



## Discriminant Analysis of Rheumatoid Factor, Anti-Cyclic Citrullinated Peptide, and C-Reactive Protein for Rheumatoid Arthritis Patients

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## ABSTRACT

**Background:** Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality with uncertain aetiology by significant morbidity and mortality. Prevalence of approximately 0.75% in India. About 40% diseased become work disabled within 5 years from onset of symptoms.

**Objectives:** The study was conducted to determine the most parsimonious way to distinguish between groups and to test the theory by which cases are classified as predicted.

**Method:** The study is based on 290 clinically suspected RA patients. Cross-sectional study design was used. Clinically suspected cases were referred by different OPD's of Sir Sunderlal Hospital for screening.

**Result:** RF, AntiCCP and CRP diagnosed RA 21.3%, 21.7% and 33.1% respectively as positive . 277/290 (92.1%) of respondents were classified correctly into 'RA' or 'non-RA' groups. Non-RA were classified with better accuracy (98.8%) than RA (58.3%).

**Interpretation & Conclusion:** Early diagnosis of these high risk suspects will help in preventing from foremost disability. The quality of life may be approved of infected subjects by providing timely diagnosis and treatments and balanced nutritional and social supports.

**Keywords:** Rheumatoid arthritis, discriminant analysis, Cross-sectional, classification,

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality<sup>1-5</sup> with uncertain aetiology by significant morbidity and mortality. The diagnosis of RA, particularly in the early course of disease is empirical and imprecise. RA treatment may be efficient if the treatment starts early (window of opportunity). At the same time, an early and accurate diagnosis may protect other types of patients who do not have RA, from aggressive therapies with potential toxicity.<sup>6</sup>

The diagnosis of rheumatoid arthritis (RA) is primarily based on clinical manifestations and serologic tests<sup>7</sup>. Serological studies form a underpinning of laboratory based patient assessment in rheumatology. The presence of "rheumatoid factor" (RF) was identified in patients with RA over 50 years ago<sup>8</sup> Although RF remains one of the American College of Rheumatology (ACR) classification criteria for RA, its value as diagnostic tool is suboptimal, due to its lack of specificity.<sup>9,10</sup>

In 1998, Schellekens et al. showed that citrullin is an essential constituent of antigenic determinant, recognized by the above-mentioned RA specific auto-antibodies<sup>11</sup>. This discovery led to the development

of anti-cyclic citrullinated peptide antibody (anti-CCP), that were discovered in 1964,<sup>12</sup> (ELISA) test to measure auto-antibodies recognizing citrullinated anti-gens as a diagnostic test for RA.<sup>13</sup>

Steuer et al. (2008) a prospective study to evaluate the role of anti-CCP antibodies for the diagnosis of RA in primary care, all patients with joints pain were tested for RF latex, particle agglutination assay (RAPA) and anti-CCP antibodies. This study suggested using RF latex as a screening test together with anti-CCP antibodies as an effective strategy for screening RA in primary care.<sup>14</sup>

Gupta et al. (2009) study conducted at AIIMS on usefulness of anti-CCP antibodies in rheumatic diseases in Indian patients, they found sensitivity of 85% and specificity of 90.19% with regard to the use of anti-CCP antibodies assay in patients with joints pain to correctly identify RA. Anti-CCP antibodies positive patients did not have more erosive disease. IgM-RF-positive patients had more erosions when compared to the IgM-RF-negative group.<sup>15</sup>

Accumulating evidence shows that anti-CCP antibodies are very useful in the diagnosis of RA<sup>16</sup>. They may be present in the very early disease course<sup>17</sup> and are also considered as a prognostic factor for articular destruction.<sup>18</sup>

Several studies have shown that anti-CCP antibodies are moderately sensitive but highly specific for the diagnosis of RA, and their specificity is higher than that of Rheumatoid factor (RF)<sup>20-22</sup>. the presence of autoantibodies, such as RF and anti-citrullinated protein antibody (ACPA) (tested as [anti-CCP]), which can precede the clinical manifestation of RA by many years.<sup>24-26</sup>

The acute-phase reactants are a class of serum proteins, mainly glycoproteins, whose concentration in the blood increases after various stimuli such as trauma or inflammation.<sup>25</sup> The magnitude of the acute-phase protein response is roughly proportional to the severity of the stimulus.<sup>28,29</sup>

Serial measurements of these proteins can therefore be used, like the erythrocyte sedimentation rate (ESR), which is largely a measure of fibrinogen,<sup>30</sup> to monitor the progress of an inflammatory disorder.

## OBJECTIVE

The primary goal this study is to find a dimension(s) on the basis of which disease groups differ and to create the classification functions for separating the disease severity groups. The objectives are to determine the most parsimonious way to distinguish between groups; to test the theory by

which cases are classified as predicted; and to determine the strength of association between group membership observed and the estimated number obtained through predictors?

