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ORIGINAL ARTICLE

Prevalence of latent tuberculosis infection in persons with and without human immunodeficiency virus infection using two interferon-gamma release assays and tuberculin skin test in a low human immunodeficiency virus prevalence, intermediate tuberculosis-burden country

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Background: The risk of tuberculosis (TB) is higher in human immunodeficiency virus (HIV)-infected patients and intravenous drug users (IDUs). We determined the prevalence and risk factors of latent TB infection (LTBI) in individuals with or without HIV infection, including IDUs, in a country with a low HIV prevalence, an intermediate TB burden, and a high Bacillus Calmette-Guérin (BCG) vaccine coverage using two interferon-gamma release assays (IGRAs) and the tuberculin skin test (TST).

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intravenous drug users;
latent tuberculosis;
QuantiFERON;
tuberculin skin test

Methods: For this prospective, cross-sectional study, HIV-infected and -uninfected patients from a regional hospital and medical center in Taiwan were enrolled. Results of the two IGRAs [QuantiFERON-TB Gold (QFT-G) and QuantiFERON-TB Gold In-Tube (QFT-GIT)] and the TST were compared. Risk factors for positivity were analyzed.
Results: We recruited 233 patients [198 (85%) men; mean age, 39.4 years]. Most patients (74%) were BCG vaccinated. The prevalence of LTBI was estimated to be 22.8% by TST, 15.9% by QFT-G, and 20.6% by QFT-GIT. HIV-infected individuals had fewer positive QFT-GIT [7.0% vs. 28.6%, $p < 0.001$, adjusted odds ratio (aOR) = 0.28, $p = 0.05$] and TST results, and more indeterminate QFT-G responses (9.3% vs. 0.7%, $p = 0.002$). Concordance between IGRAs and TST was very poor in HIV-infected patients ($\kappa < 0.05$). Independent risk factors for IGRA positivity were increasing age (QFT-G: aOR = 1.98, $p = 0.03$; QFT-GIT: aOR = 2.00, $p = 0.01$) and IDUs (aOR = 4.33, $p = 0.05$ by QFT-G).
Conclusion: HIV-infected persons had a significantly lower response to both IGRAs and TST. High discordance was found between the two generations of IGRAs and between IGRAs and TST. Increasing age, a known risk factor for LTBI, was significantly associated with IGRAs, but not with TST.

Introduction

The World Health Organization (WHO) estimated that 1.1 million (13%) of the 8.6 million people worldwide who developed tuberculosis (TB) in 2012 are those living with human immunodeficiency virus (HIV).[1] TB remains the leading cause of death in HIV-infected persons, and at least one in four deaths among people living with HIV can be attributed to TB.[2] The TB case-fatality rate is markedly increased among patients with HIV in the context of high TB incidence and HIV prevalence.[3] In 2012, 0.3 million (23%) of the 1.3 million deaths due to TB are considered to be related to HIV.

People latently infected with *Mycobacterium tuberculosis* are the major reservoir of potential, active TB disease, especially among high-risk groups.[4] Both HIV patients and intravenous drug users (IDUs) are considered high-risk groups for active TB disease. HIV-infected patients are at the greatest risk of progression from latent TB infection (LTBI) to active disease,[5] with a lifetime risk of progression of 30%, compared with 10% in the general population.[6] IDUs are also associated with a higher prevalence of LTBI and incidence of TB.[7] Among HIV-negative IDUs, the incidence of TB remains six times higher than in the general population.[8]

Therefore, identification and treatment of LTBI in high-risk groups is an important strategy that can lower the risk of developing active TB disease and effectively reduce the number of future potential sources of infection.[9,10] WHO recommends 12 collaborative HIV/TB activities, including the "Three I's for HIV/TB" [isoniazid preventive treatment (IPT), intensified case finding, and infection control for TB], as core prevention, care, and treatment services for HIV infection.[1] Antiretroviral therapy (ART) is a critical intervention for reducing the risk of TB morbidity and mortality among people living with HIV. Although ART reduces the risk of TB disease by 65%,[11] the incidence of HIV-related TB remains unacceptably high.[12,13] IPT significantly reduces TB diseases in HIV-infected patients who have a positive tuberculin skin test (TST),[14] and the combination of IPT with ART achieves an 85% reduction in the incidence of TB.[15]

