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# Anti-Citrullinated Antibodies, Radiological Joint Damages and Their Correlations with Disease Activity Score (DAS28)

**Marija Glasnović[1], Ivica Bošnjak[1], Aleksandar Včev[1], Ivan Soldo[2], Elizabeta Glasnović-Horvatić[3], Silva Soldo-Butković[4], Jasna Pavela[5] and Nikola Mićunović[1]**

[1] Department of Internal Medicine, School of Medicine, University of »J. J. Strossmayer«, Osijek, Croatia
[2] Department of Infectology, School of Medicine, University of »J. J. Strossmayer«, Osijek, Croatia
[3] Department of Pathology and Cytology, General Hospital »Tomislav Bardek«, Koprivnica, Croatia
[4] Department of Neurology, School of Medicine, University of »J. J. Strossmayer«, Osijek, Croatia
[5] Department of Medical Biochemistry, School of Medicine, University of »J. J. Strossmayer«, Osijek, Croatia

**A B S T R A C T**

*Determination of anti-citrullinated peptides (anti-CCP) specificity as a predictor of joint erosive changes, correlation between their serum level and radiological damages as well as disease activity score (DAS28). A trial has been conducted on a 211 patient sample fulfilling ACR criteria for rheumatoid arthritis (RA). There was assigned anti-CCP serum level, disease activity score by the formula for DAS28(3)-CRP and assessed radiological changes degree after Steinbrocker score. In 132 patient (62,559%) the serum anti-CCP concentration was positive for RA. Specificity of the test was 100% and sensitivity 65% (Z=0,731, p=0,465). There is a medium intensity correlation between variables representing anti--CCP and Steinbrocker score. Pearson's coefficient was 0,479 and Spearman's rank correlation coefficient was 0,614, i.e. statistically significant (p=0,000). There is no statistically significant correlation between variables representing anti--CCP and DAS28(3)-CRP. Anti-CCP are good RA predictor and their concentration correlate with radiological damages degree.*

***Key words**: anti-citrullinated antibodies, rheumatoid arthritis, disease activity score, radiological damages*

## Introduction

Rheumatoid arthritis (RA) is the most common inflammatory joint disease, affecting nearly 1% of the adult population worldwide. It is characterized by multiple deformities and is associated with considerable morbiditiy and mortality[1]. Although the precise etiology of RA remains unknown, there is a strong evidence for autoimmunity. Several auto-antibodies are associated with the disease. Besides the rheumatoid factor (RF), another group of auto-antibodies has recently been detected in the serum of patients with RA: the anti-cyclic citrullinated peptide antibodies (anti-CCP)[2]. The disease activity score (DAS) is a continuous measure of RA that is becoming increasingly utilized in clinical research and practice. DAS commonly uses a 28-count for tender and swollen joints (DAS28) and can be calculated as originally developed using ESR (DAS28-ESR) or with a more recent formula that uses CRP (DAS-CRP)[3]. Several observations have indicated that an anti-CCP positive in early RA patients may develop a more erosive disease than those without an anti-CCP, and it has been shown that anti--CCP can be an independent predictor for radiological damage and progression[4–6]. There is a question whether serum anti-CCP is able to be an objectivity test on joint changes in RA patients and if its concentration may be an indicator of inflammatory activity as DAS28(3)-CRP.

## Materials and Methods

The trial has been conducted on a 211- patient sample fulfilling ACR criteria for RA and 243 control patients with other autoimmune diseases[7]. Anti-CCP serum level

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was determined by an ELISA test of the firm Euroimmune on the MiniBos apparatus (referent range of anti--CCP ≤ 5(25) RU/mL negative, antiCCP ≥ 5(25) RU/mL positive). The Waaler Rose (WR) was determinated with the hemaglutination technique of the firm Chronolab, Switzerland. The WR test reagent sensivity hay been adjusted to detect a minimum of a 6 IU/mL of rheumatoid factors according with the WHO International Standard without previous sample dilution.

CRP was determined by the immuno-turbidimeter test of the firm Olympus on a multi-channel analyzer Olympus AU 640 (referent range 0–5 mg/L).

