Comparison of an Interferon-γ Assay with Tuberculin Skin Testing for Detection of Tuberculosis (TB) Infection in Patients with Rheumatoid Arthritis in a TB-Endemic Population

DARIO PONCE de LEON, EDUARDO ACEVEDO-VASQUEZ, SERGIO ALVIZURI, CESAR GUTIERREZ, MARIANO CUCHO, JOSE ALFARO, RISTO PERICH, ALFREDO SANCHEZ-TORRES, CESAR PASTOR, CESAR SANCHEZ-SCHWARTZ, MARIELA MEDINA, ROCIO GAMBOA, and MANUEL UGARTE

ABSTRACT. ***Objective.*** Tuberculosis (TB) in patients with rheumatoid arthritis (RA) undergoing treatment with anti-tumor necrosis factor (TNF) agents is commonly the result of reactivation of latent TB infection (LTBI); detection and treatment of LTBI is essential before treatment with anti-TNF agents. We reported previously that the tuberculin skin test (TST) is inaccurate for diagnosis of LTBI in patients with RA. Here, we compare the prevalence of LTBI in RA patients and matched controls according to positive TST and QuantiFeron-TB Gold® In-Tube version (QFT) results and determine their agreement.

Methods. A cross-sectional study of 101 RA patients and 93 controls was conducted in Lima, Perú, where the prevalence of LTBI in the general population has been estimated to be 68%. Blood was drawn for QFT assay followed by TST using 2-TU of RT 23 purified protein derivative. TST was deemed positive at ≥ 5 mm for RA patients and ≥ 10 mm for controls.

Results. There were no significant differences between RA patients and controls for age, sex, bacillus Calmette-Guérin vaccination, or history of or contact with TB. 88% of patients had active RA disease and 2 (1.9%) patients had indeterminate QFT results. The number of subjects testing positive with the QuantiFeron assay was comparable between patients and controls (44.6% vs 59.1%, respectively), whereas the TST detected significantly less LTBI among RA patients (26.7%) than controls (65.6%). Thus, the rate of LTBI in RA patients represented 75% and 41% of the rate in their controls using QFT or TST, respectively ($p = 0.008$). Poor agreement between TST and QFT was seen in RA patients, but in controls, good agreement was observed between these tests.

Conclusion. In a TB-endemic population, the QuantiFeron-TB Gold In-Tube assay seemed to be a more accurate test for detection of LTBI in RA patients compared with the TST, and may potentially improve the targeting of prophylactic therapy before treatment with anti-TNF agents. (First Release April 1 2008; J Rheumatol 2008;35:776–81)

Key Indexing Terms:
TUBERCULOSIS RHEUMATOID ARTHRITIS LATENT TUBERCULOSIS INFECTION
TUBERCULIN SKIN TEST INTERFERON-γ RELEASE ASSAY

Tuberculosis (TB) is a serious infectious complication in patients with rheumatoid arthritis (RA), especially in those undergoing treatment with anti-tumor necrosis factor-α (TNF-α) agents. Frequently, it is the result of reactivation of latent tuberculosis infection (LTBI)[1], thus detection and treatment of LTBI through prophylactic therapy is essential before prescribing anti-TNF agents[2-4]. However, diagnosis of LTBI traditionally relies on the tuberculin skin test

From the Academic Department of Rheumatology, Red Asistencial Almenara, Hospital Nacional Guillermo Almenara Irigoyen, Lima, Peru.

