Attenuated response to PPD in the diagnosis of latent tuberculosis infection in patients with rheumatoid arthritis

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ABSTRACT

Introduction*:** *With the introduction of Tumor Necrosis Factor Inhibitors (anti-TNFs) into rheumatological practice, it has become obligatory to identify cases of latent tuberculosis infection (LTBI) prior to the start of treatment, using PPD, chest radiography and clinical history of tuberculosis contact. Patients with Rheumatoid Arthritis (RA) have an abnormality of the cellular immune function, characterized by decreasing responsiveness of peripheral mononuclear cells (T Reg lymphocytes), leading to a loss in delayed hypersensitivity, which is fundamental for the recognition of antigens, such as PPD.* ***Objectives*: The purpose of our study was to evaluate the response to PPD in patients with RA, compared with healthy people, in an area where tuberculosis is endemic, as is the state of Pernambuco.* ***Methodology:*** *We studied 96 patients, 48 with RA and 48 healthy subjects, most of them females. All patients were given an interdermic injection of 0.1 mL PPD RT-23. The reading of the PPD result was carried out 72 hours after application, by way of palpation of maximum transverse diameter of induration, and the result was expressed in millimeters.* ***Results:*** *In the RA group, the average time of diagnosis was 10.2 years, the average dosage of methotrexate was 15.5 mg / week, the average dosage of prednisone 12.7 mg / day and the average activity of the disease, measured using CDAI, was 30.4. In the healthy subjects group there was a greater number of positive PPD results (33.3%) when compared with the results for the RA group (14.6%), with a statistically significant difference (p = 0.034).* ***Conclusion:*** *The performance of PPB in LTBI diagnosis is poor in patients with RA. These results suggest that more careful screening needs to be undertaken before treatment with an anti-TNF drug.*

Keywords: *rheumatoid arthritis, PPD, latent tuberculosis.*

INTRODUCTION

Due to an increasing incidence and severity of tuberculosis (TB) infections after the introduction of tumor necrosis factor inhibitors (anti-TNFs) into rheumatoid arthritis (RA) treatment, it has become obligatory to identify cases of latent tuberculosis infection (LTBI) prior to the start of treatment. Brazilian guidelines for the performance of LTBI or active disease screening recommend that assessment prior to starting anti-TNF treatment should include: the complete clinical history (previous treatment or chemoprophylaxis, domestic contact or institutional contact with TB), chest radiography and purified protein derivatives (PPD).[1-3] However, PPD use in RA patients presents a major complicating factor: an abnormality in the cellular immune function observed in such patients.[4] There is a decreasead responsiveness of peripheral mononuclear cells, leading to a loss in delayed hypersensitivity, which is fundamental for the recognition of antigens, such as PPD.[4-6] The mechanism for this change is not exactly known, but it has been demonstrated that

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it may be caused by IL-2[7] production deficiency or chronic TNF exposure.[6] Regulatory T cells (T reg) ($CD4^+CD25^+$), which has a fundamental role in autoimmunity prevention, present a reduction in number and function in RA patients,[8] and, as demonstrated in a recent Brazilian paper, the number of T $CD4^+$ (T Reg) cells is directly related to PPD response magnitude.[9]

The purpose of our study was to evaluate the response to PPD in patients with RA, compared with healthy individuals, in an area where tuberculosis is endemic, as is the state of Pernambuco, Brazil.

MATERIAL AND METHODS

A transversal study was carried out, including 96 patients using nonprobability sampling, by convenience type, divided in two groups: 48 patients with RA according to American College of Rheumatology[10] criteria (RA group) indicated for infliximab use and 48 healthy subjects, forming the comparison group (COMP group), from May to October, 2007, selected at the Rheumatology Clinic at Hospital das Clínicas at University of Pernambuco (HC-UFPE), in the state of Pernambuco.

In order to be part of this study, RA patients had to be, at least, 18 years old, with active disease and assigned for treatment with infliximab. The following items were considered as exclusion criteria: active TB; BCG vaccine less than 15 years before; previous treatment using biological agents (infliximab, etanercept or adalimumab); known diagnosis of other diseases considered risk factors for TB: AIDS, malnutrition, diabetes, kidney disease or liver disease and neoplasia; acute infections; inpatients; and pregnant women. The comparison group were patients from the Rheumatology Clinic who did not present autoimmune diseases, meeting the same exclusion criteria for RA group.