## MATERIALS AND METHODS

The present study is based on cross-sectional study design. There were 290 clinically suspected cases of rheumatoid arthritis patients out of which 110(38%) male and 180(62%) female. These subjects were screened at UGC Advanced Immunodiagnostic Training and Research Centre, Department of Pathology, IMS, BHU, Varanasi, U.P. The cases were referred by different OPD's of Sir Sunderlal Hospital. Mostly screened subjects were from eastern Uttar Pradesh, western Bihar, Madhya Pradesh and Jharkhand. About 2-ml of blood samples were collected in plain vial from each patient and each sample were tested for diagnostic tests such as RF, CRP and AntiCCP by using RF-Latex, CRP Latex and ELISA method respectively by the laboratory person and counter signed by pathology experts. The patients for rheumatoid arthritis were diagnosed according to -'The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis'<sup>29</sup> Classification criteria for RA (score-based algorithm: add score of categories A-D; a score of  $\geq 6/10$  is needed for classification of a patient as having definite RA)

**Statistical Analysis:** Initially data have been entered in MS- Excel, then transferred to trial version of SPSS 16.0 and data were presented in cross tables as RF, AntiCCP and CRP versus ACR/EULAR 2010 criteria separately, After then a classification made based on the independent variables to assess how the independent variables separate the correct categories in the classification by discriminant analysis.

## RESULT

The findings are presented on 290 clinically suspected RA cases. The univariate analysis is presented in cross tables. Consider the gross study subject, found that 48 study subject were positive (16.6%) including male and female according to ACR 2010 criteria

Given below table 1 shows the 62 / 290 (21.4%) subjects found positive for RF. In which 37(59.7%) had RA.

This compared with 63(21.7%) out of 290 subjects found positive for anti-CCP, and out of these 63 subjects, 36(57.1%) had RA.

**Table 1: Distribution of positivity rates of various tests for Rheumatoid arthritis**

Variables	ACR/EULAR 2010 Criteria		Total
	RA (%)	Non-RA (%)	
<b>RF</b>			
Positive	37 (77.1)	25 (10.3)	62 (21.3)
Negative	11 (22.9)	217 (89.7)	228 (78.6)
<b>AntiCCP</b>			
Positive	36 (75.0)	27 (11.2)	63 (21.7)
Negative	12 (25.0)	215 (88.8)	227 (78.3)
<b>CRP</b>			
Positive	45 (93.8)	51 (21.1)	96 (33.1)
Negative	3 (6.2)	191 (78.9)	194 (66.9)

**Table No. 2 Criteria for Classification Accuracy Using the Discriminant Model**

ACR/EULAR 2010 Criteria	Predicted Group Membership		Total
	Non-RA	RA	
Non-RA	239(98.8)	3(1.2)	242(100.0)
RA	20(41.7)	28(58.3)	48(100.0)

**Table 3: Discriminant Coefficients Showing the Contributions of Three Variables**

Variable	Regression Coefficients
RF	1.500
AntiCCP	1.447
CRP	1.110
(Constant)	-5.140

**Table 4: Showing the Classification Function Coefficients**

Variables	ACR/EULAR 2010 Criteria	
	Non-RA	RA
RF	6.276	11.108
AntiCCP	6.140	10.802
CRP	6.419	9.994
(Constant)	-10.941	-32.589

**Table 5: Ranking of the variable in order of contribution in discrimination**

Tests	Function 1	Ranking
RF	0.560	2
AntiCCP	0.571	1
CRP	0.429	3

In the other hand, 96 (33.1%) out of 290 subjects found positive for CRP, and out of these 96 subjects, 45(46.9%) had RA.

In the present research we have divided subjects into two categories viz non-RA as '1' and RA as '2', the SPSS has grouped the data into two groups. The total numbers of 290 subjects, which represent 100% of the observations, have been grouped for the Discriminant Analysis.

Eigen values table provides information on each of the discriminate functions (equations) produced. The maximum number of discriminant functions

produced is the number of groups minus 1. We are only using two groups here, namely 'RA' and 'non-RA', so only one function is displayed.

Fraction of variance explained i.e., Eigen value (1.443). Bigger Eigen value develops a well-built function. The canonical relation is a correlation between the discriminant scores and the levels of these dependent variables. The higher the correlations value, the better the function that discriminates the subjects. One is considered as perfect. Here, we have the correlation of 0.769 is comparatively high.

To classify a prospective case into RA and non-RA group based on three measurements namely RF, AntiCCP and CRP discriminant function analysis was done.

