Screening for Latent Tuberculosis Infection in Patients with Chronic Inflammatory Arthritis: Discrepancies Between Tuberculin Skin Test and Interferon-γ Release Assay Results

Félicie Costantino, Marcelo de Carvalho Bittencourt, Anne-Christine Rat, Damien Loeuille, Hervé Dintinger, Marie C. Béné, Gilbert Faure, and Isabelle Chary-Valckenaere

ABSTRACT. ***Objective.*** Screening for latent tuberculosis infection (LTBI) is mandatory before initiating biologics in patients with chronic inflammatory arthritis (CIA). However, few studies have evaluated the discrepancies between the results of tuberculin skin test (TST) and interferon-γ release assays (IGRA) in these patients. The purpose of our study was to investigate factors associated with TST and IGRA results in a large cohort of patients with CIA before the introduction of biologics.

Methods. A total of 563 consecutive patients with CIA (293 rheumatoid arthritis, 270 spondyloarthritis) and eligible for biologics were prospectively enrolled. Demographic, clinical, and biological data were recorded. Risk factors for LTBI were assessed. All patients underwent a TST, a chest radiograph, and an IGRA test (T-SPOT.TB).

Results. Agreement between the 2 tests was low ($\kappa = 0.16$). The bacillus Calmette-Guerin (BCG) status was significantly associated with discordance between the 2 tests ($p = 0.004$). The TST positivity rate was 34.8%. Factors associated with a negative TST were female sex ($p = 0.02$) and immunosuppressive treatment ($p = 0.003$). The only LTBI risk factor associated with TST positivity was an abnormal chest radiograph ($p = 0.02$). T-SPOT.TB was positive in 21.7% of patients and indeterminate in 15.6%. Previous active TB and chest radiograph abnormalities were associated with IGRA positivity ($p = 0.008$ and $p = 3.9 \times 10^{-5}$, respectively). The BCG vaccination was associated with negative IGRA ($p = 3 \times 10^{-4}$). Indeterminate IGRA results were associated with age, C-reactive protein, and immunosuppressive treatment ($p = 0.005$, 0.007, and 0.004, respectively).

Conclusion. Our data support the combined use of T-SPOT.TB and TST in patients with CIA before biologics introduction. However, despite these good diagnostic values, indeterminate results may complicate the use of IGRA. (First Release Oct 1 2013; J Rheumatol 2013;40:1986–93; doi:10.3899/jrheum.130303)

Key Indexing Terms:
TUBERCULOSIS BIOLOGICAL AGENTS INTERFERON-γ RELEASE TESTS
ANKYLOSING SPONDYLITIS RHEUMATOID ARTHRITIS

Tumor necrosis factor (TNF) antagonists are very effective treatments of many immune-mediated inflammatory diseases including rheumatoid arthritis (RA) and spondyloarthritis (SpA). However, anti-TNF therapy is associated with an increased risk of tuberculosis (TB)[1] most often because of reactivation of a latent infection[2]. Therefore, screening for latent TB infection (LTBI) has become mandatory before the initiation of TNF antagonists. National recommendations for LTBI screening based on medical history, clinical examination, tuberculin skin test (TST), and chest radiograph[3,4,5,6,7] have demonstrated their effectiveness to reduce TB incidence[8]. However, despite recommendations, the incidence of TB in patients treated with anti-TNF therapy still remains higher than in the general population[9,10].

Use of the TST has been questioned. Indeed, TST has well-known limitations: poor specificity owing to cross-reactivity with environmental mycobacteria or bacillus Calmette-Guerin (BCG)[11], poor sensitivity in

From the Rheumatology Department and the Immunology Department, CHU Nancy, Nancy; Université de Lorraine, Lorraine, France.

F. Costantino, MD, Rheumatology Department, CHU Nancy; M. de Carvalho Bittencourt, MD, PhD, Immunology Department, CHU Nancy, and EA RHEM4369, Université de Lorraine; A.C. Rat, MD, PhD, Rheumatology Department, and INSERM, CIC-EC CIE6, Epidémiologie et Evaluation Cliniques, CHU Nancy; D. Loeuille, MD, PhD, Rheumatology Department, CHU Nancy, and UMR 7365 (IMoPA) CNRS, Université de Lorraine; H. Dintinger, MD, Rheumatology Department, CHU Nancy; M.C. Béné, MD, PhD, Immunology Department, CHU Nancy and EA RHEM4369, Université de Lorraine; G.C. Faure, MD, PhD, Immunology Department, CHU Nancy; EA RHEM4369, Université de Lorraine; I. Chary-Valckenaere, MD, PhD, Rheumatology Department, CHU Nancy, and UMR 7365 (IMoPA) CNRS, Université de Lorraine.

Address correspondence to Dr. F. Costantino, Institut Cochin Bâtiment Hardy A, 1e étage, 27 rue du Faubourg Saint Jacques, 75014 Paris, France. E-mail: felicie.costantino@inserm.fr

Accepted for publication July 25, 2013.

immunocompromised patients[12,13], and poor reproducibility due to operator-related variability in the administration and reading of the test[11,14]. These drawbacks could lead to unnecessary preventive treatment of LTBI[15] or, on the contrary, to the development of active TB[10].

