IBBJ

Summer 2016, Vol 2, No 3

Original Article

Frequency and Accuracy of Anti-Citrullinated Protein Antibodies, Prognostic Evaluation in Overall Developing to Erosive Disease in Patients with Psoriatic Seronegative Osteoarthropathy

Dejan Spasovski*

University Clinic of Rheumatology, "Sc Ciryl and Methodius" University, Skopje, Republic of Macedonia.

Submitted 27 Sep 2016; Accepted 30 Nov Oct; Published 10 Dec 2016

Anti-cyclic peptide antibodies (CCP/ACPA) are directed towards synthetic citrullinated peptides and are specific markers in diagnosis of Rheumatoid arthritis. This study aimed 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). Using the ELISA method, sera of 70 participants were examined (35 untreated PsA patients and 35 healthy controls). RF and CRP were determined with the agglutination test. At the same time, the sensitivity, specificity, predictive value for positive and negative test, and accuracy were determined. From 35 PsA patients, 1 patient showed presence of Anti-CCP antibodies (sensitivity test 2.86%), while RF was not detected (sensitivity test 0%). In the healthy control group positive values for RF, CRP and erythroid sedimentation rate were detected in 1 participant. Therefore, ACPA antibodies have low sensitivity, but high specificity in PsA.

Keywords: Anti cyclic citrullinated peptide (Anti-CCP), psoriatic arthritis (PsA), rheumatoid factor (RF)

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 anti-perinuclear factor (APF) and anti-keratin antibodies (AKA) detected by indirect fluorescence using buccal epithelium 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. An epidermal filaggrin which is an intermediary filament involved in the epidermal cornification, serves as antigen for the anti-filaggrin antibodie (AFA) (2-3). Profilaggrin, present in keratohyalin granules of the buccal cells is proteolytically released in filaggrin subunits during cell differentiation. In this stadium, the protein is dephosphorylated and some arginine residues are converted into citrulline by peptidyl-arginine deaminase (PAD) enzyme (4). Their reactivity

*Correspondence: University Clinic of Rheumatology[1], "Sc Cyryl and Methodius" University, Skopje, Republic of Macedonia.
E-mail: drspasovski@yahoo.co.uk

depends on epitopes containing citrulline. In 1998, Schellekers et al. reported the presence of autoantibodies that reacted with linear synthetic peptides which contained the unusual amino acid citrulline. So, two types of CCP assay were developed with 2 peptides (A and B) which were recognized by antibodies present in the sera of 76% of RA patients, 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 features 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 some psoriatic arthritis (PsA) patients in whom these antibodies may be detected. The aim of this study was to determine the diagnostic value of ACPA antibodies in PsA.

Materials and methods

Patients included in the study were diagnosed according to the revised diagnostic criteria for classification of PsA, proposed by the American association of rheumatism (ARA) at 2005 (8). Clinical evaluation of disease activity and disease diagnosis was performed by a subspecialist in the field, based on the diagnostic criteria of Moll-Wright for classification of PsA (9). Patients 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 was taken into consideration when less than 5 joints were involved and polyarthritis when more than 5 joints were involved. Symmetric arthritis was considered when there was bilateral involvement and when more than 50% of joints were seized.

35 patients (18 women, 17 men) with PsA and 35 subjects (19 women, 16 men) from the healthy control group, were included in the study. Mean age was 47.18± 9.08 years (35-65 years) in the PsA group, and 40.20± 9.21 years (29-65 years) in the healthy control group. Mean disease duration was 6.27± 8.22 months (1-36 months). None of the patients received disease modification drugs. The others negated drug use before entering the study, especially baseline drugs such as methotrexate, leflunomide or sulphasalasine. Specimen were collected during 2 years period. Subjects with diseases or conditions that could influence results directly or indirectly, such as having less than 18 years, previous history of blood transfusion, disease of the spleen, thyroid gland, liver, kidneys, hematological, cardiovascular, neurological, autoimmune and lung diseases; those presenting diabetes mellitus, febrile conditions, acute infections, neoplasms, uric arthritis, SLE, mixed connective tissue disease, vasculitis; patients with increased level of glucose, serum and urine urea and creatinine, blood hypertension, blood and enzyme disorders at 0 point of recruitment; smokers, and patients who were previously treated with salycilates, antibiotics, golden salts or diuretics, were excluded from the study. All subjects took part voluntarily in this study, so the ethic criteria for this study was fulfilled.

