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THE DIAGNOSTIC VALUE OF THE SECOND GENERATION ANTI-CCP TEST IN RHEUMATOID ARTHRITIS

DIJAGNOSTIČKA VREDNOST ANTI-CCP TESTA DRUGE GENERACIJE KOD REUMATOIDNOG ARTRITISA

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Summary: The purpose of this research was to compare the diagnostic values of laboratory variables, to present quantitative evaluations of the diagnostic test with reference to sensitivity, and specificity, the predictive value of the positive and negative test and precision of the test for anti-cyclic citrullinated peptide (anti-CCP 2) antibodies, rheumatoid factor (RF), C-reactive protein (CRP), DAS 28 index, in early diagnosis of untreated rheumatoid arthritis (RA). Using the ELISA technology of DIA-STAT^{TM} Anti-CCP (Axis–Shield Diagnostics), the serum has been examined in 70 participants (35 RA who were not treated, 35 healthy controls). RF was defined with the test for agglutination (Latex RF test) in the same participants. In 23 of the 35 examined patients with RA, we found presence of anti-CCP 2 antibodies (sensitivity of the test 65.71%), while RF appeared in 17 patients (sensitivity of the test 48.57%). Twelve patients were anti-CCP 2 and RF positive, 11 were anti-CCP 2 positive but RF negative. Five patients were anti-CCP 2 negative and RF positive. Out of 18 RF negative patients, 11 were anti-CCP 2 positive. In 17 RF positive patients, anti-CCP 2 antibodies were positive in 12 patients. In the healthy control group, 1 patient was anti-CCP 2 positive, while 2 patients were RF positive. Anti-CCP 2 antibodies have higher sensitivity and specificity than RF in RA.

Keywords: cyclic citrullinated protein, rheumatoid arthritis, rheumatoid factor

Kratak sadržaj: Cilj ovog istraživanja bio je da se uporede dijagnostičke vrednosti laboratorijskih parametara za definisanje osetljivosti i specifičnosti, prediktivne vrednosti pozitivnog i negativnog testa, kao i tačnost testa za antitela anticikličnog citrulliniranog peptida (anti CCP 2), reumatoidnog faktora (RF), C-reaktivnog proteina (CRP), DAS 28 indeksa, u ranoj dijagnozi, kod netretiranog reumatoidnog artritisa. Koristeći ELISA tehniku na DIA-STAT^{TM} Anti CCP (Axis–Shield Diagnostics), ispitani su serumi 70 učesnika (35 RA netretirani i 35 kontrolna zdrava grupa). RF je određen testom aglutinacije (Latex RF test) kod istih učesnika. Od ispitanih 35 pacijenata sa RA, kod 23 pacijenta otkriveno je prisustvo anti CCP 2 antitela (osetljivost testa 65,71%), dok je RF bio prisutan kod 17 pacijenata (osetljivost testa 48,57%). Dvanaest pacijenata bili su anti CCP 2 i RF pozitivni; 11 su bili anti CCP 2 pozitivni a RF negativni; 5 pacijenata bili su anti CCP 2 negativni i RF pozitivni. Od 18 RF negativnih pacijenata, 11 su bili anti CCP 2 pozitivni. Od 17 RF pozitivnih pacijenata, anti CCP 2 antitela nađena su kod 12 pacijenata. U kontrolnoj zdravoj grupi, 1 pacijent bio je anti CCP 2 pozitivan, dok su 2 imali pozitivni RF. Anti CCP 2 antitela imaju veću osetljivost i specifičnost od RF kod RA.

Ključne reči: ciklični citrulinirani protein, reumatoidni artritis, reumatoidni faktor

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that is multi-functional in origin and is characterised by the inflammation of the membrane lining joints. The disease spreads from small to large joints, with the greatest damage in the early phase (1).

The diagnostics of RA is primarily based on clinical, radiological and immunological features. The most frequent serological test is the measurement of

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rheumatoid factor (RF). The presence of RF is one of the American College of Rheumatology's criteria for the classification of RA. The IgM class is the most common and is found in 60–80% of RA patients. RF is not specific for RA, as it is often present in healthy individuals and patients with other autoimmune diseases and chronic infections (2). It is reported that up to 30% of SLE patients with no evidence of RA are RF positive (3). Despite its low specificity, a positive RF is considered an important predictor of outcome in RA.

