

Association of disease activity score (DAS) 28 with subsets of rheumatoid factor and anti-cyclic citrullinated peptides (anti-CCP) among rheumatoid arthritis patients in North India

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Rheumatoid factor (RF), anticyclic citrullinated peptides (Anti-CCP), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) are used as disease markers for rheumatoid arthritis (RA). However, correlation of disease activity score (DAS) 28 with autoantibodies and inflammatory markers has not been studied in detail. Here, we have made an attempt to determine the association of DAS-28 with autoantibodies and inflammatory markers among the North Indian population. Further, we investigated the potential of subsets of autoantibodies in RA diagnosis. Disease activity was calculated by DAS-28-ESR calculator and levels of disease markers were estimated in serum. We noticed positive correlation between DAS-28, CRP ($r=0.63$, $p=0.00$) and ESR ($r=0.72$, $p=0.00$). Moderate and weak correlation was found with RF ($r=0.41$, $p=0.00$) and anti-CCP ($r=0.25$, $p=0.011$), respectively. Patients with positive subsets of both autoantibodies (RF+/Anti-CCP+) have shown a positive correlation with DAS-28. In our study, we found that the presence of RF and Anti-CCP in patient sera can increase the early detection rate of disease. But, for the diagnosis of disease in seronegative patients, more studies on biomarkers are required.

Keywords: Autoantibody, Electrochemiluminescence, Immunological markers, Immunoturbidimetric assay, Sensitivity, Specificity

Rheumatoid arthritis (RA) is an autoimmune disease that attacks the host's joints and causes an inflammatory effect¹. RA is characterized by the progressive destruction of joints and erosive synovitis². RA causes persistent synovitis, pain, joint destruction, and functional disability². RA affects 1% of the world's population³. Antibodies present in the serum of patients have provided many clues about the disease's pathophysiology⁴.

Rheumatoid factor (RF) and Anti-cyclic citrullinated peptide (anti-CCP) antibodies are major auto-antibodies observed in RA. According to the American College of Rheumatology (ACR) criteria, RF is taken as the gold standard for the diagnosis of RA⁵. Anti-CCP antibodies are found to be strongly associated with the development of RA in healthy individuals and can be detected in the blood before the appearance of clinical symptoms⁶.

Disease activity score (DAS) is a tool for a better understanding of disease progression by converting it

into a simple numerical value. Internationally acclaimed "core" sets of variables to be used in RA disease activity assessment include DAS 28, SDAI and CDAI. Calculation of the DAS includes the number of joints swollen or tendered, the Acute phase, the patient's general health, and an inflammatory marker for 28 joints⁷. DAS 28 score is a good source to measure changes in disease activity over some time. DAS 28 calculation takes swelling and tenderness in 28 joints, ESR/CRP, and Visual Analogue Scale (VAS) between 0-100 into account. DAS-28 helps clinicians to monitor patients' prognosis of the disease.

In the present study, we tried to evaluate the clinical association of autoantibodies and inflammatory markers with a DAS and to re-evaluate the sensitivity and specificity of autoantibodies in rheumatoid arthritis (RA) patients.

Materials and Methods

Subject recruitment

One hundred patients with confirmed rheumatoid Arthritis (RA; test population) were recruited from the Joint Clinic at a tertiary healthcare centre, in

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New Delhi. Also, 100 subjects with matched age and gender, non-related were recruited as a control population. Patients were classified as definite RA using the American College of Rheumatology 2010 criteria⁸. Recruited patients were examined for clinical features including joint temperature, change in joint colour, Early Morning Stiffness (EMS), deformity, swelling, and tenderness. All qualified patients were subjected to blood collection after signing informed consent. The inclusion criteria for the recruitment of patients consist of (i) The patient should have definite RA with a history of symptoms should be ≥ 6 weeks old; (ii) There must be evidence of currently active clinical synovitis (i.e., swelling) in at least 1 joint and not better explained by another diagnosis as determined by an expert assessor; and (iii) Patients with synovitis in the distal interphalangeal (DIP) joints, the first metatarsophalangeal (MTP) joint, and the first carpometacarpal (CMC) joint should not be recruited.

