

## Clinical Relevance and Evaluation of the Antibodies against Cyclic Citrullinated Peptide as Overall Test in Undifferentiated Psoriatic Arthropathy Sine Psoriasis Vulgaris with or without Joint Inflammation. Hallmark, Overhaling or Amplification to Rheumathoid Arthritis?

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### Abstract

**Aims:** To compare the values and acuracy of the test in anticyclic citrullinated peptides (Anti-CCP /ACPA) antibodies, Rheumatoid factor (RF), C-Reactive Protein (CRP) and disease activity index (PASI) in early diagnosis of untreated Psoriatic arthritis (PsA).

**Methods:** Using the ELISA method of DIA-STAT<sup>TM</sup> Anti CCP (Axis–Shield Diagnostics), sera of 70 participants were examined (35 untreated patients with PsA and 35 persons from the healthy control group). RF and CRP were determined with the agglutination test (Latex test) in the same participants. In the same time we determined the sensitivity, specificity, predictive value for positive and negative test and accuracy.

**Results:** From 35 pts with PsA, 1 patient showed presence of Anti-CCP antibodies (sensitivity test 2.86%), while RF was present in 0 pts (sensitivity test 0%). In the healthy control group positive values for RF, CRP and erythrocyte sedimentation rate (ESR) were detected in 1 participant.

**Conclusion:** ACPA antibodies have low sensitivity, but high specificity in PsA.

**Keywords:** Anti cyclic citrullinated peptide; Psoriatic arthritis; Rheumatoid factor.

### Introduction

Anti-cyclic peptide antibodies (CCP/ACPA) are antibodies directed towards synthetic citrullinated peptides and are specific markers in diagnosis of Rheumatoid arthritis (RA). They belong to the group of protein/peptide antibodies. There are several generations of these antibodies in their evolution. Antibodies like APF and AKA detected by indirect fluorescence using buccal epithe-

lium or rat's esophagus [1], have great specificity for RA.

Absence of donors for buccal cells limits the use of APF as a routine laboratory test. Antigen for these Anti-flaggrin antibody (AFA) is identified as an epidermal flaggrin which is intermediary filament involved in the epidermal cornification [2,3]. Profilaggrin, present in keratohyalin granules of the buccal cells is proteolytically released in flaggrin subunits during cell differentiation. In this stadium, the protein is dephosphorylated and some arginine residues are converted in citrullines from enzyme Peptidyl-Ararginine Deaminase (PAD) [4]. Their reactivity depends on epitopes which

contain amino acid citrulline. In 1998, Schellekers et al. reported about autoantibodies that reacted in linear synthetic peptides which contained unusual amino acid citrulline. So two types of CCP assay were developed with peptides A and B. They are present in 76% in RA, with 96% specificity. Antibodies in patients with RA are predominantly IgG type and have relatively high affinity [5]. The ELISA test, based on these Cyclic Citrullinated Peptides (CCP) have superior characteristics in detection of RA [6], with different sensitivity and specificity [7]. Sensitivity of the ACPA test in different populations ranges between 64% and 74%, while specificity ranges between 90% and 99%. There are PsA patients in whom these antibodies are detected. The aim of this study is to determine the diagnostic value of ACPA antibodies in PsA.

## Material and Methods

In patients included in the study the disease diagnosis was based upon revised diagnostic criteria for classification of Psoriatic arthritis from 2005, proposed by the American Association of Rheumatism (ARA) [8]. Clinical evaluation of disease activity and disease diagnosis was performed by subspecialist in the field based upon the diagnostic criteria of Moll-Wright for classification of Psoriatic arthritis [9]. They were dermatologically tested, including examination of the psoriatic changes of the nails, psoriatic areas, disease activity index (PASI) and evaluation of the peripheral and axial joints [10]. Oligoarthritis is taken in consideration when < 5 joints are involved and polyarthritis when > 5 joints are involved. Symmetric arthritis is considered when there is bilateral involvement and when > 50% of joints are seized.

In the study were included 35 pts (18 women, 17 men) with PsA and 35 pts (19 women, 16 men) from the healthy control group. Mean age was 47.18 years ( $\pm 9.08$ ) (35-65 years) in the group with PsA, while 40.2 years ( $\pm 9.21$ ) (29-65 years) in the healthy control group. Mean disease duration was 6.27 months ( $\pm 8.22$ ) (1-36 months). None of them received disease modification drugs. The others negated drug use before entering the study, especially drugs from the base line such as methotrexate, leflunomide or sulphasalazine. Specimen were collected in the period of 2 years.

### Including criteria

In the study were included patients with Psoriatic arthritis aged 18-65, newly diagnosed and previously untreated.

