Prevalence and risk factors for latent tuberculosis infection among healthcare workers in Morocco

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Abstract

Increased prevalence of latent tuberculosis infection (LTBI) has been observed among high-risk populations such as healthcare workers (HCWs). The results may depend on the method of LTBI assessment, interferon-gamma release assay (IGRA) and/or tuberculin skin test (TST). Here, we investigated the prevalence and risk factors for LTBI assessed by both IGRA and TST in HCWs living in Morocco, a country with intermediate tuberculosis (TB) endemicity and high BCG vaccination coverage. HCWs were recruited in two Moroccan hospitals, Rabat and Meknes. All the participants underwent testing for LTBI by both IGRA (QuantiFERON-TB Gold In-Tube, QFT-GIT) and TST. Different combinations of IGRA and TST results defined the LTBI status. Risk factors associated with LTBI were investigated using a mixed-effect logistic regression model. The prevalence of LTBI among 631 HCWs (age range 18–60 years) varied from 40.7% (95%CI 36.9–44.5%) with QFT-GIT to 52% (95%CI 48.2–56.0%) with TST using a 10 mm cut-off. The highest agreement between
QFT-GIT and TST \((\kappa = 0.50; \text{95\%CI 0.43–0.56})\) was observed with the 10 mm cut-off for a positive TST. For a definition of LTBI status using a double positive result for both QFT-GIT and TST, significant associations were found with the following risk factors: being male \((\text{OR} = 2.21; \text{95\%CI 1.40–3.49}; p = 0.0007)\), belonging to age groups \(35–44\) years \((\text{OR} = 2.43; \text{95\%CI 1.45–4.06}; p = 0.0007)\) and even more \(45–60\) years \((\text{OR} = 4.81; \text{95\%CI 2.72–8.52}; p = 7.10^{-8})\), having a family history of TB \((\text{OR} = 6.62; \text{95\%CI 2.59–16.94}; p = 8.10^{-5})\), and working at a pulmonology unit \((\text{OR} = 3.64; \text{95\%CI 1.44–9.23}; p = 0.006)\). Smoking was associated with LTBI status when defined by a positive QFT-GIT result \((\text{OR} = 1.89; \text{95\%CI 1.12–3.21}; p = 0.02)\). A high prevalence of LTBI was observed among HCWs in two Moroccan hospitals. Male gender, increased age, family history of TB, and working at a pulmonology unit were consistent risk factors associated with LTBI.

Introduction

Approximately one quarter of the world population is estimated to have latent tuberculosis infection (LTBI) [1]. At this stage, infected subjects do not experience clinical symptoms and are not contagious. However they have a 5–10% life-time risk of developing active tuberculosis (TB) [2], which is most pronounced during the early stage of infection [3], thereby also becoming an important source of TB contagion. LTBI screening in targeted populations is thus pivotal in order to prevent the spread of TB. Healthcare workers (HCWs) have a higher risk of contracting TB than the general population through occupational exposure [4]. Several studies have investigated the prevalence of and the risk factors for LTBI in HCWs [4–7] in countries highly endemic for TB. Reported pooled prevalences ranged from 37 to 63% in these recent meta-analyses [4–7] with a variability depending on important factors such as countries’ income, Bacillus Calmette-Guérin (BCG) vaccination coverage, and LTBI status definition.

The diagnosis of LTBI is inferred indirectly from quantitative measurements of antimycobacterial immunity, attesting to induction of acquired immunity to *Mycobacterium tuberculosis* [8]. The tuberculin skin test (TST) is done in vivo and consists of an intradermal injection of purified protein derivative (PPD) that provokes a delayed hypersensitivity reaction at the site of injection. It is a sensitive test widely used but it lacks specificity, partly due to cross-reactions with BCG, and, to a lesser extent, non-tuberculous mycobacteria [9]. Another type of tests, interferon-gamma release assays (IGRAs), are performed in vitro and detect the secretion of interferon-gamma (IFN-\(\gamma\)) by leukocytes that recognize *M. tuberculosis*-specific antigens. In particular, the QuantiFERON-TB Gold In-Tube test (QFT-GIT) is based on the specific *M. tuberculosis* antigens ESAT-6, CFP10 and TB7.7 (Qiagen, Hilden, Germany). The specificity of these tests is higher than TST and is not affected by BCG vaccination [10]. IGRA results are not fully concordant with TST findings, but they provide complementary information about infection status [11–13]. The World Health Organization recommends tailored LTBI management based on tuberculosis burden and resource availability using either the TST or IGRAs [14]. However, the performance of the two tests is not known in some specific settings. In this study, we investigated the prevalence and risk factors for LTBI assessed by both IGRAs and TST in HCWs from Morocco, a low-middle-income country with an intermediate annual incidence of TB (99/100,000 in 2017), and high BCG vaccination coverage [15–16].
**Materials and methods**

