Role of anti cyclic citrullinated peptide antibodies in erosive disease in patients with rheumatoid arthritis

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Background & objectives: Antibodies to cyclic citrullinated peptide (CCP) are a recently described marker in rheumatoid arthritis (RA), which are said to connote aggressive disease. No data on these antibodies are available from India. We undertook this study to evaluate the role of second generation anti CCP antibodies (anti CCP-2) in predicting erosive disease in Indian patients with rheumatoid arthritis and to define their role in seronegative RA.

Methods: A total of 211 patients with established RA were evaluated in this cross-sectional study for radiographic erosions. A high percentage of seronegative RA patients (40%) were included to assess the role of anti CCP-2 antibodies in this subgroup. Radiographic damage was quantified using modified Sharp score. Apart from anti CCP-2 antibodies, other factors evaluated for their ability to predict erosions included rheumatoid factor (RF) positivity, disease duration, and disease modifying anti rheumatic drugs (DMARD) naïve period.

Results: Anti CCP-2 antibodies were seen in 80 per cent patients with RA. Predictors of erosive disease included anti CCP-2 antibody positivity and DMARD naïve period. Patients positive for both RF and anti CCP-2 antibodies had a higher prevalence of erosions as compared to patients positive for only one antibody or negative for both. In seronegative RA (RF absent), anti CCP-2 antibodies were seen in over 50 per cent patients and were associated with a higher incidence of erosive disease.

Interpretation & conclusion: Our finding showed that anti CCP-2 antibodies were present in 80 per cent patients with established RA. These have an independent role in predicting erosive disease, especially in the seronegative subgroup.

Key words Anti cyclic citrullinated peptide antibodies - anti CCP-2 - erosions - rheumatoid arthritis - seronegative RA

Rheumatoid arthritis (RA) is the commonest inflammatory joint disease, affecting nearly 0.75 per cent of adult Indian population. It is characterized by multiple deformities and is associated with considerable morbidity and mortality. The progressive joint damage characteristic of the disease is reflected in radiographs by bony erosions and as joint space
narrowing\(^2,3\). Disease severity in RA may range from mild to severe which has a bearing on the intensity of the treatment offered to the patient. Several factors predict disease severity in RA including female sex, early development of erosions, genotype, acute-phase reactants, extra-articular manifestations and duration of disease\(^4,5\). Of these, erosions on radiographs of hands and feet are widely utilized in clinical practice. However, there is a need to identify markers that predict aggressive RA even before the appearance of erosions. Rheumatoid factor (RF), the time honoured serologic marker has been around for more than 50 yr. RF has a sensitivity of 70-80 per cent in diagnosis of RA but the specificity is poor as it is found in several other conditions like infections, 5-10 per cent of healthy elderly individuals, sarcoidosis, systemic lupus erythematosus, scleroderma, etc. RF positivity is an independent predictor of erosive disease as shown in cohorts followed up for 5-12 yr\(^5,6\).

Citrullinated peptides (filaggrin, keratin, vimentin, etc.) are considered to be potential autoantigens driving the immune response in RA. Autoantibodies against citrullinated antigens have been shown to be highly specific for RA\(^7\). Anti-cyclic citrullinated peptide (anti CCP) antibodies, first described in 1998, were shown to be highly specific (95%) in the diagnosis of RA and slightly less sensitive than IgM RF (60-70%)\(^8\). The second generation of anti CCP antibodies (anti CCP-2) increased the sensitivity to 80 per cent, while retaining the specificity (98\%)\(^9\). Studies that have followed up cohorts of early RA patients for up to 6 yr have shown that anti CCP antibodies are an independent factor in predicting development of erosions\(^9,14\). This suggests that these antibodies have a prognostic value as a marker of erosive disease. No published data are available from India on this aspect.

Seronegative RA is a diagnosis made only on clinical grounds, which at times can be very difficult. The availability of a serologic marker like CCP-2 antibodies in this setting can be helpful to the clinician.

