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BRIEF REPORT

Antibodies to citrullinated peptides in tuberculosis

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Abstract Rheumatoid arthritis (RA) is an autoimmune disease characterized by symmetric polyarthritis, rheumatoid factor (RF) positivity, and bone erosions. Recently, research has been conducted on anti-citrullinated peptide antibodies (ACPAs) to which there are greater sensitivity and specificity than RF. However, these antibodies have also been described in infectious diseases, particularly tuberculosis (TB), placing the high specificity of the test in doubt. The aim of this research was to study the prevalence of ACPAs in TB, RA, and healthy controls. Patients with bacteriologically confirmed pulmonary tuberculosis, RA (ACR criteria), in addition to healthy controls were included. ACPAs were researched by: anti-cyclic citrullinated peptide (CCP), anti-modified citrullinated vimentin (MCV), and RF by ELISA. The study was conducted in 50 TB patients, 50 with RA, and 20 controls. Anti-CCP antibodies were found in 39 (78 %) of the RA patients (median titer, 128 U), whereas anti-MCV antibodies were found in 25 (50 %). Of the patients with TB, two (4 %) had positivity for anti-CCP and anti-MCV and no patient in the control group tested positive for these antibodies. Sensitivity of anti-CCP for RA was 78 % (confidence interval (CI), 63 to 88 %) and specificity was 97 % (CI, 89 to 99 %) while the sensitivity of anti-MCV was 50 % (CI, 35–64 %) and specificity was 97 % (CI, 89 to 99 %). RF was positive in 40 samples (80 %) of RA, in 30 (60 %) of TB, and in 1 (5 %) of the controls. Our findings showed high sensitivity of anti-CCP and high specificity of both anti-CCP and anti-MCV antibodies for RA, even in a population with high incidence of tuberculosis. The higher frequency of positivity of ACPA in TB observed in previous studies may be attributed to methodological factors.

Keywords ACPA · Anti-CCP · Anti-MCV · Rheumatoid arthritis · Rheumatoid factor · Tuberculosis

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that affects around 1 % of the adult population [1]. In Brazil, its prevalence ranges from 0.2 to 1 % [2, 3]. The diagnosis of RA is based on clinical, radiological, and laboratory criteria [4, 5]. Advances in laboratory tests and in imagining exams made it possible to diagnosis RA at an earlier stage of the disease [6]. With respect to laboratory tests, research into antibodies against citrullinated peptides (ACPAs) has demonstrated their higher specificity and positive predictive value than rheumatoid factor (RF) [7–9]. On the other hand, recent studies have demonstrated the presence ACPAs by ELISA in several infectious diseases, particularly tuberculosis (TB) [8, 10–14]. The aim of this research was to study the prevalence of ACPAs in patients with TB, RA, and healthy controls, using two methods: anti-cyclic citrullinated peptides (CCP) and anti-modified citrullinated vimentin (MCV).

Material and methods

Patients

This was a cross-sectional study including patients with RA (ACR criteria) [5], those with pulmonary TB (diagnosed on the basis of radiologic exam and confirmed by positive sputum test for acid-fast bacillus) before or within a month of treatment, and healthy controls (C) selected from among blood donors. All patients were over the age of 18 years, voluntarily agreed to participate in the study, and signed a term of free and informed consent. Patients with RA who had an associated infectious condition were excluded. The patients were submitted to clinical evaluation to obtain data about gender, age, time of diagnosis, smoking, comorbidities, medications in use, and osteoarticular symptoms. This study was approved by the research ethics committees of the institutions involved in the project.

Laboratory tests

All sera were tested for anti-CCP (INOVA), anti-MCV, and RF by ELISA (Orgentec) in accordance with the manufacturers' instructions. Values higher than 20 U were considered positive for anti-CCP and anti-MCV and higher than 25 U for RF.

Statistical analysis

Gender and positivity of ACPAs and RF were presented in frequencies; age, time of diagnosis, and time of use of anti-TB therapy were presented in the form of mean ± standard deviation or median plus interquartile interval (IQ). Comparison of the median of ACPA titers among the groups was made by the Kruskal–Wallis test. Correlation between anti-CCP and anti-MCV in the RA population was evaluated by Spearman's test. Sensitivity and specificity of anti-CCP, anti-MCV, and RF for RA were calculated by means of the program http://faculty.vassar.edu/lowry/clin1.html and the results were presented with a confidence interval (CI) of 95 %.