D. Ponce de Leon, MD, Staff Internist, Department of Internal Medicine, Hospital Nacional Guillermo Almenara; E. Acevedo-Vasquez, MD, PhD, Chief, Department of Rheumatology, Staff Rheumatologist, Hospital Nacional Guillermo Almenara, Professor, School of Medicine, Universidad Nacional Mayor de San Marcos, Lima; S. Alvizuri, MD, Department of Clinical Immunology, Hospital Nacional Guillermo Almenara; C. Gutierrez, MD, Department of Epidemiology, Professor, School of Medicine, Universidad Nacional Mayor de San Marcos; M. Cucho, MD, Staff Rheumatologist; J. Alfaro, MD, Staff Rheumatologist; R. Perich, MD, Staff Rheumatologist; A. Sanchez-Torres, MD, Staff Rheumatologist; C. Pastor, MD, Staff Rheumatologist, Department of Rheumatology, Hospital Nacional Guillermo Almenara, Professor, School of Medicine, Universidad Nacional Mayor de San Marcos; C. Sanchez-Schwartz, MD, Staff Rheumatologist, Department of Rheumatology, Hospital Nacional Guillermo Almenara, Professor of Rheumatology, School of Medicine, Universidad Nacional Mayor de San Marcos; M. Medina, MD, Rheumatology Fellow; R. Gamboa, MD, Rheumatology Fellow; M. Ugarte, MD, Rheumatology Fellow, Department of Rheumatology, Hospital Nacional Guillermo Almenara Irigoyen.

Address reprint requests to Prof. E. Acevedo-Vasquez, Academic Department of Rheumatology, Red Asistencial Almenara, Hospital Nacional Guillermo Almenara Irigoyen, Avenida Grau 800, La Victoria, Lima 13, Peru. E-mail: edacvas@terra.com.pe

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(TST), and we have reported that only 30% of patients with RA had positive TST compared with 70% in immunocompetent controls[5]. This poor sensitivity can lead to false-negative results, with a subsequent risk of TB reactivation following anti-TNF-α therapy. There is a need for a more accurate test for *M. tuberculosis* infection in patients with RA.

Advances in mycobacterial genomics have led to the development of 2 new blood interferon-γ release assays (IGRA) in response to 2 unique antigens, ESAT-6 and CFP-10, that are highly specific for *M. tuberculosis*, and which are absent from *M. bovis*, *M. avium*, and most other nontuberculosis mycobacteria[6-9]. One assay, the enzyme-linked immunospot [Elispot (T-SPOT.*TB*®; Oxford Immunotec, Oxford, UK)] enumerates IFN-γ-secreting T cells; the other measures IFN-γ concentration in supernatant by enzyme-linked immunosorbent assay [ELISA (QuantiFeron-TB Gold®; Cellestis, Carnegie, Australia)]. The latest improvement within this technology is the QuantiFeron-TB Gold In-Tube (QFT) test (Cellestis), which incorporates another specific TB antigen (TB 7.7), and in which whole blood is drawn directly into a vacutainer tube precoated with antigens ready for incubation.

The aims of our study were to compare the prevalence of LTBI in RA patients and matched controls as estimated by TST and QFT positivity, and to determine the level of agreement between these tests.

MATERIALS AND METHODS

Patients. Consecutive RA patients and immunocompetent controls were recruited into this cross-sectional study, conducted at the Hospital Guillermo Almenara in Perú, where the incidence of TB in RA patients in this institution was 216/100,000[10] and in the general population has been estimated at 108/100,000[11]. The protocol was approved by the Peruvian Health National Institute and the institutional review board. All patients provided written informed consent before enrollment.

Patients with RA who were not receiving anti-TNF-α agents and control subjects diagnosed with noninflammatory rheumatic diseases such as osteoporosis and osteoarthritis and mechanical pain were included. Patients with diseases associated with nonspecific immunosuppression or patients who had received immunosuppressive treatment were excluded from the control group. Overall exclusion criteria included active TB, known hypersensitivity to purified protein derivative (PPD), hospitalization, and positive serology for HIV. Bacillus Calmette-Guérin (BCG) status was determined by inspection for vaccine scar, and blood was drawn for the QFT assay prior to administration of the TST. The tests were interpreted independently of each other and with no knowledge of exposure information.

Tuberculin skin test. A 2-TU dose of PPD RT 23 (Statens Serum Institut, Copenhagen, Denmark) was administered by a certified technician using the Mantoux method and induration measured after 72 h. TST was deemed positive at ≥ 5 mm for RA patients and ≥ 10 mm for controls[12].