For several years, it has been recognised that antibodies to anti-perinuclear factor (APF) and keratin (AKA) are highly specific for RA. Antibodies to APF and AKA have been detected by indirect immunofluorescence using buccal epithelium of rat oesophagus (4). The lack of availability of suitable buccal cell donors has limited the use of APF as a routine laboratory test. Recently, the antigen of both these antibodies has been identified as epidermal filaggrin, an intermediate filament-associated protein involved in the cornification of the epidermis (5, 6).

Profilaggrin, which is present in the keratohyaline granules of human buccal mucosa cells, is proteolytically cleaved into filaggrin subunits during cell differentiation. At this stage, the protein is dephosphorylated and some arginine residues are converted to citrulline by the enzyme peptidylarginine deaminase (PAD) (7).

In 1998, Schellekens and colleagues (8) reported that autoantibodies reactive with linear synthetic peptides containing the unusual amino citrulline were present in 76% of RA sera with a specificity for RA of 96%. The antibodies in patients with RA that recognized the citrulline containing epitopes were predominantly of the IgG class and of relatively high affinity (8). In a subsequent paper, Schellekens and colleagues (9) reported that an ELISA test based on cyclic citrullinated peptide (CCP) showed superior performance characteristics to one based on the linear version in the detection of antibodies to RA.

Very recently, it has been reported that, in principle, most citrullinated protein/peptides are recognized by autoantibodies in RA sera, although with differing sensitivities and specificities (10). These findings suggest an important role for citrullinated antigens in the diagnosis of RA. Sensitivity of the anti-CCP 2 test among different populations is between 64% and 74%, but the specificity is between 90% and 99% (11–16).

Patients and Methods

Among the patients examined for this study, the diagnosis of the disease was established on the basis of the revised diagnostic criteria for classification of rheumatoid arthritis, suggested in 1987 by the American Association for Rheumatism (ARA) (17). For the purpose of classification, in order for a patient to be in the group of rheumatoid arthritis patients, he/she must fulfill at least four out of seven criteria. Criteria from one to four should be present for at least six weeks. The study involved 35 patients (females 28, males 7), suffering from RA, and 35 patients (females 18, males 17) as the healthy control group.

The average age was 56.68 years (± 6.79) (40–65 years) in the RA group, but 46.2 years (± 12.49) (29–65 years) in the healthy control group. The average duration of the disease in months from the beginning was 43.97 (± 45.23), in the interval of 1–168 months. None of the patients who were in the research had any medical record of past or present renal disease. Three patients were treated with gold salts and oral corticosteroid medicines, while 6/29 had previously used NSAIL. The rest of the patients refused use of other medicines before taking part in the examinations.

The study involved patients suffering from rheumatoid arthritis, aged 18–65 years, newfound and till then not treated.

From the study were excepted all the patients with disease or condition which could directly or indirectly influence any change in the results:

1. Patients with previous medical record of disease of the spleen, thyroid gland, hepatal damage, renal, hematologic, cardiovascular, neurotic and lung damage, autoimmune disease, AIDS, age<18 years.
2. Patients with diabetes mellitus, acute infections, malignant neoplasm, febrile conditions.
3. Patients treated with antibiotics and salycilate in periods under six months from the beginning of the study.
4. Patients with hypertension arterialis, uric arthritis, uric infections, SLE, Sy Sjögren, mixed conjunction texture disease, vasculitis.
5. Patients treated with antihypertension, antidiabetic and cardiac therapy.
6. Patients with anamnesis for transfusion of blood and overweight.
7. Hypersensitive to some of the medicines or their components.
8. Excepted patients who together with these medicines took medicines from basic line.
9. Excepted patients whose results showed that in 0 spot there was a glycemia, or increased level of degraded products: creatinine in serum and urine, urea in serum and disorder of the hematologic and enzymatic status.

All patients took part in this study voluntarily, so the ethical criterion was fulfilled during our work.

Clinical evaluation of disease activity

A subspecialist in this field did the clinical evaluation. The activity of the disease was evaluated using DAS 28 index (Disease Activity Score, DAS 28) (18–21). The index is a mathematical formula that allows us to get a uniquely composed quantitative score, which constitutes from palpation painfull sensitive joints (max number 28), swollen joints (max number 28), Westergren ESR, and patient's global assessment of the activity of disease (0–100 mm Visual Analogous Scale VAS) and the morning rigid (minutes). DAS 28 index is ranked from 0 to 10 and a score under 3.2 ranks the disease as low active.

Laboratory assessment

For a clinical assessment of the basic disease, these laboratory variables needed to be measured: haemogram and differential haemoanalysis, reactors of acute phase, anti-CCP 2, C-reactive protein (CRP), rheumatic factor (RF), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine kinase (CK), lactate dehydrogenase (LDH), serum urea and creatinine.

