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Original Article

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Early Tuberculin Skin Test for the Diagnosis of Latent Tuberculosis Infection in Patients with Inflammatory Bowel Disease

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Abstract

Background and Aim: Sensitivity of tuberculin skin test [TST] during screening for latent tuberculosis infection [LTBI] is affected by steroid and/or immunosuppressant therapy. The aim of this study was to compare performance of the two-step TST in inflammatory bowel disease patients immediately before anti-tumour necrosis factor [TNF] therapy as part of routine screening for LTBI vs control patients when the TST was carried out at an early stage.

Methods: In this multicentre prospective controlled study, we evaluated the performance of two-step TST with 5-mm threshold. Factors associated with TST results were determined by logistic regression.

Results: We evaluated 243 candidates for anti-TNF therapy and 337 control patients. Overall, 105 patients [18.1%] had an induration ≥ 5 mm in the first TST or in TST retest. LTBI was diagnosed in 25% of patients by TST retest. Twenty-eight [11.5%] anti-TNF group patients vs 77 [22.8%] control patients had a positive TST (odds ratio [OR] 0.44, 95% confidence interval [CI] 0.28–0.70; $P < 0.001$]. In multivariate analysis, positive TST was associated with higher age [OR 2.63, 95% CI 1.21–5.72; $P < 0.001$] and 5-aminosalicylate therapy [OR 1.86, 95% CI 1.14–3.05; $P = 0.013$]. Negative TST was associated with steroid therapy [OR 0.36, 95% CI 0.16–0.83; $P = 0.016$], immunosuppressant therapy [OR 0.36, 95% CI 0.21–0.62; $P < 0.001$], or steroids + immunosuppressant therapy [OR 0.20, 95% CI 0.07–0.59; $P = 0.004$].
Conclusions: The sensitivity of routine TST performed just before starting anti-TNF therapy is low. TST performed at an early stage enables screening in the absence of immunosuppressive treatment and thus maximises the diagnostic yield of TST for detecting LTBI.

Key Words: Tuberculosis; tuberculin skin test; anti-TNF; ulcerative colitis; Crohn´s disease; steroid; immunosuppressant; inflammatory bowel disease

1. Introduction

Active tuberculosis [TB] will develop in 5% to 15% of persons with latent infection during their lifetimes, and this proportion is higher in the case of immunocompromised individuals.[1] Tumour necrosis factor [TNF] antagonists are highly effective treatments for many immune-mediated inflammatory diseases [IMID], including Crohn´s disease and ulcerative colitis. However, whereas TNF is a central cytokine in the pathogenesis of inflammatory bowel diseases [IBD], it is also a critical component of host defence against *Mycobacterium tuberculosis*. Therefore, use of TNF antagonist is associated with an increased risk of active TB.[2] When TB develops in patients on anti-TNF therapy, it is more commonly atypical, often occurring at extrapulmonary sites and sometimes progressing to disseminated disease with a potentially fatal outcome.[2,3] Screening and treating latent TB infection [LTBI] before use of anti-TNF therapy has decreased the risk of active TB.[4] Therefore, preventive treatment should be offered to all patients with evidence of LTBI before starting any anti-TNF agent.[3–6] In Spain, which has a low to intermediate incidence of TB, recommended TB screening before the use of anti-TNF therapy includes a careful history [including epidemiological risk factors], a two-step tuberculin skin test [TST], and a chest radiograph.[7] In rheumatic patients receiving anti-TNF agents, the incidence of active TB decreased by 78% after the adoption of official recommendations.[4] Unfortunately, despite all preventive actions, new cases of active TB still occur in patients on anti-TNF therapy.

In patients with IMID there is a pronounced negative effect of steroid and/or immunosuppressant therapy on the rate of positive TST results.[8–10] Therefore, to maximise TST sensitivity, early screening with TST for LTBI has been suggested, ideally at diagnosis or in absence of any steroid or immunosuppressant therapy, but this strategy has never been evaluated.

The aim of the study was to evaluate the results of the two-step TST in IBD patients. We compared the performance of the two-step TST in two groups of patients: those selected for initial anti-TNF therapy in whom the TST was part of the mandatory routine screening for LTBI, vs control patients in whom the TST was carried out at an early stage before prescribing anti-TNF therapy.

2. Materials and Methods

2.1. Study design

This was a multicentre prospective controlled study. The study was approved by the local ethical committees of all centres. Informed consent was obtained from each participant.

