

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

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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. (J Rheumatol First Release Oct 1 2013; 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 infection². 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 incidence⁸. 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

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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 LTBI¹⁵ or, on the contrary, to the development of active TB¹⁰.

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 BCG¹⁶. 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 LTBI¹⁷. 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 recommendations³ (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 agents²⁶. 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 calcifications²⁷.

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 instructions²⁸. 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.

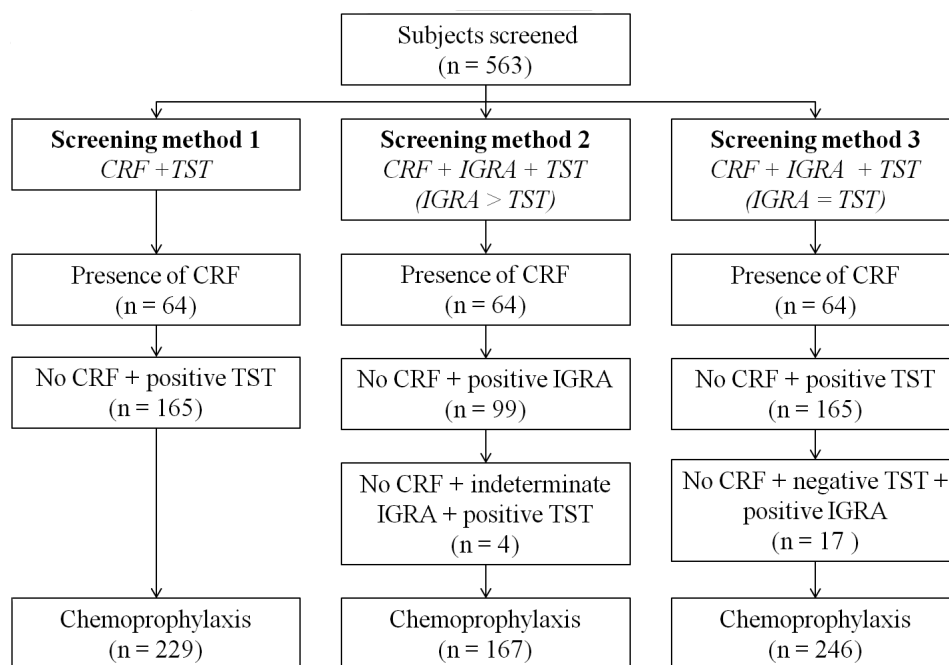


Figure 1. Latent tuberculosis infection screening flow chart. CRF: conventional risk factors; TST: tuberculin skin test; IGRA: interferon- γ release assays.

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%).

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

		TST			Total
		Positive	Negative	Missing Results	
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.

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

	N	Positive TST, n = 196			N	Positive T-SPOT.TB, n = 122		
		Bivariate p value	OR* (95% CI)	Multivariate p value**		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-naïve 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, $p = 3.2 \times 10^{-5}$) 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
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)
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.

	N	Low Positive Controls, n = 59			N	High Nil Control, n = 30		
		Bivariate p value	OR* (95% CI)	Multivariate p value**		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 results³⁴. 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 LTBI³⁷.

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 hosts³⁸.

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 drugs⁴². 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 golimumab³⁰. 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 proposed⁴⁶. 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.

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