The diagnosis of LTBI traditionally depended on TST, which involves measurement of the transverse diameter of induration after an intradermal injection of tuberculin. It is easy to perform and cheap, but has the drawbacks of cross-reacting to nontuberculous mycobacterium (NTM) and Bacillus Calmette-Guérin (BCG) vaccination, anergy in immunocompromised hosts, the need of return visit for the test result interpretation, and the boosting phenomenon.[16]

Interferon-gamma (IFN-γ) release assays (IGRAs) are recently developed *in vitro* assays that are based on IFN-γ production in response to *M. tuberculosis*-specific antigens, early secreted antigenic target 6-kDa protein (ESAT-6), and culture filtrate protein-10 (CFP-10). IGRAs have several advantages over TST, including a higher specificity without cross-reactivity with BCG strains and most NTM.[14] QuantiFERON-TB Gold (QFT-G; Cellestis Ltd, Victoria, Australia) is a whole-blood assay that uses enzyme-linked immunosorbent assay for detection of IFN-γ responses. The novel in-tube version of QFT-G contains a third of *M. tuberculosis* antigen [TB 7.7; QuantiFERON-TB Gold In-Tube (QFT-GIT)].[10] QFT-GIT had been approved for use in Europe in 2005 and received approval from the United States Food and Drug Administration in 2007 and is recommended as the diagnostic tool for LTBI by Centers for Disease Control and Prevention.[1]

Taiwan is a country with a low HIV prevalence and an intermediate TB burden. Since 1965, the universal immunization program included neonatal BCG vaccination, and the national coverage reached 99.8% in 2004.[17] By 2012, the total number of HIV patients in Taiwan was 24,239 (9725 of whom had developed full-blown acquired immunodeficiency syndrome with 3771 deaths). The annual incidence of TB infection was 53.0/100,000 individuals in 2012.[18] Of all new TB cases in 2010, 0.8% had HIV co-infection, and among those aged 15–49 years, 2.5% were HIV positive.[19] The HIV prevalence in IDUs was estimated to be between 12.3% and 25.5%, compared with 0.08% in the general population.[20]

The sensitivity of IGRAs and TST is diminished in HIV-infected patients because of anergy associated with a low cluster of differentiation 4 (CD4) cell count.[21] There are

limited data describing the difference between the two generations of QFTs in the HIV-infected population.

The purpose of our study is to determine the prevalence of LTBI in HIV-infected and HIV-uninfected individuals, in IDUs with and without HIV infection, to compare the performance of the two IGRAs in HIV-infected and HIV-uninfected patients, to determine the concordance between the three diagnostic tools for LTBI (TST, QFT-G, and QFT-GIT), and to assess the risk factors associated with positive IGRAs.

Methods

This is a prospective, cross-sectional study conducted from January 2008 to January 2010. We enrolled patients from a regional hospital (E-Da Hospital) and a medical center (Kaohsiung Veterans General Hospital) in southern Taiwan. Study patients were invited to participate by announcements from infectious diseases outpatient clinics and from the methadone clinic providing care for IDUs. They were grouped into HIV-uninfected and HIV-infected patients. The study protocol was approved by the Institutional Review Board of both hospitals.

All study patients received baseline medical evaluations and a chest X-ray to rule out active TB infection. Symptomatic patients were excluded. A questionnaire was administered to collect demographic data, including sex, age, underlying diseases, BCG vaccination status, and risk factors for LTBI, such as having a history of TB disease or contact with persons who had active TB disease. In HIV-infected patients, HIV viral load and CD4 count drawn within 3 months of study entry were recorded.

Whole blood (8 mL) was drawn for QFT-G (Cellestis Ltd. Melbourne, Australia) and QFT-GIT (Cellestis, Melbourne, Australia) prior to performing TST. Blood was processed according to the manufacturer's instructions.

The QFT-G and QFT-GIT were interpreted as positive if the IFN-γ level in response to ESAT-6, CFP-10, or TB 7.7 was $\geq$0.35 IU/mL and $\geq$25% of the background value; the values were interpreted as negative if the IFN-γ level was $<$0.35 IU/mL or $<$25% of the background value, with the IFN-γ level in response to mitogen (the positive control) reaching at least 0.5 IU/mL. The result was interpreted as indeterminate if the IFN-γ level was $<$0.35 IU/mL or $<$25% of background value with the IFN-γ level in response to mitogen $<$0.5 IU/mL, or if the negative control was $\geq$8.0 IU/mL, irrespective of the IFN-γ response to ESAT-6 or CFP-10 or TB 7.7.

