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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▽

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Gamma interferon release assays (IGRAs) are increasingly used for latent *Mycobacterium tuberculosis* infection (LTBI) screening in patients with rheumatic diseases starting anti-tumor necrosis factor (anti-TNF) therapies. We compared the performances of two IGRAs, an enzyme-linked immunospot release assay (T-SPOT.TB) and an enzyme-linked immunosorbent assay (QuantiFERON-TB Gold In Tube [QFT-GIT]), to that of tuberculin skin testing (TST) for LTBI screening of 157 consecutive rheumatic patients starting anti-TNF therapies. Among 155 patients with valid results, 58 (37%) were positive by TST, 39 (25%) by T-SPOT.TB assay, and 32 (21%) by QFT-GIT assay. IGRAs were associated more strongly with at least one risk factor for tuberculosis (TB) than TST. Risk factors for a positive assay included chest X-ray findings of old TB (TST), advanced age (both IGRAs), origin from a country with a high TB prevalence, and a positive TST (T-SPOT.TB assay). Steroid use was negatively associated with a positive QFT-GIT assay. The agreement rate between IGRAs was 81% (kappa rate = 0.47), which was much higher than that observed between an IGRA and TST. If positivity by either TST or an IGRA was required for LTBI diagnosis, then the rate of LTBI would have been 46 to 47%, while if an IGRA was performed only for TST-positive patients, the respective rate would have been 11 to 17%. In conclusion, IGRAs appear to correlate better with TB risk than TST and should be included in TB screening of patients starting anti-TNF therapies. In view of the high risk of TB in these patients, a combination of one IGRA and TST is probably more appropriate for LTBI diagnosis.

The accurate diagnosis of latent *Mycobacterium tuberculosis* infection (LTBI) is critical for patients with various autoimmune diseases who are starting anti-tumor necrosis factor (anti-TNF) therapies, since the majority of tuberculosis (TB) cases developing in this population are due to LTBI reactivation (1.5 to 4 times increased risk) (18–20). Traditional methods for LTBI diagnosis, such as the tuberculin skin test (TST), have well-known limitations regarding their sensitivity and specificity for LTBI diagnosis (18–20). Over the last decade, different gamma interferon (IFN-γ) release assays (IGRAs) have been approved for LTBI diagnosis (13). These assays detect IFN-γ secreted by peripheral mononuclear cells after *in vitro* stimulation with specific *M. tuberculosis* antigens not present in the *M. bovis* bacillus Calmette-Guérin (BCG) vaccine, either by enzyme-linked immunosorbent assay (ELISA) (QuantiFERON-TB Gold [QFT-G] and QuantiFERON-TB Gold In Tube [QFT-GIT] assays; Cellestis Limited, Carnegie, Victoria, Australia) or by enzyme-linked immunospot assay (T-SPOT.TB assay; Oxford Immunotec, Oxford, United Kingdom). Newer techniques for the diagnosis of LTBI or active TB based on the secretion of IFN-γ after specific stimulation with specific *M. tuberculosis* antigens, such as the heparin-binding hemagglutinin (HBHA), have also shown promising results (28).

Although there have been a number of studies evaluating the performance of IGRAs in comparison to TST in rheumatic patients (2, 4, 10, 23, 24, 29, 31, 32), head-to-head studies with an adequate number of patients to compare all three assays are limited (5, 21). Furthermore, combined approaches for LTBI diagnosis incorporating the results of the individual IGRAs and TST have not been explored adequately so far.

The objective of our prospective study was a head-to-head comparison between the latest IGRAs (QFT-GIT and T-SPOT.TB assays) and TST for LTBI diagnosis in rheumatic patients starting anti-TNF treatment.

MATERIALS AND METHODS

Patients. Between September 2008 and September 2010, 157 consecutive patients with various rheumatic diseases who were seen at the Outpatient Rheumatology Clinic of Hippokration General Hospital (2nd Department of Medicine, Athens University School of Medicine, Athens, Greece) and scheduled for anti-TNF treatment were included in the study. Patients with active TB, a history of treatment with anti-TB agents, including isoniazid (INH) for LTBI, or a history of previous treatment with anti-TNF agents or other biologics were excluded from the study. All patients signed an informed consent form prior to their participation in the study, and the study was approved by the Institutional Review Board.