This study was approved by Aggeu Magalhães Research Center ethics committee and all patients read and signed the informed consent.

Then patients were given a specific questionnaire, created to establish the epidemic history for TB and RA activity, measured by CDAI (*Clinical Disease Activity Index*).[11] PPD was performed by intradermal injection of 0.1 mL (2UT) of PPD RT-23, in the left mid-arm (approximately 8cm under elbow) (Figure 1).

PPD result measurement was done 72 hours after inoculation, using palpation of maximum induration diameter, with results in millimeters (Figure 2). Measurement was done by only one examiner in all patients. PPD response was analyzed according to the following:

RA group: 0 to 4 mm, negative; ≥ 5, positive

COMP group: 0 to 4 mm, negative; 5 to 10 mm, weak positive; more than 10 mm, strong positive.

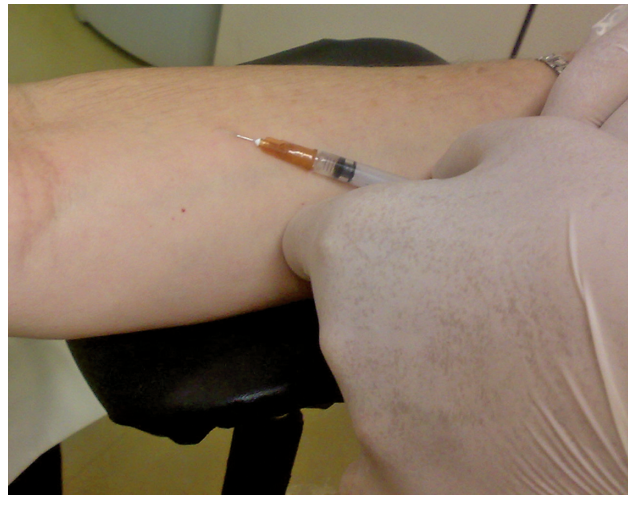

Figure 1. PPD inoculation technique performed approximately 4 fingers under the elbow.

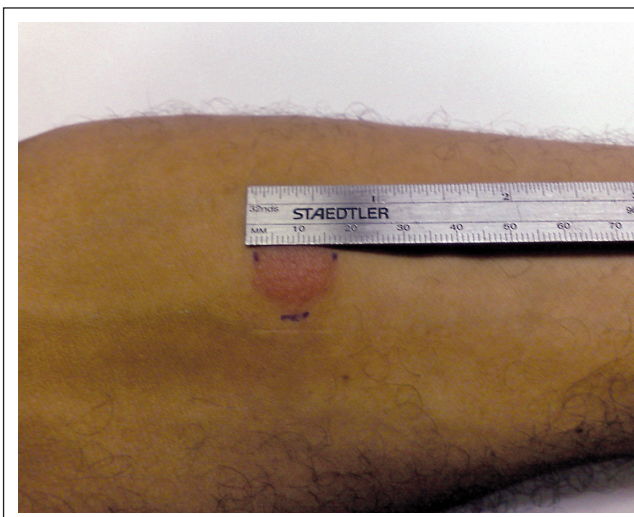

Figure 2. Measurement of PPD in millimeters where induration diameter is larger.

RESULTS

In this study, 96 patients were included, divided into RA group (48) and COMP group (48). Most of them were female in both groups (89.6% in RA group and 72.9% in COMP group). In RA group, mean age was 49.71 years old (±12.41; minimum 19 and maximum 78 years old), while in COMP group it was 46.29 years old (±13.99; minimum 21 and maximum 75 years old).

Average time of RA diagnosis was 10.2 years (±12.41). All patients were taken prednisone, with an average dosage of 12.7 mg/day (±6.7). Out of these 48 patients, only 30 were taking methotrexate (MTX), with an average dosage of 15.5 mg/week (±4.3). Clinical variables in RA group are summarized in Table 1.

