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# Diagnostic Performance of Rheumatoid Factor and Anti-Cyclic Citrullinated Peptide Antibodies in Rheumatoid Arthritis: A Comparative Study

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## ABSTRACT

**Background**: "Rheumatoid Arthritis" (RA) is a persistent autoimmune illness defined by chronic synovial inflammation, gradual joint destruction, and functional disability. Early diagnosis is crucial to prevent irreversible damage. "Rheumatoid Factor" (RF) has been widely used as a diagnostic marker, but its limited specificity reduces precision. "Anti-Cyclic Citrullinated Peptide" Antibodies" ("Anti-CCP"/ACPA) are extremely particular and may appear before clinical onset. Evaluating the comparative diagnostic performance of RF and ACPA antibodies in the Indian population is essential for improving early RA detection.

**Objectives**: To compare the diagnostic accuracy of RF and ACPA in differentiating "RA Patients" from disease controls with joint pain, assess their serum titers, and evaluate their correlation with disease activity (DAS28) and inflammatory status.

**Materials and Methods**: There were 172 participants in this 18-month case-control study: 86 verified "RA Patients" and 86 disease controls, categorised according to the 2010 ACR/EULAR standards. Serum RF and ACPA levels were measured quantitatively by ELISA. "C-Reactive Protein" (CRP) levels and DAS28 scores were recorded. Diagnostic performance was evaluated utilising the ROC curve analysis, sensitivity, and specificity.

**Results**: RF positivity was found in 75.6% of RA cases and 9.3% of controls, while ACPA positivity occurred in 72.1% of cases and 3.5% of controls. Combined RF and ACPA positivity was seen in 59.3% of "RA Patients" versus 1.4% of controls. ACPA showed a sensitivity of 72.1% and specificity of 96.5%, whereas RF demonstrated a 90.7% specificity and a 75.6% sensitivity. Increasing antibody titers reduced sensitivity but enhanced specificity for both markers. Higher titers correlated with greater disease activity. Both ROC analysis and CRP levels varied considerably between groups ($p < 0.001$) revealed superior diagnostic accuracy for ACPA. Conclusion: Both RF and ACPA are valuable in RA diagnosis. RF offers slightly higher sensitivity, while ACPA provides superior specificity. Combined testing and quantitative assessment improve early and accurate RA diagnosis.

**KEYWORDS**: "Rheumatoid Arthritis"; "Rheumatoid Factor"; "Anti-CCP"; ACPA; ELISA; DAS28; Diagnostic biomarkers; Autoimmunity; Serology; Early diagnosis

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## INTRODUCTION

"Rheumatoid Arthritis" (RA) is a long-term inflammatory condition of autoimmune origin that causes progressive structural deterioration and mainly affects synovial joints, pain, and functional impairment. Over time, persistent synovitis results in cartilage degradation, bone erosion, and joint deformity, significantly reducing quality of life. RA is not limited to joint involvement alone; it is a systemic disease associated with extra-articular manifestations involving the cardiovascular, respiratory, hematological, and nervous systems. These systemic complications contribute to increased morbidity and premature mortality among affected individuals. Epidemiological studies estimate that Roughly 0.5–1% of adults worldwide suffer from RA, with a higher incidence among women and individuals in middle age. In the Indian population, delayed diagnosis and limited access to specialist care often result in presentation at advanced stages of disease. [3,4] [1,2]

The clinical onset of RA is frequently insidious, with first signs like oedema, stiffness, and joint pain being nonspecific and

variable in intensity. In the absence of classical clinical features, early inflammatory arthritis may mimic other musculoskeletal or autoimmune conditions, making prompt diagnosis challenging. Diagnostic delay is associated with irreversible joint damage and poor long-term outcomes. Current treatment strategies emphasize the significance of early intervention, as initiation of "Disease-Modifying Antirheumatic Drugs" (DMARDs) during the early phase of disease has been shown to suppress inflammation, prevent structural damage, and improve functional outcomes. Accurate identification of RA during this critical "window of opportunity" is therefore essential. [1]

Serological markers play a vital role in the diagnosis and classification of RA. "Rheumatoid Factor" (RF), an autoantibody that targets immunoglobulin G's Fc component, has historically been the most commonly used laboratory marker. Although RF is detected in a substantial proportion of patients with established RA, it lacks disease specificity. RF positivity may also be observed in other autoimmune disorders, chronic infections, and even in healthy individuals, particularly at low titers and in older age groups. As a result, reliance on RF alone may lead to diagnostic uncertainty, especially in those with undifferentiated or early-stage arthritis. [5,6]

The identification of antibodies against citrullinated proteins has significantly improved the serological evaluation of RA. A post-translational alteration of arginine residues is called citrullination to citrulline, taking place during inflammatory processes and leading to the formation of neoantigens. In genetically predisposed individuals, an autoimmune reaction is triggered by these modified proteins, resulting in the production of "Anti-Citrullinated Protein Antibodies" (ACPA), most frequently identified as "Anti-Cyclic Citrullinated Peptide" ("Anti-CCP") antibodies. "Anti-CCP" Antibodies" demonstrate high disease specificity and are considered one of the most reliable serological markers for RA. [5,7,8]