We undertook this prospective study to assess the role of anti CCP-2 antibodies as a predictor of erosive disease in Indian patients with RA and to define its role in the subgroup of patients with seronegative RA.

**Material & Methods**

This cross-sectional study was conducted at Rheumatology outpatient services of a large tertiary care hospital in North India (All India Institute of Medical Sciences, New Delhi) over a period of 10 months (September 2004 to June 2005). Adult patients of RA satisfying the American College of Rheumatology criteria\(^15\) with a disease duration of at least 2 yr were included in the study. This is because 60-90 per cent patients with RA develop erosions within this period\(^2,16,17\). Patients having an overlap of RA with other rheumatic diseases like systemic lupus erythematosus, systemic sclerosis, etc., were excluded from the study.

Stratified sampling was done to put together a group of 60 per cent seropositive (positive for RF) and 40 per cent seronegative (RF absent) patients (henceforth termed ‘seropositive’ and ‘seronegative’ respectively). Ordinarily, seronegative patients comprise 20-25 per cent of all patients with RA\(^18\). For our study, we deemed it desirable to have a large subgroup of seronegative patients so that the effect of anti CCP antibody on erosive disease could be studied independent of RF positivity. The primary outcome measured was for the presence of erosions on plain radiographs of hands.
**Radiological assessment:** Radiographs of both hands (posteroanterior view) were scored for joint damage using modified Sharp score wherein 17 joints of hands were assessed for erosions (range 0-5 per joint, maximum score 170) and 18 joints for joint space narrowing (range 0-4 per joint, maximum score 144)\textsuperscript{19,20}. The cumulative score is obtained by the addition of these 2 components (maximum score 314). A set of 20 radiographs were scored by two observers (SS and RG) till the concordance achieved was >90 per cent. They were scored again a fortnight later (by SS) to determine intra-observer variation (0.95). A single observer (SS) scored all the X-rays thereafter. The average time to score one pair of hand radiographs was about 10 min.

**Serology:** Serum anti CCP-2 (second generation) antibodies was tested using a commercial enzyme linked immunosorbent assay (ELISA) kit from Euroimmun, Germany. A value of >5 RU (relative units) was considered positive as per the manufacturers’ recommendations. IgM RF antibody test was done using the standard latex agglutination technique\textsuperscript{21}.

**Statistical analysis:** The primary outcome variables were the presence of erosions and modified Sharp score. The explanatory variables included RF positivity, CCP positivity and titres, duration of disease and period prior to institution of disease modifying anti rheumatic drugs (DMARDs) (DMARD naïve period).

To assess the effect of each of the two autoantibodies (RF and anti CCP-2) in contributing to erosions, patients were divided into 4 groups based on their RF and CCP positivity status. These were RF+ CCP+, RF+ CCP-, RF- CCP+ and RF- CCP- groups. The percentage of patients with erosive disease and the median modified Sharp scores were compared between the 4 groups. Kappa statistic was applied to describe intra-observer variation in scoring of radiographs. Association between categorical variables was done using Chi square test. Non parametric tests (Mann Whitney, Kruskal Wallis) were performed to analyse data that were not normally distributed. Bivariate analysis of each factor was done with the presence of erosions. Regression analysis was carried out to assess the value of anti CCP-2 antibodies as an independent factor in predicting erosive disease, after adjusting for other variables. Unadjusted and adjusted odds ratio were calculated. Significance was defined at an \( \alpha \) of 0.05 and all tests were two tailed. All statistical tests were analysed using SPSS 9.0 software.

**Results**

A total of 211 patients with RA were included in the study. The mean (± SD) age of patients was 41.6 (±11.7) yr and 88 per cent (n=187) were women. The median (IQR, Interquartile range) disease duration was 5 (3-8) yr and the median (IQR) DMARD naïve period was 3 (1-5) yr. Nearly 60 per cent (n=125) patients were seropositive (RF positive). Of the 211 patients, 167 (80%) tested positive for anti CCP-2 antibodies. Erosive disease was seen in 68 per cent (n=144) patients.

