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CLINICAL SIGNIFICANCE OF ANTI-CARBAMYLATED PROTEIN ANTIBODIES IN ACPA-POSITIVE AND ACPA-NEGATIVE RHEUMATOID ARTHRITIS: A CROSS-SECTIONAL STUDY IN THE UZBEK POPULATION

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Abstract. Purpose of the study. To evaluate serum anti-CarP antibody levels in patients with rheumatoid arthritis (RA) and their association with disease activity and radiographic joint damage in ACPA-positive and ACPA-negative groups.

Materials and methods. This cross-sectional study included 120 RA patients (60 ACPA-positive, 60 ACPA-negative) and 25 healthy controls. Anti-CarP levels were measured by ELISA; radiographic joint damage was assessed using the Larsen score.

Conclusion. Serum anti-CarP levels in RA patients were significantly higher than in controls ($p < 0.001$), and their clinical relevance was phenotype-dependent: in ACPA-positive patients, anti-CarP was associated with a greater number of erosions, while in ACPA-negative patients it was associated with disease activity (DAS28), supporting anti-CarP as a complementary serological marker of rheumatoid arthritis, of greatest value in seronegative patients.

Key words: Rheumatoid arthritis, anti-CarP antibodies, ACPA, radiographic progression, disease activity.

Аннотация. Цель исследования. Изучение уровня антител к карбамилированным белкам (анти-CarP) у больных ревматоидным артритом (РА) и его связи с активностью заболевания и рентгенологическим поражением суставов в АЦЦП-позитивной и АЦЦП-негативной группах.

Материалы и методы. Одномоментное исследование включило 120 больных РА (60 АЦЦП-позитивных, 60 АЦЦП-негативных) и 25 здоровых лиц. Уровень анти-CarP определен методом ИФА; рентгенологическое поражение суставов оценено по индексу Ларсена.

Заключение. Уровень анти-CarP у больных РА статистически значимо превышал показатель в контрольной группе ($p < 0,001$), а его клиническая значимость носила фенотип-зависимый характер: у АЦЦП-позитивных больных анти-CarP ассоциирован с большим числом эрозий, у АЦЦП-негативных — с активностью заболевания (DAS28), что позволяет рассматривать анти-CarP как дополнительный серологический маркер ревматоидного артрита, наиболее ценный у серонегативных пациентов.

Ключевые слова: Ревматоидный артрит, анти-CarP антитела, АЦЦП, рентгенологическое прогрессирование, активность заболевания.

Аннотация. Тадқиқот мақсади. Ревматоид артрит (РА) билан оғриган беморларнинг АЦЦП-мусбат ва АССП-манфий гуруҳларида карбамилланган оксилларга қарши антитаначалар (anti-CarP) даражасини ҳамда унинг касаллик фаоллиги ва бўғимларнинг рентгенологик шикастланиши билан боғлиқлигини ўрганиш.

Материаллар ва усуллар. Бир босқичли тадқиқотга РА билан касалланган 120 нафар бемор (60 нафари АЦЦП -мусбат, 60 нафари АЦЦП -манфий) ва 25 нафар соғлом шахс жалб этилди. Anti-CarP даражаси ИФА усулида аниқланди; бўғимларнинг рентгенологик шикастланиши Ларсен индекси бўйича баҳоланди.

Хулоса. РА билан оғриган беморларда анти-CarP даражаси назорат гуруҳидаги кўрсаткичдан статистик аҳамиятли даражада юқори эканлиги аниқланди ($p < 0,001$). Унинг клиник аҳамияти фенотипга боғлиқ характерга эга бўлди: АЦЦП -мусбат беморларда anti-CarP кўп сонли эрозиялар билан, АЦЦП -манфий беморларда эса касаллик фаоллиги (DAS28) билан боғлиқ бўлганлиги кузатилди. Бу эса anti-CarP ни ревматоид артритнинг қўшимча серологик маркери сифатида, айниқса серонегатив беморлар учун энг қимматли маркер сифатида кўриб чиқишга имкон беради.

Калит сўзлар: Ревматоид артрит, anti-CarP антитаначалари, АЦЦП, рентгенологик прогрессиялаш, касаллик фаоллиги.

Introduction. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by persistent synovitis, progressive destruction of cartilage and bone, functional disability, and an increased risk of cardiovascular morbidity and premature mortality [1]. Although substantial advances have been achieved in the understanding of RA pathogenesis, early diagnosis and accurate prediction of disease progression remain major clinical challenges, particularly in patients lacking conventional serological markers.

