SERUM ACPA IN PERIODONTITIS ASSOCIATED WITH RHEUMATOID ARTHRITIS – RELATIVE VALUE FOR THE SEVERITY OF PERIODONTITIS

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Abstract. Antibodies against cyclic citrullinated peptides (ACPA) have significant prognostic value for the onset or progression of rheumatoid arthritis (RA). Extraarticular citrullination and the production of ACPA as an immune response have been well documented in a number of tissues, including inflamed gingiva associated with periodontal disease. The aim of this study was to analyze the relative value of serum ACPA, determined by anti-CCP test, in the periodontitis (P) associated with RA severity's assessment. The study included 60 patients with a mean age of 58 ± 10 (34 to 74 years), of whom 44 were women and 16 were men with concomitant P and RA. All patients underwent clinical and laboratory tests for the diagnosis of RA and clinical periodontal examination for the diagnosis of P, after signed informed consent. In the studied cohort we found that the average number of lost teeth was 8 ± 5 (0-18), and the average depth of periodontal pockets in mm was 4.4 ± 1.1 . In 50% of patients there was a loss of attachment > 5 mm, and in 43% we found furcation lesions. Fifty-eight patients (96.7%) had a 100% prevalence of probing bleeding (BoP), and 47 patients (78.3%) had $PISA \geq 934.71$ mm². The mean value of PISA in the studied patients was 1727.04 ± 873.64 (214.37-4324.00). We found a statistically significant association between serum ACPA levels with the number of lost teeth ($p = 0.045$, $r = 0.260$), a criterion for the periodontitis severity, but we did not find significant correlations with parameters PD ($p = 0.357$), CAL ($p = 0.589$) and PISA ($p = 0.788$). We found that the ROC-based cutoff values for ACPA were high (123.85 IU/ml) and had low sensitivity and specificity in distinguishing between severe and moderate/mild forms of periodontal disease.

Key words: Periodontitis, rheumatoid arthritis, ACPA, anti-CCP test

INTRODUCTION

Periodontitis (P) is a chronic multifactorial inflammatory disease associated with dysbiotic biofilm, characterized by progressive destruction of tooth-supporting structures [1]. Its main features are loss of attachment, loss of alveolar bone, formation of periodontal pockets and bleeding on probing [2]. Periodontal diseases can cause tooth loss and are one of the main reasons for edentulism, which negatively affects masticatory function and aesthetics. This leads to a deteriorating quality of life and social disadvantage for those suffering from the disease, as well as an overall adverse impact on their systemic health [1].

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease with unclear etiology, autoimmune pathogenesis, diverse clinical features and unpredictable clinical course [3]. Its main manifestation is progressive erosive arthritis, leading to fibrous proliferation and ankylosis of the peripheral

joints in the final stages of the disease [3]. It causes severe and irreversible disability of patients, requiring expensive and long-term treatment and special care [3].

RA is known to be associated with the formation of antibodies directed against cyclic citrullinated peptides (ACPA) [4]. Citrullination or deimination is a term used to describe a genetic protein modification based on enzymatic conversion of the amino acid arginine to the amino acid citrulline by peptidylarginine deiminases.

During the preclinical immunological phase of RA, serum autoantibodies are produced, which may exist for many years before the onset of RA clinical signs and symptoms and they can be detected in various tissues of the body. The two best-studied types of autoantibodies are rheumatoid factors (RF) and antibodies against citrullinated proteins (ACPA). ACPAs have significant prognostic value for the future development of RA and an unfavorable prognostic sign for the progressive course of

the disease. The American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) define the presence of ACPA as one of the classification criteria for RA [5]. Extraarticular citrullination and ACPA production, as a key link in the immune response, have been well documented in a number of tissues, including inflamed gingival tissues in periodontal disease [6]. ACPA is the unifying concept of reactivity targeting citrullinated peptides from fibrinogen, alpha-1 enolase, vimentin, filagrin and histones, as the anti-cyclic citrullinated peptide (anti-CCP) test reflects the presence of antibodies to mixed cyclic citrullinated peptides (ie. ACPA), as an artificial simulator of true autoantigens [7].

