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ORIGINAL ARTICLE

Evaluation of an interferon gamma assay in the diagnosis of latent tuberculosis infection in patients with rheumatoid arthritis

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Abstract The tuberculin skin test is not an ideal screening test for the patients with rheumatoid arthritis to identify cases of latent tuberculosis infection (LTBI) prior to the start of treatment with anti-TNFs, as it responds inadequately to late hypersensitivity, which is fundamental for producing a response to the inoculated antigen. Assays based on detection of the production of IFNγ in vitro by mononuclear peripheral cells stimulated by specific antigens are more specific than PPD in detecting LTBI. The aim of this study was to evaluate the performance of T-SPOT.*TB* in diagnosis of LTBI in patients with rheumatoid arthritis, comparing with the PPD. The specificity of the T-SPOT.*TB* varied from 87 to 90% and the negative-predictive value (NPV) from 94.4 to 100%. It can be concluded that the T-SPOT.*TB* showed high specificity and NPV, proving the capability of identifying false-negative cases of PPD, raising the level of safety for the use of anti-TNFs.

Keywords Rheumatoid arthritis · Tuberculosis · Diagnosis · Interferon-gamma assay

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Introduction

Given that PPD is not the ideal test for identification of cases of LTBI in patients with RA [1–3], the development of alternative tests for the identification of LTBI has been the object of countless studies. Evidence suggests that assays based on the detection of IFNγ perform better than PPD, as they have a high specificity, correlate better with indirect measures of exposure to *M. tuberculosis* and are less likely to present a cross-reaction with vaccination using BCG and other microbacterial infections [4–6].

This study aims to evaluate the performance of the T-SPOT.*TB* test in the diagnosis of LTBI, comparing it with PPD in patients with RA, by measuring its sensitivity, specificity, positive-predictive value (PPV) and negative-predictive value (NPV).

Methodology

A cross-cutting study was carried out to evaluate the diagnostic test. The study involved 96 patients selected by non-probabilistic sampling, according to convenience, divided into two groups: 48 diagnosed with rheumatoid arthritis according to the ACR criteria [7] (RA group) and 48 healthy individuals, who made up the comparison group (COMP group), between May and October 2007, selected from patients attending the outpatient Rheumatology Unit of the Clinical Hospital of the Federal University of Pernambuco (HC-UFPE). In order to be included in the study, patients with RA had to be over 18 years of age, to have the active form of the disease and to have been recommended for the use of infliximab. Exclusion criteria included: active tuberculosis infection; BCG vaccination within the past 15 years; prior treatment using biological drugs (infliximab,

etanercept or adalimumab); a known diagnosis of other diseases that are considered risk factors for TB, such as AIDS, malnutrition, diabetes, kidney or liver disease, and neoplasia; acute infections; patients in hospital; and pregnant women. The comparison group was made up of patients selected from the outpatient rheumatology unit who did not show signs of auto-immune disease, according to the same exclusion criteria used with the RA group.

The study was approved by the Ethics Committee for Research on Human Beings of the Aggeu Magalhães Research Centre (CPqAM–FIOCRUZ) and all patients read and signed the terms of free, informed consent before undergoing any procedure.

After filling in a clinical form with data on their epidemiological history of TB and RA activity, 8 mL of venous blood was collected by the way of percutaneous punction, for the purposes of conducting the T-SPOT.*TB* test. After the collection of the blood samples, the patients were inoculated with 0.1 ml (2UT) of PPD RT-23, using the Mantoux technique [8], in the middle third of the left forearm (approximately 8 cm below the elbow). The reading of the PPD result was carried out 72 h after application, by way of palpation of maximum transverse diameter of induration, the result being expressed in millimeters. The reading was carried out by a single examiner for all patients. The response to the PPD was analyzed as follows: the RA group: 0–4 mm, negative, ≥5 mm, positive; the COMP group: 0–4 mm, negative; 5–10 mm, weak reaction; over 10 mm, strong reaction. All patients were submitted to a chest X-ray.