Table 1
Clinical variables of the RA group

Variables	Average ± SD	Minimum	Maximum
Age	49.71 ± 12.41	19	78
Disease duration (in years)	10.2 ± 7.2	1	35
MTX dosage in mg/ week (n = 30)	15.5 ± 4.3	10	25
Prednisone dosage (mg/day)	12.7 ± 6.7	5	30
Duration of prednisone use (in months)	38.0 ± 42.8	1	180
Disease's activity (CDAI)	30.4 ± 16.9	0	76

MTX = methotrexate; CDAI = *clinical disease activity index*; SD= standart deviation

Frequencies of other variables, as well as comparative analysis of equivalence in both groups, are demonstrated in Table 2. We can observe that these two groups were similar, and there was no statistically significant difference in gender, race, education level, agglomeration, tuberculosis infection history or domestic transmission tuberculosis contact. Statistically significant differences ($p = 0.017$ and $p = 0.034$ respectively) were observed between the two groups only in income and origin variables. It is important to highlight that in the comparison group health professionals who worked at rheumatology clinic at Hospital das Clínicas were included, due to the latent tuberculosis infection risk that this group presents. All patients in both groups presented BCG vaccination history during childhood, which could be confirmed by a scar in the right arm, and none of them presented alcoholism history. Table 3 shows the frequency of positive PPD in both groups.

No association was found among RA clinical variables, such as duration of use of prednisone or MTX dosage, duration of RA activity or disease measured by CDAI and PPD results.

DISCUSSION

Tuberculin skin test, which uses a standard preparation of purified protein derivative (PPD), is used since 1931 to screen people infected by *M. tuberculosis*. It contains a mixture of antigens that leads to a late hypersensitivity reaction and reflects the cellular immunity against *bacillus*, and, despite its known limitations in sensitivity and specificity, it continues to be used as a standard criterion for LTBI diagnosis. Although it is occasionally used in symptomatic infection diagnosis, its primary use is LTBI detection.[12] However, screening for TB using PPD is discouraging, due to the low specificity in the test, as both BCG vaccine and nonmycobacterium tuberculosis exposure lead to a response similar to the one induced by *M. tuberculosis* infection.[13]

Mantoux method evaluates *in vivo* the immune cellular response against purified protein derivative of *M. tuberculosis,* resulting in a classical reaction of delayed skin hypersensitivity, depending on the migration of INFγ $CD4^+$ T cells to the antigen injection location. Patients with RA should present incapacity to produce a proper PPD response, even in subjects infected with *M. tuberculosis,* causing this test to be inadequate for recognizing latent forms of TB in these patients.[7] Some authors recommended that patients with RA and a negative PPD, but who have a high clinical or epidemiological risk for tuberculosis infection, should be empirically treated as they had LTBI before start using a biological agent.[12]

Our study demonstrates that the frequency LTBI diagnosis by PPD in patients with RA is lower than in healthy individuals, with statistically significant difference (OR = 0.31; 0.11 – 0.84, $p = 0.034$), similar to results already described in the literature, which could provide a false judgment of RA being a protective factor for LTBI. However, this factor probably occurs due to low PPD responsiveness in RA or due to the number of false positive PPD results in healthy individuals (cross reaction with other mycobacterium or BCG vaccine). The fact that we have included subjects who work in the health system did not influence the number of positive results, once that out of 13 people in the 48-person group only 2 presented a positive PPD result, which did not influence the final average of the total positive number in this group.

In a study carried out in Peru,[14] where TB is endemic, PPD was performed in one group with RA patients and another group of immunocompetent volunteers, matched by gender and age. A result higher or equal to 5 mm in RA group and higher or equal to 10 mm in the immunocompetent group was considered positive. A result lower than 5 mm after 72h was considered negative in both groups. A 71% PPD positivity was found in immunocompetent group against 29% in RA group. All patients in RA group were taking dosages lower than 7.5mg/day of corticosteroid, which does not abolish delayed skin hypersensitivity. This discrepancy in results has been associated to RA cellular immunity abnormality. In another study, carried out in Turkey, area where TB prevalence is relatively high, a low PPD positivity (29.8%) was observed in RA patients, while compared to patients with ankylosing spondylitis (65.9%), gouty arthritis (68.8%) and osteoarthritis (63%).[15]

Provenzano, Ferrante e Simon[3] evaluated 69 Italian patients with chronic inflammatory joint disease, who would start anti-TNF treatment. During screening for LTBI, 2.9% patients were found with previous treated TB history, 8.7% positive PPD

Table 2
Characteristics of individuals with or without rheumatoid arthritis and equivalence analysis between groups