The DIA-STAT^{TM} Anti-CCP (Axis–Shield Diagnostics) test is a semi-quantitative/qualitative enzyme-linked immunosorbent assay (ELISA) for the detection of the IgG class of autoantibodies specific to synthetic cyclic citrullinated peptide (CCP) containing modified arginine residues. The test provides an additional tool in the diagnosis of patients with RA.

The absorbance value (optical density) ratio for the positive and negative control and for each sample was calculated. The test recommended values were:

Absorbance ratio	Result interpretation
<0.95	Negative
≥0.95 to ≤1.0	Borderline recommended repeated testing
>1.0	Positive

C-reactive protein (CRP) was determined with the test of agglutination (Latex CRP test) (BioSystems S.A. Reagents&Instruments Costa Brava 30, Barcelona, Spain) (22–26).

Reference values were under 6 mg/L CRP in serum.

Rheumatic factor (RF) was assayed with the test of agglutination (Latex RF test) (BioSystems S.A. Reagens&Instruments Costa Brava 30, Barcelona, Spain) (22, 26–30).

Reference values were under 8 mg/L RF in serum.

For the estimation of red cell sedimentation rate (SER), we used the method after Westergren, and normal values were for males 7–8 mm, for females 11–16 mm.

Statistical analysis

For testing the importance of the difference between two arithmetic means, with respect to proportion, a Student's t-test was used, which compares the middle values of certain numerical parameters between two groups, as well as a Wilcoxon-matched test for independent examples. Sensitivity and predictivity for positive and negative test of examined marks were defined. P value between 0.05 and 0.1 was taken as statistically significant. Data processing was done with the statistics package Statistica 7.0

Results

Out of 35 examined patients with RA, 23 patients (65.71%) showed presence of anti-CCP 2 antibody, while RF was present in 17 patients (48.57%). Twelve patients were anti-CCP 2 and RF positive (34.28%), 11 patients (31.42%) were anti-CCP 2 positive and RF negative, while 5 patients (14.28%) were anti-CCP 2 negative and RF positive. Out of 18 RF negative patients, 11 patients (61.11%) were anti-CCP 2 positive. Out of the total of 12 anti-CCP 2 negative RA patients, 5 patients (41.66%) were RF positive.

In the total of 35 examined patients with RA, sensitivity to anti-CCP 2 was 65.71%, while RF sensitivity was 48.57%.

Out of 17 RF positive RA patients, anti-CCP 2 antibody was present in 12 patients and its sensitivity was 70.58%.

Out of 18 RF negative RA, anti-CCP 2 antibody was present in 11 patients and its sensitivity was 61.11%.

In the healthy control group, 1 patient (2.85%) was anti-CCP 2 positive, while 2 patients (5.71 %) were RF positive (*Table I*).

Diagnostic value of anti-CCP 2 antibody in patients with rheumatoid arthritis (RA)

For anti-CCP 2 antibody and RF in rheumatoid arthritis, sensitivity, specificity, predictive value of the positive and negative tests as well as their precision are shown in *Table II*.

Anti-CCP 2 antibodies have shown better diagnostic performance than RF (sensitivity 65.71% vs. 48.57%, specificity 97.14% vs. 94.28%) in the detection of RA.

Table I Anti-CCP 2 antibody and RF in RA and healthy control group.

	RA untreated group (n=35) value (M ± SD)	RA $^{sero-}$ (n=18) value (M ± SD)	RA $^{sero+}$ (n=17) value (M ± SD)	Healthy control group (n=35) value (M ± SD)
	Positive / Negative	Positive / Negative	Positive / Negative	Positive / Negative
Anti-CCP 2 + ≥ 1.26	23/12 1.71 (± 0.69) (0.92–3.0)	11/7 1.56 (±0.59) (0.93–2.6)	12/5 1.87 (± 0.77) (0.92–3.0)	1/34 0.95 (± 0.10) (0.90–1.38)
DAS 28 + ≥ 3.2	28/7 4.79 (± 1.56) (1.85–7.03)	13/5 4.56 (± 1.76) (1.85–7.03)	15/2 5.04 (± 1.33) (2.47–6.83)	0/35 0.00 (± 0.00) (0.00–0.00)
MORNING RIGID + ≥ 0 min	26/9 43.20 (± 65.13) (0–300)	14/4 57.50 (± 81.40) (0–300)	12/5 28.05 (± 38.72) (0–120)	0/35 0.00 (± 0.00) (0.00-0.00)
RF + 30 ≥ IU/mL	17/18 346.15 (± 625.22) (0.00–1920)	0/18 0.00 (± 0.00) (0.00–0.00)	17/0 712.67 (± 743.72) (30–1920)	2/33 13.71 (± 38.73) (0.00–120)
CRP + 12 ≥ mg/L	14/21 46.86 (± 79.19) (0.00–384)	3/15 8.66 (± 24.62) (0.00–96)	13/4 87.31 (± 96.44) (0.00–384)	4/31 5.48 (± 12.80) (0.00–48)
SEDIMENTATION + ≥ 16 mm/h	27/8 48.62 (± 39.81) (2.0–120)	13/5 43.94 (± 39.82) (2.0–120)	14/3 53.58 (± 40.39) (5.0–120)	4/31 9.42 (± 8.21) (2.0–44)