Laboratory evaluation

For clinical evaluation of the disease, the following parameters were assessed: complete blood count (CBC) and differential, reactants of the acute phase, ACPA antibodies, C-reactive protein (CRP), rheumatoid factor (RF) and erythrocyte sediment-ation rate (ESR), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotra-nsferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), serum urea and serum creatinine levels.

Serum creatinine was determined with the

"Jaffe" method, with reference values being 45-109 mmol/l for serum creatinine, and 7-17 mmol/dU for urine creatinine.

CRP was determined with the agglutination test (Lateks CRP test) (BioSystems S.A. Reagens &Instruments Costa Brava 30, Barcelona, Spain) with reference values being less than 6 mg/l in serum. RF was determined with the agglutination test (Lateks CRP test) (BioSystems S.A. Reagens &Instruments Costa Brava 30, Barcelona, Spain) with reference values being less than 8 IU/ml in serum. Westergren method was used for quantitative determination of ESR, and reference values were 7-8 mm/h for women, 11-16 mm/h for men.

DIA-STAT™ Anti CCP (Axis-Shield Diagnostics) test which is a semiquantitative /qualitative ELISA test, based upon the detection of IgG autoantibodies in human plasma or serum, is directed towards synthetic cyclic citrullinated peptides (CCP), which contain modified arginine residues, and was an additional test used in this study.

Principles of anti CCP test

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 bound to the surface antigen. Then, a washing is performed in order to eliminate the unbound components. During the second incubation, the conjugate, which is an enzyme of the monoclonal autoantibody for human IgG, is bound 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 in colored end product. The amount of the absorbed conjugate is expressed in absorption units. In the quantitative protocol, the amount of the conjugate bound to the sample is compared with the same bound to the reference control. In the semi-quantitative protocol the anti CCP autoantibody concentration could be estimated with interpolation of the curve based on the standard. Fresh sera or plasma were used.

Calculation and interpretation of the results for the qualitative protocol were estimated from the absorbed values (optic density) from the positive and negative controls as well as for every sample. Absorbed values <0.95 were considered as negative, $\geq$0.95$\leq$1.0 were borderline, and >1.0 were considered as positive.

Statistical analyzes

For testing the significance of differences between two arithmetical means, i.e. proportions, Student-t-test was used to compare the mean parameters of certain numerical parameters between groups, as well as Willcoxon-matched test for independent samples. Sensitivity and predictivity for positive and negative test of the examined markers were determined with the test for sensitivity and specificity. P-value of 0.01 was considered as statistically significant. Analysis of the data was performed with the statistical package Statistica 7.0.

Results

From 35 PsA patients, 1 patient (2.86%) showed presence of ACPA antibodies, while RF was not detected in PsA patients (0%) (Table 1).

In the healthy control group no subject (0%) showed ACPA positivity, while 1 patient (2.86%) had positive RF.

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 accuracy are shown in Table 2. ACPA autoantibodies have diagnostic performances similar to RF (sensitivity 2.86% vs 0%, specificity 100% vs 97.14%) for detection of untreated PsA.

Table 1. ACPA autoantibodies in PsA and healthy control groups

	Untreated PsA N= 35 Positive/negative	**Healthy control group N= 35 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 mm/h	18/17	1/34

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

	ACPA	**RF**	**CRP**	**ESR**
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

Using the Wilcoxon-matched test statistical correlation was found between ACPA and RF (P= 0.00), or CRP (P= 0.00) in PsA patients. However, no statistical correlation was found in the PsA group between ACPA and age (P= 0.04), disease duration in months (P= 0.07), PASI index (P= 0.08), and RF (P= 0.02), as well as between AAP and ESR (P= 0.06). Also, in the PsA group statistical correlation was found between CRP and age (P= 0.00), disease diuration in months (P= 0.00), PASI index (P= 0.00), and ESR (P= 0.00), but not with RF (P= 0.02).