QFT test. QFT testing was performed in the Immunology Laboratory at the Hospital Guillermo Almenara according to the manufacturer's instructions. Briefly, 1 ml of blood was drawn directly into each of 3 evacuated blood collection tubes: one containing heparin alone (as negative control), the second containing the T cell mitogen phytohemagglutinin (PHA; as a positive control), and the third with peptides of ESAT-6, CFP-10 and TB 7.7 (TB antigens) dried on the inside of the tube. After mixing, the tubes were incubated upright for 20 h at 37°C before plasma was harvested and stored frozen at –20°C until further analysis. The concentration of IFN-γ present in each plasma sample was determined using the QFT ELISA. IFN-γ release in the saline control tube (Nil) was subtracted from the TB antigen and PHA-stimulated samples. Samples with ≥ 0.35 IU/ml IFN-γ following stimulation with *M. tuberculosis*-specific antigens were considered positive, while samples < 0.35 IU/ml were considered negative. The QFT test result was considered indeterminate if the concentration of IFN-γ was < 0.35 IU/ml for TB antigens and < 0.5 IU/ml for the positive control.

Statistical analysis. Descriptive statistics include mean ± standard deviations for continuous variables and frequencies and proportions for categorical variables. Chi-square or Fisher's exact test was used to compare proportions between the 2 groups (RA and controls). Two-sided 95% confidence intervals (95% CI) were used to estimate the difference in the proportion of positive results in each group between TST and QFT. Continuous variables were compared by Student's t test. A value of $p < 0.05$ was considered significant.

Concordance between TST and QFT was evaluated using agreement and kappa statistics. The strength of this agreement was examined using Cohen's kappa (κ), with κ value > 0.75 representing excellent agreement beyond chance, 0.40 to 0.75 fair to good agreement, and < 0.40 poor agreement.

RESULTS

Study population. A total of 106 RA patients and 97 controls were recruited. Two (1.9%) RA patients had indeterminate QFT result, 1 (0.9%) had a hypersensitivity reaction to PPD, and 2 (1.9%) did not return for their TST to be examined. In control subjects, all had valid QFT results, 1 (1%) had a hypersensitivity reaction, and 3 (3%) did not return for TST reading. Therefore, 101 RA patients and 93 controls were included in the final analysis. Baseline characteristics are shown in Table 1. BCG vaccinations were present in 80% of RA and control groups; 88% of RA patients had active disease (determined by Disease Activity Score 28) and 91.1% were receiving treatment with prednisone at daily doses < 10 mg.

TST and QFT. The mean TST diameter was 3.73 ± 6.59 mm in RA patients and 11.0 ± 8.19 mm in controls ($p < 0.001$). This was also reflected by a significantly lower proportion of positive TST results in the RA group (27/101, 26.7%) as compared with controls (61/93, 65.6%) ($p < 0.001$). For the QFT test, 45/101 (44.6%) RA patients had a positive result,

Table 1. Demographic and clinical characteristics of the 101 RA and 93 control patients.

Characteristic	RA	Controls	p
Age, mean ± SD yrs	57.6 ± 12.6	56.2 ± 15.2	0.47
Female, %	90.1	84.9	0.27
BCG vaccination, %	80.2	80.6	0.93
TB contact, %	17.8	20.4	0.64
History of TB, %	5.9	5.4	0.86
DAS 28, mean ± SD	5.0 ± 1.3		
Active disease, %	88		
Prednisone, %	91.1		
Methotrexate, %	73.3		

Differences between mean values were analyzed by t test, and differences between proportions by chi-square test. DAS: Disease Activity Score; active disease > 3.2. BCG: bacillus Calmette-Guérin.

a lower rate than that for the controls (55/93, 59.1%) (p = 0.04). Overall, the rate of TST positivity in RA patients was only 41% of that for the controls, significantly lower than for the QFT assay, where the positivity rate for RA patients was 75% of that for the controls (p = 0.008; Figure 1).