2.2. Patients

The anti-TNF group included consecutive IBD patients with an indication for initial treatment with anti-TNF agents, in whom the routine screening for TB was mandatory. The control group included IBD patients for whom anti-TNF therapy was not planned in the short term. Inclusion criteria applicable to all patients were age ≥ 18 years and diagnosis of IBD according to the recommendations of the European Crohn's and Colitis Organisation [ECCO].[11,12] The exclusion criteria were previous positive TST, present or past active TB, previous anti-TNF therapy, and pregnancy.

Demographic and clinical information was recorded in a key encrypted electronic case report form designed specifically for the study. IBD patients were classified according to the Montreal classification.[13] All patients were asked about previous screening for LTBI and history and symptoms of active TB. We also sought information on accepted risk factors for LTBI such as close contact with patients with active TB [past or in last year], stays in a TB-endemic area [born or staying longer than 3 months in countries with a high TB prevalence according to the World Health Organization classification], and smoking habits. *Bacillus Calmette-Guérin* [BCG] vaccination status was recorded according to vaccination history or by evidence of a characteristic skin scar. The dose and duration of immunosuppressants [azathioprine, mercaptopurine,e and methotrexate], systemic steroids, beclometasone dipropionate, budesonide, and 5-aminosalicylates [mesalazine or sulphasalazine] were recorded before LTBI screening. We defined immunosuppressant therapy as use of immunosuppressants for at least 2 months. Steroid therapy was defined as use of systemic steroids at any dose for at least 2 weeks. C-reactive protein [CRP] value was collected at baseline and classified by the number of times above the upper limit of normal. We also recorded relevant comorbidities.

2.3. Screening for LTBI

2.3.1. Two-step tuberculin skin test

The TST was performed by trained personel. Tuberculin purified protein derivate [PPD RT23, Staten Serum Institute, Denmark] at a dose of 2 tuberculin units [0.1 ml] was injected according to the intradermal Mantoux method. The maximal diameter of the skin induration was recorded in mm 72 h after injection, and TST was considered positive if induration was ≥ 5 mm. If first test was negative [< 5 mm] a second TST ['booster' TST or retest] was administered 1 week later.

2.3.2. Chest X-ray

Chest X-rays were evaluated by experienced radiologists. Findings indicative of LTBI included pleural scarring, interstitial granulomatous calcification, apical densities, and/or hilar lymphadenopathy.

2.4. Definitions

'Positive TST' indicates an induration of ≥ 5 mm in first test or an induration of ≥ 5 mm in second test [if the first test was < 5 mm]. 'Routine TST' comprises the two-step TST indicated as part of the mandatory screening for LTBI before initiation of anti-TNF therapy. 'Early TST' means the two-step TST performed in control patients [regardless of duration of disease] in whom anti-TNF therapy was not foreseen.

2.5. Statistical analysis

Categorical variables were described by absolute and relative frequency [number, percentage, and 95% confidence interval]. Numerical variables were described by the mean and standard deviation or median and interquartile range [IQR] as appropriate. The initial comparability between groups was analysed with the chi-square test or Fisher's exact test for qualitative variables and with Student's t test or median test for quantitative data. The association between variables and the results of TST was assessed with univariate and multivariate analysis. Results are presented as crude odds ratio [OR] or adjusted OR [AOR] and their 95% CI. Candidate variables [p-value < 0.1 in univariate analysis] were initially entered in the multivariate logistic regression models. Variables retained in the final multivariate model were selected by a backward procedure. The presence of positive TST was stratified by group [anti-TNF or control] and treatment [steroids and/or immunosuppressants]. Interaction was assessed by introducing the multiplicative term in the model described.

