concentrations in individual HCW. Only cytokines for which a significant difference was observed between the LTBI-positive and LTBI-negative group after adjustment for multiple comparisons are represented. Adjusted P values are shown below the heat map. HCW are ranked according to cytokine concentrations, tuberculin skin test (TST) and QuantiFERON®-TB Gold In-Tube (QFT) results. For each parameter, an appropriate colour was assigned ranging from white representing the lowest values (TST ≤ 5 mm, QFT < 0.1 IU/ml and cytokines concentrations < median in LTBI-negative group) to black symbolizing the highest values (TST > 20 mm, QFT ≥ median in the LTBI-positive group, i.e. 1.055 IU/ml, and cytokines concentrations ≥ median in the LTBI-positive group) passing by light gray (TST [5-10 mm], QFT [0.1-0.35 IU/ml], and cytokines concentrations [median in the LTBI-negative group-threshold]) and gray (TST [10-20 mm], QFT [0.35-median in the LTBI-positive group, i.e. 1.055 IU/ml], and cytokines concentrations [threshold-median in the LTBI-positive group]). The second look LTBI diagnosis was done using IL-15 and MIG measurements after QFT assay and previously defined thresholds.