A distinguishing feature the existence of "Anti-CCP" Antibodies" early in the disease course, often preceding the emergence of clinical signs by several years. Their detection in people suffering from undifferentiated arthritis has been shown to predict progression to definite RA. In addition to their diagnostic value, "Anti-CCP" Antibodies" are connected with a more serious illness phenotype, including higher inflammatory activity, rapid radiographic progression, and poorer functional outcomes. These characteristics have established "Anti-CCP" not only as a diagnostic marker but also as a biomarker with prognostic significance. [11,15]

The importance of serological testing is reflected in the 2010 "American College of Rheumatology/European League Against Rheumatism" (ACR/EULAR) classification standards, which incorporate both RF and "Anti-CCP" Antibodies" into a weighted scoring system. Higher antibody titers are assigned greater diagnostic weight, acknowledging their stronger association with established disease. Within this framework, RF contributes diagnostic sensitivity, whereas "Anti-CCP" provides high specificity. When combined with clinical features, acute-phase reactants, and symptom duration, these biomarkers enhance diagnostic accuracy and facilitate earlier classification of RA. [1]

Despite the widespread use of RF and "Anti-CCP" in clinical practice, data evaluating their comparative diagnostic performance in the Indian population remain limited. Genetic background, environmental exposures, healthcare access, and disease presentation may influence the utility of serological markers across different populations. Furthermore, the interpretation of test results is affected by antibody titers, as low-level positivity may be observed in non-RA conditions, while higher titers are more strongly associated with true disease. Understanding the impact of varying titer thresholds on sensitivity and specificity is therefore critical for optimal clinical application.

Activity of the disease assessment is another crucial component of RA management. Combination indices such as the "Disease Activity Score" in 28 joints (DAS28), along with inflammatory markers like "C-Reactive Protein" (CRP), are routinely used to monitor disease severity and guide therapeutic decisions. Exploring the connection between RF and "Anti-CCP" Antibodies" levels and disease activity parameters may provide additional insight into their clinical relevance beyond diagnosis.

In this regard, the current investigation was conducted to evaluate and compare the performance in diagnosis of RF and "Anti-CCP" Antibodies" in patients with "Rheumatoid Arthritis" attending an Indian tertiary care facility. By including disease control individuals presenting with joint pain and applying standardized ACR/EULAR classification criteria, the study aims to assess the real-world utility of these biomarkers in differentiating RA from other causes of arthralgia. Quantitative measurement of antibody titers and correlation with inflammatory markers and "Disease Activity Scores were undertaken to provide a comprehensive assessment of their diagnostic and clinical significance. [1]

## MATERIALS AND METHODOLOGY

This case-control study was carried out in a hospital over 18 months at the Departments of Microbiology, Medicine (Rheumatology Clinic), and Orthopaedics, "Integral Institute of Medical Sciences and Research" (IIMSR), Lucknow, following the Institutional Ethics Committee's clearance. Every participant provided written informed permission.

A total of 172 subjects were enrolled and divided into two equal groups: 86 "Rheumatoid Arthritis" (RA) patients (Group C) satisfying the 2010 ACR/EULAR categorisation criteria (score ≥6), and 86 disease controls (Group DC) presenting with joint pain but not meeting RA criteria. Adults (≥18 years) attending outpatient or inpatient services were included. Patients with other autoimmune diseases, chronic infections affecting autoantibody production, or those refusing to engage were excluded. [1,2] [1]
Sample size was calculated based on reported sensitivity of "Anti-CCP" Antibodies" in previous literature and rounded to 86

participants per group.

Demographic details were recorded for all participants. Disease activity in "RA Patients" was measured using the DAS28 score, high painful and swollen joint counts, CRP, and patients worldwide evaluation. Serum CRP was measured as a marker of systemic inflammation.

Venous blood was collected under aseptic conditions clotted, then centrifuged for 15 minutes at 1000 ×g. Serum was analyzed immediately or stored at −20 °C. Hemolyzed, lipemic, or turbid samples were excluded.

"Rheumatoid Factor" (RF) and "Anti-Cyclic Citrullinated Peptide" ("Anti-CCP") antibodies were quantified using QUALISA™ ELISA kits. The threshold for RF positive was ≥30 IU/mL and Positive "Anti-CCP" as ≥25 IU/mL. Samples and controls were tested in duplicate, and optical density was read at 450 nm. Antibody titers were further categorized using multiples of the upper limit of normal (1×, 1.5×, 2×, 2.5×, and 3× ULN) to evaluate diagnostic performance at varying thresholds. [5–7]

Data were analysed using SPSS version 26.0. The expression for continuous variables was mean ± SD and categorical variables as frequencies and percentages. Group comparisons were performed using Chi-square/Fisher's exact test for categorical data and Student's t-test or Mann–Whitney U test for continuous variables. Diagnostic performance was assessed by calculating sensitivity, specificity, PPV, and NPV. "Receiver Operating Characteristic" (ROC) curves were created to compare the diagnostic accuracy of RF and "Anti-CCP". Statistical significance was defined as a p value of less than 0.05.