Table II Diagnostic performance of anti-CCP 2 antibody and RF in rheumatoid arthritis.

	Anti-CCP 2 (RA=35)	Anti-CCP 2 (RA^-=18)	Anti-CCP 2 (RA^+=17)	RF (RA=35)	RF (RA^-=18)	RF (RA^+=17)	CRP (RA=35)	CRP (RA^-=18)	CRP (RA^+=17)
Sensitivity %	65.71	61.11	70.58	48.57	0	100	66.66	16.66	76.47
Specificity %	97.14	97.14	97.14	94.28	94.28	94.28	88.57	88.57	88.57
Predictive values of the positive test %	95.83	91.66	92.30	89.47	0	89.47	77.77	42.85	76.47
Predictive values of the negative test %	26.08	17.03	12.82	35.29	35.29	0	40.38	36.60	11.42
Precision %	81.42	84.90	88.46	71.42	62.26	96.15	64.28	64.15	84.61
	SER (RA=35)	SER (RA^-=18)	SER (RA^+=17)	MORNING RIGID (RA=35)	MORNING RIGID (RA^-=18)	MORNING RIGID (RA^+=17)	DAS 28 (RA=35)	DAS 28 (RA^-=18)	DAS 28 (RA^+=17)
Sensitivity %	77.14	72.22	82.35	74.28	77.77	70.58	80	72.22	88.23
Specificity %	88.57	88.57	88.57	100	100	100	100	100	100
Predictive values of the positive test %	87.09	76.47	77.77	100	100	100	100	100	100
Predictive values of the negative test %	20.51	13.88	8.82	20.45	10.25	12.5	16.16	12.5	5.40
Precision %	82.85	83.01	86.53	87.14	92.48	90.38	90	90.56	96.15

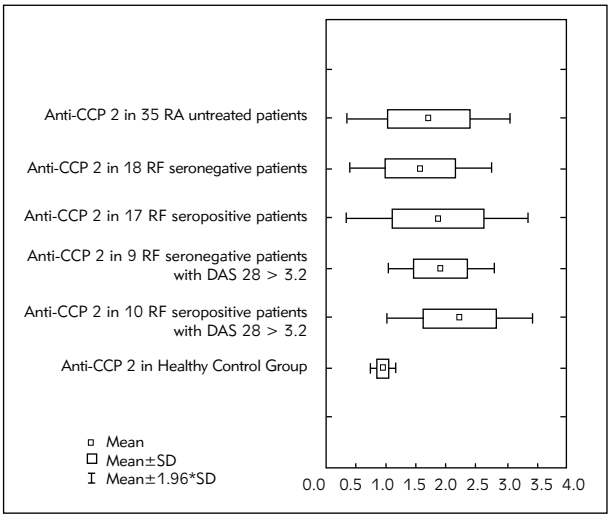

Figure 1 Distribution of anti-CCP 2 antibody.

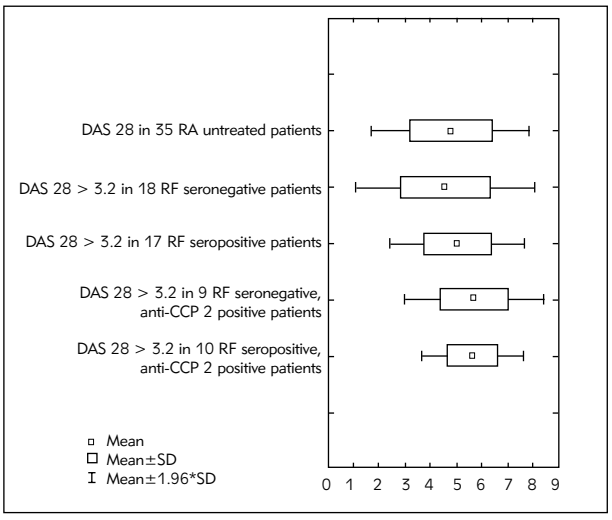

Figure 2 Distribution of DAS 28 index.