'A discriminant analysis was conducted to predict whether patients had RA or not. The classification results (Table 2) shows that 267/290 (92.1%) of respondents were classified correctly into 'RA' or 'non-RA' groups. This overall predictive accuracy of the discriminant function is called the 'hit ratio'. Non-RA was classified with better accuracy (98.8%) than RA (58.3%).

It has also been noticed that out of the 242, 239 subjects have been correctly classified as non-RA. Out of the 48 RA subjects, 28 subjects have been correctly classified as RA whereas 20 subjects have been wrongly classified as non-RA subjects. The accuracy of the model may hence be considered adequate

The descriptive technique successively identifies the linear combination of attributes known as canonical discriminant functions (equations) which contribute maximally to group separation. The discriminant function coefficients b or standardized form beta both indicate the partial contribution of each variable to the discriminate function controlling for all other variables in the equation. They can be used to assess each IV's unique contribution to the discriminate function and therefore provide information on the relative importance of each variable.

Table 3 gives the canonical discriminant function(cdf) score can be determined using the function given  $cdf = -5.140 + 1.500 RF + 1.447 \text{ AntiCCP} + 1.110 \text{ CRP}$

Two sets (one for each dependent group) of unstandardized linear discriminant coefficients are calculated, which can be used to classify cases. These unstandardized coefficients (b) are used to create the discriminant function (equation).

In this case we have (Table 4):

$$F_{\text{non-RA}} = -10.941 + (6.276 \times RF) + (6.140 \times \text{AntiCCP}) + (6.419 \times \text{CRP})$$

$$F_{RA} = -32.589 + (11.108 \times RF) + (10.802 \times \text{AntiCCP}) + (9.994 \times \text{CRP})$$

For predicting the group membership of new case, the value of both the function should be calculated. If say,  $F_{\text{non-RA}} > F_{RA}$  then cases will be classified in group 1 (non- RA). If whereas  $F_{RA} > F_{\text{non-RA}}$  then cases will be classified in group 2 (RA)

Table 5 shows that AntiCCP score was the strongest predictor while slightly low RF was next in importance as a predictor. These two variables with large coefficients stand out as those that strongly predict allocation to the RA or non-RA group. CRP attitude score were less successful as predictors.

## DISCUSSION

A discriminant analysis was performed in two other studies. Feigenbaum *et al.* studied 50 patients during an average of 5 years and divided the patients into three outcome groups.<sup>31</sup> And Young *et al.* classified 76% of 149 patients correctly with four variables in a category with or without erosions after 3 years of follow-up (latex titre, RAH A titre, haemoglobin, platelet count).<sup>32</sup> Many researchers studied correlation of possible predictive factors with outcome, but with a wide variety of conclusions.<sup>33</sup>

The conflicting results are probably due to the heterogeneous study designs. In many studies outcome was not well denned, patients with longstanding RA were included, or results were based on cross-sectional or retrospective studies.<sup>34</sup> But in the present study take in experience with the serological tests anti-CCP, RF, CRP in the early detection of rheumatoid arthritis patients.

Robert B. Burns and Richard A. Burns explain (2009) in the chapter extension on advanced techniques of book discriminant analysis characterize classification table, as a confusion table, is simply a table in which the rows are the observed categories of the dependent and the columns are the predicted categories. When prediction is perfect all cases will lie on the diagonal. The percentage of cases on the diagonal is the percentage of correct classifications. The number of observations given in this column indicates how many have been correctly and incorrectly classified. The original gives the frequencies along with their percentages of the groups in the data. The cross validated set of data is a more honest presentation of the power of the discriminant function than that provided by the original classifications and often produces a poorer outcome. The cross validation is often termed a 'jack-knife' classification, in that it successively classifies all cases but one to develop a discriminant function and then categorizes the case that

was left out. This process is repeated with each case left out in turn. This cross validation produces a more reliable function. The argument behind it is that one should not use the case we are trying to predict as part of the categorization process.<sup>35</sup>

Correlated study defines the interpretation of the standardized discriminant function coefficients in the table serve the same purpose as beta weights in multiple regression (partial coefficient) : they indicate the relative importance of the independent variables in predicting the dependent. They allow to compare variables measured on different scales. Coefficients with large absolute values correspond to variables with greater discriminating ability. Table provides an index of the importance of each predictor like the standardized regression coefficients (beta's) did in multiple regression. The sign indicates the direction of the relationship.<sup>36</sup>

## CONCLUSION

Based on the finding of the study presented under observation and the ensuing discussions, these conclusion can be arrives that detection of AntiCCP is thoroughly practicable for the diagnosis of RA, in fact even RF likewise quite valuable for diagnosis of RA and combination of testing for both RF and AntiCCP may be even more useful in comparison to individual test. AntiCCP score was the strongest predictor while slightly low RF was next in importance as a predictor.

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