3. Results

In total, 615 patients from 33 hospitals were considered for inclusion. Seven patients were excluded due to previous positive TST, and two due to previous exposure to TNF antagonists. A further 26 patients were excluded due to lack of compliance in the performance of the two-step TST: in three patients the first TST was not administered, and in 23 patients the retest was not performed despite induration < 5 mm in the first test. The study population finally comprised 580 patients (94.3% of those considered, 243 patients [41.9%] selected for anti-TNF therapy and 337 control patients [58.1%]). The characteristics of the two groups are summarised in Table 1. Anti-TNF group patients were more frequently diagnosed with Crohn´s disease [71%] than controls [53.8%, p < 0.001]. A higher proportion of anti-TNF group patients were on immunosuppressant therapy [63.4%] than controls [35.3%, p < 0.001]. At time of testing, the proportion of patients who had normal CRP was lower in the anti-TNF group [38.2%] compared with the control group [66.2%, p < 0.001]. Control group patients had slightly higher age [43.6 years vs 41.2 years, p = 0.49] and were more frequently receiving 5-aminosalicylates [55.5% vs 38%, p < 0.001]. Mean [SD] age of BCG-vaccinated population [51 ± 13 years] was significantly higher than the age of the unvaccinated population [44 ± 14 years, p < 0.001]. Mean [SD] interval between BCG vaccination and screening for LTBI was 50 [± 12] years.

3.1. Rate of positive TST

Of the 580 patients included, 79 [13.6%, 95% CI 10.7–16.5] had a first TST result ≥ 5mm. TST retest was performed in 501 patients who had a TST result < 5 mm, and TST retest result was ≥ 5mm in 26 patients (26/580 [4.5%, 95% CI 2.7–6.3]). Thus, 105 of 580 patients [18.1%, 95% CI 14.9–21.3] met criteria for LTBI in first TST or TST retest. The median TST induration in the study population was 0 mm [range 0–52]. In the 105 patients with a positive TST, the median induration was 13 mm (interquartile range [IQR] 10–18, range 5–52). Median induration was 13 mm [IQR 10–19, range 6–52] in the 79 patients with positive first TST vs 10 mm [IQR 7–15, range 5–20] in the 26 patients with positive TST retest [p = 0.009].

3.2. Rate of positive TST by groups

Twenty-eight of 243 [11.5%] anti-TNF group patients and 77 of 337 [22.8%] control patients had a positive TST [OR 0.44, 95% CI 0.28–0.70; p < 0.001]. The proportion of positive TST found in the TST retest was 3/28 in the anti-TNF group [10.7%] vs 23/77 in the control group [29.9%, p = 0.07]. Among patients with positive TST, the median TST induration was 13 mm [IQR 10–19, range 6–22] in the 28 anti-TNF group patients vs 12 mm [IQR 10–17, range 5–52] in control patients [p = 0.89]. Among patients with positive TST, there were no differences in median TST induration between patients who received or did not receive steroids and/or immunosuppressant therapy.

3.3. Univariate analysis of factors associated with TST results

In the univariate analysis [Table 2], positive TST was associated with higher age, BCG vaccination, ulcerative colitis vs Crohn´s disease, and 5-aminosalicylate therapy. A negative TST was associated with anti-TNF group vs control group, elevated CRP, and steroids and/or immunosuppressant therapy.

3.4. Multivariate analysis of factors associated with TST results

Factors showing significant association in the univariate analysis were retained in the logistic model [Table 3]. Positive TST was linearly associated with higher age [AOR 2.63, 95% CI 1.21–5.72; p < 0.001] and 5-aminosalicylate therapy [AOR 1.86, 95% CI 1.14–3.05; p = 0.013]. Conversely, a negative TST was associated with steroid therapy [AOR 0.36, 95% CI 0.16–0.83; p = 0.016], immunosuppressant therapy [AOR 0.36, 95% CI 0.21–0.62; p < 0.001], or steroids + immunosuppressant therapy [AOR 0.20, 95% CI 0.07–0.59; p = 0.004].

3.5. Impact of steroids and/or immunosuppressant therapy on TST results among groups

After stratifying by group and treatment there was a modification of effect [p = 0.034]: the rate of positive TST was 33% [57/175] for control group patients without steroids and/or immunosuppressant therapy vs 16% [9/58] for anti-TNF group patients without steroids and/or immunosuppressant therapy [OR 2.63, 95% CI 1.21–5.73; p = 0.008] [Figure 1]. The association between steroid and/or immunosuppressant therapy with negative TST remained for both groups without significant differences.

3.6. Impact of CRP on TST results among patients without steroids and/or immunosuppressant therapy

At time of testing, 58 of 243 anti-TNF group patients [24%] vs 175 of 337 control patients [52%, P < 0.001] were not on steroids and/or immunosuppressant therapy. Among them, the proportion of elevated CRP levels was 58.5% for anti-TNF group patients [22/53] vs 30% for control patients [50/167, p < 0.001]. There was a trend towards a lower rate of positive TST in patients with elevated CRP [OR 0.68, 95% CI 0.43–1.10; p = 0.07]. Among patients with no steroid and/or immunosuppressant therapy who had normal CRP, the likelihood of positive TST [adjusted by age

Table 1. Patients characteristics: anti-TNF group and control group.