Anti-CCP 2 antibody and DAS 28 index of intensity of the disease

Among 35 patients with RA, DAS 28 > 3.2 was replaced in 28 patients (80%). In 17 seropositive RF patients, replacement of DAS 28 > 3.2 was found in 15 patients (88.23%). Among these 15 patients with DAS 28 > 3.2, 10 were anti-CCP 2 positive (66.66%), and their M ± SD (2.23 ± 0.61) was extended (1.28–3.0).

In 18 seronegative RF patients, replacement of DAS 28 > 3.2 was found in 13 patients (72.22%). Among these 13 patients with DAS 28 > 3.2, 9 were anti-CCP 2 positive (69.23%) and their M ± SD (1.92 ± 0.45) was extended (1.3–2.6).

Seropositive RF patients have bigger titers of anti-CCP 2 antibody than RF seronegative (See *Table I*), (1.87± 0.77 (0.92–3.0) vs. 1.56 ± 0.59 (0.93–2.6)), and a bigger DAS 28 > 3.2 index (5.04 ±1.33 (2.47–6.83) vs. 4.56 ± 1.76 (1.85– 7.03)). Between these two groups of anti-CCP 2 antibody there is no statistical relation (p=0.265948).

Although the same representation of anti-CCP 2 positive patients with DAS 28 > 3.2 was found in seropositive and seronegative patients (10 vs. 9 patients; 66.66% vs. 69.23%), the extent of the titer of anti-CCP 2 was bigger in 10 RF seropositive patients with DAS 28 > 3.2, compared with RF seronegative patients with DAS 28 > 3.2 (2.23 ± 0.61 vs. 1.92 ± 0.45). Between these two groups there was no statistical relation (p=0.374260) (*Figure 1*).

The condition was almost equal for DAS 28 index in 9 RF seronegative, anti-CCP 2 positive patients (5.69 ± 1.37) extent (3.31–7.03) compared with 10 RF seropositive, anti-CCP 2 positive patients (5.63 ±1.01) extent (4.17–6.83).

There was no statistical relation between the DAS 28 index in RF seropositive and seronegative patients (p=0.379375) and between two groups of DAS 28 > 3.2, anti-CCP 2 positive patients, but RF seropositive and seronegative patients (p=0.905696) (*Figure 2*).

A statistical relation was found using the Wilcoxon-matched test between anti-CCP 2 in RA and healthy control group for $p<0.05$ (p= 0.000002).

A statistical relation was found using the Wilcoxon-matched test between: anti-CCP 2 in RA and DAS 28, RF and CRP, SER, morning rigid, in the same group for $p<0.05$: (anti-CCP 2 vs. DAS 28 p=0.000000; anti-CCP 2 vs. RF p=0.018345; anti-CCP 2 vs. CRP p= 0.040620; anti-CCP 2 vs. morning rigid p=0.000032; anti-CCP 2 vs. SER p=0.000000).

Discussion

The report for the sensitivity of first generation anti-CCP 2 antibody is approximately 68% (45–80%) and 98% (96–100%) for specificity (9). The report for the sensitivity of second (2) generation anti-CCP 2 antibody is approximately 64–74%, with the specificity of 90–99% (11–16, 32). The advantages of the use of anti-CCP 2 test can be seen in the early differentiation of arthritis (33). Our conclusions for sensitivity from 65.71% and specificity from 97.14% are in the frames of these studies. High specificity is useful in RF negative RA patients, where it is 61.11%. Mean sensitivity and high specificity allow anti-CCP 2 antibody to be included as a classification criterion in RA. Although the DAS 28 index, which is not only a laboratory variable but a clinical index for the estimate of the disease, has higher sensitivity (80%) and specificity (100%), anti-CCP 2 antibody, as an isolated laboratory variable, dominates with its performance in the early diagnosis of undifferentiated RA. However, we have to pay attention

to the fact that the results achieved in this study are smaller and retreat from values given by the producer DIA-STAT™ Anti-CCP (Axis-Shield Diagnostics) (sensitivity for anti-CCP 2 79%, specificity 100 %). Data given for anti-CCP 2 antibody are higher than those from previous tests by other examiners (12, 31, 34).

In conclusion, anti-CCP 2 antibodies have higher sensitivity and specificity than RF in RA. Anti-CCP 2 test is used in everyday clinical practice for the diagnosis of early undifferentiated RA.

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