Sample collection

Blood was collected into wax-coated serum separating vacutainer (REF 365074) and subjected to centrifuge at 4000 rpm for 10 min for separation of serum. Collected serum was used for detection of autoantibody levels.

Autoantibody detection

Measurement of serum RF and serum CRP was done on Roche-Cobas c311. The Roche RF assay is based on the immunological agglutination principle with the enhancement of the reaction by latex, which is known as an immunoturbidimetric assay. Serum Anti-CCP levels were detected using the Cobas Elecsys 2010 (Cobas e411) analyser, which works on the principle of electrochemiluminescence immunoassay (ECLIA). Erythrocyte Sedimentation Rate (ESR) was detected by the Westergren method. The disease activity score on 28 joints (DAS-28) was calculated using the DAS-28-ESR calculator. Cut-off values were considered as RF: ≤ 14 IU/mL; Anti-CCP: ≤ 17 IU/mL; CRP: < 5 mg/dL; ESR: 0-20 mm/hr (female) and 0-15 mm/h (male). After the estimation of RF and Anti-CCP in the patient and control population, sensitivity and specificity were calculated using the formula:

$$\text{Sensitivity} = \frac{\text{True Positive}}{(\text{True Positive} + \text{False Negative})}$$

$$\text{Specificity} = \frac{\text{True Negative}}{(\text{True Negative} + \text{False Positive})}$$

Statistical analysis

Statistical summaries for continuous data were presented as mean \pm SD or Median (Range) as per the distribution of data. Summary of categorical data presented in frequency and percentage. Scatter plots and box plots were presented for data visualization. Karl-Pearson correlation coefficient was used to find an association between two numbers. Categorical and continuous data were analysed by using chi-square and t-test, respectively. Statistical significance was assessed by applying Bonferroni's correction for multiple tests. Statistical analysis and box plots were performed using IBM SPSS version 26.

Results

Average and median age of the participant in the patient group was 48.02 ± 10.7 years and 49.50 (range: 21-78 years). The average and median ages of participants in the control group were 45.44 ± 10.5 and 43 (range: 18-71 years). Both groups consist of 84% female and 16% male. The recruited population is statistically identical with a p-value of 0.087. In the present study, the disease prevalence was found to be 5 times more among females than males. Most of the patients (73%) had complaints of a rise in the temperature of their joints. About 41% of patients have experienced early morning stiffness for 15-30 min, 25% for 1 h and 16% for more than 1 h. About 92% of patients showed characteristic deformities and/or swelling and/or tenderness.

Both patient and control groups underwent the estimation of RF and Anti-CCP. Table 1 summarizes the median value of markers in both populations and the distribution of population among different disease activity groups. Seventy-one subjects in the patient group and 4 subjects in the control group were positive for RF. We found the specificity and sensitivity of RF as 96 and 71%, respectively. Whereas, 69 subjects in the patient group and 3 subjects in the control group were positive for Anti-CCP, the specificity and sensitivity of Anti-CCP were calculated as 97 and 69%, respectively.

The correlation between the inflammatory marker, autoantibodies, Visual analog scale (VAS) and DAS-28 was calculated. DAS-28 was found in strong positive correlation with VAS ($r=0.69$, $p=0.00$), CRP ($r=0.63$, $p=0.00$) and ESR ($r=0.72$, $p=0.00$). But a moderate correlation was found between DAS-28 and RF ($r=0.41$, $p=0.00$), and very weak correlation was found with anti-CCP ($r=0.25$, $p=0.011$). The

Table 1 — Continuous variables and their stratified distribution according to disease activity score and divided into three groups presented as median (IQR)

	No. of Subjects (N)	Control	Patients	High	Moderate	Low
	Male	16	16	5	4	7
	Female	84	84	27	45	12
RF	Median	9.40	37.1	127.97	29.30	18.30
	IQR	3.30	136.43	245.60	91.90	39.10
Anti-CCP	Median	7.0	200.9	411.95	177.50	63.70
	IQR	.00	490.46	459.60	492.67	244.80
ESR	Median	20.00	33	69.50	30	10
	IQR	23.75	45.50	49.00	24	13
CRP	Median	2.25	5.7	26.73	4.46	1.54
	IQR	3.57	21.71	44.14	10.31	1.39