### Excluding criteria

From the study were excluded patients with diseases or conditions that could influence results directly or indirectly:

1. Patient younger than 18 years.

2. Patients with previous history of disease of the spleen, thyroid gland, liver, kidneys, hematological, cardiovascular, neurological, autoimmune and lung diseases.
3. Patients with diabetes mellitus, febrile conditions, acute infections, neoplasms.
4. Patients with uric arthritis, SLE, mixed connective tissue disease, vasculitis.
5. Patients with history of blood transfusion and patients with body overweight.
6. Patients with history of use of drugs from the base line.
7. Patients that in 0 point had increased level of glucose, serum and urine urea and creatinine, blood hypertension, smokers and blood and enzyme disorders.
8. Patients previously treated with salicylates, antibiotics, golden salts or diuretics.

All the patients took part in this study voluntarily, so the ethic criteria for this study are fulfilled.

## Laboratory Evaluation

For clinical evaluation of the disease, the following parameters were necessary: complete blood count (CBC) and differential, reactants of the acute phase, ACPA antibodies, C-reactive protein (CRP), Rheumatoid factor (RF) and Erythrocyte Sedimentation Rate (ESR), Alkaline Phosphatase (AP), Aspartate Amino Transferase (AST), Alanine Amino transferase (ALT), Creatine Kinase (CK), Lactate Dehydrogenase (LDH), serum urea and serum creatinine.

Serum creatinine was determined with the "Jaffe" method.

Referent values: serum creatinine 45-109 mmol/L ; Urine creatinine 7-17mmol/dU.

CRP was determined with the agglutination test (Lateks CRP test) (Bio Systems S.A. Reagents & Instruments Costa Brava 30, Barcelona, Spain). Referent values: < 6 mg/L CRP in serum.

RF was determined with the agglutination test (Lateks CRP test) (Bio Systems S.A. Reagents & Instruments Costa Brava 30, Barcelona, Spain).

Referent values: < 8 IU/ml RF in serum.

Quantitative method used for for determination of ESR was Westergren method.

Referent values: 7-8mm for women, 11-16mm for men.

DIA-STATM Anti CCP (Axis-Shield Diagnostics) test is semi quantitative/qualitative ELISA test, based upon the detection of IgG autoantibodies in human plasma or serum, directed towards

synthetic cyclic citrullinated peptides (CCP), which contain modified arginine residues. This test was an additional tool.

## Principles of work

The walls of the microtiter have highly purified synthetic cyclic peptide which contains modified arginine residues. During first incubation, the specific autoantibodies from diluted serum or plasma are connected to the surface antigen. It is washed than in order to eliminate the unconnected components. During the second incubation, the conjugate, which is an enzyme of the monoclonal autoantibody for human IgG, is connected to the surface autoantibody. After the second wash, the specific autoantibodies are incubated with the substrate. After adding the stop solution, the reaction is interrupted and this results with the colored end result. The amount of the absorbed conjugate is expressed in absorption units. In the quantitative protocol, the amount of the conjugate connected with the sample is compared with the same connected in the referent control. In the semi-quantitative protocol the anti CCP autoantibody concentration could be estimated with interpolation of the curve based on the standard. Fresh serum or plasma is used.

Calculation and interpretation of the results for the qualitative protocol are estimated from the absorbed value (optic density) from the positive and negative control as well as for every sample.

Absorbed value	Results interpretation
<0.95	Negative
>0.95 <1.0	Borderline
>1.0	Positive

## Statistical analysis

For testing the significance of differences between two arithmetical means, i.e. proportions the Student-t-test is used to compare the mean parameters of certain numerical parameters between groups, as well as Wilcoxon-matched test for independent samples. Sensitivity and predictivity for positive and negative test of the examined markers is determined with the test for sensitivity and specificity. P-value between 0.05 and 0.1 is considered statistically significant. Analysis of the data is performed with the statistical package Statistica 7.0.

## Results

From the 35 pts with PsA, 1 patient (2.86%) showed presence of ACPA antibodies, while RF was present in 0 pts (0%). In the healthy control group 0 pts (0%) showed ACPA positivity, while 1 patient (2.86%) had positive RF (Table 1).

	Untreated PsA Group NO 35 Value (M ± SD)	Healthy control group NO 35 Value (M ± SD)
	Positive/negative	Positive/negative
ACPA + > 1,26	1/34	0/35
RF +30 > IU/ml	0/35	1/34
CRP +12 > mg/L	16/19	1/34
ESR + > 16	18/17	1/34