		Anti-TNF group		Control group		
		N = 243		*N* = 337		
		Cases [*N*]	%	Cases [*N*]	%	*p*-Value
Sex [M/F]	Male	139	57.4	181	53.7	0.373
	Female	103	42.6	156	46.3	
Age mean, years		41.2	14.2	43.6	14.8	0.049
Any risk factors for LTBI	0	170	69.4	231	66.8	0.76
	1	71	29.0	110	31.8	
	≥ 2	4	1.6	5	1.4	
History of TB contact	No	233	97.5	318	94.9	0.123
	Yes	6	2.5	17	5.1	
TB contact, past year	No	235	100.0	330	99.1	0.145
	Yes	0	0.0	3	0.9	
Stay > 3months TB endemic area	No	237	99.2	326	97.6	0.160
	Yes	2	0.8	8	2.4	
Smoker	No	111	46.8	147	45.0	0.590
	Yes	67	28.3	86	26.3	
	Ex-	59	24.9	94	28.7	
BCG vaccination	No	117	79.1	153	76.5	0.572
	Yes	31	20.9	47	23.5	
Relevant c-morbidity	No	210	87.5	282	84.4	0.300
	Yes	30	12.5	52	15.6	
Inflammatory bowel disease	CD	171	71.0	178	53.8	< 0.001
	UC	70	29.0	153	46.2	
Disease duration median, years [IQR]		4	1.11	3	0.8	0.185
Baseline CRP	Normal	89	38.2	208	66.2	< 0.001
	2–5 times UNL	99	42.5	76	24.2	
	6–10 times UNL	35	15.0	14	4.5	
	> 10 times UNL	10	4.3	16	5.1	
Steroid therapy	No	187	77.0	269	80.0	0.406
	Yes	56	23.0	68	20.0	
Immunosuppressant therapy	No	89	36.6	218	64.7	< 0.001
	Yes	154	63.4	119	35.3	
Immunosuppressant, drug	Azathioprine	119	77.8	96	80.0	0.053
	Mercaptopurine	18	11.8	20	16.7	
	Methotrexate	16	10.5	4	3.3	
Steroid or/and IM therapy	None	59	24.3	181	53.7	< 0.001
	Steroids alone	30	12.3	37	11.0	
	IM alone	128	52.7	88	26.1	
	Both	26	10.7	31	9.2	
Beclometasone dipropionate	No	235	97.1	306	93.0	0.030
	Yes	7	2.9	23	7.0	
Budesonide	No	222	92.5	295	88.9	0.145
	Yes	18	7.5	37	11.1	
5-aminosalicylates	No	150	62.0	149	44.5	< 0.001
	Yes	92	38.0	186	55.5	

M/F, male/female; LTBI, latent TB infection; TNF, tumour necrosis factor; SD, standard deviation; TB, tuberculosis; BCG, *Bacille Calmette-Guérin*; CD, Crohn´s disease; UC, ulcerative colitis; IQR, interquartile range; CRP, C-reactive protein; ULN, upper limit of normal; IM, immunosuppressant [azathioprine, mercaptopurine, or methotrexate].

and 5-aminosalicylates] was significantly higher in control patients vs anti-TNF group patients [AOR 3.55, 95% CI 1.01–12.7; $p = 0.041$]. Exposure to BCG was not associated with positive TST among patients who were not on steroids and/or immunosuppressant therapy.

4. Discussion

Improvement in the accuracy of tests for the diagnosis of LTBI is crucial in countries such as Spain where, despite preventive action, new cases of active TB still occur in IMID patients on anti-TNF therapy. In our study, which included 243 IBD patients selected for anti-TNF therapy and 337 IBD control patients, we found that the sensitivity of TST is severely affected if the test is performed immediately before the initiation of TNF therapy, as mandated by Spanish recommendations for LTBI screening. Our data indicate that performing the TST at an early stage [before scheduling anti-TNF therapy and in the absence of steroids and/or immunosuppressant therapy] increases the likelihood of detecting LTBI in IBD patients.

A unified consensus statement regarding preventive actions before starting therapy with TNF antagonists is not available. Guidelines for screening for LTBI in patients on anti-TNF therapy

Table 2. Univariate analysis of factors associated with results of the tuberculin skin test.