Results

The study was conducted in 50 TB patients (52 % women), mean age of 48±17 years, and the positivity of anti-CCP was observed in only two (4 %) (86 and 105 U)—a result quite similar to anti-MCV (69 and 196 U). These patients also presented positivity of RF (352 and 224 U). Among the patients with TB, the RF was positive in 30 (60 %). Of the two patients positive for ACPA, one complained of pain in the knees and a diffuse pain in the left hand, associated with a trauma in the past. These two patients were reevaluated 1 year after initial evaluation and none presented manifestations compatible with RA. Fifty patients with RA were included (94 % women), mean age of 55±13 years and median time of diagnosis of 13 years (IQ, 6 to 17). Thirty-nine (78 %) were positive for anti-CCP, with a median titer of 128 U (IQ, 24 to 233); 25 (50 %) were positive for anti-MCV, median titer of 21 U (IQ, 10–218), and 40 (80 %) were positive for RF median titer of 368 U (IQ, 32–658). There was statistically significant difference between the mean titers of ACPAs and RF among three studied groups. The sensitivity of anti-CCP for diagnosis of RA was 78 % (CI, 63 to 88 %) and specificity was 97 % (CI, 89 to 99 %). Lower sensitivity was observed for anti-MCV (50 %; CI, 35 to 64 %), nevertheless maintaining high specificity of 97 % (CI, 89 to 99 %) for RA. Statistically significant correlation was observed between the anti-CCP and anti-MCV titers (Spearman, $p>0.001$ and $r=0.6$) in the RA population. As regards RF, sensitivity was 80 % (CI, 65 to 89 %) and specificity was 55 % (CI, 43 to 67 %). The demographic and clinical data and results of tests in the three groups of patients are presented in Table 1

Table 1 Clinical, epidemiological, and laboratorial characteristics of the three groups of patients studied

	RA (50)	TB (50)	Controls (20)
Female (%)	94	52	40
Age (years)	55 (±13)[a]	48 (±17)[a]	35 (28–42)[b]
Smoking (%)	13	42	0
Time of diagnosis (years[c] or days[d])	8 (2–18)[b, c]	8 (2–18)[b, d]	NA
Time of RIP therapy (days)	NA	8 (2–19)[b]	NA
Positivity of anti-CCP (%)	78	4	0
Anti-CCP titer (%)	128 (24–233)[b, *]	4 (2–9)[b, *]	2 (1–2)[b, *]
Positivity of anti-MCV (%)	50	4	0
Anti-MCV titer (units)	21 (10–218)[b, *]	9 (7–12)[b, *]	2 (2–3)[b, *]
RF positivity (%)	80	60	5
RF titer (units)	368 (32–658)[a, *]	30 (19–43)[a, *]	6 (3–11)[a, *]

RA rheumatoid arthritis, *TB* tuberculosis, *RIP* rifampicin/isoniazid/pyrazinamide, *RF* rheumatoid factor, *NA* not applicable

*$p<0.0001$

[a]Mean ± standard deviation

[b]Median and interquartile interval

[c]Diagnosis time in years

[d]Diagnosis time in days

Discussion

ACPAs in RA have been investigated for a long time, and initially, this was done by means of anti-perinuclear factor detection [15]. Since then, search for various antibodies against citrullinated peptides has been developed, and in 2000, the first kit for anti-CCP was launched [8]. In a recent study that reviewed 151 articles, the sensitivity of anti-CCP ranged from 40 to 93 % and specificity from 70 to 100 % for the diagnosis of RA [9]. This finding is in accordance with our previous experience as we observed a sensitivity of 79 % and a specificity of 93 % for anti-CCP for the diagnosis of RA, using a population with systemic sclerosis and primary biliary cirrhosis as control [16].

On the other hand, recent studies have demonstrated the presence of anti-CCP antibodies in infectious diseases, particularly TB. These findings may represent a disturbance to clinicians attending patients in countries such as Brazil, where there is high frequency of these diseases, mainly because osteoarticular manifestations are presented during the course of many of them. Bearing in mind this concern, we performed a systematic review on this subject, which revealed studies demonstrating a positivity of anti-CCP antibodies in TB ranging from 0 to 37 % [17]. In the present study, there was low positivity of ACPA in TB resulting in high specificity of the test for RA. The discrepancy of our results in comparison with those of previous studies may be attributed to methodological causes, particularly due to the use of different kits, or the use of "in-house" ELISA. It should be noted that one cannot exclude the hypothesis that the positivity of ACPAs in TB observed in some studies could be related to the specificity of these antibodies to non-citrullinated epitopes of the substrate, and thus, the choice of kit to perform this test assumes a relevant importance, particularly in countries where there is high prevalence of TB. Alternatively, the diversity of the studied populations may also have contributed to this difference or one should even consider the possibility of transitory positivity of these antibodies in TB, considering that no study has repeated its dosage.

In conclusion, the positivity of ACPA antibodies in TB was observed to be low. In addition, the good diagnostic performance of ACPA for the detection of RA was confirmed.

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Disclosures None.

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