Autoantibody production represents one of the earliest immunological events in rheumatoid arthritis. Rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) are currently considered the principal serological biomarkers incorporated into the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria [2]. ACPA possesses excellent specificity for RA and is associated with more aggressive structural joint damage, radiographic progression and poorer long-term outcomes. Nevertheless, approximately 20–40% of patients with clinically confirmed RA remain ACPA-negative, creating diagnostic uncertainty during the early stages of disease and limiting the prognostic value of conventional serological testing.

During the last decade, increasing attention has focused on antibodies directed against carbamylated proteins (anti-CarP antibodies), which constitute a distinct family of autoantibodies generated in response to post-translational protein carbamylation. Carbamylation is a non-enzymatic chemical modification in which cyanate reacts with lysine residues to produce homocitrulline [13]. This process differs fundamentally from citrullination, which is catalyzed by peptidylarginine deiminase enzymes. Under conditions of chronic inflammation, oxidative stress and increased myeloperoxidase activity, carbamylation becomes markedly enhanced, promoting the generation of neoepitopes capable of inducing autoimmune responses.

Unlike ACPA, anti-CarP antibodies recognize homocitrulline-containing proteins rather than citrullinated epitopes. Several experimental and clinical investigations have demonstrated that these antibodies may develop years before the onset of clinically apparent arthritis and may therefore participate in the earliest phases of autoimmune activation [4–6]. Importantly, anti-CarP antibodies are detectable not only in ACPA-positive patients but also in a substantial proportion of individuals with seronegative rheumatoid arthritis, suggesting that they represent an independent immunological pathway contributing to disease pathogenesis.

Accumulating evidence indicates that anti-CarP antibodies are associated with increased radiographic progression, erosive joint damage and long-term disability [3,7,9]. Prospective cohort studies have demonstrated that patients positive for anti-CarP antibodies exhibit a greater probability of developing persistent inflammatory arthritis and severe structural damage during follow-up. Moreover, anti-CarP antibodies appear to provide additional prognostic information beyond that obtained from RF and ACPA alone, particularly among seronegative patients in whom established laboratory biomarkers are absent [7,8].

Despite these promising observations, the relationship between anti-CarP antibodies and inflammatory disease activity remains controversial. Some investigators have reported positive associations between anti-CarP concentrations and disease activity indices, including DAS28, CDAI and SDAI [7], whereas others have failed to demonstrate independent correlations after adjustment for conventional inflammatory markers. Differences in study populations, laboratory methods, disease duration and ethnic background may partly explain these inconsistent findings.

Ethnic variability in genetic background, environmental exposures and immunological responses may substantially influence the prevalence and clinical significance of anti-CarP antibodies. However, data regarding anti-CarP antibodies in Central Asian populations remain extremely limited. To date, no comprehensive study has evaluated the diagnostic and clinical relevance of anti-CarP antibodies among patients with rheumatoid arthritis in the Uzbek population. Consequently, their potential utility as an adjunctive biomarker in routine rheumatological practice remains uncertain.

The identification of reliable biomarkers capable of distinguishing patients with more aggressive disease phenotypes is particularly important in the era of personalized medicine. Earlier recognition of patients at increased risk of structural progression may facilitate timely initiation of intensive disease-modifying therapy and improve long-term clinical outcomes.

Aim of the study. The present study aimed to evaluate serum anti-carbamylated protein antibody concentrations in patients with rheumatoid arthritis and to investigate their association with disease activity, radiographic joint damage and clinical characteristics in both ACPA-positive and ACPA-negative subtypes of rheumatoid arthritis in the Uzbek population.

Materials and methods. This cross-sectional observational study included 120 consecutive patients with rheumatoid arthritis (RA) who were treated at the Republican Specialized Scientific and Practical Medical Center of Rheumatology and the Department of Hospital Therapy of Tashkent Medical Academy between January 2023 and December 2025. The diagnosis of RA was established according to the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria [2]. Patients younger than 18 years of age, those with overlap connective tissue diseases, active infectious diseases, malignant neoplasms, severe hepatic or renal insufficiency, pregnancy, or other inflammatory arthritides were excluded from the study. Twenty-five apparently healthy volunteers without autoimmune or inflammatory diseases served as the control group.