A standard questionnaire was completed for each patient, including basic demographic data (sex, country of origin, and country of residence), rheumatic disease (type and duration), comorbid conditions, history of previous TB contact or BCG vaccination, and concurrent immunosuppressive therapy (glucocorticoids and disease-modifying anti-rheumatic drugs [DMARDs]). A baseline chest X-ray was obtained for each patient, with evaluation of findings suggestive of previous, inactive TB (calcified or noncalcified nodules or fibrotic scars) according to published guidelines (1). A thorough physical examination and a whole-blood cell count were also performed for each patient.

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TABLE 1. Patient characteristics

Characteristic	Value
n	155
Sex (no. of males/no. of females)	65/90
Age (yr) (mean ± SD)	52 ± 16
No. (%) of patients of >50 yr	88 (57)
No. (%) of patients with underlying rheumatic disease	
Rheumatoid arthritis	74 (48)
Psoriatic arthritis	35 (23)
Ankylosing spondylitis	31 (20)
Other spondyloarthropathies	13 (8)
Other diseases	2 (1)
No. (%) of patients receiving immunosuppressive therapy	
DMARDs and/or steroids	98 (63)
DMARDs	80 (52)
Steroids	66 (43)
Daily steroid dose (mg) (mean ± SD [median])	6.8 ± 3.2 (5)
Duration of steroid treatment (mo) (mean ± SD [median])	25.7 ± 37.6 (12)
No. (%) of patients with comorbid conditions	15 (21.4)
No. (%) of patients with BCG vaccination	81 (76)
No. (%) of patients with history of TB exposure or chest X-ray findings consistent with old TB	16 (15)

TST. TST was performed by intradermal injection (Mantoux method) of 0.1 ml (2 IU) of purified protein derivative (PPD RT 23; Statens Serum Institute, Copenhagen, Denmark) according to standard guidelines (1), as previously described (32). A TST was considered positive when the diameter of transverse induration was ≥5 mm.

IGRAs. The QFT-GIT assay was performed according to the manufacturer's instructions. The T-SPOT.TB assay was performed as previously described (32). The blood draw for both IGRAs was performed just prior to TST application in order to avoid potential interference with the IGRA results.

Statistical analysis. Statistical analysis was conducted using SPSS statistical software (SPSS Statistics 17.0; SPSS Inc., Chicago, IL). Two-sided Fisher's exact test was used for comparison of categorical variables, and the Wilcoxon rank test was used for comparison of continuous variables. The concordance of agreement between the different assays was assessed using Cohen's kappa test (κ of >0.75, excellent agreement; $0.4 < \kappa < 0.75$, moderate agreement; and κ of <0.4, poor agreement). Risk factors for individual assay positivity were evaluated using odds ratios (ORs) in a univariate analysis. In a multiple regression analysis model, TST or either IGRA was used as a dependent variable, and advanced age (>50 years), sex, history of previous TB contact, chest X-ray findings suggestive of previous TB, origin from a country with a high TB prevalence (non-Greek nationality), and treatment with steroids or DMARDs were selected as independent variables. *P* values of <0.05 were considered statistically significant.

RESULTS

Patient characteristics. The patient characteristics are summarized in Table 1. A total of 157 consecutive patients were included in the study. Two patients with indeterminate QFT-GIT results (1.3%) were excluded from the analysis; both had spondyloarthropathy related to ulcerative colitis and were receiving high-dose methylprednisolone (24 mg/day and 48 mg/day *per os*). None of the patients had an indeterminate T-SPOT.TB result. Among the 155 patients with results available for all assays, 57% were older than 50 years, and approximately half (48%) had rheumatoid arthritis (RA). The majority of patients had been vaccinated with BCG during adolescence or early adulthood years (76%).

TABLE 2. Risk factors for tuberculosis associated with a positive screening assay

Screening assay and risk factor	OR	95% CI	*P* value
TST			
Chest X-ray suggestive of old TB	3.5	1–12.2	0.05
T-SPOT.TB assay			
Age of >50 yr	9.99	3.1–32.3	0.0001
TST-positive result	8.06	3.08–21.1	0.00002
Non-Greek nationality	5.65	1.08–29.5	0.04
Any risk factor for TB (≥1)[a]	4.8[b]	1.75–13.2	0.002
QFT-GIT assay			
Age of >50 yr	4.51	1.58–12.1	0.005
Steroid use	0.31	0.1–0.96	0.04
Any risk factor for TB (≥1)[a]	2.68[b]	1.02–6.99	0.04

[a] Risk factors for TB included age of >50 years, chest X-ray suggestive of old/healed TB, contact with a person with TB, and birth or residence in a country with a high TB prevalence (non-Greek nationality).

