

Comparison of two interferon-gamma release assays (QuantiFERON-TB Gold In-Tube and T-SPOT.TB) in testing for latent tuberculosis infection among HIV-infected adults

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Abstract

There is currently no 'gold standard' for diagnosis of latent tuberculosis infection (LTBI), and both the tuberculin skin test and interferon-gamma release assays (IGRAs) are used for diagnosis; the latter have a higher sensitivity than tuberculin skin tests for diagnosis of LTBI in HIV-infected individuals with lower CD4 counts. No evidence base exists for selection of IGRA methodology to identify LTBI among human immunodeficiency virus-infected patients in the UK. We prospectively evaluated two commercially available IGRA methods (QuantiFERON-TB Gold In Tube [QFG] and T-SPOT.TB) for testing LTBI among HIV-infected patients potentially nosocomially exposed to an HIV-infected patient with 'smear-positive' pulmonary tuberculosis. Among the exposed patients median CD4 count was 550 cells/ μ L; 105 (90%) of 117 were receiving antiretroviral therapy, of who 104 (99%) had an undetectable plasma HIV load. IGRAs were positive in 12 patients (10.3%); QFG positive in 11 (9.4%) and T-SPOT.TB positive in six (5.1%); both IGRAs were positive in five patients (4.3%). There was one indeterminate QFG and one borderline T-SPOT.TB result. Concordance between the two IGRAs was moderate ($\kappa = 0.56$, 95% confidence interval = 0.27–0.85). IGRAs were positive in only 4 (29%) of 14 patients with previous culture-proven tuberculosis. No patient developed tuberculosis during 20 months of follow-up.

Keywords

Interferon-gamma release assays, latent tuberculosis infection, HIV, screening, tuberculin skin test, AIDS, IGRA, *Mycobacterium tuberculosis*

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Introduction

An estimated one-third of the world's population is latently infected with *Mycobacterium tuberculosis*. Screening for latent tuberculosis infection (LTBI), using either interferon-gamma release assays (IGRAs) or tuberculin skin testing (TST), is important as it permits identification of those at greatest risk of developing active tuberculosis, and treating LTBI reduces the risk of progression to active disease.¹ Among individuals co-infected with *M. tuberculosis* and human immunodeficiency virus (HIV) and without access to antiretroviral therapy (ART) the risk of progression to active tuberculosis is 10% annually.² The risk of progression is reduced among those receiving ART,^{3,4} but is still twice that of the HIV-uninfected general population, even in countries with a low prevalence of tuberculosis.^{3,4}

Currently, there is no 'gold standard' for diagnosis of LTBI among both immune competent and immunosuppressed individuals, and both TST and IGRA are used, in single or in combination^{5,6}; the latter appear to have a higher sensitivity than TST for diagnosis of

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LTBI in HIV-infected individuals with CD4 counts <200 cells/ μL , and among those who have received BCG vaccination.^{1,7,8} LTBI is diagnosed on the basis of either a positive TST or IGRA result.¹ The lack of a gold standard for diagnosis of LTBI raises methodological difficulties in interpreting results of IGRA and TST, both within and between studies.

The two commercially available IGRA are QuantiFERON-TB Gold In-Tube (QFG; Cellestis, Carnegie, Australia) and T-SPOT.TB (Oxford Immunotech Abingdon, UK). Both assays measure interferon-gamma released by peripheral blood mononuclear cells in response to *M. tuberculosis* complex-specific recombinant antigens not shared with the BCG bacillus or most environmental mycobacterial species. It has been suggested that among HIV-infected individuals T-SPOT.TB has a higher sensitivity and fewer indeterminate results than QFG.^{7,8} It is thought that this is because the T-SPOT.TB assay requires a specific number of peripheral blood mononuclear cells and so is less likely to be affected by low CD4 lymphocyte counts,⁸ however, not all studies confirm this observation.^{9–11}

Despite accumulating evidence for the utility of IGRA in detecting LTBI among HIV-infected patients from low- and middle-income countries, there is a paucity of data from high-income countries (which have a low prevalence of tuberculosis) and in the latter setting, few studies have performed 'head to head' comparisons of the two IGRA tests.^{9–12} Currently, there is no evidence on which to base choice of IGRA for identifying LTBI among HIV-infected patients in the UK (a low-prevalence setting). In the absence of an evidence-base on which to base a decision to use one IGRA, rather than the other, we prospectively evaluated both QFG and T-SPOT.TB for testing HIV-infected patients attending an inner-London treatment centre who were potentially nosocomially exposed to an HIV-infected patient with smear-positive pulmonary tuberculosis.