P and RA show a number of similarities in some pathogenetic processes, cytokine profile, inflammatory biomarkers; association with HLA-DRB1, IL-1 β , TNF- α and other gene polymorphisms; presence of citrullinated proteins and similar peptide epitopes, as well as the presence of RF [8, 9, 10]. For the first time in 1982, Snyderman and McCarty commented on common pro-inflammatory mechanisms in the development of RA and P. Both diseases are characterized by a chronic inflammatory reaction in the connective tissue and adjacent bone. In this environment, inflammatory cells and mediators cause similar clinical symptoms such as pain, swelling, redness, and eventual bone destruction [11]. Patients with active RA show a significantly higher frequency and severity of P, greater prevalence and severity of gingival bleeding, greater number of lost teeth and more alveolar bone loss compared to healthy controls [12, 13, 14], and Ramamurthy et al. [15] published in 2005 an experiment in which the induction of arthritis in mice was associated with periodontal destruction, increased production of cytokines and matrix metalloproteinases, and alveolar bone loss.

The aim of the present study was to investigate the relationship between serum ACPAs, determined by anti-CCP test and the clinical parameters of P, as well as to define their predictive value for assessing severity and activity of the periodontal disease in patients with concomitant P and RA.

PATIENTS AND METHODS

Study subjects

The study included 60 patients with a mean age of 58 ± 10 years (range 34-74), of whom 44 women and 16 men diagnosed with periodontitis (P) based on the World Workshop on the Classification of

Periodontal and Peri-Implant Diseases and Conditions of 2017 [14] and rheumatoid arthritis (RA) based on the ACR/EULAR classification criteria of 2010 [5].

Patients, participating in the study met the following inclusion criteria: age over 18 years, presence of P, defined as stage II, III and IV according to the updated classification in 2017 [14], presence of ≥ 9 teeth, presence of 6 sites in 2 quadrants with at least 2 affected teeth in each of the quadrants, each affected site having a probing pocket depth PPD ≥ 5 mm, clinical loss of attachment CAL ≥ 3 mm and bleeding on probing, defined as BOP $\geq 20\%$ of all examined sites (6 sites per tooth were examined). With regard to RA, patients are allowed to have different duration of the disease and to be treated with different therapeutic regimens, according to the standard for the treatment of the disease.

Excluding criteria: no antibiotic treatment in the last 3 months, no periodontal treatment in the last 6 months, no need for antibiotic prophylaxis during oral procedures, including periodontal probing, no presence of diabetes or other disease affecting immune status, no present severe progressive or uncontrolled somatic disease that would affect study participation, no pregnancy and lactation.

The research was ethically conducted according to the Helsinki Declaration of World Medical Association. The Institutional Council on Medical Science of Medical University – Sofia approved the study protocols including the recording of clinical measurements. All subjects signed informed consent prior to entry into the project and after reading the notification letter.

Clinical assessment

Data were collected on patient demographics, therapy, and RA activity, which was assessed by DAS28-CRP [17]. Its level was interpreted as remission or presence of low, moderate and high disease activity according to the generally accepted threshold values of DAS28-CRP < 2.6 , 2.6-3.2, 3.2-5.1 and > 5.1 , respectively. As an additional indicator, we used the inflammatory biomarker C-reactive protein (CRP).

Clinical periodontal examination was performed in all patients and the following parameters were measured: Hygiene index (HI), Papilla bleeding index (PBI); probing pocket depth (PD); clinical attachment loss (CAL); bleeding on probing (BOP); presence of recessions for each periodontal unit (R); presence of furcation lesions (F). The study

was performed with a specialized graduated periodontal probe (UNC-15 and Nabers probe for the furcation area) of each periodontal unit at six sites. The Periodontal inflamed surface area (PISA) [18], which shows the amount of inflamed periodontal surface, calculated in mm² was determined. The calculation of PISA was performed by entering periodontal measurements (CAL, PPD, BOP, gingival margin level) on a especially created file of Microsoft Excel. Two levels of disease activity and severity of P were stratified; with PISA values ≥ 934.71 mm², by analogy with the results of other authors [19], as well as PD > 5 mm and CAL > 5 mm were considered as severe P.