**Study design and population**

A cross-sectional study was conducted from March 2012 to September 2016 in two Moroccan cities, Rabat and Meknes. HCWs were recruited from two hospitals, the Military Hospital Mohammed V of Rabat and the Military Hospital Moulay Ismail of Meknes. During the four years period of enrollment, the TB incidence in Morocco was stable around 100/100,000. We included in the study all adult HCWs with an age between 18 and 60 years who accepted to participate. Exclusion criteria were ongoing pregnancy, immunosuppressive treatment, history of clinical TB disease, known chronic HIV, hepatitis B or C infections, as well as known cancer or chronic renal failure. Information on the following variables was obtained using a questionnaire: gender, age, comorbidities such as diabetes or asthma (yes/no), smoking status (yes/no), history of TB in family members (yes/no), BCG vaccination, working area and occupation. The working area variable consisted of four relevant categories: non-clinical department (such as administrative sectors or laboratories, with indirect exposure to patients), surgical department (direct exposure to patients but low risk of airborne transmission), clinical department (direct exposure to patients) and the pulmonology unit (direct exposure to high-risk patients). The occupation variable was divided into three categories: the non-medical staff (such as administrative personnel, secretaries), the paramedical staff (lab technicians and auxiliaries), and the medical staff (physicians and nurses). All HCWs declared to have been vaccinated with BCG at birth. The sample size was determined by the number of HCWs who fulfilled the inclusion criteria and responded favorably to the questionnaire. The study was approved by the ethics committee of the Faculty of Medicine of Casablanca, Morocco. Written informed consent was obtained from each participant.

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

QFT-GIT testing was performed on blood samples collected before the TST was carried out, in accordance with the kit manufacturer’s instructions. Briefly, 1 ml of peripheral blood was drawn directly into each of three blood collection tubes under vacuum: the nil tube or negative control, the mitogen tube containing phytohaemagglutinin or positive control and the TB specific antigen tube containing specific antigens (early secretory antigenic target 6, culture filtrate protein 10 and TB 7.7 antigen) for *M. tuberculosis*. The contents of each tube were mixed and the tubes were incubated at 37°C for 16 to 24 hours within 16 hours of collection. Then they were centrifuged at 2500 rpm for 15 minutes at room temperature. The supernatant was stored at -20°C. The concentration of IFN-γ in each sample was determined by QFT-GIT ELISA (enzyme-linked immunosorbsent assay) kit. QFT-GIT results, calculated as TB antigen minus nil value (IU/mL), were categorized as positive (≥0.35 IU/mL, QFT+), negative (<0.35 IU/mL, QFT-) or indeterminate following the software provided by the manufacturer. To further investigate discordant results with TST, QFT-GIT results were sub-classified as strong negative (<0.2 IU/mL), weak negative (0.2–0.34 IU/mL), weak positive (0.35–0.7 IU/mL) and strong positive (>0.7 IU/mL) [17–18].

**Tuberculin skin test**

The tuberculin skin test (TST) was performed by the medical staff, in accordance with routine hospital procedure. Two units of standardized purified protein derivative (PPD) solution (2 T. U Tuberculin PPD RT 23, Statens Serum Institut, Copenhagen, Denmark) were injected intradermally into the forearm of all the participants using the Mantoux method. Results were evaluated after 72 hours of the intradermal injection. The transversal diameter of the induration
was measured in millimeters. We considered three different cut-offs (≥ 5, 10 and 15 mm) for a positive result (TST+) to compare with QFT-GIT results. Based on our agreement results, previous studies in similar settings [5] and published guidelines [19], the 10 mm cut-off was used to assess LTBI risk factors.