The difference between the proportions of positive TST and QFT (diff = TST+% minus QFT+%) was statistically significant in RA patients [diff = –17.9 (95% CI –31.8 to –3.9; p = 0.013)] but was not statistically significant in control patients [diff = –6.5 (95% CI –8.5 to 21.4; p = 0.449)].

In RA patients, 23 of 27 TST-positive (85.2%) and 58 of 74 (78.4%) TST-negative patients were BCG-vaccinated (p = 0.58). Similarly, QFT results were not influenced by previous BCG vaccination or other covariates (Table 2).

Agreement between TST and QFT. Overall agreement between TST and QFT was low in RA patients, with positive or negative concordance in 71/101 (70.3%) patients (κ = 0.37). Agreement between the 2 tests was substantially higher in the controls, where good overall agreement was observed in 82.8% (κ = 0.635; Table 3).

TST+/QFT– results were similar in RA patients and controls [6/27 (22.2%) vs 11/61 (18.1%), respectively; p = 0.646]. However, the TST–/QFT+ result was higher in RA patients compared with that in controls [24/74 (32.4%) vs 5/32 (15.6%); p = 0.075].

Table 2. Covariates associated with QFT response in RA patients.

Characteristic	QFT+, n = 45	QFT–, n = 56	p
Age, mean ± SD yrs	57.07 ± 11.9	58.20 ± 13.3	0.65
Women, n (%)	40 (88.9)	51 (91.1)	0.71
BCG vaccine scan, n (%)	38 (84.4)	43 (76.8)	0.33
DAS 28, mean ± SD	5.10 ± 1.27	4.92 ± 1.35	0.49
Active disease, n (%)	39 (88.6)	49 (87.5)	0.86
Prednisone, n (%)	40 (88.9)	52 (92.9)	0.48
Methotrexate, n (%)	35 (77.8)	39 (69.6)	0.35
Lymphopenia, n (%)	10 (22.7)	15 (27.8)	0.56
BMI, mean ± SD	25.34 ± 4.20	25.41 ± 4.21	0.92
Albumin, mean + SD	4.26 ± 0.40	4.24 ± 0.33	0.87
Mitogen, mean ± SD	16.81 ± 9.78	13.16 ± 10.65	0.11

Differences between mean values were analyzed by t test, and differences between proportions by chi-square test. BMI: body mass index; DAS: Disease Activity Score; active disease > 32, lymphopenia < 1500/mm^3. BCG: bacillus Calmette-Guérin.

DISCUSSION

To our knowledge this is the first study demonstrating the performance of the QFT in-tube version in immunosuppressed patients with RA in a highly TB-endemic area. Our results suggest that the QFT test maintained better performance in patients with RA than the TST.

In our patients at high risk of TB, the number of patients who tested positive was more comparable between RA patients and controls with the IFN-γ assay (59.1% vs 44.6%, respectively), whereas the TST detected significantly less LTBI among patients with RA than controls (65.6% vs 26.7%). These findings suggest that the sensitivity of the QFT for the diagnosis of LTBI could be higher than that of the TST in patients with RA, even in cases of RA immunosuppression.