[b] By univariate analysis (see details in Table 3). All other results were from multivariate analysis (as described in Materials and Methods).

Individual assay positivity. (i) TST. TST was found to be positive for more than one-third of rheumatic patients (*n* = 58; 37%). By multivariate analysis, only a chest X-ray suggestive of previous TB (OR = 3.5; *P* = 0.05) was marginally associated with a positive TST (Tables 2 and 3). Birth or residence in a country with a high TB prevalence (OR = 3.7), previous TB exposure (OR = 1.73), BCG vaccination (OR = 1.43), and an age of >50 years (OR = 1.2) were also associated with a positive test, but the association did not reach statistical significance. Steroid use was negatively associated with a positive test (OR = 0.52; *P* = 0.06). By univariate analysis, the presence of at least one known risk factor for TB had an OR of 1.6, but the association was not statistically significant (*P* = 0.12) (Table 3).

(ii) T-SPOT.TB assay. The T-SPOT.TB assay was positive for fewer patients than TST was (*n* = 39; 25%). In a similar multivariate analysis (including TST), factors associated with a positive result were age of >50 years (OR = 9.9; *P* = 0.0001), non-Greek nationality (OR = 5.6; *P* = 0.04), and a positive TST (OR = 8.06; *P* = 0.00002) (Tables 2 and 3). Steroid therapy was negatively associated with a positive test (OR = 0.33), but this did not reach statistical significance (*P* = 0.07). Previous BCG vaccination did not affect the test results (OR = 0.17). The presence of at least one risk factor for TB was statistically associated with a positive T-SPOT.TB test (OR = 4.8; *P* = 0.002). Similar results were obtained when TST was not included in the multivariate analysis (data not shown).

(iii) QFT-GIT assay. Thirty-two patients (21%) exhibited a positive QFT-GIT assay. Age of >50 years (OR = 4.51; *P* = 0.005) and a positive TST (OR = 2.35; *P* = 0.03) were associated with a positive result, while current steroid therapy was negatively associated with a positive test by multivariate analysis (OR = 0.31; *P* = 0.04) (Tables 2 and 3). Similar to the T-SPOT.TB assay, the results of QFT-GIT were not influenced by previous BCG vaccination (OR = 0.72). The presence of at least one risk factor for TB was associated with a positive QFT-GIT result (OR = 2.68; *P* = 0.04). Similar results were found when TST was not included in the multivariate analysis (data not shown).

TABLE 3. Variables associated with positive test results

Variable	No. of results (unless indicated otherwise)		Univariate analysis[b]		Multivariate analysis[b]	
	Positive	Negative	Odds ratio	*P* value	Odds ratio	*P* value
Variables associated with positive TST	*n* = 58	*n* = 97				
Age (yr) (mean ± SD)	52.9 ± 17.2	51.7 ± 15				
<50 yr	26	41				
>50 yr	32	56	0.9	0.76	1.18	0.67
Diagnosis						
RA	22	52				
Non-RA	36	45	**0.5**	**0.05**	0.56	0.22
DMARD use						
Yes	26	54				
No	32	43	0.65	0.19	0.87	0.75
Steroid use						
Yes	19	47				
No	39	50	0.52	0.06	0.53	0.14
TB exposure						
Yes	10	10				
No	48	87	1.81	0.22	1.73	0.3
Chest X-ray suggestive of old TB						
Yes	9	5				
No	49	92	**3.38**	**0.04**	**3.5**	**0.05**
BCG vaccination						
Yes	41	62				
No	17	35	1.36	0.39	1.43	0.34
Nationality						
Greek	52	93				
Non-Greek	6	4	2.68	0.14	3.74	0.07
Any risk factor for TB (≥1)[a]						
Yes	42	60				
No	16	37	1.6	0.12		
Variables associated with positive T-SPOT.TB assay	*n* = 39	*n* = 116				
Age (yr) (mean ± SD)	62.9 ± 14	48.6 ± 14.8				
<50 yr	7	60				
>50 yr	32	56	**4.89**	**0.001**	**9.99**	**0.0001**
Diagnosis						
RA	21	54				
Non-RA	18	62	1.33	0.43	1.45	0.53
DMARD use						
Yes	23	57				
No	16	59	1.49	0.29	2.1	0.22
Steroid use						
Yes	14	52				
No	25	64	0.69	0.33	0.33	0.07
TB exposure						
Yes	5	15				
No	34	101	0.99	0.99	0.89	0.86
Chest X-ray suggestive of old TB						
Yes	4	10				
No	35	106	2.21	0.76	0.48	0.31
BCG vaccination						
Yes	24	79				
No	15	37	0.75	0.45	0.51	0.17
Nationality						
Greek	35	110				
Non-Greek	4	6	2.09	0.27	**5.65**	**0.04**
TST-positive result						
No	13	84				
Yes	26	32	**5.5**	**0.00002**	**8.06**	**0.00002**
Any risk factor for TB (≥1)[a]						
Yes	34	68				
No	5	48	**4.8**	**0.002**		
Variables associated with positive QFT-GIT assay	*n* = 32	*n* = 123				
Age (yr) (mean ± SD)	59.2 ± 14.6	50.4 ± 15.7				
<50 yr	8	59				
>50 yr	24	64	**2.77**	**0.02**	**4.51**	**0.005**