## RESULTS

**Demographic Characteristics**

A total of 172 people were included in the study comprising 86 "Rheumatoid Arthritis" (RA) patients (Group C) and 86 disease controls with joint pain (Group DC). The age-wise distribution of the study population revealed a distinct difference between cases and controls.

Among "RA Patients", the highest proportion belonged to the 41–50-year age group, accounting for 34 individuals (39.53%). This was followed by the 31–40-year age group with 25 patients (29.07%). No RA cases were observed between the ages of 10 and 20. In contrast, the control group included 6 individuals (6.98%) in the 10–20-year category. Both groups showed similar representation in the 31–40-year age group (29.07% each). The overall distribution indicated that RA predominantly affected individuals in the middle decades of life.

Gender distribution demonstrated a female predominance in both groups. Among RA cases, females constituted 49 patients (56.98%), while males accounted for 37 (43.02%). Similarly, in the control group, females comprised 55 individuals (63.95%) and males 31 (36.05%). This pattern reflects the well-recognized higher prevalence of RA among women.

**Inflammatory Marker Profile**

Serum "C-Reactive Protein" (CRP) levels differed significantly between RA cases and controls ($p < 0.001$). Among individuals with RA, 32 individuals (37.2%) had CRP values <0.6 mg/dL, whereas 37 (43.0%) exhibited increased CRP values ≥0.6 mg/dL; CRP values were unavailable in 17 cases (19.8%).

In the control group, a substantially higher proportion of individuals revealed high CRP levels (≥0.6 mg/dL) (69; 80.2%), while only 14 (16.3%) had CRP values <0.6 mg/dL; data were unavailable for 3 controls (3.5%). Overall, elevated CRP levels were observed in 106 of 172 participants (61.6%). The distribution of the difference in CRP levels between the two groups was statistically significant., indicating a marked difference in inflammatory status.

**"Distribution of RF and Positive "Anti-CCP"**

The serological profile of participants showed a clear distinction between RA cases and controls.

1. Only RF positive was detected in 14 "RA Patients" (16.3%) and in 7 controls (8.1%).
2. Only Positive "Anti-CCP" was seen in 11 "RA Patients" (12.8%) and in 2 controls (2.3%).
3. Both RF and Positive "Anti-CCP" was present in 51 "RA Patients" (59.3%), compared with only 1 control (1.4%).
4. Both markers negative were noted in 10 "RA Patients" (11.6%) and in 76 controls (88.4%).

Thus, the majority of "RA Patients" demonstrated dual seropositivity, whereas most controls were seronegative for both biomarkers.

**Diagnostic Performance of "Rheumatoid Factor"**

Using a 2×2 contingency analysis:

- True positives (TP): 65
- False negatives (FN): 21
- False positives (FP): 8
- True negatives (TN): 78

From these values, RF demonstrated:

- Sensitivity: 75.6%
- Specificity: 90.7%

This indicates that RF identified approximately three-quarters of "RA Patients" but produced a moderate number of false-positive results among controls.

**Diagnostic Capabilities of "Anti-CCP" Antibodies"**

For "Anti-CCP":

- True positives (TP): 62
- False negatives (FN): 24
- False positives (FP): 3
- True negatives (TN): 83

"Anti-CCP" exhibited:

- Sensitivity: 72.1%
- Specificity: 96.5%

Thus, "Anti-CCP" demonstrated slightly lower sensitivity than RF but markedly superior specificity, with very few false-positive results among controls.

**Titre-Based Analysis**

Among "RA Patients", 62 (72.1%) were "Anti-CCP" positive overall, whereas only 3 controls (3.5%) tested positive. Cumulative tire analysis revealed that:

- 58 "RA Patients" (67.4%) had "Anti-CCP" titers >1.5 × ULN
- 55 (64.0%) >2 × ULN
- 49 (57.0%) >2.5 × ULN
- 46 (53.5%) >3 × ULN

Low-titer Positive "Anti-CCP" (<3 × ULN) was observed in 16 "RA Patients" (18.6%) and 2 controls (2.3%).

For RF, 65 "RA Patients" (75.6%) were positive overall, compared with 8 controls (9.3%). Titer stratification showed:

- 53 "RA Patients" (61.6%) with RF >1.5 × ULN
- 49 (57.0%) >2 × ULN
- 46 (53.5%) >2.5 × ULN
- 43 (50.0%) >3 × ULN

Low-titer RF positivity (<3 × ULN) was observed in 22 "RA Patients" (25.6%) and 6 controls (7.0%).

Increasing titer thresholds resulted in a progressive decline in sensitivity with a corresponding improvement in specificity for both biomarkers. At titers >3 × ULN, "Anti-CCP" retained a specificity of 98.8% with a sensitivity of 53.5%, whereas RF showed a specificity of 97.7% with a sensitivity of 50.0%.