On bivariate analysis, patients with erosive disease had a significantly higher disease duration, DMARD naïve period, RF positivity, anti CCP-2 antibody positivity and higher anti CCP-2 titres than those with non erosive disease (Table I). In multiple logistic regression analysis only anti CCP-2 antibody positivity (adjusted odds ratio 3.13, 95% CI 1.3-7.57, \( P = 0.01 \)) and DMARD naïve period (adjusted OR-1.24, 95% CI 1.04-1.47, \( P = 0.01 \)) were found to be significantly associated with erosive disease.
One hundred and twenty two patients (58%) were positive for both antibodies (RF+, CCP+), 48 (23%) were positive only for one antibody (RF+ or CCP+) and 41 (19%) were negative for both antibodies (RF-, CCP-). In the 48 patients positive for only one antibody, 45 were RF- CCP+. Only 3 patients (1%) were RF positive while being negative for anti CCP-2 antibodies (RF+, CCP-). The prevalence of erosive disease was significantly higher (P<0.001) amongst patients positive for both autoantibodies as compared to those positive for one autoantibody and those negative for both autoantibodies (Fig.). The median modified Sharp score was also significantly different amongst the three groups (P<0.001) with the score being highest among patients positive for both antibodies and the least for those negative for both (Fig.).

Anti CCP-2 antibodies were present in 53 per cent (45/86) patients with seronegative RA while erosions were seen in 57 per cent (49) of these patients. When erosions were correlated with the presence or absence of anti CCP-2 antibodies, it was seen that 31 of 45 (68%) patients with anti CCP-2 positivity exhibited erosions compared to only 18 of 41 (44%) patients negative for CCP-2. The difference was statistically significant (P<0.05).

The sensitivity and specificity of anti CCP-2 antibodies to predict erosive disease was evaluated in both seropositive and seronegative RA patients (Table II). Anti CCP-2 antibodies were found to have about 99 per cent sensitivity in predicting erosive disease amongst seropositive patients with a high positive predictive value (PPV) of 97 per cent. Amongst seronegative RA patients, the sensitivity and PPV was >60 per cent in predicting erosive disease. Additionally, they had a good negative predictive value (NPV) of 60 per cent in this subgroup.

### Table I. Characteristics of patients with erosive and non erosive disease

<table>
<thead>
<tr>
<th>Characteristics*</th>
<th>Erosive disease (n=144)</th>
<th>Non erosive disease (n=67)</th>
<th>Tests used</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (yr) Median (IQR)</td>
<td>5 (3-9)</td>
<td>4 (3-5)</td>
<td>Mann Whitney U</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DMARD naïve period (yr) Median (IQR)</td>
<td>3(1.77-6)</td>
<td>2 (1-3)</td>
<td>Mann Whitney U</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RF positivity No. (%)</td>
<td>95 (66)</td>
<td>29 (43)</td>
<td>Chi square</td>
<td>0.002</td>
</tr>
<tr>
<td>CCP positivity No. (%)</td>
<td>125 (87)</td>
<td>42 (62)</td>
<td>Chi square</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Median CCP titre (RU/l) Median (IQR)</td>
<td>104 (21.7-145)</td>
<td>28.5 (2.37-117.2)</td>
<td>Mann Whitney U</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Values are expressed in number and per cent for categorical variables and median (Inter quartile range) for the continuous variables. DMARD, disease modifying anti rheumatic drugs; IQR, interquartile range; CCP, cyclic citrullinated peptide.
Anti-CCP-2 antibodies have been demonstrated to predict aggressive RA in several cohorts of patients with early arthritis\textsuperscript{22}. They can, thus, be added to several other already well defined prognostic factors for disease damage/functional outcome in RA like female sex, genotype, acute-phase reactants, and RF. However, the contribution of anti-CCP-2 antibodies towards predicting cumulative damage in RA over and above that predicted by RF has not been well documented. The present study assessed the role of anti-CCP-2 antibodies in predicting the radiological damage over and above RF in established RA (disease duration exceeding 2 yr). In addition, our study group comprised a large percentage of seronegative RA (40%) to enable us to evaluate the role of anti CCP-2 antibodies in this subgroup.