Methods

HIV-infected patients attending an inner-London outpatient HIV treatment centre who were potentially nosocomially exposed to an HIV-infected patient with smear-positive pulmonary tuberculosis were identified from electronic patient records. The index patient (CD4 = 490 cells/ μL , viral load = undetectable on ART) with known chronic obstructive pulmonary disease attended the HIV clinic with worsening respiratory symptoms. The patient spent several hours in the clinic waiting area prior to the diagnosis of tuberculosis.

Patients were screened between three and six months after their exposure. For each patient, at a 'face to face' interview, we recorded the presence or absence of

symptoms (cough, fever, night sweats, weight loss), gender, ethnicity, country of birth, duration of residence in UK, BCG status and prior treatment for active (culture-confirmed) tuberculosis, current CD4 count and plasma HIV load and receipt of ART.

All patients had a chest radiograph and those with a CD4 count <200 cells/ μL also had a TST, as per NICE guidelines.⁶ A positive TST was defined as ≥ 5 mm induration evident 48–72 h after intra-dermal injection of 0.1 mL (2 tuberculin units) of Purified Protein Derivative (PPD). IGRA results were reported as positive, negative, indeterminate or borderline.

Statistical analysis

Data were entered into Microsoft Excel and analysed using GraphPad Prism software v5.1 (GraphPad Software Inc, La Jolla, CA). Concordance between QFG and T-SPOT.TB results was assessed by kappa (κ) coefficient.¹³ Strengths of agreement were considered 'poor', $\kappa \leq 0.20$, 'fair', $0.20 < \kappa \leq 0.40$, 'moderate', $0.40 < \kappa \leq 0.60$, 'good', $0.60 < \kappa \leq 0.80$ and 'very good', $0.80 < \kappa \leq 1.00$. Correlation between median CD4 count and concordance between IGRA results in the present study and in recent studies from low income countries^{9–12} was done using Pearson's test; $p < 0.05$ was regarded as significant.

Results

One hundred and seventeen patients were potentially nosocomially exposed and were tested for LTBI with both IGRAs. Of these 117 patients, 91 (78%) were men, 48 (41%) were white British, 29 (25%) black African, 21 (18%) European, seven (6%) South American and 12 (11%) were from other ethnic groups. Individuals born outside the UK had been resident in the UK for a median of 10 years (range 3–45). The majority, 91 (78%) of 117 had received BCG vaccination and 14 (12%) of 117 had previously been treated for culture-proven tuberculosis a median of 9.5 years previously (range = 3–13 years). Of the 117 patients, 116 were asymptomatic. None of the patients had active tuberculosis at the time of screening.

Of 105 (90%) patients receiving ART, 104 (99%) had undetectable plasma HIV loads and their median (range) CD4 count was 530 (140–1250) cells/ μL . Among those not receiving ART the median (range) plasma HIV load and CD4 counts were 21,000 copies/mL (590–160,000) and 520 (250–950) cells/ μL , respectively. IGRA results are shown in Table 1. Overall there was moderate concordance between QFG and T-SPOT.TB; $\kappa = 0.49$ (95% confidence interval [CI] = 0.21–0.77). When indeterminate results were excluded concordance remained moderate, $\kappa = 0.558$

Table 1. Comparison of T-SPOT.TB and QuantiFERON results in 117 patients.

Assay	Result	QuantiFERON		
		Positive	Negative	Indeterminate
T-SPOT.TB	Positive	5	1	0
	Negative	6	103	1
	Indeterminate	0	0	0
	Borderline	0	1	0

(95% CI=0.270–0.847). When those with previous tuberculosis were excluded, concordance was lower, $\kappa=0.19$ (95% CI=0.18–0.56).