**Statistical analysis**

The concordance between QFT-GIT and TST results was calculated as the number of double positive (QFT+/TST+) plus double negative (QFT-/TST-) test results divided by the total number of test results. The agreement between QFT-GIT and TST results was also calculated using Cohen’s kappa coefficient (κ) and its 95% confidence intervals (CIs). Fisher’s exact test was used to compare the proportion of QFT-GIT subcategories between concordant QFT/TST and discordant QFT/TST results. The TST induration between QFT-/TST+ and QFT+/TST+ results and between QFT+/TST- and QFT-/TST- was compared using Mann-Whitney test. We assessed the risk factors associated with TB infection using five different definitions of the LTBI status: (i) QFT+ vs. QFT-, (ii) TST+ vs. TST-, (iii) QFT+/TST+ vs. QFT-/TST-, (iv) discordant TST positive QFT-/TST+ vs. QFT-/TST- and (v) discordant QFT-GIT positive QFT+TST+ vs. QFT-/TST-. All analyses were conducted with a univariable and a multivariable mixed-effects logistic regression with a random effect per hospital to investigate the risk factors associated with LTBI. Statistical significance was considered to be a p-value under 0.05. All analyses were carried out using R software (version 3.4.0) and related packages “psych” and “lme4” [20–22].

**Results**

**Healthcare workers**

A total of 662 healthy HCWs were recruited from the hospitals of Rabat (n = 328) and Meknes (n = 334). We excluded 31 participants because of an indeterminate QFT-GIT result. Baseline characteristics of the 631 HCWs with reliable QFT-GIT results are displayed in Table 1. Overall, 54% of HCWs were male (n = 341), 47.1% were younger than 35 years old (n = 297) and 44.1% of them worked in a non-clinical department (n = 278). The majority of HCWs belonged to the medical staff (n = 321, 50.9%). The prevalence of QFT positivity was 40.7% (95% CI 36.9–44.5%) and that of TST positivity at the 10 mm cut-off was 52.1% (95% CI 48.2–56.0%) (Fig 1). When considering the LTBI status among individuals with concordant positive (QFT+/TST+) and concordant negative (QFT-/TST-) results, the prevalence of QFT+/TST+ was 45.2% (95% CI 40.7–49.5%).

**Agreement between QFT-GIT and TST results**

The agreement between QFT-GIT and TST results was slightly higher with a 10 mm TST cut-off [κ = 0.50; 95% CI 0.43–0.56; concordance of 74.6%] than with a 5 mm cut-off [κ = 0.48; 95% CI 0.41–0.54; concordance of 72.7%] or a 15 mm cut-off [κ = 0.44; 95% CI 0.37–0.51; concordance of 73.3%] (Table 2). Therefore, we kept the 10 mm cut-off for all further analyses on TST.

We further investigated the levels of the QFT-GIT and TST results in discordant and concordant subjects (S1 Table). Among 374 HCWs with QFT- results, the proportion of weak negative QFT-GIT result (0.2–0.34 IU/mL) was significantly higher (p = 0.0005) in discordant QFT-/TST+ subjects (17/116, 14.7%) than in double negative QFT-/TST- subjects (10/258, 3.9%). When focusing on 257 HCWs with QFT+ results, we did not observe any significant difference in the proportions of weak positive QFT-GIT result (0.35–0.7 IU/mL) between
discordant QFT+/TST- subjects (5/44, 11.4%) and double positive QFT+/TST+ subjects (26/213, 12.2%). Among 302 HCWs with TST- results, the median induration size was significantly higher (p = 3.10^{-9}) in discordant QFT+/TST- subjects (median = 3 mm) than in double negative QFT-/TST- subjects (median = 0 mm). Finally, among 329 HCWs with TST+ results, the median induration size was significantly lower (p = 0.0002) in discordant QFT-/TST+ subjects (median = 15 mm) than in double positive QFT+/TST+ subjects (median = 17 mm).

Risk factors associated with LTBI status

When considering the LTBI status defined only by QFT-GIT results, univariable analyses showed that QFT+ was significantly associated with male gender (OR = 2.14; 95%CI 1.54–2.97, p = 6.10^{-6}) in discordant QFT+/TST- subjects (median = 3 mm) than in double negative QFT-/TST- subjects (median = 0 mm). Finally, among 329 HCWs with TST+ results, the median induration size was significantly lower (p = 0.0002) in discordant QFT-/TST+ subjects (median = 15 mm) than in double positive QFT+/TST+ subjects (median = 17 mm).

