ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODY
AND RHEUMATOID FACTOR AND THE RELATIONSHIP
BETWEEN PERIODONTITIS AND RHEUMATOID
ARTHRITIS

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Abstract

The purpose of this study is to determine the concentration of Rheumatic Factor (RF) and Anti-cyclic Citrullinated Peptide Antibody (ACPA) in serum and in Gingival Crevicular Fluid (GCF) and their dependence on the presence of periodontitis (P) and rheumatoid arthritis (RA). The study involved 81 patients divided into three groups: Group I – patients with P and without RA (with osteoarthritis) – 26 subjects; Group II – patients with P and RA – 30 subjects; Group III – patients with periodontal health (without P) and RA – 25 subjects. For all patients the levels of ACPA and RF (class IgM) have been analyzed by ELISA in serum and GCF. We found a significantly higher incidence of ACPA in the GCF positive individuals and significantly higher mean ACPA values in GCF in Group II patients compared to patients in Group I. We found a significant difference between the frequency of RF in the GCF positive patients in Group II compared to those in Group I as well as the RF in the GCF positive patients in Group II compared to those in Group III. In the study among the patients of Group II we found a significant correlation between the RF and ACPA concentrations in serum and the number of lost teeth. These results are

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associated with possible enhanced extraarticular synthesis of ACPA and RF in
the periodontal tissues in patients with periodontitis and predisposed to RA.
Evidence for the effect of RA on periodontitis is the correlation between the
concentration of ACPA and RF in serum – biomarkers for RA and the number
of lost teeth.

Key words: chronic periodontitis, rheumatoid arthritis, rheumatic factor,
anti-cyclic citrullinated peptide antibody

Introduction. Rheumatoid arthritis (RA) is a chronic, inflammatory joint
disease with world distribution around 5 per 1000 adult individuals, which can
lead to severe joint damage, inability to work and increased mortality [1].

Periodontitis (P) is a chronic multifactorial inflammatory disease, associated
with dysbiotic plaque biofilms and characterized by progressive destruction of the
tooth-supporting apparatus [2]. The spread of periodontitis affects between 3 and
60% of the individuals in the studied populations [3,4].

In both P and RA, inflammation is triggered by antigen stimulation (pep-
tides, lipopolysaccharides) and a subsequent cascade of inflammation leading to
the release of proinflammatory mediators interacting with the host cells. In both
diseases, resident cells (synovial cells in RA, keratinocytes, fibroblasts and os-
teoblasts in P) and migrated inflammatory cells are responsible for the tissue de-
struction. The relationship between P and RA is epidemiologically and clinically
proven [5–7].

Autoantibodies have a significant role in RA, forming immune complexes that
can activate complement and enhance the inflammatory response [8]. Rheumatoid
factor (RF) and Anti-cyclic Citrullinated Peptide Antibody (ACPA) are autoan-
tibodies that together can cause a significant inflammatory response. At the
base of the autoimmune reaction is the formation of autoantigens by posttransla-
tional protein modifications such as citrullination of arginine by peptidylarginine
deoiminase or carbamylation of lysine and creation of new epitopes of various au-
tologous proteins (collagen, vimentin, fibrinogen). This contributes to breaking
immunological tolerance, autoantibody formation against autoantigens (ACPA),
antibodies against IgG (RF), nucleic antigens or autoantigens that cross-react
with bacterial or viral antigens [1]. Serum levels of RF and ACPA are important
markers in diagnostics and are used as classification criteria for RA, according
to the American College of Rheumatology/European League Against Rheuma-
tism collaborative initiative [9]. Patients who are ACPA-positive are more likely
to suffer from severe and moderate periodontitis compared to patients who are
ACPA-negative, which supports the relationship between P and RA [10].

The aim of this study was the determination of the concentration of RF and
ACPA in serum and in Gingival Crevicular Fluid (GCF) and their dependence on
the presence of periodontitis and rheumatoid arthritis.