**"Receiver Operating Characteristic" (ROC) Analysis**

"Receiver Operating Characteristic" (ROC) curves demonstrated that RF as well as "Anti-CCP" discriminated RA cases from controls significantly better than chance. Compared to RF, the "Anti-CCP"'s area "Under the Curve" (AUC) was larger., indicating superior overall diagnostic accuracy. "Anti-CCP" provided higher specificity at comparable sensitivity levels, whereas RF demonstrated marginally higher sensitivity at lower thresholds.

**ACR/EULAR Classification Scoring**

Application of the ACR/EULAR classification standards from 2010 revealed that RA cases accumulated significantly higher scores across all domains compared with controls. [1]

- In the joint involvement domain, 43 "RA Patients" (50.0%) had involvement of over ten joints including at least one tiny joint, while none of the controls exhibited inflammatory joint involvement.
- In the serology domain, 51 "RA Patients" (59.3%) demonstrated high-positive RF or "Anti-CCP", compared with only 3 controls (3.5%).
- In the acute-phase reactants domain, abnormal CRP was observed in 62 "RA Patients" (72.1%) and only 4 controls (4.7%).
- In the symptom duration domain, 70 "RA Patients" (81.4%) reported symptoms lasting ≥6 weeks, whereas all controls had symptom duration <6 weeks.

These findings confirm that RA cases consistently met higher classification scores, validating the discriminatory capacity of this cohort's ACR/EULAR criteria. [1]

## TABLES AND FIGURES.

**Table 1. Distribution of Cases and Controls by Age**

| "Age Group (years)" | "RA Cases (n=86)" | "Controls (n=86)" |
|---|---|---|
| 10–20 | 0 (0.00%) | 6 (6.98%) |
| 21–30 | 11 (12.79%) | 9 (10.47%) |
| 31–40 | 25 (29.07%) | 25 (29.07%) |
| 41–50 | 34 (39.53%) | 22 (25.58%) |
| 51–60 | 11 (12.79%) | 16 (18.60%) |
| 61–70 | 4 (4.65%) | 7 (8.14%) |
| >70 | 1 (1.16%) | 1 (1.16%) |

**Table 2. Gender Distribution of RA Cases and Controls**

| Category | RA Cases (n=86) | Controls (n=86) |
|---|---|---|
| "Male" | 37 (43.02%) | 31 (36.05%) |
| "Female" | 49 (56.98%) | 55 (63.95%) |
| "Total" | 86 (100%) | 86 (100%) |

**Table 3. CRP Values in Cases and Controls**

| CRP level (mg/dL) | "RA Cases" | Controls | "Total" | P value |
|---|---|---|---|---|
| <0.6 | 32 (37.2%) | 14 (16.3%) | 46 (26.7%) | |
| ≥0.6 | 37 (43.0%) | 69 (80.2%) | 106 (61.6%) | <0.001** |
| Not available | 17 (19.8%) | 3 (3.5%) | 20 (11.7%) | |
| Total | 86 (100%) | 86 (100%) | 172 (100%) | |

**Table 4. Distribution of RF and ACPA Positivity Patterns**

| Pattern | RA Cases (n=86) | Controls (n=86) |
|---|---|---|
| Only RF positive | 14 (16.3%) | 7 (8.1%) |
| Only ACPA positive | 11 (12.8%) | 2 (2.3%) |
| Both RF & ACPA positive | 51 (59.3%) | 1 (1.4%) |
| Both RF & ACPA negative | 10 (11.6%) | 76 (88.4%) |
| Total | 86 (100%) | 86 (100%) |

**Table 5. Contingency Table for "Rheumatoid Factor" (RF)**

| | RA Present | RA Absent |
|---|---|---|
| RF Positive | TP = 65 | FP = 8 |
| RF Negative | FN = 21 | TN = 78 |

**Table 6. Contingency Table for "Anti-CCP" (ACPA)**

| | RA Present | RA Absent |
|---|---|---|
| ACPA Positive | TP = 62 | FP = 3 |
| ACPA Negative | FN = 24 | TN = 83 |

**Table 7. RF and ACPA Sensitivity and Specificity**

| Biomarker | Sensitivity (%) | Specificity (%) |
|---|---|---|
| ACPA | 72.1 | 96.5 |
| RF | 75.6 | 90.7 |

**Table 8. RF and ACPA Status with Titer Categories**

| Category | RA Cases (n) | RA % | Controls (n) | Controls % |
|---|---|---|---|---|
| ACPA negative | 24 | 27.9 | 83 | 96.5 |
| ACPA positive (overall) | 62 | 72.1 | 3 | 3.5 |
| ACPA >1.5× ULN | 58 | 67.4 | 2 | 2.3 |
| ACPA >2× ULN | 55 | 64.0 | 1 | 1.2 |
| ACPA >2.5× ULN | 49 | 57.0 | 1 | 1.2 |
| ACPA >3× ULN | 46 | 53.5 | 1 | 1.2 |
| RF negative | 21 | 24.4 | 78 | 90.7 |
| RF positive (overall) | 65 | 75.6 | 8 | 9.3 |