Thus, new tools were developed allowing improvement of LTBI diagnosis. The genome sequencing of *Mycobacterium tuberculosis* has enabled the identification of several genes that are absent in most environmental mycobacteria and BCG[16]. Two commercial tests evaluating interferon-γ (IFN-γ) production by T cells in the presence of these specific antigens have been developed: T-SPOT.TB (Oxford Immunotec) and QuantiFERON-TB Gold (QFT-G) In-Tube test (Cellestis Ltd.). In the general population, IFN-γ release assays (IGRA) seem to be more powerful than TST for diagnosing active TB or LTBI[17]. Consequently, some national guidelines have recommended their use in LTBI screening before anti-TNF therapy, in addition to or in replacement of TST[2,18,19,20]. Nevertheless, little is known about the underlying influence of disease activity and associated therapies on the test results.

The objective of our cross-sectional study was to compare TST and IGRA results in screening for LTBI in a large population of patients with chronic inflammatory arthritis requiring biologic treatment and to investigate predictive factors of results of these 2 tests, with special attention for indeterminate IGRA results.

MATERIALS AND METHODS

Patients. Between 2005 and 2009, consecutive patients with RA or SpA requiring TNF antagonists (first-line therapy or switch) in the Rheumatology Department of Nancy University Hospital (France) were enrolled in the study. All patients underwent clinical examination. The diagnosis of RA or SpA was based on standard criteria[21,22,23]. Disease activity was assessed with the 28-joint Disease Activity Score (DAS28) for RA and the Bath Ankylosing Spondylitis Activity Index for SpA[24,25].

Three treatment regimens were distinguished: conventional disease-modifying antirheumatic drugs (DMARD, including methotrexate, leflunomide, and others), corticosteroids, and nonsteroidal antiinflammatory drugs. No patient was treated by biologics at the time of the screening. Previous biologics were recorded but all were discontinued at least 1 month before inclusion. Moreover, patients who had already received an antituberculous chemoprophylaxis were excluded from the study.

Conventional LTBI screening. All patients underwent LTBI screening according to French national recommendations[3] (including a detailed history of TB exposure and BCG vaccination), TST, and a chest radiograph. As recommended by the American College of Rheumatology, LTBI screening was also performed in patients previously treated by biological agents[26]. On radiographic examination, features considered as suggestive of previous TB infection were pulmonary nodules, upper lobe bronchiectasis, apical pleural thickening, interstitial granulomatous calcifications, cavitations, and lymph node or pericardial calcifications[27].

The following characteristics were considered as conventional risk factors (CRF) for LTBI reactivation: history of active TB treated before 1970 or not treated for at least 6 months including 2 months with a combination of rifampicine and pyrazinamide, close contact with a patient with active TB, and chest radiograph suggestive of previous TB infection.

The TST was performed with 5 tuberculin units corresponding to 0.1 ml of purified protein derivative (Tubertest, Sanofi Pasteur MSD, SNC) according to the Mantoux method. Tuberculin was injected intradermally in the forearm, and 72 h later the diameter of skin induration was recorded. An induration diameter of 5 mm or more was considered a positive test.

IFN-γ release assay. T-SPOT.TB assays were performed according to the manufacturer's instructions[28]. To avoid any potential boosting effect of TST on IGRA results, all T-SPOT.TB assays were performed before initiating TST.

Assays were considered indeterminate if the negative control (cell suspension in medium alone) spot count yielded more than 10 spots (referred to hereafter as a high nil control) or if the positive control (cell suspension stimulated with phytohemagglutinin) spot count yielded fewer than 20 spots (low positive control). For determinate tests, T-SPOT.TB assays were interpreted according to the manufacturer's recommendations by subtracting the spot count of the negative control from the highest spot count between panels A (TB-specific antigen ESAT-6) and B (TB-specific antigen CFP-10). A test was considered positive if this difference was equal to, or higher than, 6 spots; otherwise, the test was considered negative.

Statistical analyses. Associations between studied variables and the TST or T-SPOT.TB were assessed as follows: in univariate analysis, Student's t or Wilcoxon tests were used for continuous variables and chi-square or Fisher's exact tests for categorical variables. Results are presented with OR and their 95% CI.

Multivariate logistic regression models entered candidate variables (p value < 0.1 in univariate analysis). Variables retained in the final multivariate model were selected by a backward procedure.

Concordances between TST and T-SPOT.TB were analyzed by Cohen κ coefficient.

The level of type I error used to determine statistical significance was 5%.

Statistical analyses were performed using SAS for Windows, version 9.1. (SAS Institute Inc.) and R for Windows, version 14.2.

RESULTS

Patient characteristics. There were 563 patients included in the study: 293 (52.0%) with RA (82.6% rheumatoid factor-positive, 85.7% anticitrullinated protein antibodies-positive), and 270 (48.0%) with SpA (72.3% HLA-B27+). Patient characteristics are shown in Table 1. As expected in patients requiring biologics, the level of disease activity was high. The rate of BCG vaccination was higher among patients with SpA than in patients with RA, probably because they were younger and benefited from the establishment of mandatory vaccination for all school children.

LTBI screening. Results of the LTBI screening are given in Table 1. Overall, 64 patients (11.4%) had a diagnosis of LTBI based on questioning or chest radiograph, 196 (34.8%) based on TST results, and 122 (21.7%) based on T-SPOT.TB results. Among the 94 patients previously treated with biologics, none had previously received antituberculous chemoprophylaxis. The number of patients requiring anti-TB drugs before biologics introduction was different depending on the LTBI screening method, as summarized in Figure 1. With screening method 1, taking into account CRF and TST, 229 patients (40.7%) had to be treated. Considering TST results only if T-SPOT.TB was indeterminate in screening method 2, 167 patients (29.7%) required treatment compared to 246 (43.7%) if TST results were also considered in case of negative T-SPOT.TB (screening method 3).

