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Evaluation of Anti-Cyclic Citrullinated Peptide (Anti-CCP) Positivity in RA-Negative Patients with Joint Pain

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A B S T R A C T

Rheumatoid arthritis (RA) diagnosis traditionally relies on Rheumatoid Factor (RF), but RF negativity in clinically suspected cases may delay recognition and treatment. Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies demonstrate higher specificity and may identify patients at risk for early or evolving RA even when RF is negative. To evaluate the diagnostic significance of Anti-CCP positivity in RF-negative patients presenting with joint pain and assess its implications for early RA detection. This retrospective analytical study included 1,494 patients with joint pain who underwent both RF and Anti-CCP testing. Patients were categorized based on combined serological status. Special emphasis was placed on the RF-negative Anti-CCP–positive subgroup. Chi-square (χ^2) testing assessed association between RF and Anti-CCP, while Odds Ratio (OR) and proportion analysis evaluated diagnostic likelihood and clinical relevance. A p-value <0.05 was considered statistically significant. Of 1,494 patients, 213 (14.25%) were RF positive and 1,281 (85.75%) were RF negative. Anti-CCP positivity was observed in 121 patients (8.1%). Importantly, 53 patients (4.35%) were RF-negative but Anti-CCP positive, accounting for 43.8% of all Anti-CCP–positive cases. There was a statistically significant association between RF and Anti-CCP reactivity (χ^2, $p < 0.001$). Anti-CCP–positive individuals demonstrated significantly higher odds of RF positivity, while RF-negative Anti-CCP–positive patients represented a clinically important "at-risk" population for evolving RA. A substantial proportion of Anti-CCP–positive patients in this cohort were RF negative, underscoring the value of Anti-CCP testing in suspected RA, particularly when RF is negative. Incorporating Anti-CCP testing enhances diagnostic precision, supports early disease recognition, and may facilitate timely therapeutic intervention to prevent long-term joint damage. Anti-CCP should therefore be considered an essential biomarker in evaluating patients with persistent joint pain.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disease characterized by persistent synovitis, progressive joint destruction, disability, and systemic complications if untreated (20,21). Biomarkers are central to its diagnosis and prognostication, with Rheumatoid Factor (RF) historically used as the principal serological marker. However, RF lacks specificity and may be negative in a considerable proportion of clinically suspected RA patients, creating diagnostic uncertainty, particularly during early disease or in atypical clinical presentations (7,9).

With the discovery of antibodies against cyclic citrullinated peptides (Anti-CCP), diagnostic accuracy in RA has markedly improved (1,4). Anti-CCP antibodies

demonstrate superior specificity compared with RF and often appear years before clinical onset of disease, suggesting their role in identifying patients at risk for developing RA even in the pre-clinical or undifferentiated arthritis phase (2,3,10,11). Studies have consistently shown that Anti-CCP positivity predicts transition from undifferentiated musculoskeletal symptoms to definite RA, as well as correlating with more aggressive disease and radiographic progression (11–14,17,18).

Importantly, a subset of patients presents with joint pain and inflammatory symptoms but remains seronegative for RF while testing positive for Anti-CCP antibodies. This group represents either early evolving RA, seronegative RA phenotypes, or individuals at high risk of future RA development (8,12,16–18). Recognizing Anti-CCP positivity in RF-negative joint pain patients may therefore be critical for early diagnosis, timely therapeutic intervention, and prevention of long-term disability. The present study evaluates the diagnostic significance of Anti-CCP positivity in RA-negative patients presenting with joint pain, aiming to contribute evidence toward refining clinical decision-making and improving early RA detection.

Materials and Methods

This retrospective analytical study evaluated laboratory records of patients presenting with joint pain who underwent both Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide (Anti-CCP) testing. Anti-CCP has been widely adopted as a reliable biomarker in RA diagnostics and research, owing to its high specificity and predictive value, supporting its inclusion as a primary variable in this analysis (4,6,7,9,10).

A total of 1,494 patient records were included. Patients were categorized based on serological status into four groups:

1. RF Positive / Anti-CCP Positive
2. RF Positive / Anti-CCP Negative
3. RF Negative / Anti-CCP Positive
4. RF Negative / Anti-CCP Negative

Particular emphasis was placed on the RF-negative but Anti-CCP–positive group, as this subset has been shown to represent potential early RA, at-risk individuals, or evolving seronegative disease phenotypes (8,11,12,16–18).