The TST was performed by an intradermal injection of 2 tuberculin unit (RT-23; Statens Serum Institut, Copenhagen, Denmark) on the volar surface of the forearm, and the diameter of the induration was read 48–72 hours later by the Mantoux method. In HIV-infected persons, TST was considered positive when the induration measured $\geq$5 mm. An induration of 10 mm was considered positive in HIV-uninfected persons without BCG vaccination, and $\geq$15 mm in HIV-uninfected persons who have received BCG vaccination.

Statistical analysis

Data were analyzed using the Stata Statistical Software version 10 (StataCorp LP, College Station, TX, USA). Categorical variables were compared using Pearson Chi-square analysis or the Fisher's exact test. Continuous variables were compared by Student *t* test or the Wilcoxon rank-sum test. Concordance between the TST and QFT results was assessed by the kappa (κ) coefficient. Strength of agreement was considered poor if $\kappa \leq 0.20$ or less, fair if $\kappa = 0.20$–0.40, moderate if $\kappa = 0.40$–0.60, substantial if $\kappa = 0.60$–0.80, and optimal if $\kappa = 0.80$–1.00.[22] Risk factors for QFT and TST positivity were determined by multivariable logistic regression analysis. Age, HIV status, IDUs status, and a history of TB exposure were included *a priori* in the final multivariable model. QFT-GIT was used as the reference standard as a surrogate for the diagnosis of LTBI to calculate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TST and QFT-G.

Results

A total of 233 patients were recruited into this study [most participants were men ($n = 198$), 85%], with a mean age of 39.4 years (standard deviation, 10.2 years; range, 20.1–71.3 years). The study population included 147 (63%) HIV-uninfected and 86 (37%) HIV-infected patients. Among the 233 study participants, 137 (59%) were IDUs and most were HIV uninfected (83.7% vs. 16.3%, $p < 0.001$). HIV-uninfected patients were mostly IDUs (123, 83.7%) and many were homeless, and had a history of living in a shelter or prison (109, 80%). None of the HIV uninfected, non-IDUs (24/147) recalled having exposures to TB. The median CD4 count in HIV-infected patients was 348 cells/μL. In patients with HIV infection, 53 were men-who-have-sex-with-men (61.6%), 15 were heterosexuals (17.4%), 14 were IDUs (16.3%), and four were infected by blood transfusion (4.7%). The mean age (38.9 years vs. 40.4 years, $p = 0.28$) and the BCG vaccination status (73.5% vs. 75.6%, $p = 0.72$) were not different in those with and without HIV infection. Patients with HIV more frequently had a history of TB disease or contact with patients with TB, whereas patients without HIV more commonly lived or worked with those who are homeless or those in a shelter or prison (Table 1).

Prevalence of LTBI estimated by IGRA

The overall prevalence of LTBI was estimated to be 15.9% [95% confidence interval (CI): 11.2–20.6] by QFT-G and 20.6% (95% CI: 15.4–25.8) by QFT-GIT positivity ($p = 0.19$). HIV-infected individuals were significantly less likely to have positive QFT-G results [3.5% vs. 23.1%, $p < 0.001$, adjusted odds ratio (aOR) 0.40, $p = 0.25$] and QFT-GIT results [7.0% vs. 28.6%, $p < 0.001$, aOR 0.28, $p = 0.05$] than persons without HIV infection. Indeterminate responses occurred more frequently in HIV-infected persons when using QFT-G (9.3% vs. 0.7%, $p = 0.002$), but not with QFT-GIT (2.3% vs. 2.0%, $p = 1.00$). QFT-GIT had a nonsignificantly higher rate of positivity than QFT-G in both HIV-uninfected (28.6% vs. 23.1%, $p = 0.29$) and HIV-infected patients (7.0% vs. 3.5%, $p = 0.50$; Fig. 1). Low CD4 cell counts did not have a significant impact on the results of QFT-G and QFT-GIT in HIV-infected persons (Table 2). Indeterminate results in HIV-infected persons were mostly

Table 1 Baseline demographic and clinical characteristics of HIV-infected and -uninfected patients (n = 233)