Materials and methods. This study included 81 patients (17 men and
64 women), aged 55.23 ± 10.90 years, who received treatment at the Center for
Rheumatology, St. Ivan Rilski University Hospital, Sofia. The patients included in the study are over 18 years of age, have at least nine teeth in the mouth, have not received antibiotic treatment for the last three months, have not received periodontal treatment for the past six months, do not need antibiotic prophylaxis during oral procedures. Patients who have diabetes or another condition that affects the immune status were excluded. Pregnant and lactating women were excluded. All patients were aware of the purposes and the nature of the study by providing a notification letter and have signed informed consent.

Clinical and laboratory tests have been performed in all patients in connection with the diagnosis of RA and osteoarthritis. The diagnosis of RA is based on the American College of Rheumatology classification criteria (2010). All patients have undergone a clinical periodontal examination on the basis of which they are diagnosed with periodontitis or with clinical periodontal health. The study includes: Papillary bleeding index (PBI), Hygiene index (HI), periodontal probing depth (PPD), loss of clinical attachment (AL), presence of furcation defects (F), Bleeding on probing (BOP), presence of recessions on the tooth surface (R).

The patients are divided into three groups: Group I – patients with P and without RA (with osteoarthritis) – 26 subjects; Group II – patients with P and RA – 30 subjects; Group III – patients with periodontal health (without P) and RA – 25 subjects. Peripheral blood has been collected from a cubital vein of all patients. GCF samples have been collected from all patients by micropipettes (Microcapillary tube, calibrated size 1 to 5 µL, Sigma-Aldrich). For all patients the levels of ACPA and RF (class IgM) have been analyzed by enzyme-linked immunosorbent assay (ELISA anti-CCP, Euroimmun AG; ELISA IgM rheumatoid factor human, Euroimmun AG) in serum and GCF. Depending on the levels of biomarkers in the biological fluid, patients are defined as ACPA-positive (ACPA ≥ 6 RU/ml) and ACPA-negative (ACPA < 6 RU/ml); RF-positive (RF ≥ 20 RU/ml) and RF-negative (RF < 20 RU/ml).

Statistical analysis was conducted using SPSS 13.0 software package for Windows and \( p < 0.05 \) was considered to indicate a significant difference. The used statistical methods include frequency analysis; variance analysis; crosstabulation (mutual frequency distributions of two qualitative variables). Correlation analysis is applied – parametric (Pearson Correlation) when the variables are normally distributed or nonparametric (Spearman’s rho) when the variables are not normally distributed. The Mann–Whitney test was used to compare two groups of a variable that is not normally distributed.

**Results.** Our study showed a significantly greater incidence of ACPA in GCF-positive individuals in Group II patients (with P and RA) (51.7%) compared to patients in Group I (with P and without RA) (19.2%) \( (p = 0.024) \), as well as tendency to higher incidence of ACPA in GCF-positive patients of Group II (with P and RA) (51.7%), compared to those of Group III (with RA, and without P) (32.0%), with no significant difference \( (p = 0.175) \) (Fig. 1).

We found significantly higher mean values of ACPA in GCF in patients in

Group II (with P and RA) (12.94 RU/ml) compared to patients in Group I (with P and without RA) (2.8 RU/ml) \( (p < 0.0001) \). We did not detect a significant difference between the mean values of ACPA in GCF in patients in Group II (with P and RA) (12.9 RU/ml) and patients in Group III (without P, with RA) (9.06 RU/ml) \( (p = 0.307) \).

In Group II patients (with P and RA) we found significant correlation between the mean serum ACPA values and the number of lost teeth, a marker for the severity of P \( (p = 0.011) \).

We found a significant difference between the prevalence of RF in GCF-positive patients in Group II (with P and RA) (53.3%) and those in Group I (with P and without RA) (15.4%) \( (p = 0.005) \), as well as between the prevalence of RF in GCF-positive patients in Group II (with P and RA) (53.3%) and those in Group III (without P, with RA) (24%) \( (p = 0.032) \) (Fig. 2).
In patients in Group III (without P, with RA) we found a significant correlation between the concentration of RF in serum and the concentration of RF in GCF ($R = 0.484; p = 0.014$), and in the study of the patients in Group II (with P and RA) we found a trend of dependence between these indicators ($R = 0.339; p = 0.067$). These results could be explained by concentration dependent diffusion of RF molecules from serum to GCF in patients with RA.