Carrying out the T-SPOT.TB test

The T SPOT.TB test is a simplified variant of the ELISPOT (enzyme-linked immunospot) technique for determining the effector T cells that secrete IFNγ in response to stimulation by antigens specific to *M. tuberculosis*. The kit for this test has an ELISA format, with individual 8-well strips sensitive to the antibodies for IFN-gamma. It is also accompanied by a tube containing a solution of the ESAT-6 antigen (Panel A), a tube with a solution of CFP-10 antigen (Panel B), a tube containing a solution of phytohemagglutinin (PHA) which functions as a positive control for the test. A total of four wells are needed for each sample. All the stages in the test followed the recommendations of the manufacturers. The colored spots identified on the plate after application of a colorimetric substrate were counted using the Image Acquisition program and CTL-Immunospot (BD Bioscience). The test was considered to be positive when panel A or B showed a reaction, according to the following criteria (Fig. 1).

- In cases in which the negative control presented from 0 to 5 spots:

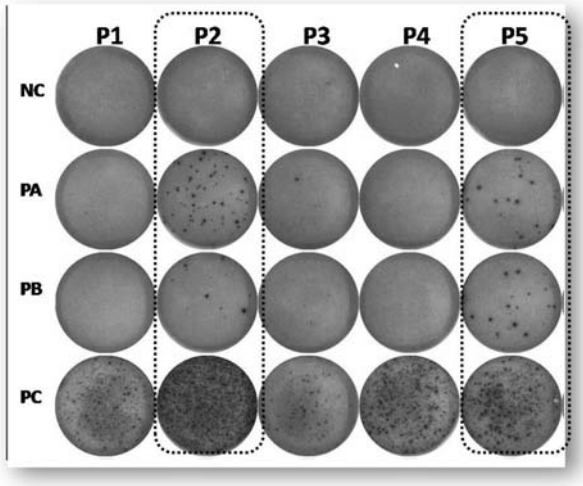

Fig. 1 Example of a T-SPOT.*TB* plate showing the test of five patients at the vertical lines. The results of the patients P2 and P5 were positive, where we can see more than five spots at Panel A (*PA*) and/or Panel B (*PB*). *NC* negative control, *PC* positive control

Panel count (A or B) – Negative control count ≥6

- In cases in which the negative control presented more than five spots:

Panel count (A or B) ≥twice the negative control count

Statistical analysis

Because the new test (T-SPOT.*TB*) is in theory, should be better than the gold standard, the PPD is classified as an "imperfect gold standard", which makes it difficult to calculate the sensibility and specificity of the new test. To minimize the problem, we opted for the use of a Standard Reference Composition technique, which involves creating a more adequate standard using a combination of the results of various imperfect tests [9].

In this way, for the statistical analysis, we set up four different models to determine whether a patient is a carrier of LTBI:

1. The PPD result (positive when ≥5 mm in the RA group and ≥10 mm in the COMP group)
2. A positive PPD + abnormalities in the chest X-ray compatible with LTBI
3. A positive PPD + contact in the home with active TB
4. A positive PPD + abnormalities in the X-ray + contact in the home.

After classification of patients, according to our "gold standard", into positives (carriers of LTBI) or negatives (absence of LTBI), was calculate the sensitivity, specificity, PPV and NPV for the new test (T SPOT.*TB*), for each of the four models used, using the Qui-square test, with a confidence interval of 95%.

Results

In the RA group, the average age was 49.71 years, while in the COMP group the average was 46.29 years and in both groups, a majority of individuals were female. Table 1 summarizes the clinical variables for the RA group.

The frequencies for the other variables, as well as the comparative analysis of the equivalence of the two groups are shown in Table 2. The two groups showed similar characteristics, there being no statistically significant difference for the age, sex, skin color, level of schooling, agglomeration, history of tuberculosis infection or contact with tuberculosis in the home. Only in the case of income and place of origin was a statistically significant difference observed between the two groups ($P = 0.017$ and $P = 0.034$, respectively). It should be pointed out that the comparison group included health professionals who were working at the rheumatology outpatient unit of the HC, due to the risk of latent tuberculosis infection (LTBI) associated with this group.