Our findings revealed that anti CCP-2 antibodies were the most important factor in predicting erosive disease. Patients positive for both RF and CCP-2 antibodies not only had a higher prevalence of erosive disease than those negative for both antibodies, but also had a higher median radiological damage score. Patients with one of two antibodies positive had intermediate scores. This suggests that both antibodies contribute towards erosive disease and possibly have synergistic effects. Anti CCP-2 antibodies were seen in almost all patients who were seropositive. In fact, of the seropositive patients, only 1.5 per cent were negative for anti CCP-2 antibodies. However, in the seronegative subgroup, more than half the patients tested positive for anti CCP-2 antibodies. Additionally, those positive for these antibodies had a significantly higher prevalence of erosive disease than those who tested negative. This suggests that anti CCP-2 antibodies are more strongly associated with erosions than RF and it is the seronegative subset where they may have the maximum utility.

Anti CCP-2 antibodies were found to have a very high sensitivity and PPV in predicting erosions in seropositive RA. Additionally, they had a good NPV in the seronegative subgroup, suggesting that if a patient is negative for both RF and anti CCP-2, the chances of developing erosive disease are significantly lesser.

Patients with erosive disease had a significantly higher anti CCP titre than those with non erosive disease. High titres of anti-CCP antibodies at baseline have been shown to be related to greater radiological progression at 2 yr in early arthritis cohorts\textsuperscript{23}. However, the antibody titres did not show a significant correlation with the modified Sharp score as our patient were heterogenous group with variable disease duration.

### Discussion

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
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<tbody>
<tr>
<td>Seropositive RA (RF+)</td>
<td>98.9</td>
<td>6.6</td>
<td>97</td>
<td>16</td>
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<tr>
<td>(n=125)</td>
<td></td>
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<tr>
<td>Seronegative RA (RF-)</td>
<td>63.2</td>
<td>62.1</td>
<td>65</td>
<td>60</td>
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<tr>
<td>(n=86)</td>
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Values are in percentage; RA, rheumatoid arthritis; RF, rheumatoid factor
and DMARD naïve periods, both of which also influence radiological damage scores in addition to anti CCP-2 titres.

Our study also showed the influence of DMARD naïve period on erosions, between diagnosis of RA and institution of DMARDs. In most western countries, all patients with RA are started on DMARD as soon as the diagnosis is established. The median DMARD naïve period was as long as 3 yr in our patients. The period was significantly higher amongst those with erosive disease than those without. In regression analysis, this was the second most important factor after presence of anti CCP-2 antibodies in predicting erosive RA. The long duration without taking DMARDs in India can be attributed to lack of awareness amongst the physicians of the beneficial effects of early initiation of DMARDs and unfamiliarity with their use.

Our study has a few limitations. A longitudinal study of an inception cohort of patients followed up for several years would have been ideal but is very challenging in terms of logistics. Also, joint damage is a function of disease specific characteristics as well as the therapy that is given. To truly elucidate the role of any one serological factor, ideally patients should be receiving a standardized therapy and be followed up over time. This is not possible most of the times as the trends of treatment change with advancement of knowledge. As such any cross-sectional study suffers from the shortcomings induced by the different interventions used. Also, our patient population comprised patients with established RA drawn from a clinic in north India and as such our results may not be applicable to early RA or to the entire Indian population.

In conclusion, our findings showed the presence of anti CCP-2 antibodies in 80 per cent patients with...
established RA. These antibodies were strong predictors of erosive disease in both seropositive and seronegative RA and had an additional role in predicting cumulative radiological damage over and above that predicted by rheumatoid factor. Their absence had a strong negative predictive value for erosive disease. The ability of this new serologic marker to predict erosive disease in the seronegative RA suggests that anti CCP-2 antibodies may have the maximum clinical utility in this subgroup of patients.

References


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