**Table 9. RA classification scoring of study participants (n = 172) by "Rheumatoid Arthritis" Classification Using 2010 ACR/EULAR Criteria [1]**

| Criteria domain | Subcategory | Score | RA Cases (n=86) | Controls (n=86) |
|---|---|---|---|---|
| "Joint involvement" | "1 large joint" | 0 | 0 | 0 |
| | "2–10 large joints" | 1 | 0 | 0 |
| | "1–3 small joints" | 2 | 15 | 0 |
| | "4–10 small joints" | 3 | 28 | 0 |
| | ">10 joints (at least 1 small)" | 5 | 43 | 0 |
| "Serology" | "Negative RF and ACPA" | 0 | 10 | 76 |

| | | | | |
|---|---|---|---|---|
| | "Low-positive RF or ACPA" | 2 | 25 | 7 |
| | "High-positive RF or ACPA" | 3 | 51 | 3 |
| "Acute-phase reactants" | "Normal CRP" | 0 | 24 | 82 |
| | Abnormal CRP | 1 | 62 | 4 |
| "Duration of symptoms" | "<6 weeks" | 0 | 16 | 86 |
| | "≥6 weeks" | 1 | 70 | 0 |

**Figure 1. "Age-wise distribution of cases and controls"**

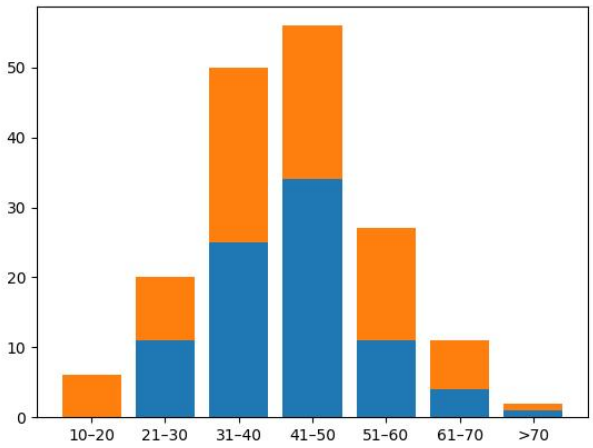



**Figure 2. "Gender distribution of RA cases and controls"**

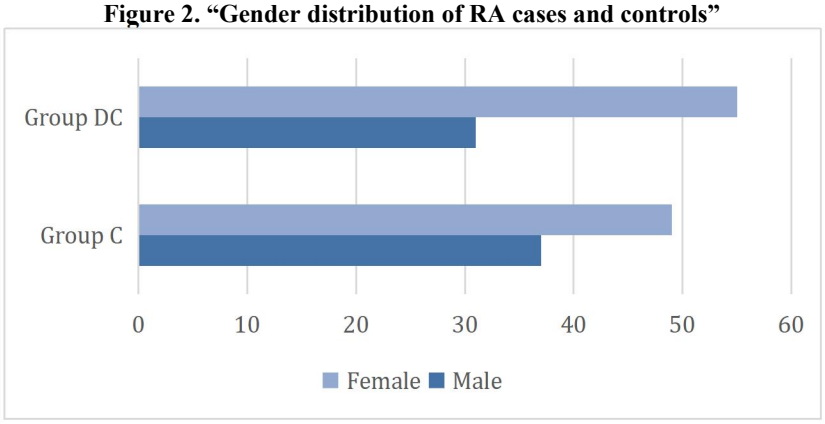



**Figure 3. CRP distribution in RA cases and controls.**

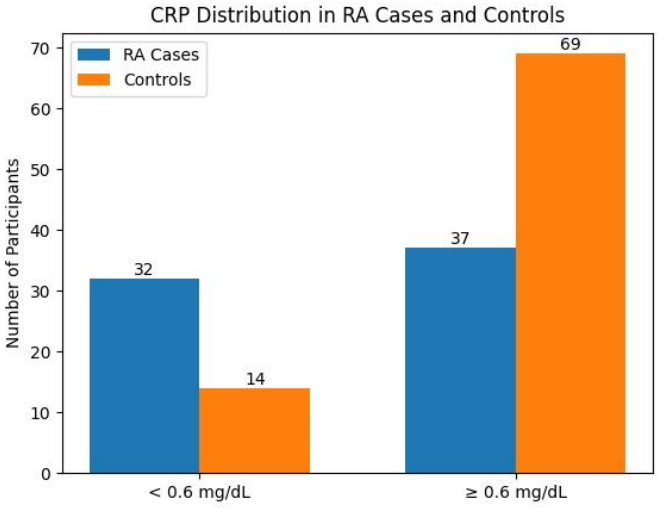


**Figure 4. Serological profile among "RA Patients" (n=86).**

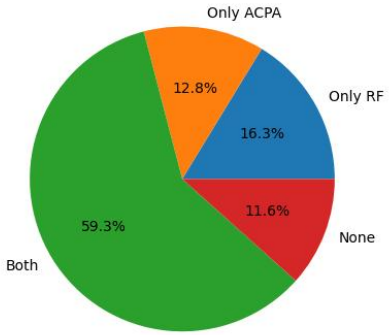



**Figure 5a. Comparative bar graph showing overall positivity of "Anti-CCP" (ACPA) and "Rheumatoid Factor" (RF) among "Rheumatoid Arthritis" cases and controls.**