### Table 1. Baseline characteristics of 631 healthcare workers.

| Characteristics          | n (%)     |
|--------------------------|-----------|
| Gender                   |           |
| Male                     | 341 (54.0) |
| Female                   | 290 (46.0) |
| Age group (years)        |           |
| 18–34                    | 297 (47.1) |
| 35–44                    | 186 (29.5) |
| 45–60                    | 148 (23.4) |
| Comorbidities            |           |
| No                       | 586 (92.9) |
| Yes                      | 45 (7.1)  |
| Smoking status           |           |
| No                       | 549 (87.0) |
| Yes                      | 82 (13.0)  |
| Family history of TB     |           |
| No                       | 588 (93.2) |
| Yes                      | 43 (6.8)  |
| Work area                |           |
| Non-clinical department  | 278 (44.0) |
| Surgical department      | 118 (18.7) |
| Clinical department      | 184 (29.2) |
| Pulmonology unit         | 51 (8.1)  |
| Occupation               |           |
| Non-medical staff        | 167 (26.4) |
| Paramedical staff        | 143 (22.7) |
| Medical staff            | 321 (50.9) |
| QFT-GIT                  |           |
| Negative                 | 374 (59.3) |
| Positive                 | 257 (40.7) |
| TST                      |           |
| <10 mm                   | 302 (47.9) |
| ≥10 mm                   | 329 (52.1) |

QFT-GIT: QuantiFERON-TB Gold In-Tube; TB: tuberculosis; TST: tuberculin skin test

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p = 0.0001) and family history of TB (OR = 3.16; 95%CI 1.63–6.12; p = 0.0007). All these variables remained significant in multivariable analysis (Table 3).

When the LTBI status was defined only by TST, the same variables as those found in the previous analysis were significantly associated with TST+ in univariable analysis, with an additional one corresponding to working at a pulmonology unit (OR = 2.31; 95%CI 1.21–4.40; p = 0.01) (Table 4). In multivariable analysis, male gender (OR = 1.70; 95%CI 1.15–2.51;
Table 2. Agreement between QFT-GIT and TST results in 631 healthcare workers according to various TST cut-offs (5 mm, 10 mm and 15 mm).

|               | TST 5 mm | TST 10 mm | TST 15 mm |
|---------------|----------|-----------|-----------|
| QFT-/TST-     | 226      | 258       | 303       |
| QFT+/TST+     | 233      | 213       | 160       |
| QFT-/TST+     | 148      | 116       | 71        |
| QFT+/TST-     | 24       | 44        | 97        |
| Concordance, %| 72.7     | 74.6      | 73.3      |
| Kappa (95% CI)| 0.48 (0.41–0.54) | 0.50 (0.43–0.56) | 0.44 (0.37–0.51) |

QFT-GIT: QuantIFERON-TB Gold In-Tube; TST: tuberculin skin test

Table 3. Risk factors associated with a positive QFT-GIT result in 631 healthcare workers.