The TST positivity rate (26.7%) in the RA patients was

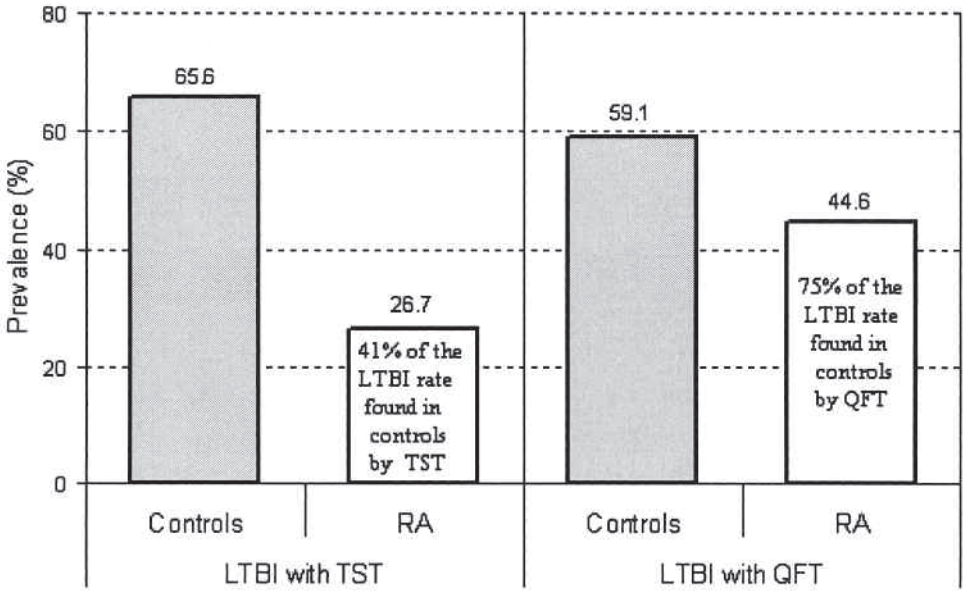

Figure 1. Prevalence of latent tuberculosis infection (LTBI) according to tuberculin skin test (TST) and QuantiFeron-TB Gold In-Tube test (QFT). The number of patients who tested positive was more comparable between RA patients and controls with the QFT (44.6% vs 59%, respectively), whereas the TST detected significantly less LTBI among patients with RA compared to controls (65.6% vs 26.7%).

Table 3. Agreement between TST and QFT assay in RA patients and controls.

Group	QFT+	QFT–	Total	Agreement, %	Kappa
Controls				82.8	0.635
TST+	50	11	61		
TST–	5	27	32		
Total	55	38	93		
RA patients				70.3	0.374
TST+	21	6	27		
TST–	24	50	74		
Total	45	56	101		

comparable to the 30% found previously in RA patients in our institution[5]; and similarly, the rate in control subjects in this study (65.6%) was commensurate with the 68% TST-positive rate among the general population of our country[13]. The TST results from this study confirm our previous findings[5] that the TST is severely compromised in patients with RA.

Significant differences between the rates of positive results with IGRA compared to those of TST have also been reported in other studies with immunosuppressed patients. Chapman, *et al*[14] compared the results of an Elispot assay and TST in 21 HIV-positive Zambian individuals and 54 HIV-negative controls. They found a better approach to control patients in the *in vitro* assay (62%) compared with TST (45%). In another study[15] among a group of 138 immunosuppressed hematology patients with documented exposure to an index case of TB, the rate of positive IGRA assay was more than twice the rate for TST (44.2% vs 17.4%, respectively). Recently, Sellam, *et al*[16] found among 13 RA patients with LTBI that sensitivity was higher with an Elispot-based IGRA than with TST (84.6% vs 61.5%), suggesting the IGRA was significantly less affected by immunosuppression than the TST in patients with RA.

It was reported that the rate of indeterminate test results was associated with severe immunosuppression. Brock, *et al* found a higher proportion of indeterminate QFT test results (24%) due to low PHA response in HIV patients with a CD4 cell count < 100 cells/μl[17]. Similarly, Luetkemeyer, *et al* found that HIV subjects with a CD4 count of < 100 cells/μl had a relative risk of an indeterminate result of 4.2 compared to those with a CD4 count ≥ 100[18]. Ferrara, *et al* also found that indeterminate results were common (21%) among severely immunosuppressed patients using a previous version of the QFT[19].

In contrast to the studies above, we found in immunosuppressed patients with RA that only 2 (1.9%) of 107 had indeterminate results. Similar findings were reported by Takahashi, *et al*[20]: in 14 RA patients only 5% of QFT tests were indeterminate; and by Sellam and colleagues[16], who found that all 68 RA patients tested responded to PHA. These findings combined argue for the selective absence of anergy in IFN-γ assays in patients with RA as compared with patients with other, perhaps more severe immunosuppressive disorders. For antigen-specific IFN-γ responses, it has been observed that there is an increase in production of IFN-γ in RA patients after stimulation with microbial antigens or autoantigens *in vitro*[21]. We previously documented this aspect of RA in a study[22] that examined the concentration of different cytokines in RA patients after stimulation *in vivo* with PPD. We found that IFN-γ was persistently elevated in PPD-anergic patients (5.49 ± 8.9 pg/ml) and responders to PPD (5.04 ± 3.68 pg/ml), concentrations that were higher in both cases than in immunocompetent controls (4.30 ± 3.47 pg/ml).