Table 1. Patients' characteristics and LTBI screening results. Data are number (%) for categorical variables and median (interquartile range) for continuous variables.

Characteristics	All Patients, n = 563	RA, n = 293	SpA, n = 270
Women	321 (57.0)	224 (76.4)	97 (35.9)
Age, yrs	51.0 (39.0–59.0)	56.0 (48.0–64.0)	42.0 (33.2–52.0)
Disease duration, yrs	8.0 (3.0–16.0)	8.0 (4.0–15.0)	8.0 (3.0–16.0)
Disease activity (DAS28/BASDAI)	NA	5.0 (4.2–5.9)	5.7 (4.4–6.9)
CRP (mg/dl)	10.4 (4.0–24.7)	12.9 (4.9–25.5)	8.8 (3.3–23.0)
Immunosuppressive treatment			
Previous biologics	94 (16.7)	71 (24.2)	23 (8.5)
DMARD	277 (49.2)	210 (71.7)	67 (24.8)
Corticosteroids	254 (45.1)	212 (72.3)	42 (15.6)
Dosage (mg/day)	10.0 (7.5–15.0)	10.0 (7.5–15.0)	10.0 (7.5–15.0)
NSAID	255 (45.4)	87 (29.7)	168 (62.2)
BCG vaccination	439 (78.0)	201 (68.6)	238 (88.1)
CRF of LTBI	64 (11.4)	38 (13.0)	26 (9.6)
History of active TB	13 (2.3)	7 (2.4)	6 (2.2)
History of TB contact	41 (7.3)	23 (7.8)	18 (6.7)
Abnormal chest radiograph	26 (4.6)	20 (6.8)	6 (2.2)
Birth in endemic zone of TB	52 (9.2)	27 (9.2)	25 (9.3)
Tuberculin skin test			
Not read	49 (8.7)	29 (9.9)	20 (7.4)
< 5 mm	318 (56.5)	180 (61.4)	138 (51.1)
≥ 5 mm	196 (34.8)	84 (28.7)	112 (41.5)
T-SPOT.TB test			
Indeterminate	88 (15.6)	44 (15.0)	44 (16.3)
Negative	353 (62.7)	185 (63.1)	168 (62.2)
Positive	122 (21.7)	64 (21.9)	58 (21.5)

RA: rheumatoid arthritis; SpA: spondyloarthritis; DAS28: 28-joint Disease Activity Score; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; DMARD: disease-modifying antirheumatic drug; NSAID: nonsteroidal antiinflammatory drug; BCG: bacillus Calmette-Guerin; TB: tuberculosis; LTBI: latent TB infection; NA: not applicable; CRF: conventional risk factors.

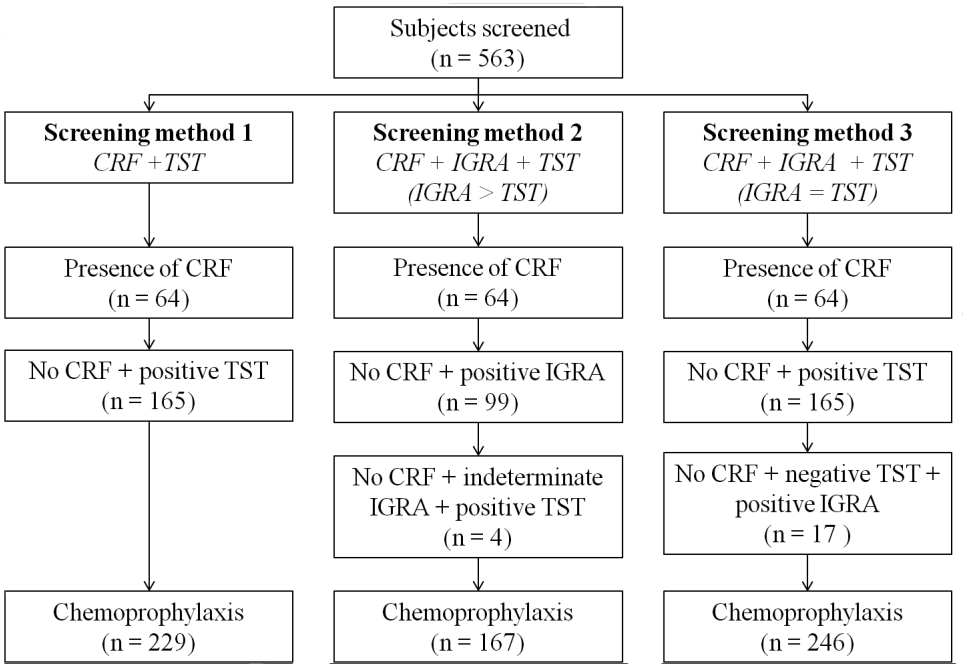

Figure 1. Latent tuberculosis infection screening flow chart. CRF: conventional risk factors; TST: tuberculin skin test; IGRA: interferon-γ release assays.