Anti-CCP tests

Commercial ELISA kits (QUANTA Lite®, USA) were used to test antibodies against CCP in the sera of the patients included in the project. The implementation of the procedure was in accordance with the recommendations of the manufacturer. The wells of the respective microtiter plate are loaded with antigens carrying epitopes recognized by the paratopes of the anti-CCP autoantibodies. After addition of the test sample, the corresponding autoantibodies, if present, would bind to the antigens fixed on the plate wells. Next, a biotinylated antibody is added, which also binds specifically to the Fc fragment of the antibody. Horse-radish peroxidase (HRP) conjugate with streptavidin is used as a detection system, which binds specifically to biotin and after the addition of the substrate tetramethylbenzidine (TMB) an enzymatic reaction takes place with the formation of a color product. The enzyme reaction was stopped with stop buffer (H₂SO₄) and spectrophotometric reading was performed (at a wavelength of 450 nm, with a correction filter for 620 nm). The intensity of the color product depends on the amount of antigen in the biological sample. A standard curve is constructed according to the calibrators used in the commercial kit and the amount of anti-CCP antibodies in the test samples is determined by a regression equation in a certain confidence interval. The samples were repeated, and the final result was the arithmetic mean between two measurements for each patient serum.

Statistical analysis

Statistical analyzes were performed using the Statistical Package for the Social Sciences (SPSS)

version 22.0 (SPSS Inc., Chicago, IL). Spearman correlation coefficients were calculated to assess the associations between clinical and laboratory parameters. Intergroup comparisons were performed using the Mann-Whitney test for continuous variables. Receiver Operating Characteristic (ROC) analysis was performed to assess the diagnostic potential of ACCP for the P severity. The area under the curve (AUC) was calculated, with the diagnostic test approaching 1 show the best discriminant ability. Sensitivity and specificity were calculated according to the ACPA cut offs associated with the severity and activity of the periodontal disease. In all analyzes, results with a significance level of $p < 0.05$ were considered statistically significant.

RESULTS

Description of the studied cohort

A total of 60 patients with concomitant periodontitis and rheumatoid arthritis were included in the study. 83.3% of patients were positive for anti-CCP antibodies (anti-CCP > 20 IU/ml), with mean serum levels of this immunological marker 182.0 ± 179.1 IU/ml. Table 1 presents baseline demographics and clinical characteristics of study participants.

In the studied cohort we found worsened periodontal status: the average number of lost teeth was 8 ± 5 (0-18) and the average depth of periodontal pockets in mm was 4.4 ± 1.1 . 50% of patients had an attachment loss > 5 mm and in 43% we found furcation lesions. 58 patients (96.7%) had a 100% distribution of probing bleeding (BoP) and 47 patients (78.3%) had PISA ≥ 934.71 mm². The mean PISA value in the studied patients was 1727.04 ± 873.64 (214.37-4324.00). These PISA values are of interest in view of published data for threshold values of ≥ 130.33 mm² with a predictive value for the presence of P and of ≥ 934.71 mm² for severe periodontitis [19].

Comparison of mean anti-CCP antibodies serum levels according to the severity and activity of periodontitis, assessed by periodontal parameters PD, CAL and PISA

We found no statistically significant difference in serum levels of anti-CCP antibodies depending on the severity of periodontal parameters PD < 5 or ≥ 5 ($p = 0.357$), CAL < 5 or ≥ 5 ($p = 0.589$) and PISA < 934.71 or ≥ 934.71 ($p = 0.788$) (Fig. 1, Tables 3 and 4).

Table 1. Main characteristics of the studied patients with periodontitis and rheumatoid arthritis (n = 60)