DAS28-CRP was determined by the formula:

$$\text{DAS28-(3)CRP} = [0.56 \times \text{sqrt(TJC28)} + 0.28 \times$$
$$\times \text{sqrt(SJC28)} + 0.36 \times \ln(\text{CRP}+1)] \times 1.10 + 1.15.$$

CRP is a more direct measure of inflammation compared to ESR and is more sensitive to short term changes.[8]

The degree of joint radiological changes has been estimated by the Steinbrocker score. Data input and manipulation was done in a computer table calculator Microsoft Excel 2003 whereas software packages SPSS 13.0 for Windows and Statistica 6.0 were used in the statistical analysis.

## Results

Out of 211 patients included in our study who fulfilled all the ACR criteria for RA 23.69% of patients were male and 76.30% were female. The average age was 52.963± 12.45, with a median length of illness of 4 years. In all the patients diagnosed with RA, DMARD therapy was included.

In 132 patients (62.559%) the serum level of anti-CCP was higher than 5 RU/mL and in 79 patients (37.441%) was lower than 5 RU/mL. The specificity of the test was 100% as there was no positive result in any case of the control group. A positive Waaler Rose test was proven in 95 anti-CCP positive patients. According to the degree of radiological changes on the joints we had 50 patients in the first group or 37.878%, 49 patients in the second group or 37.712%, 31 patients in the third group or 23.484% and 2 patients in the forth group or 1.515%.

The testing showed that it is possible to accept a selected hypothesis on the proportion of patients in the basic group out of which a concentration of anti-CCP in the serum -a predictor for rheumatoid arthritis (RA) is 0.65 (Z=0.731, p=0.465).

A simple linear regression model where anti-CCP is defined as an independent variable and dependent Steinbrocker score, runs like this. (Figure 1)

A constant member in a regression equation shows a possible expected Steinbrocker score value in RA patients if anti-CCP in serum is 0. Based upon the computed regression coefficient it can be concluded that a patient serum anti-CCP increase by 1 resulted in the mean Steinbrocker score of 0.007.

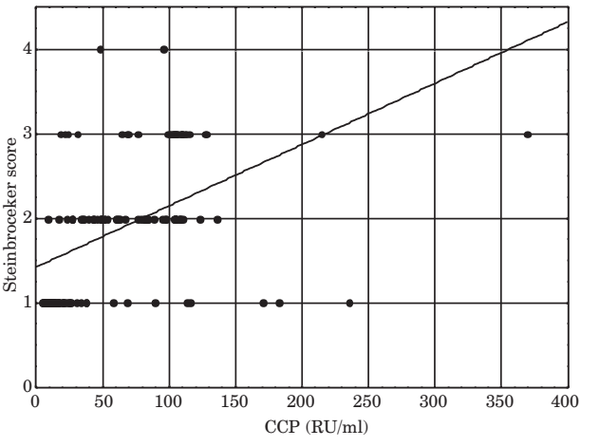


*Fig 1. Diagram of anti-citrullinated antibodies value and Steinbrocker score with pertaining regression line*

Pearson's correlation coefficient values are 0.479 in this case. Thus, there is a positive mean-intensive correlation between variables representing anti-CCP and Steinbrocker score. The hypothesis where the computed correlation coefficient is statistically significant is accepted (p=0.000). The determination coefficient shows that only 22.97% of total deviations have been explained by the applied linear regression model. The computed equation of linear regression cannot be considered as a representative one. Testing of the hypothesis on variable significance representing anti-CCP and conducted by the F-test shows that it can be a possible hypothesis on the variable significance in the applied model (F=38.766, p=0.000). The hypothesis on the computed parameter excess was rejected after t-test had been performed (t= 6.226, p=0.000). The standard parameter assessment error alongside variable designating anti-CCP is 0.001. With 95% of reliability anti-CCP increase by 1 is expected