In our cohort, chemoprophylaxis was initiated in the presence of at least 1 CRF of LTBI or T-SPOT.TB positivity or TST positivity (TST was arbitrarily considered positive if the result was missing). This represented 319 patients (56.7%). No case of active TB was reported during the 5 years of the study.

Comparisons between TST and IGRA results. Combined TST and T-SPOT.TB results are summarized in Table 2. There is a significant difference for positive/negative comparisons (p = 0.0004). This significance is essentially due to the high number of TST-positive patients with negative T-SPOT.TB, likely because of BCG vaccination. Indeed, agreement between the TST and IGRA results measured by the κ coefficient was low (0.16, 95% CI 0.07–0.25), yet higher in patients without BCG vaccination than in vaccinated patients (0.22 compared to 0.15). Indeed, BCG-vaccinated patients had a positive TST more frequently than did unvaccinated patients (37% vs 27%), but conversely, less often had a positive IGRA (18% vs 34%).

Table 2. Combined TST and T-SPOT.TB results. Data are number (%).

		TST			
		Positive	Negative	Missing Results	Total
T-SPOT. TB test	Positive	59 (10.5)	51 (9.1)	12 (2.1)	122 (21.7)
	Negative	114 (20.2)	220 (39.1)	19 (3.4)	353 (62.7)
	Indeterminate	23 (4.1)	47 (8.3)	18 (3.2)	88 (15.6)
Total		196 (34.8)	318 (56.5)	49 (8.7)	563 (100)

TST: tuberculin skin test.

Factors associated with TST/IGRA results. The factors associated with TST or T-SPOT.TB results in multivariate analysis are detailed in Table 3. TST and T-SPOT.TB positivity were both associated with CRF, i.e., history of active TB, history of TB contact, or abnormal chest radiograph. In univariate analysis, if we considered independently each CRF, TST results were associated only with chest radiograph abnormalities, whereas T-SPOT.TB results were associated with chest radiograph and history of active TB.

Importantly, immunosuppressive treatment (previous biologics, current use of conventional DMARD, or corticosteroids) had a negative influence on TST results but no influence on IGRA. Negative influence of corticosteroids on TST results was independent of dosage (11.47 mg and 12.0 mg a day in TST– and TST+, respectively; p = 0.63). Of note, BCG vaccination indeed was highly associated with a significantly low OR (0.39) to negative T-SPOT.TB, confirming the data presented above. Female sex was also associated with TST negativity.

A group of 94 patients previously treated by biologics were included in our study. The performances of TST and T-SPOT.TB were similar in this group compared to

Table 3. Factors associated with positive TST or T-SPOT.TB. Data are number (%) for categorical variables and median (interquartile range) for continuous variables.

	Positive TST, n = 196				Positive T-SPOT.TB, n = 122			
	N	Bivariate p value	OR* (95% CI)	Multivariate p value**	N	Bivariate p value	OR* (95% CI)	Multivariate p value**
Female sex	92	0.002	0.57 (0.4–0.82)	0.02	68	0.82		
Age		0.11				0.03	1.02 (1–1.03)	
Diagnosis (RA vs SpA)	112/84	0.002	0.57 (0.40–0.83)		58/64	0.99		
Disease duration		0.12				0.51		
DAS28		0.06				0.18		
BASDAI		0.89				0.34		
CRP		0.72				0.88		
Immunosuppressive drugs	118	0.0003	0.5 (0.34–0.73)	0.003	79	0.17		
Previous biologics	22	0.03	0.57 (0.33–0.96)		17	0.35		
DMARD	82	0.006	0.6 (0.42–0.86)		62	0.85		
Corticosteroids	72	0.003	0.57 (0.4–0.83)		53	0.64		
NSAID	94	0.60			54	0.76		
CRF	31	0.02	1.95 (1.13–3.36)	0.007	23	0.0008	2.7 (1.49–4.89)	0.006
History of active TB	6	0.55			7	0.008	5.31 (1.53–18.47)	
History of TB contact	20	0.08			12	0.18		
Abnormal radiograph	14	0.02	2.64 (1.12–6.22)		13	3.9×10^{-5}	5.89 (2.29–15.15)	
BCG vaccination	162	0.11			80	5.3×10^{-5}	0.39 (0.24–0.62)	0.0003
Birth in TB-endemic area	19	0.83			13	0.67		

* OR of the bivariate analysis for the variable entered in the multivariate model (p < 0.1); ** p value of the variable entered in the stepwise regression analysis. TST: tuberculin skin test; DAS28: 28-joint Disease Activity Score; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; RA: rheumatoid arthritis; SpA: spondyloarthritis; CRP: C-reactive protein; DMARD: disease-modifying antirheumatic drug; NSAID: nonsteroidal antiinflammatory drug; CRF: conventional risk factors of LTBI; TB: tuberculosis; LTBI: latent TB infection; BCG: bacillus Calmette-Guerin.

biologics-naive patients (supplementary table available from author on request).

Table 4 shows characteristics of the patients with concordant positive TST and T-SPOT.TB and of those with discordant test results compared to the patients with both negative tests. Factors associated with concordant positive tests were CRF (OR 5.93; 95% CI: 2.56-13.71, p = 3.2×10^{-5}) and immunosuppressive drugs (OR 0.32, 95% CI: 0.16–0.66, p = 0.002). Female sex (OR 0.54, 95% CI: 0.33–0.89, p = 0.02) and immunosuppressive drugs (OR = 0.53, 95% CI: 0.29–0.98, p = 0.04) reduced the probability to be TST+/T-SPOT.TB–, while BCG vaccination reduced the probability to be TST–/T-SPOT.TB+ (OR 0.37; 95% CI: 0.19–0.73, p = 0.004).