Characteristics	Value
Sex, n (%)	
men	16 (26.7%)
women	44 (73.3%)
Age, mean $\pm$ SD (range)	58 $\pm$ 9.9 (34-74)
Education, n (%)	
higher	16 (26.7%)
secondary	37 (61.7%)
primary	7 (11.6%)
Smoking, n (%)	
Non smoker	29 (48.3%)
Former smoker	4 (6.7%)
< 20 cigarettes daily	22 (36.7%)
> 20 cigarettes daily	5 (8.3%)
Rheumatoid factor, n (%)	
positive	44 (73.3%)
negative	16 (26.7%)
ACCP, n (%)	
positive	50 (83.3%)
negative	10 (16.7%)
DAS28-CRP, mean $\pm$ SD (range)	4.27 $\pm$ 1.67 (1.80-7.56)
DAS28-CRP < 2.6, n (%)	9 (15.0%)
DAS28-CRP 2.6-3.2, n (%)	12 (20.0%)
DAS28-CRP 3.2-5.1, n (%)	17 (28.3%)
DAS28-CRP > 5.1, n (%)	22 (36.7%)
Number of lost teeth, mean $\pm$ SD (range)	8 $\pm$ 5 (0-18)
PD mm, mean $\pm$ SD (range)	4.4 $\pm$ 1.1 (2.5-7.6)
Number of teeth with PD $\leq$ 3 mm	5 $\pm$ 8 (0-27)
Number of teeth with PD 3.1-5 mm	12 $\pm$ 8 (0-26)
Number of teeth with PD 5.1-7 mm	3 $\pm$ 4 (0-21)
Number of teeth with PD > 7 mm	1 $\pm$ 2 (0-12)
CAL mm, mean $\pm$ SD (range)	5.1 $\pm$ 1.4 (2.5-8.0)
Furcations, n (%)	
No furcation lesions	34 (56.6%)
F1	7 (11.7%)
F2	19 (31.7%)
F3	0 (0%)
Number of recessions, mean $\pm$ SD (range)	6 $\pm$ 7 (0-22)
BoP %, mean $\pm$ SD (range)	98.5 $\pm$ 8.6 (40-100)
PISA, mean $\pm$ SD (range)	1727.04 $\pm$ 873.64 (214.37-4324.00)

ACPA = antibodies against cyclic citrullinated peptides; DAS28-CRP = Disease Activity Score 28-joint count C reactive protein; DAS28-CRP < 2.6 = remission; DAS28-CRP 2.6-3.2 = low disease activity; DAS28-CRP 3.2-5.1 = moderate disease activity; DAS28-CRP > 5.1 = high disease activity; PD = pocket depth; CAL = loss of clinical attachment; F1 = first degree furcation involvement (up to 3 mm horizontal penetration of the probe); F2 = second degree furcation involvement (3 mm to 6 mm horizontal penetration of the probe); F3 = third degree furcation involvement (the probe passes through-and-through the entire furcation area); BoP = bleeding on probing; PISA = periodontal inflamed surface area

Fig. 1. Serum levels of ACPA depending on pocket depth (PD), loss of attachment (CAL) and PISA**Table 2. Correlations between ACPA and clinical parameters of the periodontal disease (Spearman's rho)**

	Number of lost teeth	PD mean	CAL mean	BoP	PISA	Recessions
ACPA	p = 0,045 r = 0,260	p = 0,943 r = 0,009	p = 0,795 r = 0,034	p = 0,118 r = 0,204	p = 0,406 r = -0,109	p = 0,161 r = 0,183

Table 3. Mean serum ACPA levels (IU/ml) depending on the severity of clinical periodontal parameters

		Number (N)	Mean value	Std. deviation
ACPA	PD			
	< 5 mm	45	176,5822	184,90803
	> 5 mm	15	198,1407	165,30972
ACPA	CAL			
	< 5 mm	30	180,7233	196,55843
	> 5 mm	30	183,2203	163,12669
ACPA	PISA			
	< 934,71 mm ²	13	190,4554	175,65703
	> 934,71 mm ²	47	179,6253	181,82378

Table 4. Association between the serum levels of ACPA and severity and activity of the periodontal disease, based on PD, CAL u PISA

	ACCP	
Periodontal status	Spearman's correlation coefficient	p-value
PD < 5 mm (n = 45)	-0.122	0.423
PD $\geq$ 5 mm (n = 15)	-0.132	0.639
CAL < 5 mm (n = 30)	-0.050	0.794
CAL $\geq$ 5 mm (n = 30)	-0.059	0.756
PISA < 934.71 mm ² (n = 13)	0.445	0.128
PISA $\geq$ 934.71 mm ² (n = 47)	-0.155	0.297

Association between serum ACPA and clinical parameters of the periodontal disease

Serum ACPA levels showed a weak positive association only with the number of teeth lost ($r = 0.260$, $p = 0.045$), but not with PD, CAL, BoP, PISA and recessions, assessing the severity and activity of P ($p > 0.05$) (Table 2). No correlation was found with different degrees of periodontitis severity based on PD, CAL and PISA.