		+ TST [N]	Prevalence [%]	Total [N]	OR	95% CI		*p*-Value
Group	Anti-TNF	28	11.5	243	0.44	0.28	0.70	< 0.001
	Control	77	22.8	337	1			
Sex [M/F]	Male	66	20.6	320	0.68	0.44	1.05	0.084
	Female	39	15.1	259	1			
Age, years	< = 31	7	4.9	143	1			< 0.001
	32–41	17	11.4	149	2.50	1.00	6.23	
	42–55	44	27.7	159	7.43	3.22	17.14	
	> 55	30	25.9	116	6.78	2.85	16.11	
Any risk factors for LTBI	0	41	15.6	263	1			0.149
	1	57	19.4	294	1.30	0.84	2.02	
	≥ 2	7	30.4	23	2.37	0.92	6.12	
History of TB contact	No	97	17.6	551	1			0.118
	Yes	7	30.4	23	2.05	0.82	5.11	
TB contact, past year	No	102	18.1	565	1			0.452
	Yes	1	33.3	3	2.27	0.20	25.27	
Stay > 3months TB endemic area	No	100	17.8	563	1			0.071
	Yes	4	40.0	10	3.09	0.86	11.14	
Smoker	No	40	15.5	258	1			0.218
	Yes	26	17.0	153	1.12	0.65	1.91	
	Ex-	34	22.2	153	1.56	0.94	2.59	
BCG vaccination	No	31	11.5	270	1			< 0.001
	Yes	23	29.5	78	3.22	1.74	5.96	
Relevant comorbidity	No	84	17.1	492	1			0.111
	Yes	20	24.4	82	1.57	0.9	2.73	
Inflammatory bowel disease	CD	52	14.9	349	1			0.008
	UC	53	23.8	223	1.78	1.16	2.73	
Chest X-ray suggestive LTBI	No	99	17.5	567	1			0.443
	Yes	2	28.6	7	1.89	0.36	9.89	
Baseline CRP	Normal	63	21.2	297	1			0.030
	2–5 times UNL	30	17.1	175	0.77	0.47	1.24	
	6–10 times UNL	6	12.2	49	0.52	0.21	1.27	
	> 10 times UNL	0	0.0	26	NE		.	
Steroid therapy	No	92	20.2	456	1			0.013
	Yes	13	10.5	124	0.52	0.28	0.96	
Immunosuppressant therapy	No	75	25.0	300	1			< 0.001
	Yes	28	10.3	273	0.46	0.25	0.86	
Steroid or/and IM therapy	None	66	64.1	233	1			< 0.001
	Steroids alone	9	8.7	67	0.39	0.18	0.84	
	IM alone	24	23.3	216	0.32	0.19	0.53	
	Both	4	3.9	57	0.19	0.07	0.55	
Beclometasone	No	96	17.0	566	1			0.086
	Yes	9	29.0	31	2.00	0.89	4.48	
Budesonide	No	92	17.8	517	1			0.686
	Yes	11	20.0	55	1.16	0.58	2.32	
5-aminosalicylates	No	38	12.7	299	1			< 0.001
	Yes	67	24.1	278	2.18	1.41	3.38	

M/F, male/female; TST, tuberculin skin test; OR, odds ratio; CI, confidence interval; LTBI, latent tuberculosis infection; BCG, *Bacille Calmette-Guérin*; CD, Crohn´s disease; UC, ulcerative colitis; IQR, interquartile range; CRP, C-reactive protein; UNL, upper normal level; IM, immunosuppressant [azathioprine, mercaptopurine, or methotrexate].

differ significantly between countries because they take into account local TB rates and other factors. In Spain, with a relative high risk of active TB in IMID patients under anti-TNF therapy,[14] a preventive strategy [which included careful history, a two-step TST, and a chest radiograph] was associated with a 78% decrease in active TB in rheumatic patients receiving TNF antagonists.[4,15] However, new cases of active TB still occur in anti-TNF treated IBD patients despite compliance with recommended preventive measures.[16,17]

One difficulty in evaluating performance of LTBI screening methods is the lack of a gold standard for defining LTBI. Ultimately, the aim is to prevent progression to active TB, but this event is too infrequent in IBD patients following current preventive recommendations to be useful as an endpoint in studies of performance of LTBI screening. The only way to assess the specificity of test is therefore to assess association of positive results with classic risk factors of LTBI.[18] In our patients, positive TST was independently associated with higher age, which supports the robustness of our results. We also found that male patients or patients with risk factors for LTBI showed a trend towards a higher rate of positive TST. BCG vaccination correlated with positive TST in univariate analysis, but this effect depended on age in the multivariate analysis. The age of the BCG-vaccinated population was significantly higher than the age of the unvaccinated population. The difference in age completely explained the higher rate of positive TST in the BCG-exposed population. BCG exposure

Table 3. Multivariate analysis of factors associated with results of the tuberculin skin test.