Characteristics	Non-HIV (n = 147)	HIV (n = 86)	p
Demographics			
Age (y, mean ± SD) (range)	38.9 (8.4) (21.1–62.9)	40.4 (12.6) (20.1–71.3)	0.28
Sex, male	119 (81.0)	79 (91.9)	0.03
HIV risk factor			
IDU	123 (83.7)	14 (16.3)	<0.001
MSM	—	53 (61.6)	—
Heterosexual	—	15 (17.4)	—
Transfusion	—	4 (4.7)	—
BCG vaccination	108 (73.5)	65 (75.6)	0.72
Risk factors for TB disease			
Smoking	90 (61.2)	30 (34.9)	<0.001
Lived or worked in homeless shelter or prison	103 (70.1)	11 (12.8)	<0.001
Exposure to TB[a]	4 (2.7)	26 (30.2)	<0.001

[a] History of exposure to TB includes past TB disease or contact with patients who had TB.
Data are presented as n (%), unless otherwise indicated.
BCG = Bacillus Calmette-Guérin; HIV = human immunodeficiency virus; IDU = intravenous drug users; MSM = men who have sex with men; SD = standard deviation; TB = tuberculosis.

due to a lack of response to mitogen (7/8, 87.5% in QFT-G and 2/2, 100% in QFT-GIT).

Prevalence of LTBI estimated by TST

TST was performed in 92 patients (18 HIV-uninfected and 74 HIV-infected patients). The mean diameter of TST induration was 4.2 mm (range, 1.0–20.0 mm). Fig. 2 showed the distribution of the size of TST induration in the two groups. When using a 5-mm cutoff criterion, the TST result was positive in 17 HIV-infected patients (23.0%) and 18 HIV-uninfected patients (100.0%). When using a 10-mm cutoff criterion, the TST result was positive in 10 HIV-infected patients (13.5%) and 14 HIV-uninfected patients (77.8%). TST, interpreted at any cutoff criteria, was significantly less frequently positive in HIV-infected persons compared with HIV-uninfected persons (Fig. 2).

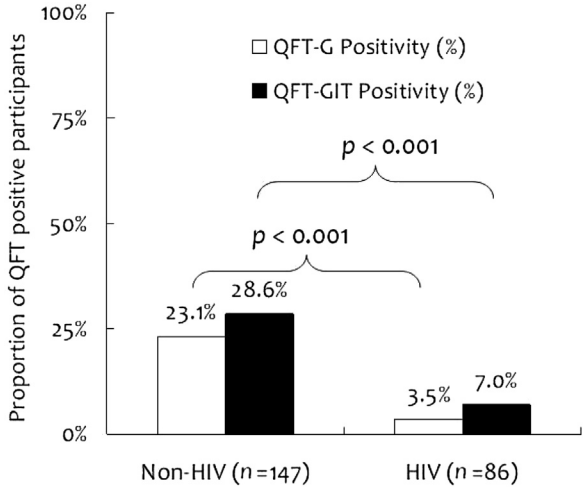

Figure 1. The prevalence of latent tuberculosis infection estimated using two interferon-γ release assays (QuantiFERON TB-Gold and QuantiFERON-TB Gold In-Tube test) in HIV-infected (n = 86) and HIV-uninfected patients (n = 147). HIV = human immunodeficiency virus; QFT-G = QuantiFERON-TB Gold; QFT-GIT = QuantiFERON-TB Gold In-Tube.

Table 2 Results of QuantiFERON-TB Gold (QFT-G) and QuantiFERON-TB Gold In-Tube (QFT-GIT) stratified by CD4 cell counts in HIV-infected persons (n = 86)

Test	Result	CD4 < 200 (n = 22)	CD4 ≥ 200 (n = 64)	p
QFT-G	Positive	0 (0.0)	3 (4.7)	0.61
	Indeterminate	1 (4.6)	7 (10.9)	
QFT-GIT	Positive	1 (4.6)	5 (7.8)	0.10
	Indeterminate	2 (9.1)	0 (0.0)	

Data are presented as n (%).
CD4 = cluster of differentiation 4; HIV = human immunodeficiency virus; QFT-G = QuantiFERON-TB Gold; QFT-GIT = QuantiFERON-TB Gold In-Tube.