Discussion

While introducing a 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, many methods were introduced. Although the subjective estimation of the clinician 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 quantitative expression of every method will allow methods classification according to their efficacy. ACPA autoantibodies are specific markers in diagnosis of RA and have a role in the disease pathophysiology. Psoriasis vulgaris is found in 3% of the common population. PsA is found in 7% of psoriasis vulgaris cases. ACPA autoantibodies have greater prevalence (8%) in PsA patients in comparison with the common population (11). ACPA autoantibodies could also be isolated from synovial fluid (12). Unlike RA, the presence of ACPA autoantibodies in PsA could be explained only as an epiphenomenon 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 destructions-fractures. In the present study, performences 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 diagnosis 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™ Anti CCP (Axis-Shield Diagnostics) for PsA (5% sensitivity for anti ACPA, 100% specificity).

In conclusion, ACPA autoantibodies have low sensitivity and high specificity in diagnosis 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 do not always correlate with the disease severity.

Conflict of interest

The authors declared no conflict of interest.

References

1. Nakamura R M. Progress in the use of biochemical and biological markers for evaluation of rheumatoid arthritis. J Clin Lab Anal. 2000;14:305-13.

2. Simon M, Girbal E, Sebbag M, et al. The cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies," autoantibodies specific for rheumatoid arthritis. J Clin Invest. 1993;92:1387-93.

3. Sebbag M, Simon M, Vincent C, et al. The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1995;95:2672-9.

4. Girbal-Neuhauser E, Durieux J J, Arnaud M, et al. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. J Immunol. 1999;162:585-94.

5. Schellekens G A, de Jong B A, van den Hoogen F H, et al. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1998;101:273-81.

6. Schellekens G A, Visser H, de Jong B A, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000;43:155-63.

7. van Boekel M A, Vossenaar E R, van den Hoogen F H, et al. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. Arthritis Res. 2002;4:87-93.

8. Helliwell P S, Taylor W J. Classification and diagnostic criteria for psoriatic arthritis. Ann Rheum Dis. 2005;64 Suppl 2:ii3-8.

9. Moll J M, Wright V. Psoriatic arthritis. Semin Arthritis Rheum. 1973;3:55-78.

10. Schmitt J, Wozel G. The psoriasis area and severity index is the adequate criterion to define severity in chronic plaque-type psoriasis. Dermatology. 2005;210:194-9.

11. Vander Cruyssen B, Hoffman I E, Zmierczak H, et al. Anti-citrullinated peptide antibodies may occur in patients with psoriatic arthritis. Ann Rheum Dis. 2005;64:1145-9.

12. Spadaro A, Riccieri V, Scrivo R, et al. Anti-cyclic citrullinated peptide antibody determination in synovial fluid of psoriatic arthritis. Clin Exp Rheumatol. 2007;25:599-604.

13. Huynh D, Etzel C, Cox V, et al. SAT0268 Anti Citrullinated Peptide Antibody (ACPA) in Patients with Psoriatic Arthritis (PSA): Clinical Relevance. Ann Rheum Dis 2013;72:A673

14. Palazzi C, Olivieri I, Petricca A, et al. Rheumatoid arthritis or psoriatic symmetric polyarthritis? A difficult differential diagnosis. Clin Exp Rheumatol. 2002;20:3-4.

15. Helliwell P S. Relationship of psoriatic arthritis with the other spondyloarthropathies. Curr Opin

Rheumatol. 2004;16:344-9.

16. Alenius G M, Berglin E, Dahlqvist S R. Antibodies against cyclic citrullinated peptide (CCP) in psoriatic patients with or without joint inflammation. Ann Rheum Dis. 2006;65:398-400.

17. Korendowych E, Owen P, Ravindran J, et al. The clinical and genetic associations of anti-cyclic citrullinated peptide antibodies in psoriatic arthritis. Rheumatology (Oxford). 2005;44:1056-60.

18. Inanc N, Dalkilic E, Kamali S, et al. Anti-CCP antibodies in rheumatoid arthritis and psoriatic arthritis. Clin Rheumatol. 2007;26:17-23.

19. Abdel Fattah N S, Hassan H E, Galal Z A, et al. Assessment of anti-cyclic citrullinated peptide in psoriatic arthritis. BMC Res Notes. 2009;2:44.