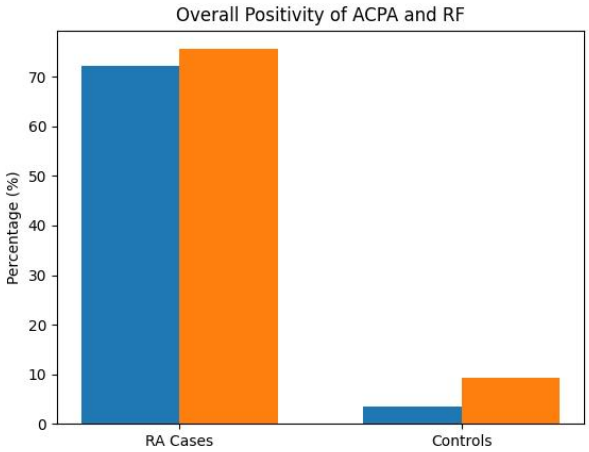



**Figure 5b. Bar graph depicting proportion of "Rheumatoid Arthritis" cases with high-titer antibodies (>3 × ULN) for ACPA and RF.**

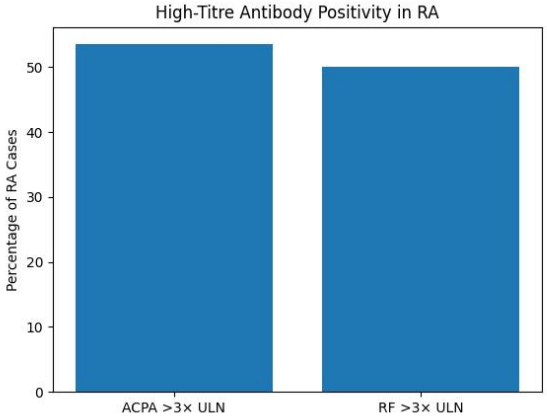


**Figure 6a. Sensitivity of ACPA and RF at different titer cut-offs (× ULN).**

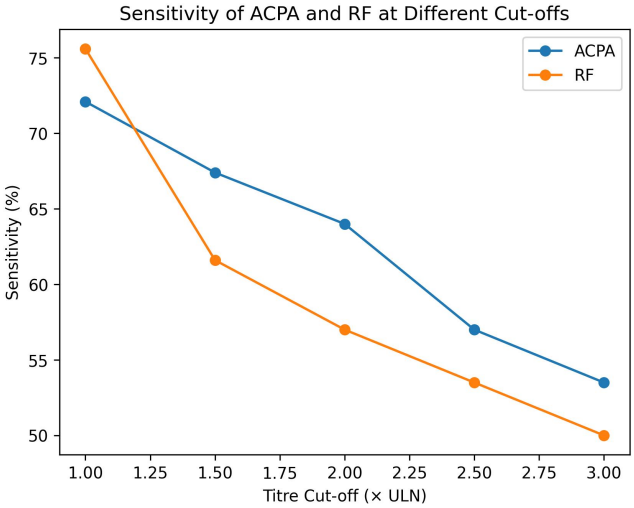



**Figure 6b: Specificity of ACPA and RF at different titer cut-offs (× ULN).**

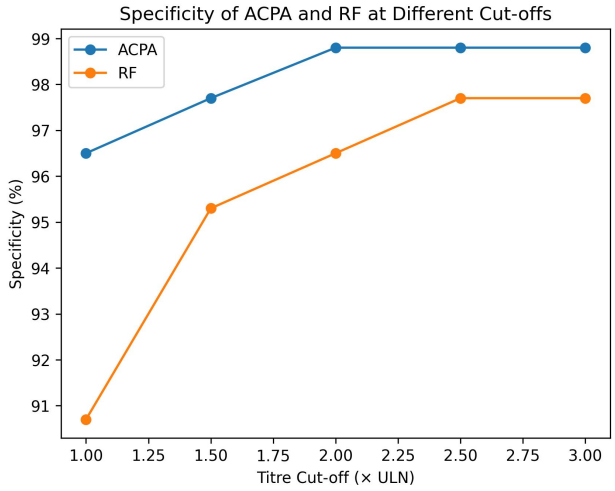
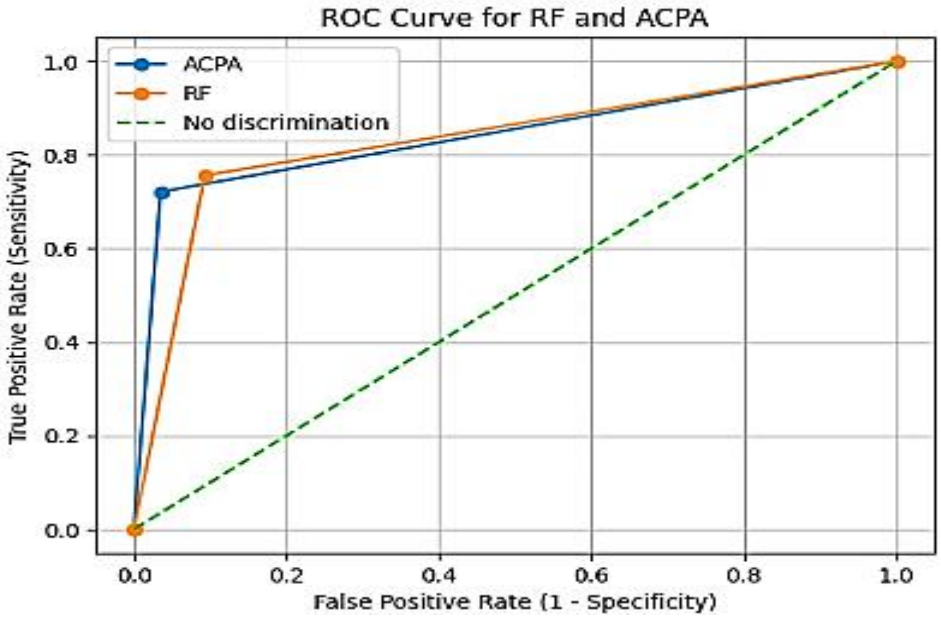