Agreement between the two IGRAs and TST and IGRA in HIV-infected and HIV-uninfected patients

The concordance between QFT-G and QFT-GIT was very poor in HIV-infected patients ($\kappa = -0.05$); however, the concordance was moderate in HIV-uninfected patients ($\kappa = 0.50$). The overall agreement between TST and QFT-G ranged from 17.7% to 82.4% in HIV-uninfected and from 75.7% to 94.3% in HIV-infected persons for TST cutoffs varying between 5 mm and 15 mm, and the concordance was fair ($\kappa < 0.5$). The overall agreement between TST and

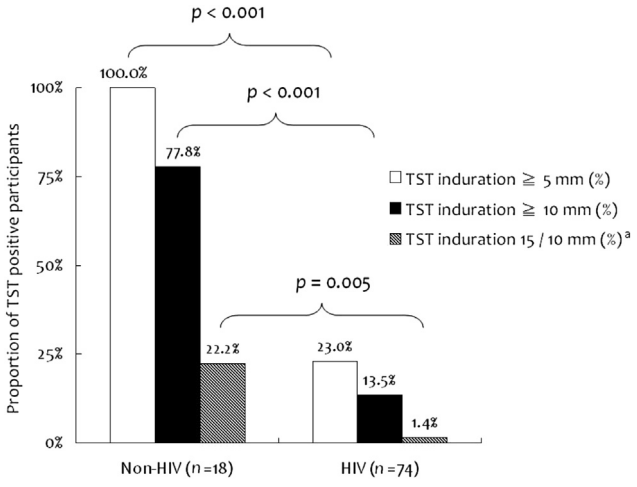

Figure 2. Tuberculin skin test (TST) positivity using different cutoff values in HIV-infected and HIV-uninfected patients (n = 92). [a] TST cutoff value was 15 mm for BCG-vaccinated and 10 mm for BCG-unvaccinated persons. HIV = human immunodeficiency virus.

QFT-GIT ranged from 23.5% to 76.5% in HIV-uninfected and from 81.9% to 91.7% in HIV-infected persons for TST cutoffs varying between 5 mm and 15 mm, but the concordance was poor ($\kappa < 0.5$). Excluding those with both indeterminate QFT-G and QFT-GIT results, 14 (15.4%) had discordant TST+/QFT− and five (5.5%) had discordant TST−/QFT + results; 3/5 had nonreactive TST indurations. There was poor concordance between the TST and IGRA (both QFT-G and QFT-GIT) results using any of the TST cutoff values, especially in HIV-infected patients (Table 3).

Risk factors for a positive IGRA

In multivariate analysis, significant risk factors for QFT-G positivity included age (aOR = 1.98, $p = 0.03$) and IDUs (aOR = 4.33, $p = 0.05$), after adjusting for HIV infection and history of exposure to TB (Table 4). The risk factor for QFT-GIT positivity included age (aOR = 2.00, $p = 0.01$), after adjusting for IDU and history of exposure to TB. HIV-infected patients were less likely to have positive QFT-GIT (aOR = 0.28, $p = 0.05$; Table 4).

Sensitivity, specificity, PPV, and NPV

Using QFT-GIT as a reference standard for the diagnosis of LTBI, both TST and QFT had low sensitivity (66.7% for TST and 51.5% for QFT-G). However, QFT had a higher specificity than TST (TST = 82.5% vs. QFT = 92.5), a higher PPV (64.9% vs. 30.0%) but a lower NPV (87.4 vs. 95.7%) than TST (Table 5).

Discussion

This study compared two IGRA tests with TST for the diagnosis of LTBI in HIV-infected and HIV-uninfected adults in a country with a low incidence of HIV, an intermediate prevalence of TB, and a high BCG vaccination coverage. We found that HIV-infected persons had a significantly lower response to both IGRAs and TST than HIV-uninfected persons. IDU is a significant risk factor for LTBI using QFT-G. We demonstrated that the two generations of IGRAs were not comparable, and a high rate of discordance was found between IGRAs and TST at any cutoff criteria of TST induration. Increasing age, a known risk factor for LTBI, was significantly associated with IGRAs on multivariable analysis, but not with TST.[23]

Our study demonstrated that HIV-infected persons were significantly less likely than HIV-uninfected persons to have a positive QFT-GIT. Previous studies reported a lower response rate in HIV-infected persons with low CD4 counts of <200 cells/μL.[21,24–26] Because IGRAs depend predominantly on the CD4 recognition of *M. tuberculosis* antigens,[27] absolute CD4 cell counts affect test performance in HIV-infected persons.[28] In our study, 25.6% (22/86) had CD4 cell counts <200 cells/μL, and had lower IGRA-positive rates, but these were nonsignificant. Factors other than CD4 counts may influence QFT positivity. It is advisable that a repeat testing of IGRAs should be performed when CD4 counts are >200 cells/μL in individuals with an initially low CD4 cell count and a nonreactive IGRA result.