	Adjusted odds ratio	95% CI		*p*-Value
Age, years				< 0.001
≤ 31	Reference			
32–41	2.94	1.11	7.81	0.033
42–55	9.93	4.00	24.63	< 0.001
> 55	7.74	3.04	19.71	< 0.001
Steroid and/or immunosuppressant therapy				< 0.001
None	Reference			
Steroids alone	0.36	0.16	0.83	0.016
Immunosuppressants alone	0.36	0.21	0.62	< 0.001
Both	0.20	0.07	0.59	0.004
5-aminosalicylates	1.86	1.14	3.05	0.013

Candidate variables: group, age, inflammatory bowel disease, *Bacille Calmette-Guérin* vaccination, baseline C-reactive protein, steroid and/or immunosuppressant therapy, beclometasone, 5-aminosalicylates.

CI, confidence interval.

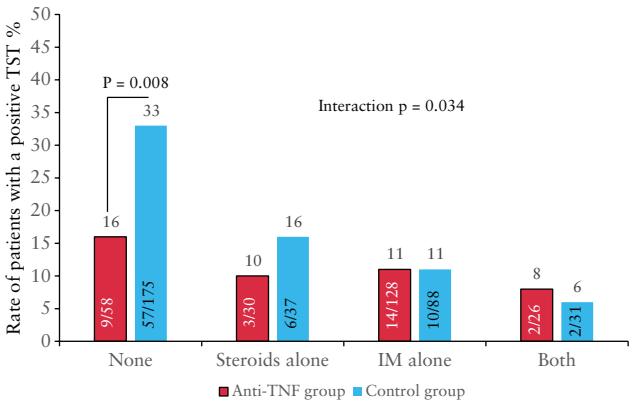

Figure 1. Proportion of patients with a positive tuberculin skin test (TST) by steroids or/and immunosuppressant (IM) therapy stratified according to study group. TNF: tumour necrosis factor.

did not modify the effect of age and steroid and/or IMM treatments on the TST results. Therefore BCG vaccination did not influence TST results in our study. This is to be expected, given that BCG was administered during the first year of life and that the interval between vaccination and TST was longer than 15 years in all patients.[19,20]

Lack of accuracy of LTBI screening methods could be associated with incident cases of active TB after a negative screening. In the high-risk setting resulting from the use of immunosuppressive therapies in a population with high rates of LTBI, implementation of strategies to increase sensitivity of LTBI screening would seem appropriate. To maximise TST sensitivity, we used a two-step TST with 5-mm threshold. In our study, LTBI was detected by a 'booster' TST retest in 25% of patients, which is a higher rate than in a previous study in IBD patients in Spain [14%].[21] Importantly, rheumatologists in Spain found that new cases of active TB occur due to lack of compliance with screening recommendations, with an inadequate two-step TST procedure being a major pitfall.[15] Therefore in Spain, a positive result in the two-step TST is a strong indication for prophylaxis in patients scheduled for anti-TNF therapy, regardless of BCG vaccination history.

Current therapies for IMID include immunosuppressive medications that may affect T cell function, resulting in anergy to TST and other skin tests.[5] Unfortunately, individuals for whom the TST has limited sensitivity are often the patients at increased risk of progression to active TB if infected. Therefore, minimising the influence of factors known to adversely affect TST is a critical factor in the performance of the test for LTBI.[22] In the present study, we clearly demonstrate that results from TST are severely affected by steroids or immunosuppressant therapy, leading to a lower rate of positive TST. Of note, patients treated with both steroids and immunosuppressant therapy had an 80% reduction in the rate of positive TST. Previous studies assessing the effect of immunosuppressants on TST results in IBD patients have been contradictory, reporting either that TST results are negatively affected[23] or not affected by immunosuppressant therapy.[24–26] Conversely, the negative influence of steroids on TST performance has been demonstrated in several studies.[6,27,28]