**Figure 7: "Receiver Operating Characteristic" (ROC) curves for "Anti-Cyclic Citrullinated Peptide" Antibodies" (ACPA) and "Rheumatoid Factor" (RF) in the diagnosis of "Rheumatoid Arthritis". The diagonal line represents no discrimination. ACPA demonstrates a higher area "Under the Curve" compared to RF, indicating superior diagnostic accuracy.**




## DISCUSSION

"Rheumatoid Arthritis" (RA) remains a significant contributor to chronic inflammatory morbidity worldwide, with significant implications for functional capacity, quality of life, and long-term survival. The current investigation was conducted to evaluate the diagnostic utility of "Rheumatoid Factor" (RF) and "Anti-Cyclic Citrullinated Peptide Antibodies" ("Anti-CCP") in an Indian tertiary-care setting and to analyze their connection to illness activities. By employing standardized ACR/EULAR classification criteria and quantitative serological assays, this study provides population-specific evidence on the comparative performance of these biomarkers in distinguishing RA from other causes of joint pain. [1,2]

The demographic profile observed in this cohort is consistent with established epidemiological trends. The peak occurrence of RA in the 41–50-year age group and the clear female predominance reflect the known predilection of RA for middle-aged women. Similar age and gender distributions have been reported in both Indian and international studies, reinforcing The current findings' external validity. The absence of RA cases in the youngest age group further highlights the rarity of RA in adolescence and early adulthood. [1,2]

Serological analysis demonstrated that Most "RA Patients" tested positive for at least one autoantibody, with more than half exhibiting dual positivity for RF and "Anti-CCP". In contrast, most controls were seronegative for both markers. This marked separation between cases and controls underscores the value of serological testing in patients presenting with joint symptoms. The high proportion of dual positivity among "RA Patients" aligns with earlier reports indicating that RF and "Anti-CCP" tests together offers superior diagnostic yield compared with either marker alone.

"Rheumatoid Factor" demonstrated a 90.7% specificity and a 75.6% sensitivity. These figures are similar to those documented in the literature, where RF sensitivity in established RA ranges from 60% to 80%. However, the relatively lower specificity of RF reflects its propensity for false-positive results in non-RA conditions. In the present study, 9.3% of controls were RF-positive, illustrating the limited discriminatory power of RF when used in isolation, particularly in populations with a high background prevalence of infections and inflammatory conditions. [5,6]

The sensitivity and specificity of "Anti-CCP" Antibodies" were 72.1% and 96.5%, respectively. Although slightly less sensitive than RF, "Anti-CCP" demonstrated markedly superior specificity, with only 3.5% of controls testing positive. This finding is consistent with multiple meta-analyses reporting "Anti-CCP" specificities exceeding 95%. The great specificity of "Anti-CCP" makes it particularly valuable in excluding non-RA causes of arthritis and in strengthening diagnostic confidence in early or undifferentiated disease. [5,7,14]

The titer-based analysis provides important insights into the clinical interpretation of serological results. Increasing antibody thresholds were associated with a progressive decline in sensitivity and a corresponding increase in specificity for both "Anti-CCP" and RF High-titer "Anti-CCP" retained excellent specificity (>98%), even at thresholds exceeding three times the typical upper limit, whereas RF required higher titers to approach comparable specificity. Low-titer RF positivity was more frequent among controls than low-titer "Anti-CCP", reinforcing the notion that low-level RF results must be interpreted with caution in clinical practice.