A high prevalence of LTBI was observed among IDUs, reaching 37.2%. This was similar to previous studies, where the prevalence of LTBI in IDUs was 14–28% using TST[29,30] and 34% by IGRA.[31] IDU is a known risk factor for LTBI due to increased exposure in crowded environments and incarceration in correctional facilities.[26,32] IDU was associated with a fourfold risk of LTBI using QFT-G. Co-infection with HIV in IDUs was associated with a reduced rate of IGRA positivity using either QFT-G (26.0% vs. 14.3%, $p = 0.57$) or QFT-GIT (31.7% vs. 7.1%, $p = 0.06$). IGRAs are effective screening tools for IDU, and can avoid the confounding factors that limit the usefulness of TST, including HIV co-infection and difficulty in returning for the TST reading.

In the current study, we found that the QFT-GIT assay may be more sensitive than the QFT-G assay, as it had a higher overall rate of positivity (20.6% vs. 15.9%, $p = 0.19$); in addition, indeterminate responses in those with HIV

Table 3 Agreement between TST and interferon-gamma release assays in HIV-infected and -uninfected patients

Tests	Overall agreement % (kappa value, 95% CI)	
	Non-HIV (n = 147)	HIV (n = 86)
QFT-G vs. QFT-GIT	80.6 (0.50, 0.34–0.66)	89.5 (−0.05, −0.27–0.17)
	Non-HIV (n = 18)	HIV (n = 74)
QFT-G vs. TST (at various cutoff values)		
≥ 5 mm	17.7 (0.00, 0.00–0.00)	75.7 (0.04,−0.12–0.19)
≥ 10 mm	41.2 (0.12,−0.12–0.35)	85.7 (0.11, −0.09–0.31)
≥ 15 mm	82.4 (0.46, −0.004–0.93)	94.3 (−0.02, −0.22–0.18)
15/10 mm[a]	82.4 (0.46, −0.004–0.93)	94.3 (−0.02, −0.22–0.18)
QFT-GIT vs. TST (at various cutoff values)		
≥ 5 mm	23.5 (0.00, 0.00–0.00)	81.9 (0.31, 0.12–0.50)
≥ 10 mm	47.1 (0.17, −0.09–0.44)	88.9 (0.37, 0.15–0.59)
≥ 15 mm	76.5 (0.35, 0.13–0.82)	91.7 (−0.02, −0.19–0.15)
15/10 mm[a]	76.5 (0.35, 0.13–0.82)	91.7 (−0.02, −0.19–0.15)

[a] TST cutoff value is 15 mm for BCG-vaccinated and 10 mm for BCG-unvaccinated patients.
CI = confidence interval; HIV = human immunodeficiency virus; QFT-G = QuantiFERON-TB Gold; QFT-GIT = QuantiFERON-TB Gold in-Tube; TST = tuberculin skin test.

Table 4 Risk factors for latent tuberculosis infection based on QuantiFERON-TB Gold and QuantiFERON-TB Gold In-Tube test positivity