RF testing was performed using standard immunoturbidimetric or latex agglutination assays, commonly applied in RA diagnostics globally (7,9). Anti-CCP antibody detection was performed using commercially available second- or third-generation ELISA-based assays validated in the literature for diagnostic accuracy and clinical utility in RA (6,7,9,10). Anti-CCP testing is recognized as more specific than RF and is incorporated into modern RA classification frameworks (8,19).

Interpretation of Anti-CCP and RF status was guided by international RA diagnostic evidence establishing their prognostic and diagnostic utility (7–10,19,20,21). The relevance of Anti-CCP positivity in patients who are RF negative has been emphasized in multiple cohort and predictive studies, forming the scientific rationale for analyzing this subgroup separately (11–14,17,18).
Data were compiled and analyzed using standard statistical software.

Chi-square (χ^2) test was performed to evaluate the association between RF and Anti-CCP status, as commonly applied in RA biomarker studies to assess diagnostic associations (7,9).

Odds Ratio (OR) was calculated to determine the likelihood of RF positivity among Anti-CCP–positive individuals. Previous RA research has demonstrated the clinical significance of such comparative metrics in evaluating disease probability and risk prediction (2,3,7,11).

Proportion analysis was conducted to quantify the percentage of Anti-CCP–positive patients within the RF-negative joint pain group, given its documented value in identifying patients who may progress to inflammatory arthritis or definite RA (11, 12, 16–18).

A p-value of <0.05 was considered statistically significant.

Results and Discussion

A total of 1,494 patients with joint pain who underwent both Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide (Anti-CCP) testing were included. Of these, 213 (14.25%) were RF positive and 1,281 (85.75%) were RF negative. Anti-CCP positivity was observed in 121 patients (8.10%), while 1,373 (91.89%) were Anti-CCP negative (Table 1 and Fig 1).

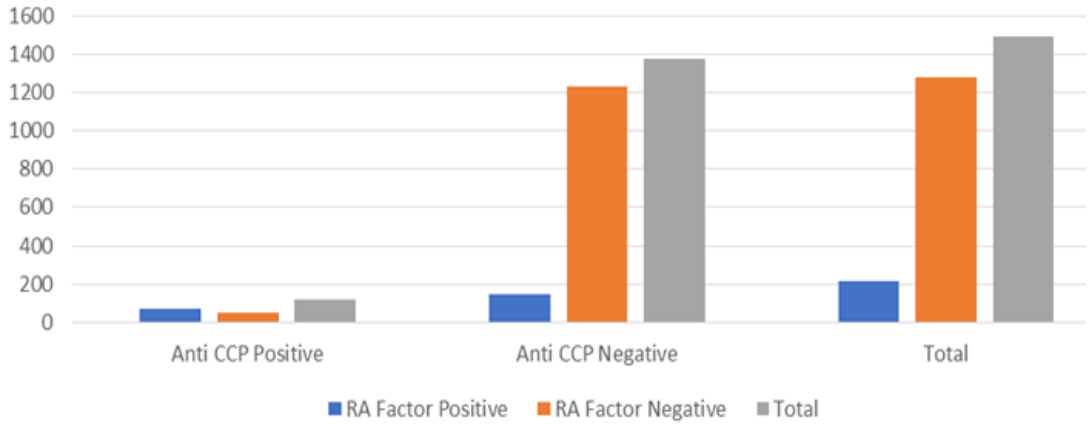
Distribution of Anti-CCP and RF Status

The combined distribution of RF and Anti-CCP test reactivity is shown in Table 1. Notably, 53 patients (4.35% of total) were RF-negative but Anti-CCP positive. This subgroup is clinically important, as Anti-CCP positivity in RF-negative patients has been strongly associated with early evolving rheumatoid arthritis, at-risk states, and seronegative inflammatory phenotypes in multiple cohort and predictive studies (8, 11–14, 16–18).

Table.1 Distribution of RA Factor and Anti-CCP Test Results

Test Status	RF Positive	RF Negative	Total
Anti-CCP Positive	68	53	121
Anti-CCP Negative	145	1228	1373
Total	**213**	**1281**	**1494**

Table.2 Comparison between RA and Anti-CCP Tests



Association Between RF and Anti-CCP Positivity

There was a statistically significant association between RF and Anti-CCP results (χ^2 test; $p < 0.001$), indicating that Anti-CCP positivity was more commonly associated with RF positivity. This is consistent with earlier literature reporting strong serological concordance due to shared autoimmune pathogenic pathways (4,6–9).