**Table 1:** ACPA autoantibodies in PsA and healthy control group.

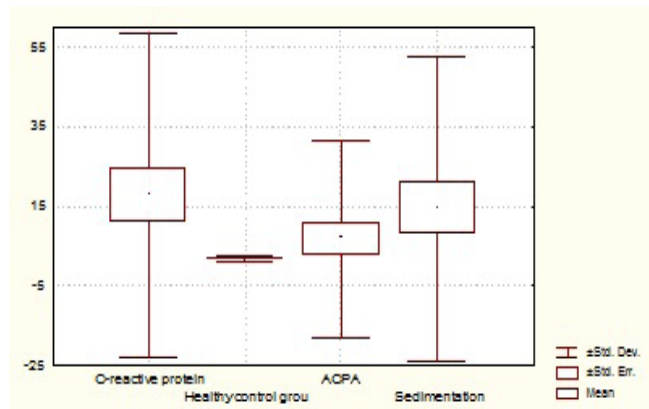
## Diagnostic value of ACPA autoantibodies in Psoriatic arthritis

For ACPA autoantibodies and reactants of the acute phase in PsA, sensitivity, specificity, predictive value for positive and negative test and their acuracy are shown on Table 2. ACPA autoantibodies have equal diagnostic performances as RF (sensitivity 2.86% vs 0%, specificity 100% vs 97.14% in detection of untreated PsA (Graf 1).

- Using the Wilcoxon-matched test we found statistical correlation between ACPA in PsA and healthy control group for  $p < 0.05$  ( $p = 0.01$ ). In the PsA group there was statistical correlation between ACPA and RF for  $p < 0.05$  ( $p = 0.00$ ); ACPA and CRP ( $p = 0.00$ ).
- Using the Wilcoxon-matched test we found no statistical correlation in the PsA group between ACPA and age, disease diuration in months, PASI index, RF and CRP for  $p < 0.05$ : (ACPA vs age  $p = 0.04$ ; ACPA vs disease diuration in months  $p = 0.07$ ; ACPA vs PASI  $p = 0.08$ ; ACPA vs RF  $p = 0.02$ ; AAP vs CRP  $p = 0.05$ ; AAP vs ESR  $p = 0.06$ ).
- Using the Wilcoxon-matched test we found statistical correlation in the PsA group between disease diuration in months, PASI index, RF and CRP for  $p < 0.05$ : (CRP vs age  $p = 0.00$ ; CRP vs disease diuration in months  $p = 0.00$ ; CRP vs PASI  $p = 0.00$ ; CRP vs RF  $p = 0.02$ ; CRP vs ESR  $p = 0.00$ ).

	ACPA	RF	CRP	ESR
	PsA No 35	PsA No 35	PsA No 35	PsA No 35
Sensitivity (%)	2.86	0	45.71	51.43
Specificity (%)	100	97.14	97.14	97.14
Predictive value for positive test (%)	100	0	94.12	94.74
Predictive value for negative test (%)	50.72	49.28	64.15	66.67
Accuracy (%)	51.42	48.57	71.42	74.28

**Table 2:** Diagnostic performances of ACPA and other laboratory variables in PsA.



**Graph 1:** Distribution of ACPA, Sedimentation and CRP in PsA and healthy control group.

## Discussion

While introducing some new diagnostic method it is necessary to estimate its quality i.e. to find out the utility of information compared to the risk for the patient and the price of the test. This became more interesting recently when due to the development in technology a lot of methods were introduced. Although the subjective estimation of the doctor who is responsible for the patient would be crucial in the choice of the available diagnostic methods, objective quantitative estimation of every method would help him in the most rational approach.

The contribution of every method quantitatively expressed would be able to classify the methods according to their efficacy.

ACPA autoantibodies are specific markers in diagnose of RA and have role in the disease pathophysiology. Psoriasis vulgaris is found in 3% of the common population. PsA in Psoriasis vulgaris is found in 7%. ACPA autoantibodies in PsA have greater prevalence of 8% in comparison with the common population [11]. Isolation of ACPA autoantibodies could be from synovial fluid also [12]. Unlike RA, the presence of ACPA autoantibodies in PsA could be explained only as an epifenomenon in this disease [13]. It is noted in the literature that these antibodies have significance in PsA for the evaluation of osteoporosis, erosive changes and bone destructiones - fractures. Data obtained for ACPA autoantibodies are not higher than previously tested kits from other researches [14-19]. The ACPA autoantibody as an isolated laboratory variable does not dominate with its performances in diagnose of early, undifferentiated PsA.

However, it should be mentioned that obtained values in this study are equal and do not digress from the values obtained from

the producer DIA-STAT<sup>TM</sup> Anti CCP (Axis-Shield Diagnostics) for PsA (sensitivity for anti ACPA 5%, specificity 100%).

## Conclusion

ACPA autoantibodies have low sensitivity and high specificity in diagnose of PsA. Every positive result should be interpreted together with the clinical evaluation of the disease and diagnoastic procedures designated for it. It should be mentioned that elevated values of these antibodies could appear in persons without clinical signs of disease. The concentration of these antibodies not always correlate with the disease severity. They could be applied in pediatric cases with great caution, because they are not designated for them.

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