

## ORIGINAL PAPER

doi: 10.5455/medarch.2020.74.183-186

MED ARCH. 2020 JUN; 74(3): 183-186

RECEIVED: MAR 12, 2020 | ACCEPTED: JUN 02, 2020

# Anti-Cyclic Citrullinated Peptide Antibody as a Predictor of Rheumatoid Arthritis Complications

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**ABSTRACT**

**Introduction:** Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease, with more frequent occurrence in the female gender, it primarily affects the lining of the synovial joints, and is associated with lower quality of life, inability to work, progressive disability, and all of these patients are more likely to develop other comorbidities. **Aim:** To display the role of anti-cyclic citrullinated peptide antibody (anti-CCP) in evaluating RA complications during a one-year follow-up, and compare its values with values of rheumatoid factor (RF). **Methods:** The study included 40 patients with RA, out of which 6 were excluded during the 1-year follow-up. All patients were treated with anti-rheumatics, methothrexate 15-25mg, occasionally corticosteroids at the same doses. **Results:** Anti-CCP values were also significantly higher during the second examination and were  $5.0 \pm 1.9$  (range 0.5-7.6) compared to the first examination when they were  $4.2 \pm 1.3$  (range 0.4-6.2) indicating a higher sensitivity of Anti-CCP in detecting of disease progression ( $t = -2.064$ ;  $p = 0.043$ ). Anti-CCP values were statistically significant in patients with complications compared to those without during the first examination and at follow-up after one year ( $t = 5.382$ ;  $p = 0.0001$ ). **Conclusion:** The positivity of anti-CCP antibodies is a useful marker in terms of predicting the course and prognosis of the RA. A higher titer of anti-CCP antibodies represents a poorer prognosis for the disease. Determination of the presence of anti-CCP antibodies should be performed as a routine examination in all patients with suspected rheumatoid arthritis.

**Keywords.** arthritis, citrulline, antibody, prognosis.

**1. INTRODUCTION**

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease, with more frequent occurrence in the female gender, it primarily affects the lining of the synovial joints, and is associated with lower quality of life, inability to work, progressive disability, and all of these patients are more likely to develop other comorbidities (1-3). The prevalence increases with age and the gender difference is reduced in older patients. (1, 3). Rheumatoid arthritis was first mentioned in 1800 by Augustine Jacob Landré-Beauvais (who first recorded the signs and symptoms of RA as a resident) (4). The immunological parameters of RA are rheumatoid factor (RF), antinuclear antibodies (ANA), immune complexes, characteristic complement levels, anti-cyclic citrullinated peptide antibody (anti-CCP), and CD4+ T lymphocyte antigen. If RA becomes clinically suspected, it is confirmed by RF (RF-specific antibody; antibodies directed against the Fc region of immunoglobulin G) (5). Negative RF does not rule out RA as a diagnosis, in some cases we can have seronegative RA (5). During the first year of the disease, RF is usually negative. However, RF determination is useful for the differential diagnosis of rheumatoid diseases as well as the prognostic factor because its high titer is associated with rapid joint destruction and extraarticular manifestations (subacute rheumatoid nodes, polyneuropathies). RF positivity has also been reported in the healthy population (up to 4% of young Caucasians may be RF positive) (6, 7). Antinuclear antibodies (ANA) are detected in 25 and up to 50% of patients with RA (8,9,10). They are usually found in patients with advanced disease, but when it comes to manifestations of the disease there is no difference between ANA-positive and ANA-negative patients (8, 9). Antinuclear antibodies can be immunoglobulins of all classes. Citrulline antibody (anti-cyclic citrullinated peptide antibody) is an immune protein (antibody) that binds to a non-standard amino acid (citrulline), formed from amino groups released from a natural amino acid called agrinine. (10, 11).

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Citrulline antibodies are present in most RA patients. It is used to diagnose rheumatoid arthritis at a time when joint inflammation is not registered. The citrulline antibody test is most useful in identifying cases of previously undiagnosed inflammatory arthritis when the standard test for rheumatoid arthritis is negative. Thus, citrulline antibodies are suitable for the recognition of the early stage of the disease. (11,12). They are more specific than RF, with the same sensitivity (11).

## 2. AIM

To display the role of anti-CCP in evaluating RA complications during a one-year follow-up, and compare its values with values of RF.