All patients in both groups had a history of BCG vaccination in infancy, which was confirmed by the presence of a scar on the right arm and none of them had a history of alcoholism. Of the 48 patients in the RA group, 25% tested positive using the T-SPOT.TB, while only 14% tested positive when PPD was used in isolation. In the COMP group, on the other hand, 35% of results were found to be positive using the PPD in isolation and 18% using the T-SPOT.*TB*. As can be seen in Table 3, the frequency of diagnosis of LTBI for PPD in isolation in the RA group was lower than that found in the COMP group, although difference was not found when the diagnosis was carried out using the T-SPOT.*TB*.

Sensitivity and specificity

Tables 4 and 5 allow us to compare the results for sensitivity, specificity, and positive and NPV of the four models used with the gold standard in the two groups.

Table 1 Description of clinical variables for the RA group

Variables	Mean ± DP	Minimum	Maximum
Age	49.71 ± 12.41	19	78
Time since diagnosis (years)	10.2 ± 7.2	1	35
MTX dose, in mg/week ($n = 30$)	15.5 ± 4.3	10	25
Prednisone dose (mg/day)	12.7 ± 6.7	5	30
Duration of use of prednisone (months)	38.0 ± 42.8	1	180
Disease activity (CDAI)	30.4 ± 16.9	0	76

MTX methotrexate, *CDAI* clinical disease activity index [10]

Table 2 Characteristics of individuals with and without rheumatoid arthritis and analysis of equivalence of the two groups

Variables	Groups				Total	
	RA		COMP		*N*	%
	N	%	*N*	%		
Sex					$P = 0.067$	
Female	43	89.6	35	72.9	78	81.3
Male	05	10.4	13	27.1	18	18.8
Age group					$P = 0.056$	
Under 40	08	16.7	18	37.5	26	27.1
40–59	31	64.6	21	43.8	52	54.2
60 and over	09	18.8	09	18.8	18	18.8
Place of origin					$P = 0.031$	
Recife and metropolitan region	32	66.7	41	85.4	73	76.0
Zona da Mata, Agreste and Sertão Regions	16	33.2	7	14.5	23	24.0
Skin color					$P = 0.512$	
White	14	29.2	18	37.5	32	33.3
Black	07	14.6	04	8.3	11	11.5
Mixed	27	56.3	26	54.2	53	55.2
Level of schooling					$P = 0.100$	
University	02	4.2	09	18.8	11	11.5
Completed secondary school	14	29.2	18	37.5	32	33.3
Secondary school incomplete	08	16.7	07	14.6	15	15.6
Completed primary school	14	29.2	09	18.8	23	24.0
Illiterate or barely literate	10	20.9	05	10.5	15	15.6
Income (in minimum wages)					$P = 0.017$	
<1	17	35.4	07	14.6	24	25.0
1 to <3	24	50.0	23	47.9	47	49.0
3 to <5	05	10.4	08	16.7	13	13.5
Over five	02	4.1	10	20.8	12	12.6
Agglomeration					$P = 0.99$	
2 individuals	41	85.4	42	87.5	83	86.5
3 or more individuals	07	14.6	06	12.6	13	13.6
History of TB					$P = 0.677$	
No	44	91.7	46	95.8	90	93.8
Yes	04	8.3	02	4.2	06	6.2
History of contact with TB						
No	47	97.9	46	95.8	93	96.9
Yes	01	2.1	02	4.2	03	3.1
Total	48	100.0	48	100.0	96	100.0

Analysis of univariate association

Comparisons of association were carried out for all the variables under study, in both groups, and none of these was significantly associated with diagnosis of LTBI using T-SPOT.*TB* or using any other of the models used as a gold

Table 3 Comparison of the frequency of diagnoses of LTBI using PPD with that using T-SPOT.*TB* in the groups with and without RA

LTBI using PPD	Frequency (%)	OR (IC)	*P* value
			0.034
AR	14.6	0.31 (0.11–0.84)	
COMP	33.3	1.0	
LTBI using T-SPOT.*TB*	Frequency (%)	OR (IC)	*P* value
			0.4602
AR	25.0	1.44 (0.54–3.83)	
COMP	18.8	1.0	

standard. This suggests that there is no stratification for analysis of sensitivity and specificity.