[RF, Rheumatoid Factor; Anti-CCP, Anti citrullinated Protein; CRP, C-Reactive Protein; and ESR, Erythrocyte Sedimentation Rate]

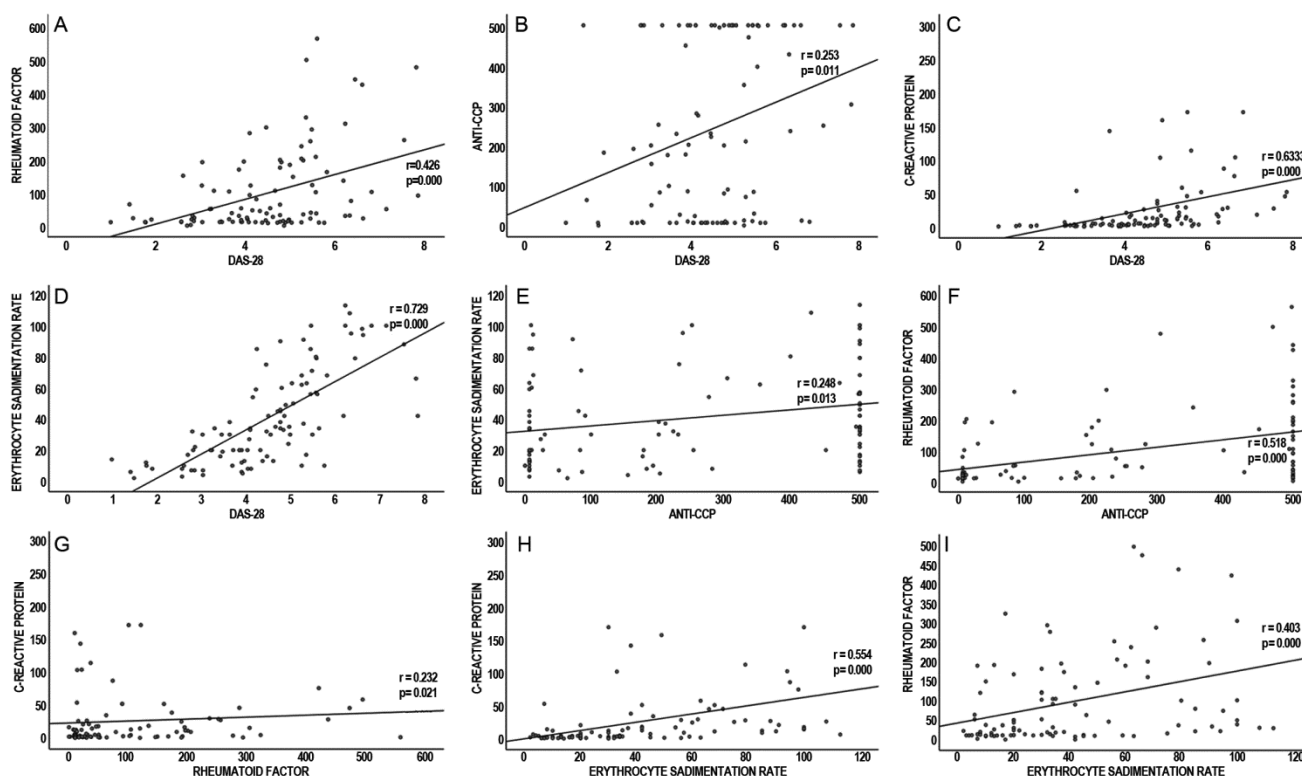


Fig. 1 — Correlation between different immunological markers

correlation between immunological markers and DAS-28 is shown in Fig. 1 (A-I).

Based on the serological analysis, patients were divided into four categories: RF positive (RF+), Anti-CCP positive (Anti-CCP+), RF negative (RF-), and Anti-CCP negative (Anti-CCP-). Out of total, 58% patients were double-positive (RF+ Anti-CCP+) and 17% of patients were double negative (RF- Anti-CCP-) also known as seronegative.