| Variables                  | Total n = 631 | QFT- n = 374 | QFT+ n = 257 | OR<sup>a</sup>(95%CI) | p<sup>b</sup> | OR<sup>c</sup>(95%CI) | p<sup>d</sup> |
|----------------------------|--------------|--------------|--------------|-------------------|------|-------------------|------|
| Gender                     |              |              |              |                   |      |                   |      |
| Female                     | 290          | 200          | 90           | Ref               |      | Ref               |      |
| Male                       | 341          | 174          | 167          | 2.14 (1.54–2.97)  | 6.10<sup>-6</sup> | 1.69 (1.17–2.46) | 0.006|
| Age group (years)          |              |              |              |                   |      |                   |      |
| 18–34                      | 297          | 207          | 90           | Ref               |      | Ref               |      |
| 35–44                      | 186          | 104          | 82           | 1.80 (1.23–2.63)  | 0.003| 1.57 (1.04–2.37)  | 0.03 |
| 45–60                      | 148          | 63           | 85           | 3.02 (1.99–4.57)  | 2.10<sup>-7</sup> | 2.74 (1.74–4.31) | 1.10<sup>-5</sup> |
| Comorbidities              |              |              |              |                   |      |                   |      |
| No                         | 586          | 348          | 238          | Ref               |      | Ref               |      |
| Yes                        | 45           | 26           | 19           | 1.08 (0.58–1.99)  | 0.82 | 0.95 (0.49–1.85)  | 0.88 |
| Smoking status             |              |              |              |                   |      |                   |      |
| No                         | 549          | 342          | 207          | Ref               |      | Ref               |      |
| Yes                        | 82           | 32           | 50           | 2.57 (1.59–4.15)  | 0.0001| 1.89 (1.12–3.21) | 0.02 |
| Family history of TB       |              |              |              |                   |      |                   |      |
| No                         | 588          | 360          | 228          | Ref               |      | Ref               |      |
| Yes                        | 43           | 14           | 29           | 3.16 (1.63–6.12)  | 0.0007| 3.41 (1.70–6.85) | 0.0006|
| Work area                  |              |              |              |                   |      |                   |      |
| Non-clinical department    | 278          | 160          | 118          | Ref               |      | Ref               |      |
| Surgical department        | 118          | 79           | 39           | 0.94 (0.64–1.38)  | 0.74 | 1.40 (0.84–2.34)  | 0.19 |
| Clinical department        | 184          | 106          | 78           | 0.68 (0.43–1.07)  | 0.10 | 0.73 (0.41–1.27)  | 0.26 |
| Pulmonology unit           | 51           | 29           | 22           | 1.09 (0.59–2.01)  | 0.77 | 1.80 (0.88–3.69)  | 0.11 |
| Job category               |              |              |              |                   |      |                   |      |
| Non-medical staff          | 167          | 89           | 78           | Ref               |      | Ref               |      |
| Paramedical staff          | 143          | 80           | 63           | 0.91 (0.58–1.43)  | 0.68 | 1.00 (0.58–1.74)  | 0.99 |
| Medical staff              | 321          | 205          | 116          | 0.65 (0.44–1.05)  | 0.10 | 0.70 (0.41–1.19)  | 0.19 |

CI: confidence interval; QFT-GIT: QuantIFERON-TB Gold In-Tube; TB: tuberculosis

<sup>a</sup>OR<sub>uni</sub>: odds ratio obtained in a univariable regression  
<sup>b</sup>p<sub>uni</sub>: p-value corresponding to the coefficient obtained in a univariable regression  
<sup>c</sup>OR<sub>mult</sub>: odds ratio obtained in a multivariable regression including all the variables explored in univariable analyses  
<sup>d</sup>p<sub>mult</sub>: p-value corresponding to the coefficient obtained in a multivariable regression including all the variables explored in univariable analyses  
Bold text indicates a statistically significant result

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Table 4. Risk factors associated with a positive TST result in 631 healthcare workers.