Continued on following page

TABLE 3—*Continued*

Variable	No. of results (unless indicated otherwise)		Univariate analysis[b]		Multivariate analysis[b]	
	Positive	Negative	Odds ratio	*P* value	Odds ratio	*P* value
Diagnosis						
RA	16	59				
Non-RA	16	64	1.08	0.84	1.16	0.79
DMARD use						
Yes	16	64				
No	16	59	0.92	0.84	0.73	0.56
Steroid use						
Yes	10	56				
No	22	67	0.54	0.15	**0.31**	**0.04**
TB exposure						
Yes	3	17				
No	29	106	0.64	0.5	0.55	0.41
Chest X-ray suggestive of old TB						
Yes	14	10				
No	28	113	1.61	0.44	1.29	0.72
BCG vaccination						
Yes	22	81				
No	10	42	1.14	0.76	1.05	0.9
Nationality						
Greek	29	116				
Non-Greek	3	7	1.7	0.45	3.5	0.12
TST-positive result						
No	15	82				
Yes	17	41	**2.35**	**0.03**	2.02	0.11
Any risk factor for TB (≥1)[a]						
Yes	26	76				
No	6	47	**2.68**	**0.04**		

[a] Risk factors for TB included age of >50 years, chest X-ray suggestive of old/healed TB, contact with a person with TB, and birth or residence in a country with a high TB prevalence (non-Greek nationality).
[b] Statistically significant differences between groups ($P < 0.05$) are shown in bold.

Agreement between assays. (i) TST and T-SPOT.TB assay. A poor level of agreement (71%) was found between the T-SPOT.TB assay and the TST ($\kappa = 0.34$; 95% confidence interval [95% CI] = 0.17 to 0.5), as described previously by our group for the same ethnic population (32). The majority of discordant results ($n = 45$; 29%) were due to TST-positive and T-SPOT.TB-negative results (32/45 results [71%]).

(ii) TST and QFT-GIT assay. The agreement rate was much lower when the QFT-GIT test was compared to TST (64% agreement) ($\kappa = 0.15$; 95% CI = −0.02 to 0.33). A similar proportion of discordant results were due to TST-positive and QFT-GIT-negative results (41/56 results [73%]).

(iii) T-SPOT.TB and QFT-GIT assays. The agreement rate between the 2 IGRAs was 81% ($\kappa = 0.47$; 95% CI = 0.3 to 0.65), which is considered moderate agreement. One hundred five patients had negative results with both tests (68%), while for 21 patients both tests were positive (14%). Discordant results were found for 29 patients (19%). Patients with discordant results as a whole were older (mean age = 61.5 ± 17 years versus 50.1 ± 14.8 years; $P = 0.0003$) and had RA more frequently (72% versus 43%; $P = 0.007$) than patients with concordant results. In a multivariate analysis, though, none of these factors were associated with a discordant result (data not shown). Among the 29 patients with discordant results, 18 were T-SPOT.TB assay positive and QFT-GIT assay negative (12%), and 11 were T-SPOT.TB assay negative and QFT-GIT assay positive (7%). When the characteristics of these two groups of patients with discordant results were compared (Table 4), only the rate of TST positivity differed significantly between the two groups. Specifically, TST was positive for 10/18 patients (56%) with positive T-SPOT.TB and negative QFT-GIT results, compared to only 1/11 patients (9%) with positive QFT-GIT and negative T-SPOT.TB tests ($P = 0.02$). Since the results of the QFT-GIT assay could potentially be influenced by the total number of circulating peripheral lymphocytes (in contrast to T-SPOT.TB assay, where a predetermined number of lymphocytes are cultured with the specific *M. tuberculosis* antigens [1×10^5 cells/well]), the absolute numbers of lymphocytes were compared between groups. As shown in Table 4, no significant difference between the two groups was found ($P = 0.88$).