## 3. METHODS

The study included 40 patients with RA, out of which 6 were excluded during a 1-year follow-up. All patients were treated with anti-rheumatics, methotrexate 15-25mg, occasionally corticosteroids at the same doses. They were examined in the Department of Rheumatology, Clinic for Heart, blood vessels and Rheumatology, Clinical Centre University of Sarajevo. Criteria for inclusion were: patients 30-60 years of age, patients who met the «An American College of Rheumatology/European League Against Rheumatism Collaborative Initiative 2010 Rheumatoid Arthritis Classification Criteria», patients who had a positive RF and positive anti-CCP finding (13). Patients suffering from other serious chronic diseases (liver and kidney failure, heart disease) as well as those with acute illness were excluded from the study. The study excluded also those subjects who previously met the inclusion criteria for inclusion and who during the study had an acute illness after which therapy with antirheumatic drugs, corticosteroids and methotrexate was contraindicated.

Anti-CCP was performed by the enzyme-linked immunosorbent assay (ELISA) assay, read on a spectrophotometer at 450 nm (The Immunoscan CCPlus). An immunofluorescence test (IFT) was used for the determination of ANA. The test for citrulline antibodies in the blood of a rheumatoid arthritis patient is extremely specific. When citrulline antibodies were found, the likelihood of the subject suffering from RA was 90-95%. (14) Reference ranges for anti-CCP were as follows: negative <0.95 units per milliliter of blood (U/ml), borderline positive > 0.95 <1.0 U/ml, positive > 1.0 U/ml, while for RF reference range is up to 20 U/ml. There are three generations of ELISA tests for anti-CCP (CCP1; CCP2; CCP3) of which the specificity of anti-CCP2 for RA ranges from 90.4% -97.3% (14).

Software IBM SPSS v19.0 (Chicago, Illinois, USA) was used for statistical analysis. All data collected are presented in tables and graphs by the number of cases, percentages, arithmetic mean with standard deviation, standard error of the mean and range of values. Student's t-test for paired samples or chi-square test depending on the data type was used to test differences between individual groups, while Pearson's linear correlation coefficient was used to test the interaction of individ-

ual parameters. The results of all tests at  $p < 0.05$  were considered statistically significant or at 95% confidence level. Ethical approval was obtained from the Ethical Committee of the Clinical Center University of Sarajevo.

## 4. RESULTS

The average age of the patients was  $47.4 \pm 5.3$  years and they were divided into two groups according to anti-CCP values (first group anti-CCP <4 u/ml and second group CCP > 4 u/ml). Out of the total number, 82.4% of the respondents were females. Analysis of the limit values of anti-CCP according to the first and control results showed that during both examinations, 12 subjects (64.7%) had anti-CCP values over 4 without a statistically significant difference between two examinations ( $p > 0.05$ ). Anti-CCP values were also significantly higher during the second examination and were  $5.0 \pm 1.9$  (range 0.5-7.6) compared to the first examination when they were  $4.2 \pm 1.3$  (range 0.4-6.2) indicating a higher sensitivity of Anti-CCP in detecting of disease progression ( $t = -2.064$ ;  $p = 0.043$ ). The values of RF were higher during the control examination ( $563.04 \pm 744.3$ ; range 9.2-3092) compared to the first examination ( $560.8 \pm 740.8$ ; range 9.2-3092) with no statistically

	N	Mean	SD	SEM	Minimum	Maximum
Yes	14	5.243	.5945	.1589	4.2	6.2
No	20	3.460	1.1311	.2529	.4	4.7
Total	34	4.194	1.2917	.2215	.4	6.2

Table 1. Anti-CCP values according to presence of complications—first examination (SD-standard deviation, SEM—standard error of the mean) (in U/ml).  $t=5.382$ ;  $p=0.0001$

	N	Mean	SD	SEM	Minimum	Maximum
Yes	14	6.863	.6539	.1748	5.6	7.6
No	20	3.748	1.5146	.3387	.5	6.4
Total	34	5.031	1.9775	.3391	.5	7.6

Table 2. Anti-CCP values according to presence of complications—control examination (SD-standard deviation, SEM—standard error of the mean) (in U/ml).  $t=7.023$ ;  $p=0.0001$

	N	Mean	SD	SEM	Minimum	Maximum
Yes	14	975.231	1039.2706	288.2418	9.8	3090.0
No	20	291.500	219.8438	49.1586	9.8	712.0
Total	34	560.848	740.8300	128.9620	9.8	3090.0

Table 3. RF values according to presence of complications—first examination (SD-standard deviation, SEM—standard error of the mean) (in U/ml).  $t=2.868$ ;  $p=0.007$

	N	Mean	SD	SEM	Minimum	Maximum
Yes	14	986.223	1040.0010	288.4444	9.2	3092.0
No	20	287.975	218.5092	48.8601	9.2	714.0
Total	34	563.042	744.3057	129.5670	9.2	3092.0