"Receiver Operating Characteristic" (ROC) analysis further demonstrated superior overall "Anti-CCP" diagnostic performance in comparison to RF. Although RF showed marginally higher sensitivity at lower thresholds, "Anti-CCP" provided better discrimination across the entire range of values. These findings support the complementary use of both biomarkers: RF contributes sensitivity, while "Anti-CCP" offers high specificity. Combined testing maximizes diagnostic accuracy and reduces

the likelihood of misclassification.

An important aspect of this investigation is the noted correlation between antibody titers and disease progression. Higher RF and “Anti-CCP” titers were predominantly seen in “RA Patients” with elevated CRP levels and higher DAS28 scores, suggesting a relationship between autoantibody burden and inflammatory activity. This association reinforces the prognostic relevance of these biomarkers and supports their use not just in diagnosis but also in disease stratification. Patients with high-titer positivity may represent a subgroup with more aggressive disease, warranting closer monitoring and earlier therapeutic escalation. [11,12,15]
Using the 2010 ACR/EULAR classification criteria demonstrated clear discrimination between cases and controls across all domains. “RA Patients” consistently accumulated higher scores for mutual participation, serology, acute-phase reactants, and symptom duration. The strong contribution of serological markers to overall classification highlights their central role in modern RA diagnosis and validates the integration of RF and “Anti-CCP” into routine evaluation protocols. [1]

The results of this study have significant clinical ramifications for practice in resource-limited settings. Delayed presentation and diagnostic uncertainty remain common barriers to optimal RA management in India. Reliance on clinical features alone may be insufficient in early disease. Incorporation of both RF and “Anti-CCP” tests into the diagnostic algorithm can facilitate earlier recognition, improve diagnostic confidence, and enable timely initiation of DMARD therapy within the therapeutic window of opportunity.

This study's advantages include its case–control design, use of standardized classification criteria, quantitative antibody measurement, and comprehensive titre-based analysis. The inclusion of disease controls presenting with joint pain reflects real-world diagnostic challenges and enhances the clinical relevance of the findings. However, the study is restricted by its single-centre design and the absence of long-term follow-up to assess radiographic progression and treatment outcomes. Future multicenter studies incorporating longitudinal data would further elucidate the prognostic value of these biomarkers in the Indian population.

Overall, this study reinforces the complementary roles of “Anti-CCP” and RF in the diagnosis and assessment of “Rheumatoid Arthritis”. While RF remains a sensitive screening marker, “Anti-CCP” provides unparalleled specificity. Their combined use, particularly with consideration of antibody titers, offers a robust and clinically meaningful approach to RA diagnosis and risk stratification.

## CONCLUSION

The persistent, incapacitating autoimmune disease known as "rheumatoid arthritis" in which early and accurate diagnosis is essential to avoid permanent joint damage and long-term disability. The present study demonstrates that both “Rheumatoid Factor” (RF) and “Anti-Cyclic Citrullinated Peptide” Antibodies” (“Anti-CCP”) are valuable serological markers for the RA diagnosis in the Indian population. [5–7]

RF exhibited slightly higher sensitivity, identifying a greater proportion of affected individuals; however, its lower specificity resulted in an increased false-positive rate results among disease controls. In contrast, “Anti-CCP” Antibodies” showed markedly superior specificity, making them highly reliable for distinguishing RA from other causes of joint pain. Combined RF and “Anti-CCP” testing significantly enhanced diagnostic yield, with the majority of “RA Patients” demonstrating dual positivity, while most controls remained seronegative.

Titer-based analysis revealed that increasing antibody levels were associated with improved specificity at the cost of reduced sensitivity for both biomarkers. High-titer “Anti-CCP” retained excellent specificity, reinforcing its value in confirming RA, particularly in early or diagnostically ambiguous cases. Furthermore, increased RF and “Anti-CCP” titers, correlated with increasing disease activity and inflammatory burden, underscoring their prognostic relevance.

These findings support the routine incorporation of both RF and “Anti-CCP” testing into diagnostic algorithms for patients presenting with joint symptoms. In resource-limited settings, such as many regions in India where delayed presentation is common, the combined and quantitative use of these biomarkers can facilitate earlier diagnosis, enable timely initiation of disease-modifying therapy, and ultimately improve patient outcomes. Integration of serological markers with clinical assessment and inflammatory indices provides a robust framework for the accurate and early diagnosis of “Rheumatoid Arthritis”.

Taken together, the results establish that RF and ACPA are not competing but synergistic biomarkers. RF contributes sensitivity and remains valuable as a screening tool, while ACPA provides unparalleled specificity and diagnostic certainty. Their combined, quantitative use—especially with consideration of antibody titers—significantly enhances diagnostic precision.

In a healthcare environment where delayed presentation and diagnostic uncertainty are common, particularly in resource-limited settings, this evidence strongly supports the routine incorporation of both RF and ACPA into the evaluation of patients with joint symptoms. Early, accurate diagnosis enabled by these biomarkers facilitates timely initiation of disease-modifying therapy within the critical therapeutic window, thereby preventing irreversible joint damage, reducing disability, and improving long-term outcomes for patients with “Rheumatoid Arthritis” in the Indian population.

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