Combination of screening assays for LTBI diagnosis. Using different combinations of the available assays, the rate of possible LTBI and thus the number of patients who needed treatment were estimated (Fig. 1). As shown in Fig. 1, similar results were obtained when one or the other IGRA was used for screening. If positivity by either TST or IGRA was required for LTBI diagnosis, then the rate of LTBI would have been 46% (TST or T-SPOT.TB assay) to 47% (TST or QFT-GIT assay). If an IGRA was performed only for TST-positive patients, then the rate of LTBI would have been much lower, ranging from 11% to 17% (for TST or QFT-GIT assay and TST or T-SPOT.TB assay, respectively). An intermediate rate of positivity would be expected if a positive IGRA was required

TABLE 4. Discordant IGRA results

Variable	No. of discordant results (unless indicated otherwise)		*P* value
	T-SPOT.TB$^+$ QFT-GIT$^-$ (n = 18)	T-SPOT.TB$^-$ QFT-GIT$^+$ (n = 11)	
Age (yr) (mean ± SD)	65 ± 17.3	55.6 ± 18.1	0.11
<50 yr	3	4	
>50 yr	15	7	0.37
Sex			
Female	12	9	
Male	6	2	0.67
Diagnosis			
RA	13	8	
Non-RA	5	3	1
DMARD use			
Yes	13	6	
No	5	5	0.43
Steroid use			
Yes	9	5	
No	9	6	1
TB exposure			
Yes	2	0	
No	16	11	1
Chest X-ray suggestive of old TB			
Yes	1	1	
No	17	10	0.41
BCG vaccination			
Yes	11	9	
No	7	2	0.68
Nationality			
Greek	17	11	
Non-Greek	1	0	1
Positive TST result (≥5 mm)			
Yes	10	1	
No	8	10	**0.02**
Lymphocyte count (absolute; cells/μl) (mean ± SD)	2,247 ± 816	2,217 ± 728	0.88
Any risk factor for TB (≥1)[a]			
Yes	15	7	
No	3	4	0.37

[a] Risk factors for TB included age of >50 years, chest X-ray suggestive of old/healed TB, contact with a person with TB, and birth or residence in a country with a high TB prevalence (non-Greek nationality).

for LTBI diagnosis only for TST-negative and vaccinated TST-positive patients in addition to nonvaccinated TST-positive patients (according to Spanish guidelines) (17) (28 to 29% for TST or QFT-GIT assay and TST or T-SPOT.TB assay, respectively).

DISCUSSION

This is the largest study in the literature to compare the performances of all currently available assays for the diagnosis of LTBI in rheumatic patients starting anti-TNF therapy. The study, which was performed in a country with a high rate of past TB exposure and BCG vaccination, showed that both IGRAs correlate better with the existence of the known risk factors for TB than the traditional TST. Using different combination strategies based on the IGRA and TST results, it was also found that the T-SPOT.TB or QFT-GIT assay can be used with similar results for LTBI screening in this population.

Our results should be interpreted in the context of our patient population. This is a population highly exposed to TB in the past, with studies showing high incidences of TB (30 to 60/100,000 individuals between 1960 and 1980) (7) and TST positivity (7 to 14% in the 1980s and 1990s) (6). Over the last decade, the incidence of TB has declined significantly (TST positivity among army recruits is <3%, and TB incidence is 5.4 cases/100,000 individuals) (16, 33). Since most of our patients were older than 50 years (57%), a relatively high rate of TB exposure (and potentially LTBI) in past decades was assumed for our patient population. Furthermore, BCG vaccination was common in our population (76%) and was usually performed after the age of 6 years, which is well known to correlate with a positive TST in adult life (20 to 40%) (14).