Risk factor	QuantiFERON-TB Gold (QFT-G)[a]				QuantiFERON-TB Gold in-Tube (QFT-GIT)[b]			
	Crude odds ratio (95% CI)	*p*	Adjusted odds ratio[c] (95% CI)	*p*	Crude odds ratio (95% CI)	*p*	Adjusted odds ratio[c] (95% CI)	*p*
Sex (male)	2.34 (0.68–8.09)	0.18	1.44 (0.38–5.50)	0.59	1.52 (0.55–4.18)	0.42	1.21 (0.39–3.72)	0.74
Age[d]	1.90 (1.11–3.27)	0.02	1.98 (1.08–3.64)	0.03	1.93 (1.19–3.13)	0.007	2.00 (1.18–3.42)	0.01
HIV	0.13 (0.04–0.44)	0.001	0.40 (0.09–1.89)	0.25	0.19 (0.08–0.46)	<0.001	0.28 (0.08–0.97)	0.05
IDU	9.44 (2.80–31.83)	<0.001	4.33 (1.04–18.08)	0.05	4.57 (2.03–10.32)	<0.001	1.77 (0.63–4.99)	0.28
Sexual transmission[e]	0.06 (0.01–0.45)	0.006	0.30 (0.02–5.79)	0.42	0.22 (0.08–0.59)	0.002	3.63 (0.27–48.74)	0.33
BCG vaccination	1.65 (0.68–3.99)	0.27	1.51 (0.59–3.88)	0.40	1.67 (0.75–3.69)	0.21	1.65 (0.71–3.83)	0.25
Smoking	8.90 (1.16–68.32)	0.04	2.29 (0.24–22.05)	0.47	6.76 (1.54–29.75)	0.01	3.15 (0.55–17.96)	0.20
Lived or worked in homeless shelter, prison	4.28 (1.78–10.32)	0.001	0.55 (0.14–2.14)	0.39	3.61 (1.71–7.59)	0.001	1.04 (0.29–3.76)	0.96
Exposure to TB	0.16 (0.02–1.25)	0.08	0.30 (0.03–2.80)	0.29	0.37 (0.11–1.30)	0.11	0.74 (0.18–3.13)	0.69
TST positivity[f]	0.88 (0.24–3.18)	0.85	6.26 (0.87–45.27)	0.07	1.69 (0.61–4.67)	0.31	10.15 (2.17–47.49)	0.003
CD4 < 200	0.69 (0.00–6.64)	0.77	0.33 (0.00–3.66)	0.39	0.62 (0.07–5.65)	0.67	0.62 (0.01–6.07)	1.00

[a] Nine patients with indeterminate results on the QuantiFERON-TB Gold test were excluded.
[b] Five patients with indeterminate results on the QuantiFERON-TB Gold In-Tube test were excluded.
[c] Multivariate analysis was adjusted for age, HIV, IDU, and exposure to TB.
[d] Age was divided into three groups (<30 years old, 30–39 years old, and ≥40 years old). There was a significantly increasing trend of QFT-G positivity with increasing age (OR = 1.79, 95% CI = 1.11–2.90, $p = 0.02$).
[e] Risk factors for sexual transmission included MSM and heterosexuals.
[f] In HIV-infected persons, TST was considered positive when the induration measured 5 mm or greater. An induration of 10 mm was considered positive in HIV-uninfected persons without BCG vaccination, and 15 mm or greater in HIV-uninfected persons who have received BCG vaccination.
BCG = Bacillus Calmette-Guérin; CD4 = cluster of differentiation 4; CI = confidence interval; HIV = human immunodeficiency virus; IDU = intravenous drug users; MSM = men who have sex with men; TB = tuberculosis; TST = tuberculin skin test.

infection were not increased, as was observed in QFT-G. The overall agreement was poor between the two IGRAs. A previous study found a fair to good agreement between QFT-G and QFT-GIT (75.4%, $\kappa = 0.51$) in unemployed workers, and suggested that discordance between the two generations of IGRA may arise from increased sensitivity due to either the extra *M. tuberculosis*-specific antigen used with the in-tube method (TB antigen 7.7) or immediate incubation of whole blood in tube with the antigens.[33] Other possible causes for inter-test discordance are small variations between duplicate IGRAs,[28] variable immune responses due to changing CD4 cell counts, medications, stress, and infection. The explanations for indeterminate results that may impair QFT-G performance are improper collection, storage, incubation, and processing of blood, as well as a time lag between drawing blood samples. Based on our study, QFT-G and QFT-GIT results were incomparable, in both HIV-infected and -uninfected persons.