RF+ patients were observed to have a positive correlation with DAS-28 ($r=0.361$, $p=0.002$) and

Anti-CCP ($r=0.367$, $p=0.002$). No correlation was found between RF- and any of the autoantibody, inflammatory marker, or DAS-28. Anti-CCP+ patient shows moderate positive correlation with DAS-28, RF ($r=0.292$, $p=0.015$) and ESR ($r=0.299$, $p=0.013$). The difference in DAS-28 between RF+ and RF- patients was highly significant ($p=0.002$) as compared to the difference between Anti-CCP + and Anti-CCP- patients. Further, the age of onset of the disease was significantly early in the autoantibody-positive patient when compared to the antibody-negative patient as shown in Table 2.

Table 2 — Stratification of the patients as per their immunological status

Parameter	RF+	RF-	p-value	Anti-CCP+	Anti-CCP-	p-value
Total Number (%)	71	29		69	31	
Male (%)	11	5	0.752	11	5	0.981
Female (%)	61	23		58	26	
Age of Onset (Mean ± S.D.)	38.77±10.9	43.57±10.7	0.052 [#]	38.2±10	44.14±10.37	<0.001 [#]
DAS-28 (Mean ± S.D.)	4.73±1.4	3.77±1.2	0.002 [#]	4.61±3.4	3.86±1.52	0.109 [#]

Parameter	Anti-CCP+		p-value	Anti-CCP-		p-value
	RF+	RF-		RF+	RF-	
Total Number (%)	58	11	-	14	17	-
RF Median (range)	104.5 (15.82-560)	10.8 0-13.6	0.010*	29.9 (0-13.6)	10.2 (0-14)	0.438*
Anti-CCP Median value (range)	499.1 (30-500)	154 (23.97-500)	0.013*	7.33 (7-12.82)	7.0 (0.5-10.36)	0.10*

[*Mann-Whitney U test. [#] Independent T-test. p-values <0.01 are considered as significant. RF+/RF- indicates Rheumatoid factor positive and negative populations. Anti-CCP+/- indicates Anti-CCP positive and negative population]

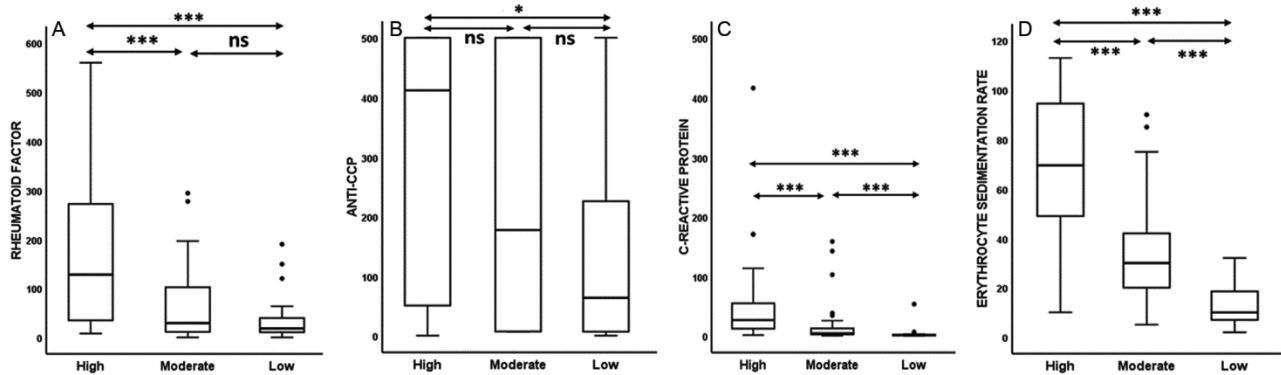


Fig. 2 — Change in disease markers levels in different disease activity groups. [Level of significance: *($P < 0.05$), **($P < 0.001$) and ***($P < 0.001$) and 'ns', non-significant]

Further, depending up on the disease activity score-28 (DAS-28) at the presentation to the rheumatology clinic, the patients were classified into High (DAS >5.1), Moderate $3.2 \leq \text{DAS} \leq 5.1$, and Low-disease activity (DAS ≤ 3.2) groups. There were 32%, 49%, and 19% patients in each group, respectively. It was found that changes in immunological markers and RF among various disease activity groups were significant. But no difference in Anti-CCP expression of auto-antibodies and inflammatory markers among different Disease Activity groups is shown in Fig. 2.