Discussion

Although widely accepted, the screening procedures carried out before the use of anti-TNFs have been criticized, since, in various situations, they are not able to identify LTBI. Problems described include the possible uncertainty on the part of the patient as to his or her medical history of contact with TB, the absence of specific radiographic signs for LTBI, as well as difficulties in using PPD. Thus, the use of these tests may lead to a failure to carry out chemoprophylaxis using INH, due to the low sensitivity of the skin test in a patient with RA or even to unnecessary treatment (in the case of a false-positive PPD) [11].

The use of antigen-specific tests for the detection of INFγ has revolutionized the diagnosis of LTBI in countries with a low prevalence of TB, and greater specificity than PPD has already been shown in immunocompetent individuals [11, 12].

The evaluation of the performance of these new tests was flawed by the lack of a gold standard to differentiate between true latent infection and a cross-reaction with the BCG vaccine or with infection by microbacteria other than *M. tuberculosis* [13]. In order to overcome this imperfect gold standard problem, studies that analyzed the performance of these tests used varying techniques to determine the presence of LTBI [14, 15].

The present study is the first to evaluate the performance of the T-SPOT.*TB* in Brazil in patients with RA. The use of these new tests to screen RA patients before the use of anti-TNFs has already occurred in some European countries, principally in cases where there is a negative PPD and radiographic abnormalities suggesting latent infection [16].

Our aim in setting up four different models for identification of latent infection was to minimize the problem created by the imperfect gold standard. We aggregated the results of various imperfect tests in order to improve the overall performance in identification of such patients. According to our results, irrespective of which model was used, the figures for sensitivity, specificity, PPV and NPV were fairly similar for both groups (RA and COMP), with a high specificity in the RA group and NPV. This results give us reason to suggest that, in our sample, the T-SPOT.*TB* is more useful in identifying false negative PPD results (12 positives for the T-SPOT.*TB* compared with 7 for the PPD), owing to its higher level of specificity. Apart from this, it is able to confirm with a higher degree of certainty, in cases where the PPD result is negative, that LTBI is indeed absent.

Our results are similar to those already described in the literature, although it is worth pointing out that none of the studies published hitherto used a similar methodology. These similarities are more worthy of note when we compare studies carried out in countries with a high incidence of TB and a broad vaccination program [11, 12].

Two patients in our study who showed radiological abnormalities suggesting LTBI tested negative using the

Table 4 Comparison of sensitivity, specificity, PPV and NPV of the four models adopted with the gold standard in the RA group

T-SPOT.*TB*	Model 1	Model 2	Model 3	Model 4
Sensitivity (%)	100.0 (59.0–100.0)	77.8 (40.2–96.1)	100.0 (59.8–100.0)	80.0 (44.2–96.5)
Specificity (%)	87.0 (73.0–95.0)	87.2 (71.8–95.2)	90.0 (75.4–96.7)	89.5 (74.3–96.6)
PPV (%)	58.0 (27.0–84.0)	58.3 (28.6–83.5)	66.0 (35.4–88.7)	66.7 (36.4–86.7)
NPV (%)	100.0 (90.0–100.0)	94.4 (88.0–99.0)	100.0 (88.0–100.0)	94.4 (80.0–99.0)

Table 5 Comparison of sensitivity, specificity, PPV and NPV of the four models adopted with the gold standard in the COMP group

T-SPOT.*TB*	Model 1	Model 2	Model 3	Model 4
Sensitivity (%)	23.0 (6.0–49.0)	26.0 (9.0–51.0)	22.0 (7.4–48.1)	25.0 (8.0–49.0)
Specificity (%)	83.0 (66.0–94.0)	86.0 (68.0–96.0)	83.3 (64.5–93.7)	85.0 (67.0–95.0)
PPV (%)	44.0 (13.0–78.0)	55.0 (71.0–94.0)	44.4 (15.3–77.3)	55.0 (21.0–86.0)
NPV (%)	65.0 (48.0–79.0)	64.0 (47.0–78.0)	64.1 (47.2–78.3)	61.0 (44.0–76.0)

T-SPOT.*TB*, which leads us to wonder whether these abnormalities really were related to latent tuberculosis, as, according to the data presented in the literature, the results of the T-SPOT.*TB* show greater specificity than radiological abnormalities [17]. This could be explained by the low-sensitivity of the X-ray in detecting small lesions, or even, by the lack of correlation between the immunological response to antigens specific for TB (including PPD) and the extent of pulmonary lesions [17, 18].