Variables	Groups				Total	
	RA		COMP			
	N	%	N	%	N	%
Gender						***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
From 40 to 59	31	64.6	21	43.8	52	54.2
60 years old or more	09	18.8	09	18.8	18	18.8
Origin						***p* = 0.031**
Recife and Metropolitan Area	32	66.7	41	85.4	73	76.0
Zona da Mata, Agreste and Sertão	16	33.2	7	14.5	23	24.0
Race						***p* = 0.512**
Caucasian	14	29.2	18	37.5	32	33.3
African-american	07	14.6	04	8.3	11	11.5
Hispanic	27	56.3	26	54.2	53	55.2
Education level						***p* = 0.100**
University	02	4.2	09	18.8	11	11.5
High school (complete)	14	29.2	18	37.5	32	33.3
High school (incomplete)	08	16.7	07	14.6	15	15.6
Fundamental education (complete)	14	29.2	09	18.8	23	24.0
With some alphabetization level or illiterate	10	20.9	05	10.5	15	15.6
Income (in minimum wages)						***p* = 0.017**
Less than 1	17	35.4	07	14.6	24	25.0
1-3	24	50.0	23	47.9	47	49.0
3-5	05	10.4	08	16.7	13	13.5
5 or more	02	4.1	10	20.8	12	12.6
Agglomeration						***p* = 0.99**
2 people	41	85.4	42	87.5	83	86.5
3 or more	07	14.6	06	12.6	13	13.6
Previous TB						***p* = 0.677**
No	44	91.7	46	95.8	90	93.8
Yes	04	8.3	02	4.2	06	6.2
Contact history						
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

RA= rheumatoid arthritis; COMP= comparison group; TB= tuberculosis

Table 3
Comparison between LTBI frequency diagnosed by PPD in the groups with and without RA

LTBI by PPD	Frequency (%)	OR (IC)	*p* - value
RA	14.6	0.31 (0.11 – 0.84)	0.034
COMP	33.3	1.0	

LTBI= latent tuberculosis infection; RA= rheumatoid arthritis; COMP= comparison group

and radiographic changes compatible with TB scar in 20.3%, demonstrating that PPD failed in identifying all patients with LTBI, which indicated that a chest radiography procedure to be is mandatory.

Bahr *et al*.[16] researched the HLA type in established RA and its relation with 4 types of skin tests for mycobacteria (tuberculin, leprosin A, scrofulin and vaccine), injected in forearm, in an Arabic population. Three groups of patients were analyzed: established RA (46), active tuberculosis infection (111) and healthy individuals (79). Responsiveness to mycobacterium skin tests was significantly lower in the RA patients group and it is related to haplotype DR7, which did not happen in the other two groups.

Although it is widely accepted, screening procedure before anti-TNF use has been criticized, because, in several situations, it is not able to identify LTBI. Among the problems described, there is the uncertainty of patient´s medical history or TB contact, lack of specific LTBI radiological signals, additionally to difficulties already described in PPD use. Thus, the use of these tests may lead to both lack of TB chemoprophylaxis, due to low sensitivity of skin test in RA, and unnecessary treatment (in case of false positive PPD).[17]

The use of specific antigen tests to detect INFγ has caused a radical change in LTBI diagnosis in countries with low TB prevalence, and has already shown a better specificity than PPD in immunocompetent people. In countries with a high TB prevalence, there is also an uncertainty about the possibility to help in latent infection diagnosis. The evaluation of these new tests performances is damaged due to a lack of golden standard to make it possible to differentiate true cross reaction between latent infection and BCG vaccine or mycobacterium infection other than *M. tuberculosis*.[18]

While diagnosing and treating LTBI in developed countries is an essential component for TB control, this is a less important strategy in developing countries. In these countries, due to the high risk of infection characterized by the high incidence of involvement of the pediatric population, the health system should prioritize treatment of active cases, reducing the risk of transmission. Additionally, a substantial part of the population in these countries has LTBI and, once the risk of these subjects to develop active infection is 5% and almost 90% of chemoprophylaxis is completely performed, it is unnecessary to add this cost (LTBI diagnosis) to TB control program in developing countries. Thus, the use of these tests in such locations should be restricted to situations that include: Epidemiologic surveillance, malnourished children and patients with tuberculosis, tuberculosis infection in patients with AIDS and RA.[18] In clinical practice, we should know that the performance of PPD for LTBI screening is poor in patients with RA. These results suggest that more careful screening need to be undertaken before treatment with an anti-TNF drug, and a good clinical history and radiological changes should be added to the PPD result until we have more specific tests available, such as those based on INFγ production.

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