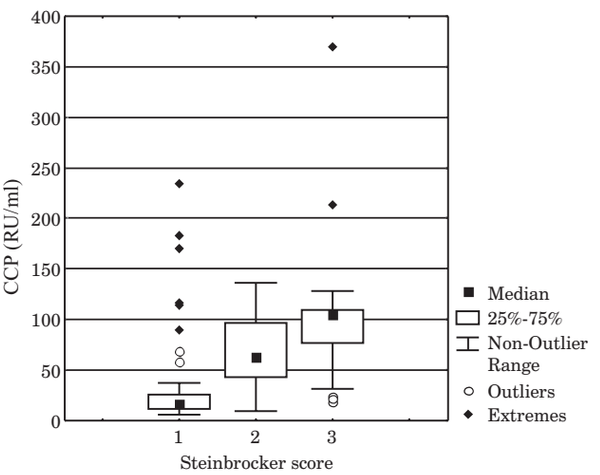


*Fig. 2 Box plot of anti-citrullinated antibodies and Steinbrocker score*

**TABLE 1**
RESULTS OF THE INTERVAL VALUE ASSESSMENT OF THE VARIABLE DEFINED AS ANTICITRULLINATED ANTIBODIES POSITIVE TO STEINBROCKER SCORE

| Steinbrocker score | Mean | Standard error | Assessment interval | |
|---|---|---|---|---|
| | | | Low bound | Upper bound |
| 1 | 33.824 | 6.869 | 20.021 | 47.627 |
| 2 | 67.902 | 4.493 | 58.872 | 76.932 |
| 3 | 103.901 | 11.489 | 80.404 | 127.398 |

to result in the mean Steinbrock score increase by more than 0.005 and less than 0.01. According to Spearman's rank correlation coefficient of 0.614, there is a positive mean-intensive correlation between the observed variables. The computed Spearman's rank correlation coefficient is statistically significant (p=0.000). With 95% reliability the mean anti-CCP result is expected to be higher than 20.021 and lower than 47.627 in persons having Steinbrocker 1 (Table 1 and Figure 2). Since the hypothesis on variance homogeneity by Leveneo's test had been accepted, the one-factor variance analysis was applied in testing the hypothesis on arithmetic means equality. It appeared to be a feasible hypothesis on the presence of statistically significant differences in the mean values of CCP three patient groups designed by the Steinbrocker score (p=0.000, Table 2).

**TABLE 2**
RESULTS OF THE LEVENS TEST HOMOGENEITY OF VARIANCES AND HYPOTHESIS TEST ON EQUALITY OF ANTICITRULLIINATED ANTIBODIES MEANS OF THREE PATIENT GROPUP FORMED BY THE STEINBROCKER SCORE

| Leven's test homogeneity of variances | | Anova | |
|---|---|---|---|
| Leven's indicator | p-value | F-rate | p-value |
| 0.133 | 0.876 | 21.315 | 0.000 |

Of 133 anti-CCP positive patients DAS28(3)-CRP was calculated in 78 (58.64%). There is a slight positive correlation between variables representing anti-CCP and DAS28(3)-CRP. Pearson's correlation coefficient was 0.0475, and the determination coefficient amounted to 0.206%. The hypothesis whereby the computed correlation coefficient is significant (p=0.693) has been rejected. Testing the hypothesis on the significance of the variable representing anti-CCP conducted by the test shows that the hypothesis on the significance of this applied model variable cannot be accepted (F=0.125, p=0.693). The hypothesis on the computed parameter excess was not rejected after t-test had been performed (t=0.396, p=0.693). By Spearman's rank, the correlation coefficient of the value 0,042 showed a slight positive correlation between the observed variables. The computed rank correlation coefficient (is not statistically significant (p=0.717).

## Discussion

In our study we wanted to show the patients who fulfilled the currently valid ACR criteria for RA. By determining a degree of radiological changes on the hand joints, as RA objectivity tests, as well as by dividing the patients in 4 groups by Stainbrocker score, which might be an obsolete but still a frequently used degree method in everyday medical practice, we determined the serum level of anti-CCP and put them in an interactive correlation.