To investigate the clinical significance of anti-carbamylated protein antibodies (anti-CarP) in different serological phenotypes of RA, patients were divided into two equal groups according to anti-cyclic citrullinated peptide antibody (ACPA) status. The first group included 60 ACPA-positive patients, whereas the second group consisted of 60 ACPA-negative patients. Demographic characteristics, disease duration, clinical manifestations, laboratory findings, and radiographic data were obtained from medical records and verified during the study visit.

Disease activity was evaluated using the Disease Activity Score in 28 joints (DAS28), which combines the tender and swollen joint counts, erythrocyte sedimentation rate (ESR), and patient global assessment. Functional impairment was additionally assessed using the Health Assessment Questionnaire (HAQ). Structural joint damage was evaluated on standard radiographs of the hands and feet using the Larsen scoring system [14], and the total number of bone erosions was recorded independently by two experienced rheumatologists blinded to laboratory findings.

Peripheral venous blood samples were obtained after overnight fasting. Serum was separated by centrifugation and stored at -80°C until laboratory analysis. Serum anti-CarP antibody concentrations were measured using a commercially available enzyme-linked

immunosorbent assay (ELISA) kit (BlueGene Biotech, Shanghai, China) according to the manufacturer's instructions. Quantitative determination of ACPA was performed using commercially available ELISA kits (Axis-Shield Diagnostics, United Kingdom, and Orgentec Diagnostika GmbH, Germany). Rheumatoid factor (RF), C-reactive protein (CRP), and ESR were determined using routine laboratory methods employed at the institutional clinical laboratory.

Statistical analysis was performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA) and MedCalc Statistical Software version 22.0. Distribution normality was assessed by the Shapiro-Wilk test. Continuous variables are presented as median and interquartile range (IQR), whereas categorical variables are expressed as frequencies and percentages. Comparisons between two independent groups were performed using the Mann-Whitney U-test or Student's t-test where appropriate. Categorical variables were compared using Pearson's χ^2 test or Fisher's exact test. Correlations between anti-CarP concentrations and clinical variables were evaluated using Spearman's rank correlation coefficient. Receiver operating characteristic (ROC) analysis was performed to determine the diagnostic performance of anti-CarP antibodies and to identify the optimal diagnostic cut-off value. A two-sided p-value below 0.05 was considered statistically significant.

The study protocol was approved by the Local Ethics Committee of Tashkent Medical Academy and conducted in accordance with the ethical principles of the Declaration of Helsinki [15]. Written informed consent was obtained from all participants before enrolment.

Results. The study included 120 patients with rheumatoid arthritis, including 60 ACPA-positive and 60 ACPA-negative individuals, together with 25 apparently healthy volunteers. The study groups were generally comparable regarding demographic characteristics, whereas laboratory and immunological parameters reflected the expected differences according to serological status.

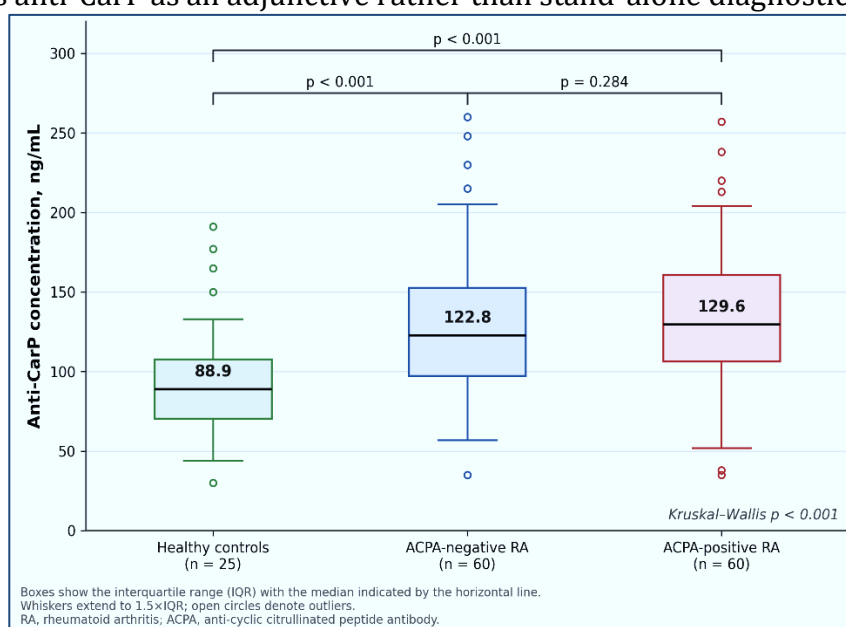
Serum anti-CarP antibody concentrations were significantly elevated in patients with rheumatoid arthritis compared with healthy controls. The median anti-CarP level in the RA cohort was 126.2 [100.83–157.41] ng/mL, whereas healthy individuals demonstrated substantially lower concentrations (88.89 [70.53–107.75] ng/mL), corresponding to a highly significant difference ($p < 0.001$). Anti-CarP positivity was detected in 40 of 120 patients (33.3%), while only one individual (4.0%) in the control group tested positive ($p = 0.002$), confirming the close association between anti-CarP antibodies and rheumatoid arthritis.