Discussion

Biomarkers serve as measuring tools to study disease status. In the case of RA, RF, Anti-CCP, ESR and CRP have been proven as important indicators. The present study was conducted to evaluate an association of immunological markers (Anti-CCP and RF) with inflammatory markers (CRP and ESR) in Indian RA patients with high, moderate, and low disease activity.

Correlation analysis of disease markers showed that all immunological markers and inflammatory

markers were in correlation with each other. DAS-28 was in positive correlation with RF, ESR, CRP and VAS scores, but less affected by Anti-CCP. A similar finding was reported by Sulemainet *al.*⁹ among the Malaysian population and Onderet *al.*¹⁰ among the Turkish population. In their study, they mentioned that Anti-CCP is not associated with ESR and CRP. While Serdaroglu *et al.*¹¹ have shown no correlation between Anti-CCP with inflammatory markers. Some studies have reported the presence of high levels of Anti-CCP in the serum well before the appearance of symptoms¹². Various literature also Anti-CCP's association with severe and early deformities¹³. Aletaha & Blüml¹⁴ discussed the association of Anti-CCP with early diagnosis and structural progression. Results of the presented study indicate that screening of ESR, CRP and RF is an important tool to observe disease progression whereas screening of Anti-CCP in addition to other markers can make disease diagnostics easy at early stages. Our above observations are supported by Brashi *et al.*¹⁵.

In the presented study, the majority of patients belong to moderate or high disease, activity group.

There are significant changes in the levels of RF, ESR and among different disease activity groups. But no significant change was observed in the level of Anti-CCP. Recently, Abdelhafiz *et al.*¹⁶ have also observed no substantial changes in Anti-CCP within different disease activity groups. It was reported that anti-CCP antibodies tend to remain stable or decline slightly with treatment¹⁷.

Further, it was observed that patients with RF+ and positive Anti-CCP (RF+ Anti-CCP+) show a positive correlation with the disease activity and the age of disease onset. A study conducted on the Tunisian population concluded that patients with positive Anti-CCP and RF show early onset of disease followed by severe joint destruction¹⁸. According to Rocha *et al.*¹², Anti-CCP positive patients are known to have severe erosion and a slow remission rate. Some reports also show that joint destruction, cardiovascular comorbidities, and other extra-articular manifestations are prominent in the subset of patients positive for both rheumatoid factor and anti-CCP antibodies¹⁹. Abolghasemi *et al.*²⁰, reported the presence of autoantibodies (RF and Anti-CCP) in association with night pain, VAS, and high disease activity.

Specificity and sensitivity of any biological marker are important considerations for the diagnosis of a disease. In the present study, the sensitivity and specificity of RF and Anti-CCP in recruited population were calculated and the specificity of RF was found less than the reported values. Many groups had reported specificity and sensitivity of RF as 85% and 60-90%, respectively²¹, whereas specificity and sensitivity of Anti-CCP as 95-96% and 53-71%, respectively²².

It is worth noting that positive RF and Anti-CCP, along with other disease markers, can give a tool for early diagnosis and progression of disease²³. Moreover, we need to study and validate more marker pools for the seronegative RA patients, as according to Wahab *et al.*²⁴, seronegative patients show different genetic predispositions²⁴. Also, Kumar *et al.*²⁵, suggests the need for reevaluation of existence of Anti-CCP in RF negative populations.

Limitations of the study

The present study is a cross-sectional study where all data was collected at a single time point, we have not collected data for changes in patients' disease activity or if any patient has undergone sero-

conversion. As a result of which we are not able to calculate the disease of recruited patients was moving towards remission or if there was any flare up.

Conclusion

Above study has demonstrated that Inflammatory markers and autoantibodies are clinically associated with DAS-28. Both the auto-antibodies (RF & anti-CCP) have high specificity but lower sensitivity in the North Indian population. Positive autoantibodies with other markers may indicate disease progression. However, seronegative patients need to undergo more careful screening. There is an unmet need to evaluate new markers for disease progression.

Ethical Approval

Protocols were approved by the Institutional Ethics Committee, ISIC, with approval number ISIC\RP\2015\045.

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Conflict of Interest

Authors declare no competing interests.

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