Relative value of serum ACPA for the assessment of the activity and severity of periodontitis in the studied patients with rheumatoid arthritis

To analyze the predictive accuracy of the anti-CCP test to differentiate severe and active periodontal lesions from moderate/mild ones, with the best combination of sensitivity and specificity values, we calculated the ROC curves. Figures 2, 3 and 4 show the ROC of the ACCP against the severity of periodontal status. The ROC-based cutoff values are significantly higher than the upper anti-CCP reference limit – 123.85 IU/ml. The ability of ACPA to differentiate patients with severe from moderate to mild P based on PD (PD < 5; PD ≥ 5) has AUC = 0.420, sensitivity = 44.4%, specificity = 33.3%; based on CAL (CAL < 5; CAL ≥ 5) has AUC = 0.459, sensitivity = 46.7%, specificity = 46.7% and based on PISA

(PISA < 934.71; PISA ≥ 934.71) has AUC = 0.525, sensitivity = 46.2%, specificity = 49.0%. In general, the anti-CCP test has low sensitivity and specificity in distinguishing between severe and moderate/mild periodontal lesions (Tables 5 and 6).

Table 5. ROC analysis

Clinical periodontal parameters	AUC	SE	p	95% CI	
PD < 5 mm; PD ≥ 5 mm	0.420	0.085	0.357	0.253	0.587
CAL < 5 mm; CAL ≥ 5 mm	0.459	0.076	0.589	0.311	0.608
PISA < 934.71 mm ² ; PISA ≥ 934.71 mm ²	0.525	0.085	0.788	0.358	0.691

AUC = area under the curve; SE = standard error; p = p value; CI = confidence interval

Table 6. ROC analysis

Clinical periodontal parameters	Cut-off ACPA (IU/ml)	Sensitivity (%)	Specificity (%)
PD < 5 mm; PD ≥ 5 mm	123.85	44.4	33.3
CAL < 5 mm; CAL ≥ 5 mm	123.85	46.7	46.7
PISA < 934.71 mm ² ; PISA ≥ 934.71 mm ²	123.85	46.2	49.0

ACPA cut-off value, sensitivity, specificity

Fig. 2. ROC curve of serum ACPA according PD in mm (PD < 5 mm; PD ≥ 5 mm)

Fig. 3. ROC curve of serum ACPA according CAL in mm (CAL < 5 mm; CAL ≥ 5 mm)

Fig. 4. ROC curve of serum ACPA according PISA in mm² (PISA < 934.71 mm²; PISA ≥ 934.71 mm²)

DISCUSSION

Recent studies indicate that periodontitis is associated with rheumatic diseases and in particular with rheumatoid arthritis, which determines the growth of research in this area. The two diseases share common risk factors, in particular smoking. Proinflammatory cytokines, including TNF- α , cause bone destruction in both diseases. The specific immune response to citrullinated proteins is a key element in the pathogenesis of rheumatoid arthritis. In this regard, *Porphyromonas gingivalis*, a major periodontal pathogen, is of particular interest as it is the only known prokaryote that produces the enzyme peptidylarginine deiminase, which is able to citrullinate its own peptides and host proteins. Unlike the human enzyme, the bacterial enzyme deiminates both free arginine and C-terminal arginine residues. The different mechanisms of citrullination probably underlie the breakdown of immune tolerance and the development of an immune response to endogenous citrullinated peptides in susceptible individuals, through the generation of new epitopes [20].

