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THE DIAGNOSTIC VALUE OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES (anti-CCP) IN PATIENTS WITH RHEUMATOID ARTHRITIS

DIJAGNOSTIČKO ZNAČENJE ANTI-CIKLIČNIH CITRULINIRANIH PEPTIDNIH ANTITELA (anti-CCP) KOD PACIJENATA SA REUMATOIDNIM ARTRITISOM

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Summary: A cross-sectional study was carried out to analyze the prevalence of anti-CCP antibodies and IgM RF in the sera of 160 randomly selected patients from the Rheumatology Department: 60 with RA, 50 with other rheumatic diseases (non-RA), 50 healthy controls (HC). The mean age of the group was 50.06+/–11.9 years. There were 141 females (88.1%) and 19 males (11.9%). RA patients fulfilled the revised ACR criteria. The mean duration of the disease was 82.4 months. Anti-CCP ELISA kit and IgM RF Latex test were used. The mean anti-CCP values were as follows: RA 60.4+/–57.6, non-RA 2.1+/–3.6, HC 1.3 +/–0.4 U/mL. Respectively, the mean values of IgM RF were: RA 515.8+/–525, non-RA 102+/–294, HC 15+/–57.5. Forty out of 60 (66.6%) RA patients were anti-CCP positive. Forty one out of 60 (68.3%) RA patients were positive for IgM RF. As expected, anti-CCP showed comparable sensitivity (66.8% vs. 68.3%) and higher specificity (98% vs. 87%) than IgM RF, at optimal cut-off values. The presence of either anti-CCP or IgM RF increased the testing sensitivity for the diagnosis of RA to 76.6%. AUC was greater for anti-CCP than for IgM RF (0.92 vs. 0.82).

Keywords: anti-cyclic citrullinated peptide antibodies, rheumatoid factor, rheumatoid arthritis

Kratak sadržaj: Radi utvrđivanja dijagnostičke senzitivnosti i specifičnosti anti-cikličnih citruliniranih peptidnih antitela anti-CCP, kao novog testa za RA urađena je »cross-sectional« studija na Reumatološkoj klinici u Skoplju. Ispitivana grupa sastojala se od 60 bolesnika s reumatoidnim artritisom koji su ispunjavali ACR kriterijume. Kontrolnu grupu 1 sačinjavali su bolesnici sa drugim reumatskim bolestima (50 non-RA), a kontrolnu grupu 2 zdrave osobe (50 ZO). Obuhvaćeni su bolesnici oba pola, 141 žena (88,1%) i 19 muškaraca (11,9%) prosečne starosti 50,06+/–11,9 godina, dužine trajanja bolesti 82,4 meseca. Upotrebljeni su anti-CCP ELISA kit i IgM RF Latex test. Srednje vrednosti za anti-CCP antitela bile su: RA 60,4+/–57,6, non-RA 2,1 +/–3,6, zdravi 1,3 +/–0,4 U/mL. Srednje vrednosti za IgM RF bile su sledeće: RA 515,8+/–525, non-RA102+/–294, zdravi 15 +/–57,5. Četrdeset RA bolesnika (40/60, 66,6%) bili su anti-CCP pozitivni, a 41/60 68,3% bili su IgM RF pozitivni. Kao što smo očekivali, anti-CCP antitela pokazala su skoro identičnu senzitivnost (66,8% vs. 68,3%), ali višu specifičnost (98% vs. 87%) u poređenju sa IgM RF. Pozitivnost za anti-CCP ili IgM RF rezultira povećanjem dijagnostičke senzitivnosti za RA (76,6%). Oblast pod krivom bila je veća kod anti-CCP antitela u odnosu na IgM RF (0,92 vs. 0,82).

Ključne reči: anti-ciklična citrulinirana peptidna antitela, reumatoidni faktor, reumatoidni artritis

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune inflammatory disorder which affects 0.5–1% of the population worldwide (1). Once viewed as inexorably progressive, RA has become a potentially treatable disease with the early, aggressive use of disease modifying anti-rheumatic drugs DMARDS (2).

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With the availability of more sophisticated and effective therapies and with the understanding that early intervention is crucial for preventing irreversible joint damage, it is widely accepted that early and accurate diagnosis of RA is critical for the management of the disease (3).

The diagnosis of RA depends primarily on the clinical manifestations of the disease, with very limited serological support. The only serological test included in the American College of Rheumatology (ACR) revised classification criteria is rheumatoid factor (RF) which is not specific for RA (1). RF is also detected in the sera of the patients with other rheumatic diseases, infectious diseases, as well as in healthy elderly individuals (4).