Patients with positive IGRAs

Of all, 12 (10.3%) patients had positive IGRAs; 11 (9.4%) patients had a positive QFG result and 6 (5.1%) patients had a positive T-SPOT.TB. In five (4.3%) both IGRAs were positive (Table 1). Their median CD4 count was 550 (range=190–920) cells/ μL ; 11 patients were receiving ART. Four patients had previously treated culture-proven tuberculosis, making interpretation of the positive IGRA results uncertain. Of these four patients, three had a positive T-SPOT.TB and four had a positive QFG. Of the 12 patients, four (33%) were from the UK, four (33%) from Central and sub-Saharan Africa, two (17%) from West Africa and two (17%) from South/Central America. Of these 12 patients, two opted to have chemoprophylaxis and the others were closely monitored. None of the patients have gone on to develop active tuberculosis after 20 months of follow-up.

Patients with discordant results

Seven (6%) patients had discordant IGRA results (one positive T.SPOT TB result, six positive QFG results). All seven were receiving ART with a median (range) CD4 count of 575 (190–910) cells/ μL ; one had previously been treated for culture-proven tuberculosis.

Patients with CD4 <200 cells/ μL

Four patients (all receiving ART) had CD4 counts <200 cells/ μL ; all had a negative TST. One patient had a positive QFG result. All had previous BCG vaccination and none had previously been treated for culture-proven tuberculosis.

Indeterminate and borderline results

One patient had an indeterminate QFG result and one had a borderline T-SPOT-TB result: there were no

borderline QFG results (Table 1). Both patients had received ART for more than two years, had CD4 counts >200 cells/ μL , and neither had received BCG vaccination nor were previously treated for tuberculosis.

Previous tuberculosis

Among the 14 patients previously treated for culture-proven active tuberculosis, 10 (71%) had received BCG; four (29%) had a positive IGRA (in three both IGRAs were positive, in one QFG was positive). The median time between treatment of tuberculosis and IGRA testing was 9.5 years (range 3–13) and did not differ between those who had a positive IGRA and those who did not. All 14 individuals previously treated for tuberculosis had undetectable HIV viral loads; 13 had received ART for more than two years, one for 18 months. Median CD4 counts in those with a positive IGRA and in those without were 895 and 670 cells/ μL , respectively. Two of the four patients with a positive IGRA result had abnormal chest radiographs (see below). If those patients with a positive IGRA and a past history of culture-confirmed tuberculosis are excluded, then seven individuals (6%) had positive QFG result and three (2.6%) had a positive T.SPOT-TB.

Chest radiographs

Of 116 asymptomatic patients, the chest radiograph was normal in 101, showed signs of previous 'inactive' tuberculosis in eight (all had previously been treated for culture-proven tuberculosis) and evidence of other non-infectious pathology including chronic obstructive pulmonary disease and dilated cardiomyopathy in seven. One patient from a country with a high-prevalence of tuberculosis, recently arrived in the UK, and not in receipt of ART, was symptomatic. Both QFG and T-SPOT.TB were positive, the chest radiograph showed hilar and mediastinal lymphadenopathy. A whole body positron emission tomography-computed tomography scan showed multiple ^{18}F fluorodeoxyglucose-avid intra- and extra-thoracic lymphadenopathy. Histology of a lymph node biopsy showed HIV reactive changes: mycobacterial staining and culture were negative. With institution of ART the patient's symptoms and chest radiographic abnormalities resolved. The patient declined chemoprophylaxis.

Correlation between CD4 count and concordance between IGRA results

Examining data from recent studies^{9–12} and the present study showed there was evidence of a correlation between median CD4 count and concordance between IGRA results; $r=0.92$, $p=0.02$.

Discussion

The major findings of this study were: first, there was a low prevalence of positive IGRA results among this ethnically diverse population attending an inner-London HIV clinic who had potential nosocomial exposure to a smear-positive patient with tuberculosis. Twelve patients (10.3%) had a positive IGRA result; QFG being positive in 9.4% and T-SPOT.TB positive in 5.1%; both IGRAs were positive in 4.3%; second, concordance between the two IGRAs was only moderate; third, indeterminate results were uncommon; and fourth, IGRAs were positive in only four of 14 (29%) with previously treated culture-proven tuberculosis.