The distribution of anti-CarP positivity according to ACPA status demonstrated that 18 of 60 ACPA-positive patients (30.0%) and 22 of 60 ACPA-negative patients (36.7%) had detectable anti-CarP antibodies. Although anti-CarP positivity was observed slightly more frequently in ACPA-negative patients, the overall prevalence remained comparable between the two serological subgroups, indicating that anti-CarP antibodies develop independently of ACPA production in a considerable proportion of patients.

Table 1. The demographic, clinical, and laboratory characteristics of the study population

Variable	ACPA-positive RA (n=60)	ACPA-negative RA (n=60)	Healthy controls (n=25)	p
Age, years	51.6 ± 11.8	49.8 ± 12.6	48.9 ± 10.4	0.631
Female, n (%)	49 (81.7)	47 (78.3)	20 (80.0)	0.724
Disease duration, years	7.3 (3.8–11.2)	6.9 (3.2–10.5)	—	—
DAS28	5.43 ± 1.18	5.27 ± 1.24	—	—
ESR, mm/h	39 (28–54)	36 (24–51)	10 (7–14)	<0.001
CRP, mg/L	18.5 (8.4–35.7)	15.9 (7.8–31.2)	2.8 (1.4–4.9)	<0.001
Rheumatoid factor positive, n (%)	54 (90.0)	23 (38.3)	0	<0.001
ACPA, U/mL	176.8 (94.4–312.7)	4.3 (2.6–7.8)	2.5 (1.8–4.0)	0.184
Anti-CarP, ng/mL	129.6 (106.5–160.8)	122.8 (97.3–152.6)	88.9 (70.5– 107.8)	<0.001
Anti-CarP- positive, n (%)	18 (30.0)	22 (36.7)	1 (4.0)	<0.001
HAQ	1.58 ± 0.63	1.49 ± 0.59	—	—
Larsen score	48 (26–71)	39 (19–63)	—	—
Number of erosions	9 (4–17)	6 (2–12)	—	—

ROC analysis demonstrated acceptable diagnostic performance of anti-CarP antibodies for RA (AUC 0.783; 95% CI 0.691–0.874; $p < 0.001$). At the optimal cut-off of 143 ng/mL, sensitivity was 36.7% and specificity 96.0%; this combination of high specificity and modest sensitivity supports anti-CarP as an adjunctive rather than stand-alone diagnostic marker.

**Figure 1.** Serum anti-CarP concentrations across study groups.

Serum anti-CarP concentrations differed significantly across the three study groups (Kruskal-Wallis, $p < 0.001$; Figure X). Both RA subgroups demonstrated markedly higher anti-CarP levels than healthy controls (ACPA-negative RA: 122.8 ng/mL [IQR 97.3–152.6] vs. 88.9 ng/mL [IQR 70.5–107.8] in controls, $p < 0.001$; ACPA-positive RA: 129.6 ng/mL [IQR 106.5–160.8], $p < 0.001$). In contrast, anti-CarP concentrations did not differ significantly between

ACPA-negative and ACPA-positive patients ($p = 0.284$), indicating that elevated anti-CarP production occurs largely independently of ACPA serostatus and may represent a distinct, rather than overlapping, humoral autoimmune pathway in rheumatoid arthritis.

These findings indicate that the clinical relevance of anti-CarP antibodies is phenotype-dependent: in ACPA-positive RA they identify a more destructive disease course, while in ACPA-negative RA they reflect inflammatory activity and may help reclassify a subset of seronegative patients as immunologically active.

Discussion. This study examined the clinical significance of anti-CarP antibodies in RA and their relationship with disease activity and structural damage across serological subtypes. Serum anti-CarP concentrations were significantly higher in RA patients than in healthy controls, and antibodies were detected in approximately one-third of patients regardless of ACPA status — supporting carbamylation-driven autoimmunity as an independent pathogenic pathway rather than an epiphenomenon of anti-citrullinated immunity.