In our study, the likelihood of a positive TST was almost double in the control group ['early TST', regardless of duration of disease] than in the anti-TNF group ['routine TST']. This difference is partially explained by the greater proportion of anti-TNF group patients who were receiving immunosuppressants. Although immunosuppressant therapy equally affects the performance of the TST in both cohorts, 63% of anti-TNF group patients vs 35% of controls were on immunosuppressants at the time the TST was performed [$p < 0.001$].The problem is that in clinical practice, IBD patient candidates for anti-TNF therapy are too severely ill to stop steroids and/or immunosuppressants before anti-TNF therapy is started. On the other hand, in the subpopulation without steroid or immunosuppressant therapy, the likelihood of positive TST was also more than twice in control patients that in anti-TNF group patients. This difference is partly explained by anti-TNF group patients having almost double the proportion of elevated CRP levels compared with control patients. Among patients without steroid or immunosuppressant therapy, those with elevated CRP had a non-significant 32% reduction in the likelihood of positive TST. None of the 26 patients with a CRP level > 10 times the upper normal level had a positive TST. Significant depression of delayed-type hypersensitivity response to skin antigens has been associated with systemic inflammation measured by erythrocyte sedimentation rate in patients with rheumatoid arthritis.[29] To our knowledge this study is the first to link CRP with decreased response to TST.

Therefore, to maximise sensitivity, TST should preferably be performed in the absence of immunosuppressive treatment and/or significant systemic inflammation. The sensitivity of TST is highest in patients who meet the above criteria and who are treated with 5-aminosalicylates, and this occurs more frequently in the control

patients. Among patients with no steroid and/or immunosuppressant therapy and with a normal CRP, the likelihood of positive TST [adjusted by age] was also significantly higher in control patients. Therefore lower age, immunosuppressive therapy, or inflammatory activity do not completely explain the lower rate of positive TST in the anti-TNF group. In previous studies, scarcely known factors inherent to IMID by itself, or with associated malnutrition, negatively affected TST performance.[26,30,31]

It has been suggested that screening for LTBI in IMID patients should be performed early, preferably before initiation of any steroid or immunosuppressant therapy,[9,24] but this strategy has never been evaluated. To our knowledge, our study is the first to demonstrate that performing TST before scheduling anti-TNF therapy increased sensitivity in the detection of LTBI in IBD patients. We should expect a high rate of false-negative results if we perform the 'routine TST' just before initiation of TNF antagonists, because at that time the vast majority of patients are inevitably receiving immunosuppressive drugs and/or have a high inflammatory activity. By contrast, 'early TST' can be performed either at diagnosis before starting immunosuppressive therapy, or at any time in the disease after steroids have been completely tapered.

In our study we have not included an interferon-γ release assay [IGRA] since, when the study was designed, it was not part of Spanish recommendations for screening LTBI before starting anti-TNF therapy.[4,15,21] IGRAs could not replace two-step TST since no study has demonstrated benefit of LTBI treatment based on IGRA-positive results in our setting, and therefore it would be unethical to withhold prophylaxis in TST-positive and IGRA-negative patients.[15,25,32] In addition, the sensitivity of IGRAs also seems to be significantly affected by immunosuppressive therapy,[9,10,24,25] and a study evaluating the performance of 'early IGRA' would be desirable. Given the low sensitivity of both TST and IGRAs, new diagnostic strategies need to be evaluated.[33] Several studies have shown that diagnostic performance for LTBI in IMID improves if an IGRA is used in addition to TST.[10,34-36] Therefore, in patients with TB risk factors such as immunosuppressant use and increased risk of progression from infection to disease, a dual strategy based on both TST and IGRA would seem to improve diagnostic yield and should be recommended.[18,22,32] Indeed, two recent guidelines recommended that a dual strategy of TST and IGRA should be pursued in our setting.[37,38] Recommendations in Spain for screening LTBI in patients before anti-TNF therapy may not necessarily apply to countries with lower incidence of TB.