| Variables                  | Total n = 631 | TST- n = 302 | TST+ n = 329 | ORuni (95%CI) | Puni | ORmult (95%CI) | Pmult |
|----------------------------|---------------|--------------|--------------|--------------|------|---------------|-------|
| Gender                     |               |              |              |              |      |               |       |
| Female                     | 290           | 165          | 125          | Ref          |      | Ref           |       |
| Male                       | 341           | 137          | 204          | 2.10 (1.50–2.94) | 2.10−5 | 1.70 (1.15–2.51) | 0.008 |
| Age group (years)          |               |              |              |              |      |               |       |
| 18–34                      | 297           | 185          | 112          | Ref          |      | Ref           |       |
| 35–44                      | 186           | 73           | 113          | 2.58 (1.74–3.84) | 3.10−6 | 2.80 (1.82–4.30) | 3.10−6 |
| 45–60                      | 148           | 44           | 104          | 3.50 (2.25–5.45) | 3.10−8 | 3.68 (2.26–5.99) | 2.10−7 |
| Comorbidities              |               |              |              |              |      |               |       |
| No                         | 586           | 280          | 306          | Ref          |      | Ref           |       |
| Yes                        | 45            | 32           | 23           | 0.98 (0.52–1.85) | 0.95  | 0.82 (0.41–1.65) | 0.58  |
| Smoking status             |               |              |              |              |      |               |       |
| No                         | 549           | 273          | 276          | Ref          |      | Ref           |       |
| Yes                        | 82            | 29           | 53           | 1.83 (1.10–3.05) | 0.02  | 1.27 (0.71–2.26) | 0.42  |
| Family history of TB       |               |              |              |              |      |               |       |
| No                         | 588           | 294          | 294          | Ref          |      | Ref           |       |
| Yes                        | 43            | 8            | 35           | 4.04 (1.79–9.10) | 0.0008 | 4.89 (2.04–11.74) | 0.0004 |
| Work area                  |               |              |              |              |      |               |       |
| Non-clinical department    | 278           | 144          | 134          | Ref          |      | Ref           |       |
| Surgical department        | 118           | 57           | 61           | 1.07 (0.72–1.59) | 0.75  | 1.41 (0.83–2.41) | 0.21  |
| Clinical department        | 184           | 81           | 103          | 1.28 (0.81–1.92) | 0.29  | 1.29 (0.73–2.29) | 0.39  |
| Pulmonology unit           | 51            | 20           | 31           | 2.31 (1.21–4.40) | 0.01  | 3.79 (1.77–8.13) | 0.0006 |
| Job category               |               |              |              |              |      |               |       |
| Non-medical staff          | 143           | 60           | 83           | Ref          |      | Ref           |       |
| Paramedical staff          | 167           | 82           | 85           | 1.46 (0.91–2.35) | 0.12  | 1.51 (0.84–2.71) | 0.17  |
| Medical staff              | 321           | 160          | 161          | 1.02 (0.69–1.51) | 0.93  | 1.07 (0.61–1.88) | 0.81  |

CI: confidence interval; TB: tuberculosis; TST: tuberculin skin test

^a^ ORuni: odds ratio obtained in a univariable regression

^b^ Puni: p-value corresponding to the coefficient obtained in a univariable regression

^c^ ORmult: odds ratio obtained in a multivariable regression including all the variables explored in univariable analyses

^d^ Pmult: p-value corresponding to the coefficient obtained in a multivariable regression including all the variables explored in univariable analyses

Bold text indicates a statistically significant result

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P = 0.008), older age between 35–44 years (OR = 2.80; 95%CI 1.82–4.30; p = 3.10−6) or 45–60 years (OR = 3.68; 95%CI 2.26–5.99; p = 2.10−7), family history of TB (OR = 3.79; 95%CI 1.77–8.13; p = 0.006) were still associated with TST+. However smoking was no more a risk factor when adjusting for other covariates (OR = 1.27; 95%CI 0.71–2.26; p = 0.42) in particular gender, probably explained by the fact that all smokers were males (n = 82).

We then considered a more stringent definition of the LTBI status by focusing on the double positive QFT+/TST+ (n = 213) vs. the double negative QFT−/TST− subjects (n = 258). The same risk factors as those identified in the previous analysis focusing on TST only were found to be significantly associated with LTBI in a multivariable analysis (Table 5): being a male (OR = 2.21; 95%CI 1.40–3.49; p = 0.0007), having an older age between 35–44 years (OR = 2.43; 95%CI 1.45–4.06; p = 0.0007) or 45–60 years (OR = 4.81; 95%CI 2.72–8.52; p = 7.10−8), family history of TB (OR = 6.62; 95%CI 2.59–16.94; p = 8.10−7) and working at a
Risk factors associated with discordant QFT-GIT and TST results

Finally, we performed a specific analysis on the individuals with discordant results, QFT-/TST+ (n = 116) and QFT+/TST- (n = 44) that we compared to QFT-/TST- subjects (n = 258) (Table 6). For QFT-/TST+ individuals, we obtained in multivariable analysis results similar to those observed when considering all TST+ subjects with significant associations for male gender (OR = 1.73; 95% CI: 1.02–2.93; p = 0.04), older age between 35–44 years (OR = 4.18; 95% CI: 2.34–7.44; p = 1.10 \times 10^{-6}) or 45–60 years (OR = 4.35; 95% CI: 2.16–8.75; p = 2.10 \times 10^{-7}) and working at a pulmonology unit (OR = 3.95; 95% CI: 1.43–10.88; p = 0.008) without any effect of smoking (OR = 0.98; 95% CI: 0.40–2.38; p = 0.97).