Table 4. RF values according to presence of complications -control examination (SD-standard deviation, SEM—standard error of the mean) (in U/ml).  $t=2.928$ ;  $p=0.006$

significant difference between examinations ( $t = -0.012$ ;  $p = 0.990$ ) There was no significant difference in RF values in patients with antiCCP  $<4$  and  $> 4$  values during the first and control examinations ( $351,983 + 226,8127$  vs  $683,648 + 903.3774$ ;  $t = -1.242$ ,  $p = 0.224$ , respectively  $354,392 + 228.0128$  vs.  $678.824 + 899.6352$ ,  $t = -1.219$ ,  $p = 0.232$ ) Only one ANA positive case was recorded. Anti-CCP values were statistically significant in patients with complications compared to those without during the first examination and at follow-up after one year ( $t = 5,382$ ;  $p = 0.0001$ ) (Table 1, Table 2). Patients with complications had higher Anti-CCP in 71% of cases. Anti-CCP values were statistically significant in patients with complications compared to those without it during both examinations, in sense that they had higher values ( $t = 2.868$ ;  $p = 0.007$ ;  $t = 2.928$ ;  $p = 0.006$ ). The values of RF were statistically significant in patients with complications compared to those without both during the first and after the one-year examination. Statistically significant positive correlations were observed in the relation of antiCCP and RF, which means that an increase in the value of Anti-CCP leads to an increase in the value of RFa and vice versa.

## 5. DISCUSSION

Since 2003, numerous studies have been published to examine the diagnostic significance of the anti-CCP test for RA. The sensitivity of anti-CCP in subjects with advanced RA ranges from 64% to as high as 96%, while in those with early or still undifferentiated arthritis, it ranges from 14.4% to 83.5% (15). The specificity of the test is reflected in the choice of the control group. The same effect is achieved with a control group consisting of subjects with the same disease. The actual diagnostic properties of a test will only come from research involving patients representing the actual population to which the test will be administered in clinical practice. Therefore, considering only the studies in which the control group most closely fits this assumption, the specificity of anti-CCP for RA ranges from 90.4% to 97.3% (15). In addition to those with RA, anti-CCP antibodies were also found in about 9% of patients with systemic lupus erythematosus (SLE), 5% of patients with Sjogren's syndrome, 8% of those with psoriatic arthritis, and 2-5% of patients with juvenile idiopathic arthritis (16).

When it comes to the predictive value of the anti-CCP test for early diagnosis of RA, we did not have healthy volunteers in our study who would subsequently retrospectively test for the presence of anti-CCP in serum. Many studies were done on groups of healthy volunteers who, after the onset of the disease, retrospectively determined that anti-CCP was positive in the blood sample long before the first symptoms of the disease appeared.

Rantapaa-Dahlqvist et al determined the presence of anti-CCP antibodies many years before the onset of the first symptoms of RA (17). Retrospective blood analysis of voluntary blood donors identified 83 patients who subsequently developed RA (17). Anti-CCP antibodies represent a predictive marker that is positive and 1.5 years before the first visit to the doctor. The sensitivity of

anti-CCP antibodies tend to increase from 4% to 52% in the last 9 years (18,19). The predictive value of anti-CCP antibodies to RA has also been confirmed in longitudinal studies in cohorts of subjects with non-specific early inflammatory arthritis (undifferentiated arthritis) (19).

Our research showed that the test positivity is highly specific in terms of poorer disease prognosis.

The predictive value of anti-CCP antibodies in combination with other variables such as sedimentation, radiologically assessed joint damage, RA-risk HLA alleles, or RF, allows very early identification of patients at high risk for the development of progressive erosive arthritis in which aggressive antirheumatic therapy is indicated (20). Alexiou et. stated that anti-CCP antibodies are a better diagnostic value than RF in correlation with radiological joint damage and are therefore useful in everyday rheumatology practice (21).

New diagnostic methods, such as nuclear magnetic resonance imaging and ultrasonography, make it possible to detect erosions on joint surfaces well before they become visible on classical radiography. Therefore, in modern rheumatology, the finding of radiographically visible erosions on the joints of patients with RA is considered to be a consequence of delayed or failed treatment. There seems to be a need to revise the applicable criteria for the diagnosis of RA, which would include anti-CCP antibodies and early erosive changes visible by sensitive visualization methods (echosonography and / or magnetic resonance imaging) in the diagnostic criteria.

## 6. CONCLUSION

The positivity of anti-CCP antibodies is a useful marker in terms of predicting the course and prognosis of the RA. A higher titer of anti-CCP antibodies represents a poorer prognosis for the disease. Determination of the presence of anti-CCP antibodies should be performed as a routine examination in all patients with suspected rheumatoid arthritis.

- **Acknowledgment:** We are grateful to Institute of Immunology and the Institute of Biochemistry, Clinical Center of the University of Sarajevo, for help with research.
- **Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms
- **Author's contribution:** Each author gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work. Each author have role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
- **Conflicts of interest:** There are no conflicts of interest.
- **Financial support and sponsorship:** Nil.

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