Factors associated with indeterminate IGRA results. Among the 88 indeterminate IGRA results (15.6%), there was insufficient response to stimulation in 59 cases ("low positive control") and excessive responsiveness of the negative control in 30 cases ("high nil control"). Both causes coexisted in 1 case.

Factors associated with indeterminate results were different between low positive control and high nil control (Table 5). Age and CRP were positively associated with low positive control tests. Risk of high nil control test was significantly reduced in patients taking immunosuppressive drugs.

DISCUSSION

Given the constant increase in the number of patients treated with anti-TNF therapy, accurate diagnosis of LTBI is critical in daily practice but can be challenging. In our study, which included 563 patients with immune-mediated inflammatory diseases who were screened for LTBI before biologics introduction or switch, we confirm that there is poor agreement between TST and IGRA results, especially in a population largely vaccinated by BCG[29,30,31]. Our data indicate that replacement of TST by IGRA in the screening would have led to a 27% reduction of antibiotics prophylaxis introduction, in agreement with data recently published by Mariette, *et al*[29].

Comparison of accuracy between IGRA and TST is difficult because there is currently no gold standard for diagnosing LTBI. An alternative way to evaluate the performance of the 2 tests is through comparison with CRF of LTBI reactivation. In our study, TST and T-SPOT.TB positivity were both associated with the presence of at least 1 risk factor of LTBI but the association with IGRA was stronger than with TST (OR 2.7 vs 1.95), confirming previously published results[32,33,34]. These results suggest a better sensitivity of T-SPOT.TB compared to TST.

One possible explanation for the better sensitivity of T-SPOT.TB in our study is that its positivity is not influ-

Table 4. Factors associated with combined TST and T-SPOT-TB test results. Data are number (%) for categorical variables and median (interquartile range) for continuous variables.

	Concordant Results, n = 279			Discordant Results, n = 155			
	TST–/T-SPOT.TB–, n = 220	TST+/T-SPOT.TB+, n = 59	Multivariate p value*	TST+/T-SPOT.TB–, n = 114	Multivariate p value*	TST–/T-SPOT.TB+, n = 51	Multivariate p value*
Female sex	138 (62.7)	34 (57.6)		49 (43.0)	0.02	26 (51.0)	
Age	51.0 (38.0–58.0)	53 (45–60.5)		46.5 (38.0–54.7)		54 (38.5–62.0)	
Diagnosis (RA/SPA)	126/94 (57.3/42.7)	32/24 (54.2/45.8)		68/46 (59.6/40.4)		25/25 (49.0/51.0)	
Disease duration	8.0 (3.0–16.0)	9.0 (4.0–14.0)		7.0 (3.0–13.0)		7.0 (3.0–17.0)	
DAS28	4.98 (4.16–5.93)	4.58 (4.23–5.45)		5.31 (3.79–5.45)		4.86 (4.19–5.91)	
BASDAI	60 (46–72)	53 (44–70)		60 (48–69)		58.0 (45–61)	
CRP	10.5 (4.1–23.0)	8.5 (3.8–17.9)		13.2 (4.3–26.5)		7.6 (3.9–20.5)	
Immunosuppressive drugs	170 (77.3)	37 (62.7)	0.002	67 (58.8)	0.04	34 (66.7)	
Previous biologics	45 (20.5)	8 (13.6)		11 (9.6)		2 (3.9)	
DMARD	123 (55.9)	29 (49.2)		45 (39.5)		27 (52.9)	
Corticosteroids	110 (50.0)	22 (37.3)		43 (37.7)		25 (49.0)	
NSAID	95 (43.2)	24 (40.7)		60 (52.6)		28 (54.9)	
CRF	13 (5.9)	17 (28.8)	3.2×10^{-5}	12 (10.5)		5 (9.8)	
History of active TB	3 (1.4)	5 (8.5)		1 (0.9)		2 (3.9)	
History of TB contact	11 (5)	10 (16.9)		9 (7.9)		2 (3.9)	
Abnormal chest radiograph	2 (0.9)	9 (15.3)		4 (3.5)		3 (5.9)	
BCG vaccination	180 (81.8)	41 (69.5)		99 (86.8)		32 (62.7)	0.004
Birth in TB-endemic area	20 (9.1)	5 (8.5)		11 (9.6)		6 (11.8)	

* P value of the variable entered in the stepwise regression analysis comparing each group with the TST–/T-SPOT.TB– reference group. TST: tuberculin skin test; RA: rheumatoid arthritis; SpA: spondyloarthritis; DAS28: 28-joint Disease Activity Score; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; DMARD: disease-modifying antirheumatic drug; NSAID: nonsteroidal antiinflammatory drug; CRF: conventional risk factors of LTBI; TB: tuberculosis; LTBI: latent TB infection; BCG: bacillus Calmette-Guerin.

Table 5. Factors associated with indeterminate T-SPOT.TB results. Data are number (%) for categorical variables and median (interquartile range) for continuous variables.