A high percentage of patients received prednisone at doses of 10 mg/day. However, it is known that patients receiving corticosteroids < 15 mg/day will not suppress cutaneous delayed hypersensitivity, including tuberculin reactivity[23].

Although previous studies showed BCG vaccination interferes with the interpretation of a positive skin test[24,25], previous BCG vaccination had little influence on TST in our study. The effect of BCG on subsequent TST responses is a complicated phenomenon affected by several factors including BCG strain used, dosage, age, and time elapsed since vaccination. We studied adults who received BCG vaccine at birth and not afterward, and it was demonstrated that TST given 15 years or more after vaccination had no influence on TST reactivity[26]. Recent studies from countries with a high burden of TB have also shown that BCG vaccination at birth rarely confounds the interpretation of a TST several years later[27,28].

The QFT assay was unaffected by BCG vaccination status, as shown in our study by similar proportions of BCG-vaccinated RA patients who were QFT-positive (84.4%) and QFT-negative (76.8%). The QFT assay was also unaffected by disease activity and immunosuppressive drugs. This does not preclude false-positive results, and therefore sensitivity and specificity cannot be reliably inferred. The QFT may give more positive results and hence more patients will receive potentially toxic chemoprophylactic therapy. This needs to be considered before advocating its use, especially as it is unknown how many patients with positive QFT progress to active disease.

As with all evaluations of IGRA, our study used a cross-sectional design, which is fundamentally limited by the lack of a "gold standard" for identifying LTBI. The only gold standard for LTBI is the later development of active TB, and this can be ascertained only through longitudinal cohort studies.

We found good agreement between TST and QFT in immunocompetent controls (k = 0.63); however, discordance is expected in immunosuppressed RA patients, that is, TST+/QFT– if the QFT is more specific, and TST–/QFT+ if the QFT is more sensitive. TST–/QFT+ was found in 32.4% of RA patients, twice the 15.6% finding in controls. These

findings suggest there is greater sensitivity of the QFT to detect *M. tuberculosis* infection in immunosuppressed RA patients. Identifying more people who are likely to be infected and providing prophylactic therapy for LTBI before starting anti-TNF treatment should have a positive epidemiological influence in RA patients, who are at high risk of progression to active TB if truly infected.

A limitation of our study was the lack of a gold standard method for diagnosing LTBI. We attempted to compensate for this by evaluating both diagnostic tests in RA patients and matched controls. Our data indicate that QFT was more accurate than the TST in RA patients, but we cannot determine the absolute sensitivity of both tests.

Although it detected significantly more RA patients than the TST, it is likely that the QFT test does fail to identify some RA patients with LTBI. For this reason care should be taken when interpreting a negative QFT result in someone about to undertake anti-TNF therapy. Prophylactic anti-TB therapy before using anti-TNF agents is obviously necessary in the presence of certain clinical and/or radiographic findings of LTBI. However, in the absence of these findings, the QFT test should replace the TST when choosing prophylactic treatment in RA patients in a high-burden context.

One potential limiting factor for QFT, particularly in high-burden, resource-limited countries, is their higher material costs and the need for laboratory infrastructure and trained personnel. However, the higher the putative proportion of false-negative TST results due to immunsuppression, the more cost-effective the use of the QFT, and this might outweigh the higher cost factor.

Our study showed that the QuantiFeron-TB Gold In-Tube test was more accurate than the tuberculin skin test in diagnosing latent TB infection among a high-risk population of patients with RA, appropriately enabling the identification of more candidates for LTBI prophylaxis. Traditionally, a positive TST has been used to diagnose and define LTBI. However, with the advent of the QFT assay, this conventional definition of LTBI will need to be reconsidered.

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