The performance of each screening assay was evaluated by measuring its association with at least one of the established risk factors for TB, and IGRAs performed better than TST in this setting. These findings, which are in accordance with recent studies (2, 21, 23, 24, 31), establish the value of IGRAs for LTBI screening in rheumatic patients receiving anti-TNF agents.

Multivariate analysis revealed that TST positivity was marginally associated only with chest X-ray findings of previous TB, while birth or residence in a country with a high TB prevalence and a positive TST were associated with a positive T.SPOT-TB result. Advanced age was the strongest risk factor for both IGRAs (most likely reflecting past TB exposure), in accordance with recent reports from Italy (2) and Ireland (23). Low-dose steroid use was negatively associated with a positive screening assay, although this reached statistical significance only for the QFT-GIT assay (OR = 0.31 by multivariate analysis). Similar results were recently reported by Bartalesi et al. (2), who observed a suppressive effect of steroids on the results of the QFT-GIT test (OR = 0.4).

The novelty of our study lies in the direct comparison of the two different IGRAs for our rheumatic patients starting anti-TNF treatment. In contrast to two previous studies (21, 23) in which small numbers of patients with positive IGRAs were included (8 to 10%), a relatively large number of patients with positive IGRA results was found in our study population (21 to 25%). A moderate rate of agreement (81% agreement rate; $\kappa = 0.47$) was observed between the two IGRAs, which is close to what has been reported in the literature for rheumatic populations (0.48 to 0.67) (5, 21). In contrast to a recent study by Kleinert et al. (21), who utilized the QFT-G assay and found a high rate of indeterminate results (18%), the rate of indeterminate results in our study was only 1.3%. Discordant results between IGRAs were observed in approximately 20% of the cases, compared to discordance rates of 10 to 15% in previous studies (5, 21). Among our patients, more had positive T-SPOT.TB and negative QFT-GIT results, and this group more frequently had a positive TST. It remains unclear,

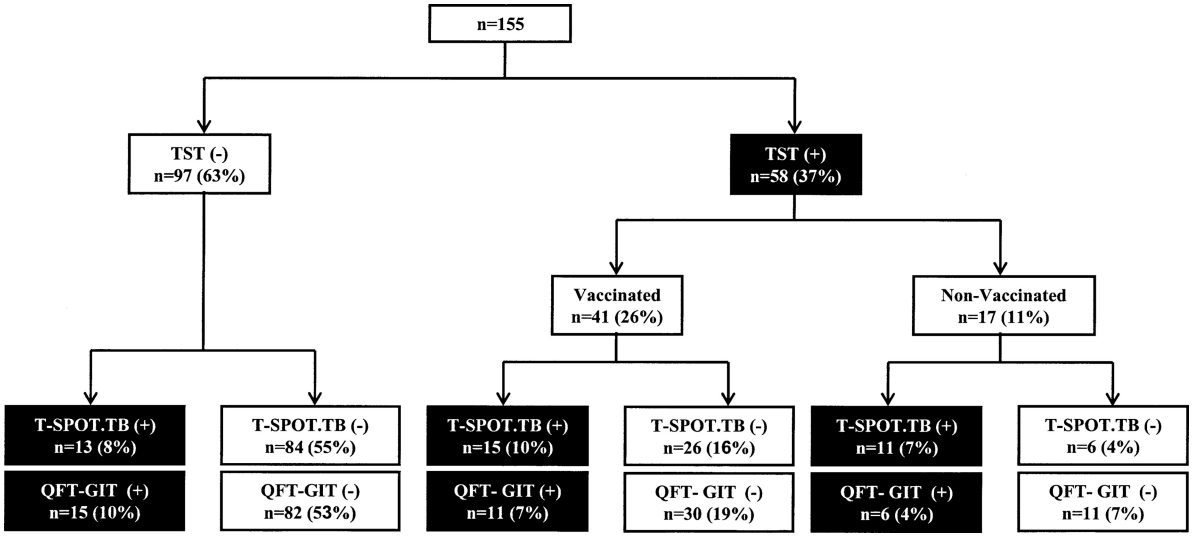

FIG. 1. Distribution of patients according to TST result and BCG vaccination status.

though, how many of these results were due to "false-positive" or "false-negative" results of each IGRA. The literature data overall suggest that the T-SPOT.TB assay is more sensitive (81 to 90%) than the QFT-GIT assay (70 to 81%), whereas the QFT-GIT assay appears to be more specific (96 to 99% versus 86 to 93% for the T-SPOT.TB assay) for active TB diagnosis (13, 27, 30). Data regarding the performances of these tests for patients with potential LTBI are limited due to the absence of a gold standard for its diagnosis. Extrapolating from data studying the rate of progression to TB among TB contacts, similar results were obtained for both assays (3.3 to 10% for T-SPOT.TB assay versus 2.8 to 14.3% for QFT-GIT assay) (11).