An Odds Ratio (OR) analysis demonstrated that patients with positive Anti-CCP antibodies were significantly more likely to have RF positivity compared to Anti-CCP–negative individuals, similar to earlier predictive diagnostic studies (2,3,7,9,11).

Clinical Significance of RF-Negative Anti-CCP-Positive Patients

Among the Anti-CCP positive population (n=121), 43.8% (n=53) were RF negative. This subset represents an important diagnostic population, since Anti-CCP antibodies have been shown to:

- predict future development of RA in symptomatic individuals (2,3,11,17,18)
- appear earlier than RF during disease evolution (2,3,10,11)
- correlate with higher likelihood of progressive inflammatory joint disease even when RF is absent (8,11–14,17,18)

Thus, the proportion of Anti-CCP positive but RF-negative individuals in this cohort underscores the importance of Anti-CCP testing in suspected RA patients, particularly when RF is negative.

Summary of Key Observations

- Anti-CCP positivity rate in symptomatic joint pain patients was 8.1%, aligning with previously reported prevalence trends in high-risk musculoskeletal cohorts (7, 9, 10, 17).
- RF-negative but Anti-CCP-positive patients accounted for 4.35% of all tested individuals, highlighting a clinically relevant “at-risk” population comparable to findings in predictive RA progression studies (11–14, 17, 18).

- RF and Anti-CCP demonstrated a strong statistical association, consistent with published international diagnostic evidence (4, 6–9).

This study evaluated the diagnostic relevance of Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies in patients presenting with joint pain, with particular emphasis on those who were Rheumatoid Factor (RF) negative. Anti-CCP antibodies have emerged as one of the most specific serological biomarkers in rheumatoid arthritis (RA), outperforming RF in diagnostic accuracy and prognostic utility (4, 6–9). Our findings reaffirm this clinical significance, demonstrating a statistically meaningful association between Anti-CCP and RF positivity, consistent with previous international reports (4, 6–9).

A key observation in the present cohort was the substantial proportion of Anti-CCP–positive but RF-negative patients. Nearly half of the Anti-CCP–positive population in this study lacked RF reactivity, a phenomenon well-documented in literature, where Anti-CCP antibodies frequently precede RF appearance and may identify early or pre-clinical RA (2, 3, 10, 11). Multiple longitudinal and cohort studies have shown that Anti-CCP positivity in symptomatic individuals significantly predicts future transition to definite RA, even in the absence of RF (11–14, 17, 18). Moreover, Anti-CCP positivity has been associated with more aggressive disease course and radiographic damage, further underscoring its value in early recognition and timely therapeutic decision-making (8, 11–14).

The presence of Anti-CCP antibodies in RF-negative patients therefore represents a clinically important subgroup. These individuals may reflect early evolving RA, “at-risk” states, or potential seronegative phenotypes with future disease progression (8, 12, 16–18). Early identification of such cases aligns with modern RA management philosophy that prioritizes prompt diagnosis and initiation of treatment to prevent irreversible joint damage and disability (19–21).

Overall, the findings of this study reinforce the necessity of incorporating Anti-CCP testing into routine evaluation of patients with persistent joint pain, particularly when RF is negative. Doing so enhances diagnostic precision, improves early disease detection, and supports evidence-based clinical decision pathways.
In conclusion, this study underscores the diagnostic value of Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies in patients presenting with joint pain, particularly in those who are Rheumatoid Factor (RF) negative. A significant proportion of Anti-CCP–positive individuals in this cohort were RF negative, highlighting a clinically important subgroup that may represent early evolving rheumatoid arthritis, an “at-risk” state, or potential seronegative disease profiles. The strong association observed between Anti-CCP positivity and RF reactivity reinforces existing evidence supporting Anti-CCP as a superior and highly specific biomarker in rheumatoid arthritis diagnostics. Incorporating Anti-CCP testing alongside RF in routine clinical evaluation improves diagnostic accuracy, enables earlier identification of disease, and may facilitate timely therapeutic intervention, thereby reducing the risk of delayed diagnosis and long-term joint damage. Overall, Anti-CCP testing should be considered an essential component in the assessment of patients with persistent joint pain, especially when RF results are negative, to enhance early recognition and informed clinical decision-making.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

Jayant Ramawat: Investigation, analysis, writing original draft, Deepak Kanjani: Methodology, investigation, writing-reviewing, Amrin Khan: Conceptualization, methodology, writing and funding acquisition protocol validation.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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