Most data about IGRA use among HIV-infected patients come from studies done in high TB prevalence regions, where patients are also likely to have more advanced HIV disease. This poses difficulties in generalising these data to patients in the UK, where TB prevalence is lower and HIV care infrastructure is better developed. This study was conducted within a cosmopolitan and ethnically diverse population: almost 90% patients were receiving ART, the majority had undetectable HIV loads and the median CD4 count was 550 cells/ μ L; a situation which reflects treatment outcomes among HIV-infected patients without TB, under the care of HIV specialists throughout the UK.¹⁴

Few studies have compared 'head to head' IGRA testing for detection of LTBI in HIV-infected patients in high-income countries.⁹⁻¹² In none of these studies is there a gold standard for diagnosis of LTBI. Poor, $\kappa=0.06$,⁹ $\kappa=0.19$,¹¹ fair, $\kappa=0.351$,¹⁰ and good, $\kappa=0.635$ ¹² concordance between QFG and T-SPOT.TB have been described in studies done in high-income settings. In the present study, concordance was moderate, and analysis of data from these studies shows concordant IGRA results are more likely with higher CD4 counts. It has previously been suggested that QFG is less sensitive than T-SPOT.TB⁵ for detection of LTBI, however, we demonstrated that more subjects were QFG positive than T-SPOT.TB positive when both tests were done in the same population. This may indicate that QFG is superior to T-SPOT.TB for diagnosis of LTBI, or it might be that the QFG is generating more false positive results, which is potentially a significant issue in low-prevalence populations such as white UK-born HIV-infected persons who are in receipt of ART and who have good CD4 counts.

Surprisingly, in the present study, only one-third of individuals who had a past history of culture-proven tuberculosis had a positive IGRA result. An explanation for this observation is not immediately apparent as the interval between TB and IGRA testing, and individuals' CD4 counts were not different among those with and those without a positive IGRA result, but it may be a reflection of the flaws of using blood to detect

distant memory cell populations. Similar findings have been reported in a study from Barcelona where seven of 42 HIV-infected patients being evaluated for LTBI using both IGRA assays had a past history of TB. Only three of the seven had a positive IGRA, and there were no differences among those with and without a positive IGRA result in interval between TB and IGRA testing, nor in CD4 count.¹²

One of the limitations of IGRA testing among HIV-infected patients is the rate of indeterminate results. Previous reports from the UK describe indeterminate T-SPOT.TB results in 2-7.4%¹⁵⁻¹⁷ of HIV-infected patients; indeterminate results were no more likely among those with CD4 counts <200 cells/ μ L.^{16,17} There are no UK data for QFG, but pooled analysis of studies from high-income countries (that did not include UK) found that indeterminate results with QFG were more likely when patients had CD4 counts <200 cells/ μ L, however, individual studies gave inconsistent results.⁷ Recently, a study from Alicante also found that indeterminate QFG results were more likely when patients had CD4 counts <200 cells/ μ L, no such relationship was observed for T-SPOT.TB.¹⁰ We have shown a very low rate of indeterminate IGRA results (<1% for QFG and 0% for T-SPOT.TB), most likely due to very few patients having low CD4 counts.

Limitations of our study include the small study population, the low rate of IGRA positivity, which precludes an analysis to identify predictors of both positive tests and discordant results, the short duration of follow-up (less than two years), and the fact that TST was not done in every participant, which limits comparison of our findings with those from other studies. Studies of HIV-infected persons with larger numbers and with longer follow-up (to five years or longer), in both higher TB prevalence settings and within Europe and the United States, for example that proposed by the Tuberculosis Epidemiologic Studies Consortium,¹⁸ may better help in identification of factors predictive of a positive IGRA, and will also help address the issue of the relative usefulness of the TST and IGRA in predicting the risk of developing active TB among HIV-infected persons.

In the absence of a gold standard we have adopted a pragmatic approach to detect LTBI among our current, ethnically diverse, HIV-infected clinic population which is based on guidelines developed by NICE⁶ and by BHIVA.¹⁹ We proactively screen for LTBI using QFG, among the following risk-groups: those presenting with newly diagnosed HIV infection who are from areas with a high- or medium-prevalence of tuberculosis: those presenting with low CD4 counts <200 cells/ μ L, irrespective of their country of origin: those with known HIV infection, who are from a high- or medium-prevalence region and who have not received

ART for two years or more. In those with a CD4 count <200 cells/ μ L, we also perform a TST.^{6,19}

This study provides evidence of a low rate of IGRA positivity and a low-prevalence of LTBI among patients in an ethnically diverse HIV-infected population attending an inner-London outpatient treatment centre, moderate concordance between QFG and T.SPOT.TB in this patient group, and very low rates of indeterminate IGRA results. It also contributes to the evidence base for use of IGRA tests for detection of LTBI in HIV-infected patients in the UK.