Despite this, the possibility that the presence of LTBI was under-estimated cannot be ruled out. In studies carried out among healthy individuals in Korea and Gambia, which are areas where TB is endemic, sensitivity was low compared with the known overall prevalence of TB in these regions [19, 20]. This low sensitivity may be related to the fact that, although specific to *M. tuberculosis*, these antigens do not represent the whole range of antigens for the TB bacillus. Furthermore, in cases of short periods of exposure, memory T cells are not capable of releasing sufficient quantities of INFγ to give rise to a positive result for an ex vivo test [15, 16].

It is interesting to note that, when we used PPD in isolation to identify LTBI, the frequency was higher in the COMP group than in the RA group. This may give the false impression that RA provides protection against LTBI (OR = 0.31; 0.11–0.84, $P = 0.034$), probably as a result of the low responsiveness of PPD in people with RA or the number of false-positive results obtained using PPD (cross-reaction with other microbacteria or with BCG vaccine), such as did not occur when we used the T-SPOT.*TB,* probably owing to its greater specificity.

In the COMP group, the results for sensitivity were fairly low (under 30%) in all four models. This may be explained by the fact that a positive result for PPD and/or X-ray and/or history of contact is much more likely in this group, whichever model is used (17, 19, 18 and 20 cases for models 1, 2, 3 and 4, respectively). Analysis of the results of the T-SPOT.*TB* shows that the numbers of positive results are lower (9 for all models). This probably indicates the presence of false-positive results, mainly due to the use of PPD, which tested positive when used in isolation on 17 occasions. In this case, the PPD shows greater sensitivity, although it is not capable of determining the presence of real latent infection.

In all the studies carried out to evaluate the performance of the T-SPOT.*TB*, the great advantage was shown to be its high level of specificity and NPV, allowing us to be certain that the PPD result was negative, there is no latent infection and, therefore, there is no risk of active infection during the use of anti-TNFs. Furthermore, it identifies patients with RA who present false negative results for the PPD. Our study produced similar results, since, depending on the model used, 4 (models 3 and 4) or 5 patients (models 1 and 2) were not identified using our gold standard but tested positive using the T-SPOT.*TB*. These results could lead us to the interpretation that these are cases of false positives. However, as recombinant antigens are specific to *M. tuberculosis,* these are probably cases of false negatives for PPD. Had these patients been screened using conventional methods (PPD, chest X-ray and clinical history), they would have been classified as normal and would be at great risk of developing active infection.

Although diagnosing and treating LTBI in developed countries is an essential component of the control of TB, this strategy is of less importance in developing countries. In these countries, owing to the high risk of infection, due to the high incidence and the fact that it affects the pediatric population, the health system's priority should be the treatment of active cases, which leads to a reduction in the risk of transmission. Furthermore, a substantial proportion of the population in these countries have LTBI and, since the risk of these individuals developing an active infection is 5% and around 90% of chemoprophylaxes are carried out in full, there is no need to add to the cost of diagnosis of LTBI to that of the TB control program in developing countries. Hence, the use of these tests in these localities should be restricted to situations such as epidemiological surveillance, children and malnourished patients with tuberculosis, and tuberculosis infection in patients with AIDS and RA [11].

Conclusions

The sensitivity of the T-SPOT.*TB* is equivalent to that of the gold standard in our population. Thus, in the case of a positive result for PPD, we can dispense with the specific test and carry out chemoprophylaxis using INH before commencing anti-TNF therapy. The T-SPOT.*TB* test is more specific than the PPD, whether it is associated with an X-ray and a clinical history or not. Thus, in cases where the PPD is negative, and the X-ray shows abnormalities or there is a history of contact with TB in the home, the T-SPOT.*TB* test should be carried out to identify probable false-negative results.

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