The agreement was poor to fair between TST and QFT (agreement = 17.6–94.3, $\kappa = -0.22$–0.46), with particularly lower kappa coefficients in the HIV-infected group ($\kappa = -0.02$–0.37). Discordance between QFT and TST may be due to variation in the amount and type of tuberculin solution used as well as due to heterogenicity in BCG vaccination rates, exposure to atypical mycobacteria, local TB incidence, estimated time since LTBI, and immune status.[21,33–35]

Discordant TST+/QFT− results were found in 15.4% (14/91) of our study patients, most of whom had received BCG vaccination (12/14, 85.7%) and were HIV infected (12/14, 85.7%). Only three HIV-infected persons had CD4 counts <200 cells/μL, and all were receiving ART. Discordance between TST and IGRA with TST+/IGRA− results was strongly associated with cross-reactivity with NTM and BCG vaccination status, especially in low TB endemic areas and in populations with low risk for TB exposure.[34] A clear proportional relationship was observed between T cell count and the level of IFN-γ response in those with CD4 cell count <500 cells/μL.[36] The higher prevalence of LTBI among HIV infected, using TST (23.0%, 95% CI: 13.2–32.8) compared with IGRA (10.5%, 95% CI: 3.9–17.1), in our study may be due to false reactivity associated with BCG

Table 5 Sensitivity, specificity, PPV, and NPV of TST and QFT-G using QFT-GIT as the reference standard for latent TB infection

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
TST (%)	66.7 (29.9–92.5)	82.5 (72.4–90.1)	30.0 (11.9–54.3)	95.7 (87.8–99.1)
QFT-G (%)	51.5 (36.1–65.9)	92.5 (87.5–95.9)	64.9 (47.5–79.8)	87.4 (81.7–91.9)

CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value; QFT-G = QuantiFERON-TB Gold; QFT-GIT = QuantiFERON-TB Gold in-Tube; TB = tuberculosis; TST = tuberculin skin test.

vaccination.[16,34,35] False-positive TST results increase the cost of TB screening with additional procedures such as chest radiographs and unnecessary drug treatment.[37]

Discordant TST−/QFT + results (5/91, 5.5%) may be attributable to either false-positive QFT results or better sensitivity of IGRAs over TST. A negative TST may have been due to anergy in HIV infection (3/5, 60%). When using the QFT-GIT as a reference standard for diagnosis of LTBI, both QFT and TST had a low sensitivity, but QFT had a higher specificity, higher PPV, and lower NPV than TST. The reliability and validity of TST and IGRAs can be influenced by several confounding factors, including the immune status, cross-reactions to BCG or NTM, and the prevalence local TB burden.[34] To increase the PPV and reduce the number of false positives, the use of a risk-stratified interpretation, as has been used for TST, was suggested.[38] We suggest that the use of TST alone may not be accurate in the diagnosis of LTBI in HIV-infected persons, especially in patients who had received BCG vaccination. Use of IGRAs may be more accurate in the diagnosis of LTBI in this high-risk group.

There are several limitations in this study. First, the control group consisted of mostly IDUs rather than healthy individuals, which may augment the negative association of an IGRA response in HIV-infected persons and may not be generalizable to non-IDU populations. However, the selection of HIV-uninfected IDUs as a control group allowed for the clarification of the risk of LTBI in IDUs with and without HIV infection. Second, other risk factors associated with LTBI, such as diabetes mellitus, hemodialysis, or other immunocompromised status, were not analyzed due to the small number of patients harboring the risk factor. However, this study was aimed to investigate the risk factors, HIV infection, and IDU. Third, not all patients underwent TST, especially the HIV-uninfected patients. Based on our study and previous reports, anergy is frequently observed in HIV-infected persons and therefore TST is of limited use in this group. Finally, we did not record the initial or nadir CD4 cell counts and the use of antiretroviral agents in the HIV-infected patients, and therefore, the impact of nadir CD4 cell counts on IGRA and TST response cannot be evaluated.

In conclusion, this study demonstrated that IGRAs are useful diagnostic tests in HIV-infected and -uninfected persons in countries with a large number of BCG-vaccinated individuals. However, HIV infection is associated with false-negative IGRA results, and a negative or indeterminate result should be interpreted with caution. Based on our study, TST alone may be unreliable in the diagnosis of LTBI in BCG-vaccinated and/or HIV-infected persons, and we recommend the use of IGRAs in these populations. IGRAs are better correlated with risk factors for LTBI, such as age, compared with TST. Further longitudinal studies need to be conducted to determine the predictive value for the development of active TB disease in HIV-infected patients and IDUs, and identify individuals who will benefit from LTBI treatment, especially in intermediate TB and low HIV-burden countries.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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