	Low Positive Controls, n = 59				High Nil Control, n = 30			
	N	Bivariate p value	OR* (95% CI)	Multivariate p value**	N	Bivariate p value	OR* (95% CI)	Multivariate p value**
Female sex	38	0.25			16	0.29		
Age		0.003	1.03 (1.01–1.05)	0.005		0.63		
Diagnosis (RA vs SpA)	23/36	0.27			22/8	0.008	0.33 (0.14–0.76)	
Disease duration		0.89				0.58		
DAS28		0.01	1.46 (1.08–1.95)			0.30		
BASDAI		0.14				0.01	0.97 (0.94–0.99)	
CRP		0.003	1.01 (1–1.02)	0.007		0.76		
Immunosuppressive drugs	48	0.06			13	0.003	0.33 (0.16–0.7)	0.004
Previous biologics	10	0.95			5	0.99		
DMARD	31	0.72			8	0.01	0.36 (0.16–0.83)	
Corticosteroids	34	0.07			5	0.002	0.24 (0.09–0.64)	
NSAID	24	0.48			16	0.40		
CRF	8	0.51			5	0.36		
History of active TB	1	1			1	0.52		
History of TB contact	3	0.78			4	0.27		
Abnormal chest radiograph	5	0.18			1	1		
BCG vaccination	42	0.20			25	0.53		
Birth in TB-endemic area	4	0.47			2	1		

* OR of the bivariate analysis for the variable entered in the multivariate model ($p < 0.1$). ** P value of the variable entered in the stepwise regression analysis. RA: rheumatoid arthritis; SpA: spondyloarthritis; DAS28: 28-joint Disease Activity Score; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; DMARD: disease-modifying antirheumatic drug; NSAID: nonsteroidal antiinflammatory drug; CRF: conventional risk factors of LTBI; TB: tuberculosis; LTBI: latent TB infection; BCG: bacillus Calmette-Guerin.

enced by immunosuppressive drugs, contrary to TST. The negative influence of immunosuppressive drugs on TST results has already been demonstrated in several studies[29,35,36], especially for corticosteroids. On the contrary, immunosuppressive treatment seems to have no influence on IGRA results[29,31,35,36] except in the study by Matulis, *et al*, showing a negative influence of anti-TNF therapy on QFT-G results[34]. So to improve TST sensitivity, it would be necessary to perform the test before introduction of any immunosuppressive drugs, i.e., as soon as possible after diagnosis. If the LTBI screening is performed under immunosuppressive drugs, IGRA seems preferable to TST. Although we did not evaluate this factor, T-SPOT.TB would probably be superior to QFT-G, because the test is performed after isolation of peripheral blood mononuclear cells, therefore washing out immunosuppressive drugs that can potentially be present in a whole-blood assay such as QFT-G.

The risk of false-positive results of TST and IGRA must also be considered, because antibiotic prophylaxis for LTBI may be associated with severe adverse events. Two main factors are known to affect TST specificity: environmental mycobacteria and BCG vaccination. In our study, there was no association between BCG vaccination and TST positivity, contrary to Matulis, *et al* and Bartalesi, *et al*[34,36]. However, BCG status was significantly associated with discordance between TST and T-SPOT.TB and the agreement between the 2 tests was better in the population not vaccinated by BCG. Interestingly, the BCG vaccination was associated with negative T-SPOT.TB, suggesting that the vaccination can play a protective role on LTBI, as previously observed in pediatric LTBI[37].

One well-known limitation of TST use in daily practice is the necessity of a second visit to read the test. In our study, 8.7% of the TST results were missing and thus hampered therapeutic decisions. A second visit is not required for IGRA; however, indeterminate results may limit its usefulness. In a metaanalysis comparing T-SPOT.TB and QFT-G performances, Diel, *et al* reported a pooled rate of indeterminate results of 2.1% for the QFT-G and 3.8% for the T-SPOT.TB, increasing to 4.4% and 6.1%, respectively, among immunosuppressed hosts[38].

In our study, the rate of indeterminate results amounted to 15.6%, which clearly represents a limit on the possibility to propose replacing TST with T-SPOT.TB. Indeterminate results could be explained by internal (i.e., test-related) or external (patient-related) factors. Several studies have highlighted the importance of technical procedures on test results, such as the number of cells added to wells and any delay in cell processing before stimulation[39,40]. By reducing the delay in blood sample processing to < 4 h, the indeterminate rate decreased from 20% in 2005 to < 10% in 2009.

Indeterminate results can also be the consequence of immunosuppression. Indeed, indeterminate IGRA were

found more frequently in immunocompromised patients than in the general population, whatever the type of immunosuppression[13,41]. Several factors have been associated with indeterminate results, such as anemia, lymphopenia, hypoalbuminemia, or immunosuppressive drugs[42]. In our study, "low positive controls" were more frequent in patients with a high level of systemic inflammation (CRP) or high disease activity (DAS28). This is consistent with the effect of immunosuppression on IFN-γ production established in other studies[43,44]. It may also explain why significantly fewer high nil control tests were observed in patients with RA, those treated with DMARD, or those treated with corticosteroids.

To our knowledge, our current study is one of the largest to have compared IGRA and TST results in patients with chronic inflammatory arthritis who are eligible for biotherapy in "real-life conditions". Hsia, *et al* enrolled more patients (n = 2282) in their study, but they included only patients from phase III trials of golimumab[30]. All those trials except 1 excluded patients with a history of latent or active TB prior to screening. Patients taking more than 10 mg of prednisone per day were also excluded. So results of that study could not be generalized in real-life conditions.