Conflict of interest

The authors declare no conflict of interest.

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References

- Chee CB-E, Sester M, Zhang W, et al. Diagnosis and treatment of latent infection with *Mycobacterium tuberculosis*. *Respirology* 2013; 18: 206–216.
- Corbett EL, Watt CJ, Walker N, et al. The growing burden of tuberculosis: global trends and interaction with the HIV epidemic. *Arch Intern Med* 2003; 163: 1009–1021.
- Horsburgh CR. Priorities for the treatment of latent tuberculosis infection in the United States. *N Engl J Med* 2004; 350: 2060–2067.
- Redelman-Sidi G and Sepkowitz KA. Interferon gamma release assays in the diagnosis of latent tuberculosis infection among immunocompromised adults. *Am J Respir Crit Care Med*. Epub ahead of print 21 December 2012.
- CDC. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States, 2010. *MMWR Morbid Mortal Wkly Rep* 2010; 59(RR-5): 1–25.
- National Institute for Health and Clinical Excellence. *Clinical diagnosis and management of tuberculosis, and measures for its prevention and control*. CG117. London: National Institute for Health and Clinical Excellence, 2011. <http://publications.nice.org.uk/tuberculosis-cg117> (accessed 23 February 2013).
- Cattamanchi A, Smith R, Steingart KR, et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. *J Acquir Immune Defic Syndr* 2011; 56: 230–238.
- Sester M, Sotgiu G, Lange C, et al. Interferon-gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2011; 37: 100–111.
- Talati NJ, Seybold U, Humphrey B, et al. Poor concordance between interferon-gamma release assays and tuberculin skin tests in diagnosis of latent tuberculosis infection among HIV-infected individuals. *BMC Infect Dis* 2009; 9: 15.
- Ramos JM, Robledano C, Masiá M, et al. Contribution of interferon gamma release assays testing to the diagnosis of latent tuberculosis infection in HIV-infected patients: a comparison of QuantiFERON-TB Gold In Tube, T-SPOT.TB and tuberculin skin test. *BMC Infect Dis* 2012; 12: 169.
- Richeldi L, Losi M, D'Amico R, et al. Performance of tests for latent tuberculosis in different groups of immunocompromised patients. *Chest* 2009; 136: 198–204.
- Rivas I, Latore I, Sanvisens A, et al. Prospective evaluation of latent tuberculosis with interferon-gamma release assays in drug and alcohol abusers. *Epidemiol Infect* 2009; 137: 1342–1347.
- Landis JR and Koch GC. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159–174.
- Harte D, Dosekun O, Sethi G, et al. Immunosuppression among HIV-1 positive patients attending for care: experience from 2 large HIV centres in the UK. *HIV Med* 2010; 11: 114–120.
- Clark SA, Martin SL, Pozniak A, et al. Tuberculosis antigen-specific immune responses can be detected using enzyme-linked immunospot technology in human immunodeficiency virus (HIV)-1 patients with advanced disease. *Clin Exp Immunol* 2007; 150: 238–244.
- Dheda K, Lalvani A, Miller RF, et al. Performance of a T-cell-based diagnostic test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count. *AIDS* 2005; 19: 2038–2041.
- Kall MM, Coyne KM, Garrett NJ, et al. Latent and sub-clinical tuberculosis in HIV infected patients: a cross-sectional study. *BMC Infect Dis* 2012; 12: 107.
- Garrett D, Katz D and Chideya S. Prospective comparison of the tuberculin skin test and interferon-gamma release assays in diagnosing infection with *Mycobacterium tuberculosis* and in predicting progression to tuberculosis. <http://www.clinicaltrials.gov/ct2/results?term=NCT01622140> (accessed 23 February 2013).
- Pozniak AL, Coyne KM, Miller RF, et al. BHIVA Guidelines Subcommittee. British HIV Association guidelines for the treatment of TB/HIV coinfection 2011. *HIV Med* 2011; 12: 517–524.