The observed anti-CarP prevalence (25–45%) is consistent with prior reports. Shi et al. [3,4] first showed that anti-CarP antibodies precede clinical RA onset and independently predict radiographic progression; this was subsequently corroborated by Brink et al. [5], Humphreys et al. [7], and Verheul et al. [8], who linked anti-CarP positivity to a more severe disease phenotype and accelerated joint destruction.

ROC analysis indicated acceptable diagnostic performance (AUC 0.783, specificity 96%, sensitivity modest), positioning anti-CarP as an adjunctive rather than stand-alone biomarker — in line with Pecani et al. [10] and Gan et al. [6], who proposed that adding anti-CarP testing to routine serology improves diagnostic accuracy, particularly in seronegative disease.

Notably, anti-CarP positivity was similarly prevalent in ACPA-positive and ACPA-negative patients, with roughly one-third of ACPA-negative individuals testing anti-CarP-positive. This supports carbamylation as immunologically distinct from citrullination: homocitrullinated proteins generate neoepitopes that activate autoreactive B-cells independently of peptidylarginine deiminase activity [13], implicating anti-CarP antibodies in disease pathogenesis among patients lacking conventional autoantibodies.

Within the ACPA-positive subgroup, anti-CarP positivity was associated with significantly greater erosive damage, consistent with data from the Leiden Early Arthritis Clinic [3] and the Norfolk Arthritis Register [7] linking anti-CarP to accelerated structural progression — plausibly through osteoclast activation, amplified cytokine production, and sustained synovial autoimmunity.

Among ACPA-negative patients, anti-CarP concentrations correlated weakly but significantly with DAS28, indicating an association with inflammatory burden rather than structural damage. Despite its modest strength, this correlation is clinically relevant given the scarcity of severity-stratifying biomarkers in seronegative RA. Humphreys et al. [7] similarly reported persistently higher disease activity and functional disability among anti-CarP-positive patients independent of conventional prognostic factors, reinforcing the complementary prognostic value of this marker.

The mechanisms underlying anti-CarP production remain incompletely defined. Chronic inflammation, oxidative stress, and increased myeloperoxidase activity promote carbamylation and the accumulation of homocitrullinated proteins within inflamed synovium; the resulting neoepitopes drive anti-CarP production, which may further amplify inflammation via immune complex formation, complement activation, and macrophage stimulation — a plausible link between anti-CarP and both inflammatory activity and joint damage [13].

This study is among the first to characterize anti-CarP antibodies in an Uzbek RA cohort. Given that ethnic differences in genetic background and environmental exposure may shape

autoantibody profiles, these findings extend the evidence base for anti-CarP beyond the predominantly European populations studied to date.

The cross-sectional design precludes assessment of anti-CarP as a predictor of future radiographic progression or treatment response; subgroup analyses may be underpowered despite an adequate overall sample size; and anti-CarP was measured using a single commercial ELISA in the absence of international assay standardization. Prospective, multicenter studies with serial measurements are needed to define its prognostic value and establish clinically validated thresholds.

Overall, anti-CarP antibodies represent a complementary serological biomarker in RA. Their value appears greatest in ACPA-negative disease, where they may reclassify a subset of seronegative patients as immunologically active, and in ACPA-positive disease, where they identify a more erosive phenotype. Incorporating anti-CarP testing into routine practice may thus enhance risk stratification and support individualized therapeutic decisions.

Conclusions. Serum anti-CarP antibodies were significantly elevated in patients with RA compared with healthy controls and were detected in approximately one-third of patients independent of ACPA status, supporting carbamylation-driven autoimmunity as a pathway distinct from anti-citrullinated immunity. Anti-CarP demonstrated high specificity but modest sensitivity for RA, indicating utility as an adjunctive rather than stand-alone diagnostic marker. Clinically, anti-CarP positivity was associated with more erosive joint damage in ACPA-positive disease and with greater inflammatory activity in ACPA-negative disease, suggesting phenotype-dependent prognostic relevance. These findings support incorporating anti-CarP testing into serological assessment of RA — particularly in ACPA-negative patients, where it may help identify immunologically active, under-recognized disease — while prospective multicenter studies are needed to confirm its predictive value and to establish validated diagnostic thresholds.

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