In recent years, the introduction of anti-cyclic citrullinated peptide antibodies (anti-CCP) showed some promise as a diagnostic tool in RA. These antibodies are detected by ELISA technique where a synthetic cyclic citrullinated peptide is used as a substrate. Citrulline can be formed by the posttranslational enzymatic conversion of arginin residues, catalyzed by peptidylarginin deiminase. The sensitivity of the anti-CCP 2 test ranges between 64 and 74% whereas the specificity ranges between 90 and 99% (5).

The objective of the study was to determine the diagnostic value, specificity and sensitivity of ACPA as a new test for rheumatoid arthritis.

Materials and Methods

A cross-sectional analysis was carried out on 160 serum samples: 60 from RA patients (RA) diagnosed according to the ACR criteria; 50 from patients with other rheumatic diseases, mostly from patients with CTDs and other inflammatory arthropathies (non-RA) and 50 healthy controls matched for age and gender. Patients and controls were consecutively recruited from the Rheumatology outpatient department, Clinical Center Skopje, Macedonia. An informed consent was obtained from the participants and the study was performed according to the Helsinki Declaration of ethical guidelines.

Serum antibodies directed to cyclic citrullinated peptide were analyzed using DIASTAT anti-CCP ELISA kit, Axis-Shield, UK, second generation, carried out according to the manufacturer's instructions. The recommended cut-off value was 5 U/mL. IgM RF was analyzed using Latex agglutination test, Biosystems, Spain. Each of these tests was performed and evaluated by operators who were blinded to other serological results and unaware of the patient's clinical data.

Acute phase reactants were measured by erythrocyte sedimentation rate (ESR, Westergreen method, mm/h) and C-reactive protein (agglutination test, mg/L). The disease activity was assessed by the disease activity score, using DAS28.

Statistical analysis was done using SPSS software, version 14, as well as Statistica for Windows. Sensitivity, specificity, positive and negative predictive values were calculated using logistic regression analysis. In addition, receiver operating curve analysis (ROC) was carried out to compare test characteristics independently of predefined cut-off points.

Area under the curve (AUC) was also calculated.

Results

Demographic characteristics of the RA patients and controls are shown in *Table I*.

Non-RA group consisted of 26 patients (52%) with SLE, 5 patients (10%) with systemic sclerosis, 10% with seronegative spondyloarthropathies, 4 patients (8%) with polymyalgia rheumatica, 3 patients (6%) with vasculitides and few patients with Sjögren's sy, chronic juvenile arthritis and dermato/polymyositis.

The mean anti-CCP values were as follows: RA 60.4+/–57.6, non-RA 2.1 +/–3.6 , HC 1.3 +/–0.4 U/mL. Respectively, the mean values of IgM RF were: RA 515.8+/–525, non-RA 102+/–294, HC 15 +/–57.5.

Among patients with RA, 40/60 (66.6%) were anti-CCP positive and 41/60 (68.3%) were IgM RF positive. Thirty five (58.3%) of the RA patients were positive for both antibodies (anti-CCP and IgM RF). Anti-CCP or RF positivity was present in 46 patients (76.6%).

Among 19 RF negative patients, 5 (26%) were anti-CCP positive. Among 20 anti-CCP negative patients, 6 (30%) were positive for IgM RF.

In the non-RA group, only 2/50 patients (4%) were anti-CCP positive, one patient with SLE and one patient with dermatomyositis. Respectively, 13/50 patients (26%) in the non-RA group were IgM RF positive. In the HC group, none of the patients was anti-CCP positive and 4 patients (8%) were IgM RF positive.

Table I Demographic characteristics of the patients.

	RA (n= 60)		Non-RA (n=50)		HC (n=50)	
	No	%	No	%	No	%
Females	55	91.7	43	86	43	86
Males	5	8.3	7	14	7	14
Age	54.1 +/– 10.9		44.84 +/–12.8		50.42+/–10.9	
Disease duration	82.4		71.3		/	

Table II Diagnostic performance of anti-CCP and IgM RF.

Anti-CCP	RA vs. HC	RA vs. non-RA	RA vs. non-RA+HC
AUC	0.92	0.89	/
Sensitivity	66.6%	66.6%	66.6%
Specificity	100%	96%	98%
PPV	100%	95%	95.2%
NPV	71.4%	70.5%	83%
Positive likelihood ratio	/	16.65%	33.3
Negative likelihood ratio	0.3	0.3	0.3
Accuracy	79%	80%	86.2%

IgM RF	RA vs. HC	RA vs. non-RA	RA vs. non-RA+HC
AUC	0.82	0.76	/
Sensitivity	68.3%	68.3%	68.3%
Specificity	92%	74%	87%
PPV	91.1%	75%	75.9%
NPV	70%	66%	85.2%
Positive likelihood ratio	8.5	2.62%	5.25
Negative likelihood ratio	0.3	0.4	0.3
Accuracy	79%	70.9%	76.2%

PPV – positive predictive value, NPV – negative predictive value

Sensitivity, specificity, positive and negative predicted values were calculated for both anti-CCP and IgM RF (*Table II*).