In Group II patients (with P and RA) we found significant correlation between the mean serum RF values and the number of lost teeth ($R = 0.374; p = 0.042$).

**Discussion.** Recent studies have found the presence of citrullinated proteins and ACPA in periodontal tissues and their increase with increased inflammation and in periodontitis, which is a reason for the association of periodontitis with the course and progression of RA $^{[11,12]}$.

In our study, we found significantly more patients, positive for ACPA in GCF and significantly higher mean ACPA values in GCF in the group of patients with P and RA than in the group of patients with P and osteoarthritis. Given the knowledge of increased production of citrullinated proteins in the periodontium under the action of bacterial infection, we assume that in some individuals there is a predisposition to the production of antibodies against the increased citrullinated proteins in P and as a result local increase of ACPA in GCF. This assertion is consistent with the established higher concentration of ACPA in gingival tissues in rats with RA and P compared to control groups that are with only one of these diseases or without disease $^{[13]}$. It can be assumed that in the predisposed individuals the local antibody response stimulates the general antibody response and favours the development of RA. In these cases P may be an additional risk factor in the development of RA. In our study these patients are in the second group (with P and RA). In individuals who are not predisposed to RA, although local production of citrullinated proteins may be increased in the periodontium, no active antibody production is stimulated nor increased levels of ACPA in GCF are found. In these individuals RA is not detected. In our study ACPA in GCF-negative individuals are more common in Group I (with P and without RA). Another possible mechanism is the diffusion of ACPA molecules from the serum to GCF in patients with RA.

We found a significant difference between the prevalence of patients-positive for RF in GCF in Group II (with P and RA) compared with patients-positive for RF in GCF in Group III (without P, with RA) and patients-positive for RF in GCF in Group I (with P and without RA). Similar to our study, in the experimental study of rats with RA and P, the serum levels of RF and ACPA in gingival tissues significantly increase and the cytokine balance changes compared to the other animal groups – with experimental arthritis, with experimental periodontitis and the control group $^{[13]}$. In our study the presence of periodontitis affects the increase of RF levels in serum, confirming the two-way relationship between RA and P. One hypothesis may be the presence of periodontal pathogens in the
periodontal pocket and their properties. According to the study of Thé and Ebersole [14] RF of seropositive patients (RF-positive) shows a cross-reaction with bacterial epitopes expressed on the surface of *P. gingivalis* and *F. nucleatum*. These findings suggest that bacterial epitopes may be related to IgG determinants and may induce cross-reactive RF–IgM. Our study is the first which reflects the direct influence of periodontitis on the RF concentrations in the biological fluid GCF. Based on the significantly higher incidence of patients positive for RF in GCF in Group II patients (with P and RA), compared with patients in Group III (without P, with RA), we confirm the effect of the influence of periodontitis on the local RF synthesis and its eventual entry into the serum. On the other hand, the higher incidence of patients positive for RF in GCF in Group II (with P and RA) compared to patients in Group I (with P and without RA) could be related to eventual diffusing of the RF molecules from the bloodstream through the endothelium of the peripheral blood vessels of the gingiva to the GCF in patients with established RA and P.

The established significant positive correlation between ACPA and RF concentration in serum and the number of lost teeth in the study of patients in Group II (with P and RA) was associated with the influence of RA on P. Increased immune complexes levels in RA increase general proinflammatory mediators that can activate the destruction in patients with already established periodontitis, can increase its severity and can lead to additional tooth loss.

**Conclusion.** Our study shows significantly more frequent ACPA and RF positive individuals with respect to these parameters in GCF in the group of patients with P and RA compared to the group of patients with P and without RA, as well as significantly higher mean ACPA concentrations in patients with P and RA compared to P and without RA patients. These results are associated with possible enhanced extraarticular synthesis of ACPA and RF in periodontal tissues in patients with periodontitis, which in patients genetically predisposed to RA may be a part of the pathogenetic mechanisms and may be an impetus in development of RA. Evidence for the effect of RA on P is the correlation between the concentration of ACPA and RF in serum, which are biomarkers for RA and the number of lost teeth.

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