Conclusively, based on our data, no clear recommendation in favor of one or the other IGRA can be made with certainty at this point. While definitive data from prospective studies looking at TB progression in different subsets of anti-TNF-treated patients are becoming available, issues such as the availability of each assay, the laboratory expertise required, and the cost should be taken into consideration before a decision is made. TST has known limitations regarding the need for two office visits and the subjectivity of injection and reading techniques. On the other hand, both IGRAs are more labor-intensive, requiring isolation and enumeration of peripheral blood mononuclear cells (T-SPOT.TB assay), overnight culture of the specimens (both IGRAs), and additional equipment for measurement of IFN-γ levels by ELISA (QFT-GIT assay).

Another significant issue that remains to be clarified is whether IGRAs should be used instead of or combined with TST for TB screening. Different national guidelines propose that IGRAs should either replace (Germany [12] and Switzerland [3]), be used interchangeably with (France [15]), or be combined with (United States [25], Spain [17], United Kingdom [26], and Canada [8]) TST.

If an individual strategy approach was followed for our patients, then 21 to 25% would have been IGRA positive and 37% would have been TST positive. If only the results of an IGRA were considered diagnostic for LTBI, then 21 to 26% of our patients who were IGRA positive and TST negative would not have received LTBI treatment. Although TST's limitations are known, its sensitivity, especially in immunosuppressed patients (e.g., HIV patients), does not seem to differ significantly from that of IGRAs (9). Furthermore, data regarding the rate of progression to TB in immunocompromised patients who are IGRA negative and TST positive without LTBI treatment are still limited. In a recent study from Switzerland, 10 vaccinated patients with psoriasis who tested TST positive and T-SPOT.TB assay negative were treated with anti-TNF agents for 64 weeks without TB reactivation (22).

If a dual strategy was implemented, different results would have emerged. If the IGRAs were performed only for TST-positive patients and then LTBI treatment administered to those found positive, then only 11 to 17% would have required such treatment. This approach has the disadvantage that it excludes the 8 to 10% of patients who are potentially falsely negative by TST (IGRA positive) from LTBI therapy. If all TST-positive patients (regardless of their vaccination status or IGRA results) and TST-negative, IGRA-positive patients were treated, then 46 to 47% of our high-risk patients would have required LTBI treatment in accordance with recent U.S. and United Kingdom guidelines (25, 26). With this strategy, the maximum sensitivity for LTBI diagnosis is achieved, but this has to be balanced against the potential side effects of unnecessary LTBI treatment (loss of specificity). In our institution, following this strategy, no cases of TB were observed during long-term follow-up (unpublished data).

An intermediate rate of positive LTBI would have been seen (28 to 29%) if the Spanish guidelines (17) were followed (only nonvaccinated TST-positive, vaccinated TST-positive plus IGRA-positive, and TST-negative plus IGRA-positive patients are treated). Nevertheless, even with this strategy, 17 to 19% of our patient population (vaccinated TST-positive and IGRA-negative patients) would have been denied LTBI therapy. Although it is well known that TST results are influenced by BCG vaccination, it is unclear if all TST-positive results can be

explained by a previous BCG exposure, particularly in patients with advanced age (>10 to 15 years after vaccination) who were potentially exposed to TB in the past.

Collectively, our data indicate that in a patient population with a high risk for TB reactivation, such as anti-TNF-treated patients, strategies that combine the results of TST and an IGRA for LTBI diagnosis should probably be implemented. Although this is particularly true in countries with intermediate to high prevalences, where approaches which are designed to enhanced sensitivity for LTBI diagnosis are necessary, the same strategy can be applied in low-prevalence countries. In the absence of longitudinal data on large populations of TST-positive and IGRA-negative patients indicating no or low risk for TB progression, recommendations to abandon the TST assay cannot be made definitely at this point. Such data from prospective studies are clearly needed before definite guidelines can be made.

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