The lack of a universal definition of periodontitis case definition for epidemiological and clinical trials led to the publication of a consensus report by a working group of the Centers for Disease Control and Prevention (CDC) and the American Academy of Periodontology (AAP) with recommendations for the use of periodontal pockets depth (PD) and clinical attachment loss (CAL) for the presence and severity of P, and bleeding on probing (BoP) to determine periodontal disease activity [21]. The possibility of local periodontal inflammation spreading system-

ically is the biological plausibility for a possible link between P and some systemic diseases (e.g. RA). In this sense, the discussion of P as a potential risk factor for a given systemic condition is related to the overall inflammatory burden of periodontal disease. In 2008, Nesse et al. developed a method for determining the amount of inflamed periodontal surface (PISA), which is based not only on linear measurements such as CAL, PD and gingival recession (R), but also takes into account P activity assessed by BoP [18]. Leira et al. (2017) supplement the above-cited publication by setting PISA thresholds for the presence of P (PISA ≥ 130.33 mm²) and for severe P (PISA ≥ 934.71 mm²) [19].

In our study, we found no association between serum levels of anti-CCP antibodies and patients periodontal status as assessed by PD, CAL, and PISA. Similar results were published by Yamashita and co-authors [22] in 2019, finding no correlation between periodontal status and RA-indicators ACPA and DAS in 54 patients with P and RA, divided into two groups, based on a threshold a value of 79.10 mm² for PISA. Serum ACPA levels did not differ significantly in the subgroups with high or low PISA values, but a strong positive correlation was found between lower PISA values and the better effect of treatment with TNF-inhibitors and IL-6R-inhibitors, evaluated with the Clinical Disease Activity Index (CDAI). Published results in 2012 by De Smith et al. showed patients with RA and severe P, assessed by periodontal parameters PD and CAL, had higher DAS28 values for disease activity than patients with RA and moderate/mild P, but serum ACPA levels in the two groups of patients did not show statistically significant differences [23]. We found a positive association of serum ACPA levels with the number of lost teeth, considered one of the criteria for the severity of periodontitis (1). We assume influence of the RA severity, based on serum ACPA values, on tooth loss, which is most often the result of periodontal destruction. We do not consider that this result is sufficient to conclude a relationship between serum ACPA and periodontitis severity, as the comparison of serum ACPA with other periodontal indicators – PA, CAL, PISA does not show significant correlations.

In addition, we evaluated the diagnostic utility of the anti-CCP test for the severity of periodontal disease in concomitant RA. ROC analyzes revealed that the cutoff values for serum ACPA based on ROC were high, in the range of 123.85 IU/ml. The area under the curves as a measure of the usefulness of the test is generally small. The sensitivity and spec-

ificity of the anti-CCP test, an important feature in clinical practice, are equally low for all three clinical periodontal parameters.

Loutan et al. in 2019 published a study which found that ACPA-positive patients with RA and P had significantly higher mean PD and CAL values, a greater number of periodontal pockets with PD > 4 mm, a higher rate of BoP prevalence as well as a higher incidence of a family history of periodontal disease compared with ACPA-negative patients with RA and P (24). On the other hand, data on the presence of citrullinated proteins and antibodies against them in periodontal tissues are known, as well as a positive relationship between the severity of periodontal inflammation and their concentration [25, 26]. Confirmation of these facts is another study of ours, in which we found a significant positive correlation between the salivary ACPA levels and the severity of some periodontal indicators (PD and BoP) in patients with P, as well as a significant positive relationship between the ACPA concentration in saliva and RA-indicators ACPA in serum, RF in serum and DAS 28 (CRP) in patients with concomitant P and RA [27]. It is likely that the local production of ACPA in inflamed periodontal tissues could lead to a change in immune tolerance to citrullinated peptides and in predisposed individuals and existing rheumatoid disease to aggravate its course by stimulating systemic production of ACPA.

We consider the small number of patients studied as the main limitation of our study and as a presumed explanation for the absence of significant relationships between serum ACPA levels and periodontal parameters. We believe that some discrepancies in the comparison of our results with the results of other studies may be due to differences in periodontal measurements and in the case definition of the severity of periodontal disease. In addition, patients were treated with various therapeutic regimens for RA, which could affect the condition of periodontitis. On the other hand, research in this area is scarce, so our analysis is a good first step in studying the issue.

CONCLUSIONS

Serum ACPA determined by the anti-CCP test are not a biomarker that can well differentiate severe from mild periodontal lesion in patients with concomitant P and RA. The predictive value of anti-CCP antibodies for assessing the severity of periodontal disease in patients with RA is low.

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