Table 5. Risk factors from multivariable regressions associated with positive concordant results (QFT+/TST+) results compared to the reference group (negative concordant results QFT-/TST-) in healthcare workers.

| Variables              | Total n = 471 | QFT- TST- n = 258 | QFT+ TST+ n = 213 | OR_{mult}^{a} (95%CI) | p_{mult}^{b} |
|------------------------|---------------|-------------------|-------------------|------------------------|-------------|
| Gender                 |               |                   |                   |                        |             |
| Female                 | 229           | 152               | 77                | Ref                    |             |
| Male                   | 242           | 106               | 136               | 2.21 (1.40–3.49)       | 0.0007      |
| Age group (years)      |               |                   |                   |                        |             |
| 18–34                  | 245           | 170               | 75                | Ref                    |             |
| 35–44                  | 121           | 56                | 65                | 2.43 (1.45–4.06)       | 0.0007      |
| 45–60                  | 105           | 32                | 73                | 4.81 (2.72–8.52)       | 7.10^{-6}   |
| Comorbidities          |               |                   |                   |                        |             |
| No                     | 440           | 241               | 199               | Ref                    |             |
| Yes                    | 31            | 17                | 14                | 0.80 (0.34–1.91)       | 0.62        |
| Smoking status         |               |                   |                   |                        |             |
| No                     | 414           | 240               | 174               | Ref                    |             |
| Yes                    | 57            | 18                | 39                | 1.83 (0.90–3.74)       | 0.10        |
| Family history of TB   |               |                   |                   |                        |             |
| No                     | 436           | 251               | 185               | Ref                    |             |
| Yes                    | 35            | 7                 | 28                | 6.62 (2.59–16.94)      | 8.10^{-5}   |
| Work area              |               |                   |                   |                        |             |
| Non-clinical department| 204           | 115               | 89                | Ref                    |             |
| Surgical department    | 90            | 54                | 36                | 1.46 (0.78–2.72)       | 0.23        |
| Clinical department    | 43            | 73                | 70                | 0.87 (0.44–1.72)       | 0.68        |
| Pulmonology unit       | 34            | 16                | 18                | 3.64 (1.44–9.23)       | 0.006       |
| Job category           |               |                   |                   |                        |             |
| Non-medical staff      | 118           | 61                | 57                | Ref                    |             |
| Paramedical staff      | 107           | 52                | 55                | 1.31 (0.66–2.62)       | 0.44        |
| Medical staff          | 246           | 145               | 101               | 0.82 (0.43–1.56)       | 0.54        |

CI: confidence interval; QFT: QuantiFERON-TB Gold In-Tube; TB: tuberculosis; TST: tuberculin skin test

\(^{a}\)OR_{mult}: odds ratio obtained in a multivariable regression including all the variables explored in univariable analyses

\(^{b}\)p_{mult}: p-value corresponding to the coefficient obtained in a multivariable regression including all the variables explored in univariable analyses

Bold text indicates a statistically significant result

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For QFT+/TST- individuals, we observed in multivariable analysis results close to those with the QFT+ analysis, with less significant p-values due to the smaller sample size. An association was observed with male gender (OR = 2.37; 95%CI 1.07–5.25; p = 0.03), older age which is borderline non-significant for the group 35–44 years (OR = 2.41; 95%CI 0.99–5.88; p = 0.05) and significant for the group 45–60 years (OR = 3.17; 95%CI 1.18–8.50; p = 0.02) and smoking (OR = 3.35; 95%CI 1.17–9.60; p = 0.02). We also noted a protective effect of working at a clinical department (OR = 0.17; 95%CI 0.04–0.72; p = 0.02).

Discussion

We report here the first study of LTBI conducted in HCWs from Morocco, a middle-income country with intermediate endemicity for TB and high BCG vaccination coverage. The prevalence of LTBI ranged from 40.7% (36.9–44.5%) with QFT+ to 52.1% (48.2–56.0%) with TST+ and was 45.2% (40.7–49.5%) when considering the double positive QFT+/TST+ status.
These values are consistent with prevalences observed in HCWs living in high TB burden countries such as Brazil (37%), China (54%), and India (43%) [6]. The higher proportion of TST+ compared to QFT+ in Morocco is consistent with a meta-analysis showing the same pattern in 24 out of 25 studies comparing IGRAs with TST in HCWs [23]. This observation may be explained in part by the higher specificity of IGRAs [9], and an extensive BCG vaccination coverage as it is the case in Morocco. The overall agreement between QFT-GIT and TST results ($\kappa = 0.50$ at a 10 mm cut-off) was also similar to previous estimates in high TB incidence countries ($\kappa = 0.38$ in a recent meta-analysis ranging from 0.19 in China to 0.61 in India [24]).