Our study was not designed to guide the approach to a positive TST. TST is designed to identify an adaptive immune response against, but not necessarily a latent infection with, *M. tuberculosis*. Therefore a majority of individuals with positive TST results had no viable TB bacilli and would not progress to active disease.[39] Considering also that TB preventive treatment is not free of adverse effects, therapy should be restricted to patients who are known to be at higher risk of developing TB.[40] Thus in our setting, all patients scheduled for anti-TNF therapy who had a positive TST should receive preventive therapy, regardless of BCG vaccination history. Conversely, since only a minority of control group patients will need anti-TNF therapy during their lives, there is a risk of overtreating a majority of this population. Furthermore, giving prophylaxis after a positive 'early TST' protects against LBTI reactivation but not against TB contacts in the interval between screening and initiation of anti-TNF therapy. Therefore, in case of a positive 'early TST', it seems advisable to delay preventive therapy until the patient requires biological treatment. All patients with a negative 'early TST' who finally need anti-TNF therapy should be completely rescreened before initiation of treatment.

Our study had other limitations. The lack of a gold standard for LTBI diagnosis makes it impossible to know the actual accuracy of two-step TST. On the other hand, we have not recorded body mass index, albumin, or pre-albumin values, and therefore cannot assess the influence of nutritional status on TST results. Finally, assessment of inflammatory activity based solely on CRP levels is weak, and the study is not powered to detect differences based on CRP in the subpopulation of patients without immunosuppressive treatment.

In conclusion, steroid and/or immunosuppressant therapy had a pronounced negative effect on the rate of positive two-step TST in IBD patients. Since most patients scheduled for anti-TNF therapy are already receiving steroids and/or immunosuppressants, we should expect a high rate of false-negative results if we perform the 'routine TST' just before initiation of TNF antagonists. 'Early TST', performed before planning anti-TNF therapy, allows us to screen patients in the absence of immunosuppressive treatment and/or significant inflammatory activity and thus maximize the sensitivity of TST for detecting LTBI in IBD patients. 'Booster' TST retesting increases sensitivity for detecting LTBI by 20%.

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Conflict of Interest

CT has served as a speaker, a consultant, and advisory member for or has received research funding from MSD, Abbvie, Hospira, Pfizer, Takeda, Ferring, Faes Farma, Shire Pharmaceuticals, Dr Falk Pharma, and Gebro Pharma. FB has served as a speaker, a consultant, and advisory member for or has received research funding from MSD, Abbvie, Hospira, Pfizer, Takeda, Ferring, Faes Farma, Shire Pharma, Tillotts Pharma, Chiesi, and Gebro Pharma. MDM has served as a speaker, a consultant, and advisory member for MSD, Abbvie, Takeda, Janssen, Ferring, Faes Farma, Shire Pharmaceutical, Tillots Pharma, and Chiesi. JLC has served as a consultant for or has received research funding from MSD, Abbvie, Kern Pharma, Pfizer, and Takeda. MBA has served as a speaker, a consultant, and advisory member for or has received research funding from MSD, Abbvie, Hospira, Pfizer, Kern Pharma, Biogen, Takeda, Ferring, Faes Farma, Shire Pharmaceuticals, Dr Falk Pharma, Tillotts Pharma, Chiesi, Gebro Pharma, Otsuka Pharmaceutical, and Vifor Pharma. EI has served as a speaker and consultant for MSD and AbbVie. IMJ has served as a speaker, a consultant, and advisory member for or has received research funding from MSD, Abbvie, Hospira, Takeda, Janssen, Ferring, Faes Farma, Shire Pharmaceuticals, Dr Falk Pharma, Chiesi, Gebro Pharma, Otsuka Pharmaceuticals, Astrazeneca, and Tillotts Pharma. MVD has served as a speaker for AbbVie, Takeda, and Shire. JPG has served as a speaker, a consultant, and advisory member for or has received research funding from MSD, Abbvie, Hospira, Pfizer, Kern Pharma, Biogen, Takeda, Janssen, Roche, Ferring, Faes Farma, Shire Pharmaceuticals, Dr Falk Pharma, Tillotts Pharma, Chiesi, Casen Fleet, Gebro Pharma, Otsuka Pharmaceutical, and Vifor Pharma. The remaining authors declare that they have nothing to disclose.

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Author Contributions

CT designed and carried out the study, and drafted the manuscript. FB, SR, and JPG designed the study, and contributed by critical revision of the manuscript.

IMJ treated study patients, acquired data, and contributed by critical revision of the manuscript. DO performed statistical analyses, acquired data, and contributed by critical revision of the manuscript. The remaining authors and collaborators treated study patients and also acquired data. All authors read and approved the final manuscript.

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