We also compared the results of anti-CCP and IgM RF for sensitivity and specificity. Anti-CCP showed similar sensitivity, but higher specificity than IgM RF.

As shown in *Table II*, the sensitivity of the anti-CCP antibodies assay was 66.6%, which was very similar with the sensitivity of IgM RF – 68.3%. The specificity of the anti-CCP assay was 96% in regard to the non-RA control group and 100% for HC. Overall, the specificity for anti-CCP was 98% in regard to both control groups. The specificity of IgM RF was significantly lower, 74% for non-RA controls, 92% for HC and 87% in regard to both control groups.

The presence of either antibody (anti-CCP or IgM RF) increased sensitivity to 76.6%, whereas the presence of both antibodies (anti-CCP and IgM RF) increased specificity to 99%.

When sensitivity and specificity were considered simultaneously, the AUC was greater for anti-CCP (AUC 0.92 for HC, 95% CI 0.86–0.97) than for IgM RF (AUC 0.82 for HC, 95% CI 0.74–0.90) (*Figure 1*).

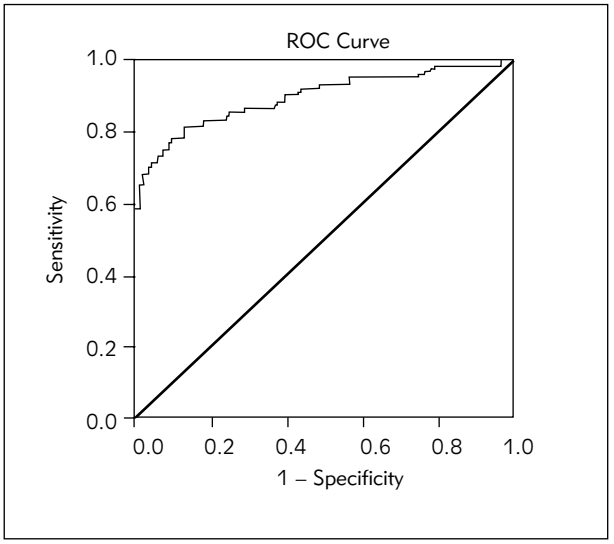

Figure 1 Receiver Operating Characteristic (ROC) curve of the anti-CCP test AUC 0.92 (95% CI 0.86–0.97).

Table III Diagnostic characteristics of the anti-CCP test (first and second generation).

Author	Year	Anti-CCP	
*CCP second generation		Sensitivity (%)	Specificity (%)
Schellekens	1998	76	96
Bizzaro	2001	41	97.8
Bas	2002*	68	96
Lee & Shur	2003*	66	90.4
Vallbracht	2004*	64.4	97.1
Dubucquoi	2004*	80	96.4
Choi	2005*	72.8	92
Alexiou	2007*	63.6	95

Discussion

First described by Schellekens, the anti-CCP antibody test has aroused great interest in the past few years. The first generation of the test showed reasonable sensitivity (48–68%) and excellent specificity (98%) (6).

The second generation of the test was introduced in 2002. Cyclic citrullinated peptide was used as an epitope in order to achieve a better sensitivity.

The sensitivity of the anti-CCP2 test varied from 64.4% to 96%. The specificity values reported ranged from 88.9% to 100%. Anti-CCP test was positive in 5% of the non-RA patients with CTDs (7). The highest specificity (100%) was seen in the studies in which the control group was rather small and consisted of healthy individuals. These results are shown in *Table III*. Our findings are in agreement with the results of

others, with the sensitivity of 66.6% and specificity of 98%.

The emergence of the anti-CCP antibody assay, as a new serological marker for RA, is a significant advance in rheumatological care. The presence of anti-CCP antibodies is linked with early aggressive RA, a greater risk for erosive disease, greater disease activity, and there is a possible association with the shared epitope. Anti-CCP antibody titres are positively correlated with the disease activity. Anti-CCP titres have been found to decrease with anti-TNF treatment (8–10).

Considering costs, it might be advisable to determine IgM RF first. In patients with low titres (<50 IU/mL) or negative IgM RF, anti-CCP determination helps to identify the additional patients with RA (11).

Conclusion

Anti-CCP antibody test is an additional serological marker to assist in the early diagnosis of RA. Sensitivity of the second generation of the anti-CCP test is close to that of rheumatoid factor, with a higher specificity for distinguishing between RA and other rheumatic diseases. Thus, anti-CCP antibody test represents a valuable addition to the diagnostic armamentarium of the rheumatologists.

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