By analyzing factors influencing TST and IGRA results, several things could be done to improve IGRA screening in patients with immune-mediated inflammatory diseases who are candidates for anti-TNF therapy. First, to limit the influence of immunosuppressive drugs and especially of corticosteroids, it would be useful to screen for LTBI as soon as possible after the diagnosis. If the screening must be performed under immunosuppressive therapy, then T-SPOT.TB should be preferred to TST. To limit indeterminate IGRA results, optimal preanalytical conditions are required, and it would be better to perform the test when disease activity is as low as possible. If the test is indeterminate despite these precautions, it may be useful to perform a new test, as demonstrated by Hsia, *et al* and Beffa, *et al*[30,45].

Our results suggest that IGRA should be included in the strategy to identify LTBI in patients with chronic inflammatory diseases before starting anti-TNF therapy. Several screening algorithms have already been proposed. One of them has suggested replacing TST with a dual IGRA strategy (T-SPOT.TB and QFT-G)[29]. Another dual testing strategy based on both TST and IGRA results has also been proposed[46]. However, further studies are needed to validate this strategy and to compare the performance of the 2 IGRA (T-SPOT.TB and QFT-G). Longitudinal followup is also required to reveal the influences of disease activity and treatment on test results.

REFERENCES

1. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001;345:1098–104.
2. Solovic I, Sester M, Gomez-Reino JJ, Rieder HL, Ehlers S, Milburn HJ, et al. The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement. Eur Respir J 2010;36:1185–206.
3. Mariette X, Salmon D. French guidelines for diagnosis and treating latent and active tuberculosis in patients with RA treated with TNF blockers. Ann Rheum Dis 2003;62:791.
4. Gómez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum 2003;48:2122–7.
5. Fonseca JE, Canhão H, Silva C, Miguel C, Mediavilla MJ, Teixeira A, et al. Tuberculose em pacientes reumáticos tratados com antagonistas do fator de necrose tumoral alfa: a experiência de Português [Portuguese]. Tuberculosis in rheumatic patients treated with tumour necrosis factor alpha antagonists: the Portuguese experience. Acta Reumatol Port 2006;31:247–53.
6. British Thoracic Society Standards of Care Committee. BTS recommendations for assessing risk and for managing *Mycobacterium tuberculosis* infection and disease in patients due to start anti-TNF-alpha treatment. Thorax 2005;60:800–5.
7. Centers for Disease Control and Prevention (CDC). Tuberculosis associated with blocking agents against tumor necrosis factor-alpha—California, 2002-2003. MMWR Morb Mortal Wkly Rep 2004;53:683–6.
8. Carmona L, Gómez-Reino JJ, Rodríguez-Valverde V, Montero D, Pascual-Gómez E, Mola EM, et al. Effectiveness of recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists. Arthritis Rheum 2005;52:1766–72.
9. Dixon WG, Hyrich KL, Watson KD, Lunt M, Galloway J, Ustianowski A, et al. Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR). Ann Rheum Dis 2010;69:522–8.
10. Tubach F, Salmon D, Ravaud P, Allanore Y, Goupille P, Bréban M, et al. Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy than with soluble tumor necrosis factor receptor therapy: the three-year prospective French Research Axed on Tolerance of Biotherapies registry. Arthritis Rheum 2009;60:1884–94.
11. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. Clin Infect Dis 1993;17:968–75.
12. Ponce de León D, Acevedo-Vásquez E, Sánchez-Torres A, Cucho M, Alfaro J, Perich R, et al. Attenuated response to purified protein derivative in patients with rheumatoid arthritis: study in a population with a high prevalence of tuberculosis. Ann Rheum Dis 2005;64:1360–1.
13. Kim EY, Lim JE, Jung JY, Son JY, Lee KJ, Yoon YW, et al. Performance of the tuberculin skin test and interferon-gamma release assay for detection of tuberculosis infection in immunocompromised patients in a BCG-vaccinated population. BMC Infect Dis 2009;9:207.
14. Pouchot J, Grasland A, Collet C, Coste J, Esdaile JM, Vinceneux P. Reliability of tuberculin skin test measurement. Ann Intern Med 1997;126:210–4.
15. Yew WW, Leung CC. Antituberculosis drugs and hepatotoxicity. Respirology 2006;11:699–707.
16. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, et al. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc Natl Acad Sci USA 2002;99:3684–9.
17. Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the

immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 2004;4:761–76.
18. Diel R, Hauer B, Loddenkemper R, Manger B, Krüger K. Empfehlungen für Tuberkulose-Screening vor Beginn der TNF-alpha-Hemmer Behandlung bei rheumatischen Erkrankungen [German]. Recommendations for tuberculosis screening before initiation of TNF-alpha-inhibitor treatment in rheumatic diseases. Pneumologie 2009;63:329–34.
19. Beglinger C, Dudler J, Mottet C, Nicod L, Seibold F, Villiger PM, et al. Screening for tuberculosis infection before the initiation of an anti-TNF-alpha therapy. Swiss Med Wkly 2007;137:620–2.
20. Kavanagh PM, Gilmartin JJ, O'Donnell J, O'Flanagan D. Tumour necrosis factor-alpha and tuberculosis: guidance from the National TB Advisory Committee. Ir Med J 2008;101:6–7.
21. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580–8.
22. Rudwaleit M, van der Heijde D, Landewé R, Listing J, Akkoc N, Brandt J, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis 2009;68:777–83.
23. Rudwaleit M, van der Heijde D, Landewé R, Akkoc N, Brandt J, Chou CT, et al. The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Ann Rheum Dis 2011;70:25–31.
24. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.
25. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21:2286–91.
26. Singh JA, Furst DE, Bharat A, Curtis JR, Kavanaugh AF, Kremer JM, et al. 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis Care Res 2012;64:625–39.
27. American Thoracic Society, Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 2000;161:S221–47.
28. T-SPOT Oxford Immunotec. Oxford, UK. [Internet. Accessed August 21, 2013.] Available from: www.oxfordimmunotec.com
29. Mariette X, Baron G, Tubach F, Lioté F, Combe B, Miceli-Richard C, et al. Influence of replacing tuberculin skin test with ex vivo interferon γ release assays on decision to administer prophylactic antituberculosis antibiotics before anti-TNF therapy. Ann Rheum Dis 2012;71:1783-90.
30. Hsia EC, Schluger N, Cush JJ, Chaisson RE, Matteson EL, Xu S, et al. Interferon-γ release assay versus tuberculin skin test prior to treatment with golimumab, a human anti-tumor necrosis factor antibody, in patients with rheumatoid arthritis, psoriatic arthritis, or ankylosing spondylitis. Arthritis Rheum 2012;64:2068–77.
31. Martin J, Walsh C, Gibbs A, McDonnell T, Fearon U, Keane J, et al. Comparison of interferon {gamma} release assays and conventional screening tests before tumour necrosis factor {alpha} blockade in patients with inflammatory arthritis. Ann Rheum Dis 2010;69:181–5.
32. Vassilopoulos D, Tsikrika S, Hatzara C, Podia V, Kandili A, Stamoulis N, et al. Comparison of two gamma interferon release assays and tuberculin skin testing for tuberculosis screening in a cohort of patients with rheumatic diseases starting anti-tumor necrosis factor therapy. Clin Vaccine Immunol 2011;18:2102-8.
33. Bocchino M, Matarese A, Bellofiore B, Giacomelli P, Santoro G, Balato N, et al. Performance of two commercial blood IFN-gamma release assays for the detection of *Mycobacterium tuberculosis* infection in patient candidates for anti-TNF-alpha treatment. Eur J Clin Microbiol Infect Dis 2008;27:907–13.
34. Matulis G, Jüni P, Villiger PM, Gadola SD. Detection of latent tuberculosis in immunosuppressed patients with autoimmune diseases: performance of a *Mycobacterium tuberculosis* antigen-specific interferon gamma assay. Ann Rheum Dis 2008;67:84–90.
35. Murakami S, Takeno M, Kirino Y, Kobayashi M, Watanabe R, Kudo M, et al. Screening of tuberculosis by interferon-gamma assay before biologic therapy for rheumatoid arthritis. Tuberculosis 2009;89:136–41.
36. Bartalesi F, Vicidomini S, Goletti D, Fiorelli C, Fiori G, Melchiorre D, et al. QuantiFERON-TB Gold and the TST are both useful for latent tuberculosis infection screening in autoimmune diseases. Eur Respir J 2009;33:586–93.
37. Basu Roy R, Sotgiu G, Altet-Gómez N, Tsolia M, Ruga E, Velizarova S, et al. Identifying predictors of interferon-gamma release assay results in pediatric latent tuberculosis: a protective role of BCG? Am J Respir Crit Care Med 2012;186:378-84.
38. Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a metaanalysis. Chest 2010;137:952–68.
39. Smith SG, Joosten SA, Verscheure V, Pathan AA, McShane H, Ottenhoff TH, et al. Identification of major factors influencing ELISpot-based monitoring of cellular responses to antigens from *Mycobacterium tuberculosis*. PLoS ONE 2009;4:e7972.
40. Herrera V, Yeh E, Murphy K, Parsonnet J, Banaei N. Immediate incubation reduces indeterminate results for QuantiFERON-TB Gold in-tube assay. J Clin Microbiol 2010;48:2672–6.
41. Lange B, Vavra M, Kern WV, Wagner D. Indeterminate results of a tuberculosis-specific interferon-gamma release assay in immuno-compromised patients. Eur Respir J 2010;35:1179–82.
42. Helwig U, Müller M, Hedderich J, Schreiber S. Corticosteroids and immunosuppressive therapy influence the result of QuantiFERON TB Gold testing in inflammatory bowel disease patients. J Crohns Colitis 2012;6:419–24.
43. Mortensen RF, Gewurz H. Effects of C-reactive protein on the lymphoid system. II. Inhibition of mixed lymphocyte reactivity and generation of cytotoxic lymphocytes. J Immunol 1976;116:1244-50.
44. Stolzenburg T, Binz H, Fontana A, Felder M, Wagenhaeuser FJ. Impaired mitogen-induced interferon-gamma production in rheumatoid arthritis and related diseases. Scand J Immunol 1988;27:73–81.
45. Beffa P, Zellweger A, Janssens J-P, Wrighton-Smith P, Zellweger J-P. Indeterminate test results of T-SPOT.TB performed under routine field conditions. Eur Respir J 2008;31:842–6.
46. Winthrop KL, Weinblatt ME, Daley CL. You can't always get what you want, but if you try sometimes (with two tests—TST and IGRA—for tuberculosis) you get what you need. Ann Rheum Dis 2012;71:1757–60.