The results of our study show that there is a correlation between anti-CCP serum level and joint changes. Their level, with 95% of reliability, indicates a degree of radiological lesions. The first degree of Steinbrocker score described radiological changes that were characterized by the largest number of extremely high anti-CCP results. This perhaps is not consistent with radiological lesions degree. However, this is where anti-CCP show their significance since these patients are shown to have the highest risk of erosive changes development. The determination of anti-CCP and x-rays of hand joints, as erosive lesions objective tests, are of special importance for setting RA diagnosis, and making decisions on the introduction of DMARD medicines as well as patient monitoring. Furthermore, although Steinbrocker score recognizes only 4 degrees of radiological changes, as we had in our group of patients only 2 such patients, we could not bring any conclusions. Nevertheless, their serum level of anti-CCP was lower than expected, probably due to severe deterioration of the cartilage with the bone damage and joint deformity. Anti-CCP is a good serological RA marker. Due to a high specificity being 100% in our study, it is valuable to differentiate RA from other arthritis forms that can also be erosive. However, 18 anti-CCP positive patients had clinical and serous inclinations of autoimmune or any other disease. (SEL, ReA, PsA), but as they also fulfilled the ACR criteria for RA, it is possible that there was a syndrome of aliasing. In these situations it would be advisable to make HLA typification to determine the final diagnosis. The introduction of anti-CCP positive into RA diagnosis criteria would certainly make its diagnosis much easier[9]. However, in spite of anti-CCP great prognostic importance for the prediction of joint erosive changes, there is a question whether there is a correlation between serum anti-CCP level and joint-change degrees as well as the possibility of using their level as an indicator of inflammatory activity instead of DAS28. Great controversies and debates are raised upon this issue. In the estimation of the disease activity, we used DAS28(3)-CRP because CRP can be used as an alternative for ESR in determination of inflammation. CRP is a more direct measure of inflammation than ESR. CRP production is associated with radiological progression in RA[10] and is considered at least as valid as ESR to

measure RA disease activity[11,12]. Another advantage of the determination of CRP is that the waiting time for the laboratory result is shorter and that in case of multicenter studies a central laboratory can be used. By putting in a correlation the patients who were anti-CCP positive and the patients had their DAS28(3)-CRP determined, we could not prove any statistic connection. The serum level itself will, therefore, not determine the degree of the very activity of the illness nor the number of the joints which would be affected by the disease. A small number of patients have been found to change their anti-CCP status in the 5 year period.[4,6,13–15] The latest papers, also approved by our study, show that changes in antibody concentration do not reflect disease activity degree and cannot be used for disease activity assessment. The aforementioned can depend on the treatment fashion[1]. Alessandri et.al. showed that anti-CCP concentration measures may be useful in effect assessment of treating RA patients with anti-TNFα preparations[17]. Most research has shown that progression of radiological lesions correlates with initial anti-CCP level[15,18,19].

In summary, anti-CCP characterized by specificity and early positive is shown to be an excellent RA diagnostic marker. Its serum level correlates with a radiological change degree. Thus, based upon 95% reliability, it can be used for erosive lesions assessment instead of the objectivity test. Still, their serum level can not be a substitute parameter in assessing disease activity degree instead of DAS28-(3)-CRP. Therefore, previously defined parameters should be determined for the aforementioned purpose.

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*M. Glasnović*

*Clinic of Internal Medicine, School of Medicine, University »J. J. Strossmayer« Osijek, Huttlerova 4, 31 000 Osijek, Croatia*
*e-mail: glasnovic.marija@kbo.hr*

## ANTICITRULINKSA PROTUTIJELA I STUPANJ RADIOLOŠKIH PROMJENA TE NJIHIVA KORELACIJA SA STUPNJEM AKTIVNOSTI BOLESTI (DAS28)

## SAŽETAK

Cilj rada bio je odrediti specifičnost anticitrulinskih protutijela (anti-CCP) kao prediktora erozivnih promjena na zglobovima, korelaciju njihove serumske razine i radioloških promjena kao i stupnjem aktivnosti bolesti (DAS28). Studija je obuhvatila 211 pacijenata koji su ispunjavali ACR kriterije za reumatioidni artritis (RA). Određena im je razina anti-CCP u serumu, stupanj aktivnosti bolesti te stupanj radioloških promjena po Steinbrocker scoru. U 132 pacijenta (62.559%) serumska razina anti-CCP bila je indikativna za RA. Specifičnost testa bila je 100%, a osjetljivost 65% (Z= 0.731, p= 0.465). Postoji korelacija srednje jakosti između varijabli anti-CCP i Stinebrocker score. Paersonov koeficijent je 0.479 a Spearmanov koeficijent korelacije 0.614; oboje statistički značajni (p=0.000). Nema statističke značajnosti između varijabli anti-CCP i DAS28(3)-CRP. Anti-CCP je dobar prediktor za RA i njihova serumska razina korelira s radiološkim promjenama na zglobovima.