Interestingly, we identified risk factors that were consistently associated with LTBI, whatever its definition, namely gender, age, and familial history of TB. Male gender is a well-known risk factor for active TB disease due at least in part to socio-cultural causes as well as biological differences between sexes [25–26] but this gender disparity has not been fully investigated for LTBI. A significant higher proportion of LTBI in adult men than in adult women was reported in several studies [27–29] except one in a moderate TB burden country (Taiwan) that did not find gender as a risk factor while taking into account potential confounders [30]. The increase of LTBI prevalence with age is a well-known observation which has been previously reported either with QFT-GIT [31–32] or TST [33]. In our study, age was extremely correlated with the length of employment as almost all HCWs in military hospitals are recruited when they are young adults (between 18–21 years). Therefore, the increased risk of LTBI in the age groups belonging to 35–44 and 45–60 years is probably explained by a longer cumulative exposure that could be occupational and/or non-occupational.

We also identified other risk factors that reflect a close contact to infectious TB patients either through household exposure with a family TB history or an occupational one as working at a pulmonology unit, as already reported [27,31,34]. Beside the work area, we did not find any association with the job category and other potential occupational exposures may have not been captured. We also observed some interesting effects related to smoking. First, the smoking effect observed in univariable analyses decreased (QFT-GIT) or disappeared (TST) when adjusting for other covariates, in particular gender, probably because all smokers were males. In addition, when defining LTBI status by QFT-GIT, the smoking effect appeared to be stronger when considering discordant QFT+/TST- than concordant QFT+/TST+. Smoking was previously reported to be a risk factor for LTBI when assessed by QFT-GIT and/or TST [27–28,35], and also to affect the performance of the QFT-GIT test itself [36]. Further specific investigations should be conducted to investigate the relationship between smoking and IGRAs results with particular attention to confounding factors such as age, gender or socioeconomic status.

Finally, we focused on the discordant results between the two tests. As expected, QFT-/TST+ results were more frequent (116/631, 18.4%) than QFT+/TST+ (44/631, 7.0%) [23]. The QFT-/TST+ discordance is often attributed in part to BCG vaccination and non-tuberculous mycobacteria. Interestingly, QFT-/TST+ subjects shared the same risk factors as those identified in the analysis for the QFT+/TST+ subjects. Moreover, we found a stronger proportion of weak negative QFT-GIT results in QFT-/TST+ than in QFT-/TST- (14.7% vs. 3.9%) indicating that some of these individuals may have a negative result due to the substantial variability of IGRA tests [10]. We also observed that the proportion of QFT-/TST+ was higher (79/334, 23.7%) in the oldest age group 35–60 years, when compared with the younger age group 18–34 (37/297, 12.5%) (S2 Table), consistent with the view that discordant QFT-/TST+ results may be due in part to temporally more remote infections [37]. Regarding the other discordant group QFT+/TST-, we also observed risk factors similar to those obtained with the QFT+ outcome, and this group had larger TST reactions than those of the QFT-/TST- group. Also, we
cannot exclude that some of these QFT+/TST- subjects displayed a complete nonspecific cutaneous anergy that may explain a negative TST [38]. All these observations suggest that a substantial proportion of QFT+/TST- and QFT-/TST+ discordant HCWs may be truly infected individuals. Overall, the results we observed in terms of prevalence and risk factors for LTBI in HCWs living in Morocco were similar when using TST or QFT-GIT, suggesting that either could be used for a screening.

Supporting information

S1 Table. Comparison of discordant QFT-/TST+ vs. double negative QFT-/TST- results and QFT+/TST- vs. double positive QFT+/TST+ results according to QFT-GIT TB antigen minus nil categories.
(DOC)

S2 Table. Agreement between QFT-GIT and TST results in 631 healthcare workers according to age category and a TST cut-off at 10 mm.
(DOC)

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