Concise report

Anti-cyclic citrullinated peptide antibodies and rheumatoid factor in rheumatoid arthritis patients and relatives from Brazil

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Abstract

Objectives. To evaluate the prevalence of anti-cyclic citrullinated peptide (anti-CCP) antibodies and RF in RA patients and their relatives from Southern Brazil.

Methods. Anti-CCP2 and IgM-RF were evaluated in 156 RA patients and 200 relatives. Sera from 100 healthy unrelated individuals were used as control. The anti-CCP2 was detected by ELISA and the IgM-RF using the latex agglutination test.

Results. We identified 117 anti-CCP2 (75%)-positive and 106 RF (67.9%)-positive patients. Anti-CCP2 was increased in relatives (5.5%; 11/200) when compared with unrelated individuals (1%; P = 0.050). Titre of anti-CCP2 in RA patients did not differ from relatives [140.4 (75.7) vs 115.6 (84.2) U, respectively; P = 0.30]. Positive relatives were younger than patients for anti-CCP2 (P = 0.0081), RF (P < 0.001) and both concomitantly (P = 0.012), and although there was no difference for anti-CCP2 positivity according to gender, increased RF positivity and concomitant anti-CCP2/RF were observed in the female relatives (P = 0.067 and 0.082, respectively). No difference regarding the relative degree of tobacco use in relatives was detected. Among the 11 anti-CCP2-positive relatives, 2 females had RA diagnosis established and 6 individuals presented with joint symptoms suggestive of RA.

Conclusion. The results demonstrate a significant positivity of anti-CCP2 in relatives of RA patients from Brazil and reinforce the importance of serological tools to identify undiagnosed RA.

Key words: Rheumatoid arthritis, Anti-cyclic citrullinated peptide antibody, Family.

Introduction

RA is a common autoimmune disease (AID), affecting ~1% of the world’s population [1]. Since precocious treatment improves its overall outcome [2], an early diagnosis as well as screening studies in relatives of RA patients are of importance [3].

The assessment of autoantibodies before clinical onset of different AIDs, has been helpful in establishing an early diagnosis [4, 5]. Recent studies on RA described several immune events that precede joint inflammation, which include the pathogenic role of anti-cyclic citrullinated peptide (anti-CCP) antibodies [6]. Data from preclinical serum samples of RA patients demonstrated that anti-CCP and RF are present months to years before the disease onset [7]. Among the different autoantibodies described in RA patients, anti-CCP displays the strongest specificity for RA (95–98%), with 70–80% sensitivity [6, 8]. The concomitant evaluation of RF and anti-CCP represents the most powerful prognostic marker for RA [7, 9, 10].

Since genetic factors contribute to the development of RA, relatives of RA patients are suggested to be at higher risk of developing the disease [3, 11, 12]. Familial RA has been reported as a key risk factor for severe joint damage in various populations. This effect is dependent on additive and non-additive genetic factors, as well as on environmental conditions that are characteristic of each region [3]. So far, there have been no reports on the
influence of familial RA in the Brazilian population. The present study aims to investigate the presence of anti-CCP antibodies and RF in RA patients and their relatives from southern Brazil, and to associate the findings with demographic and clinical features, as well as to establish early RA diagnosis.

**Patients and methods**

**Subjects and healthy controls**

This study was approved by the local Ethics Research Committee from the Evangelical Beneficent Society, Curitiba, Paraná, Brazil and informed consent was obtained from all the subjects. One-hundred and fifty-six adult RA patients meeting the ACR criteria [13] (87.2% female, 12.8% male; mean age 51.3 years; range 24–84 years) and 200 relatives of RA patients (61% female, 39% male; mean age 36.7 years; range 7–91 years) from the Rheumatologic Unit of the Evangelical Hospital of Curitiba, Paraná, Brazil, were consecutively included in the study in the period from August 2007 to April 2009. These relatives belong to 78 families (2.56 relatives per family). Table 1 summarizes the main demographic features of the RA patients and their relatives. All patients included in this study were >18 years old; the mean age of disease onset was 42.4 years and the mean disease duration was 8.9 years.

The RA patient’s relatives were submitted to a standard questionnaire applied by a physician, inquiring about tobacco smoking, articular symptoms, anaemia, other AIDs, as well as other significant previous and present medical conditions and treatments. One-hundred and eighty-eight (94%) were first-degree relatives and 12 (6%) were second-degree relatives (Table 1).

Sera from 100 healthy individuals from the same geographical area (82.0% female, 18.0% male; mean age 47.6 years; range 23–81 years) were used as a control group. From each patient and the participating family members 5 ml of venous blood was collected, the serum aliquots were stored at −80°C until the assays had been performed.

**Detection of anti-CCP2 antibodies and IgM-RF**

Serum levels of anti-CCP2 were measured using the QUANTA Lite CCP2 IgG ELISA kit (INOVA Diagnostics, San Diego, CA, USA). The cut-off point was set to 20 U/ml. IgM-RF was measured by the latex agglutination test (BioSystems S.A., Barcelona, Spain) in which a particulate latex suspension coated with human gamma-globulin agglutinates in the presence of IgM-RF in the patient serum. RF values <30 IU/ml were considered negative. The cut-off values for both anti-CCP2 and IgM-RF followed the respective manufacturer’s instructions.

**Statistical analysis**

Variables were analysed using Fisher’s exact, chi-square, Mann–Whitney and Kruskal–Wallis tests, as adequate. Spearman’s test was used for correlation study. Statistical significance was accepted when \( P < 0.05 \). The statistical analysis was undertaken using the Statistica 5.1 software package (StatSoft Inc., Tulsa, USA).

**Results**

**Anti-CCP and RF**

Figure 1 displays the positivity of anti-CCP2 and IgM-RF antibodies in the studied groups. Among the 156 RA patients, 75% (117/156) were anti-CCP2 positive and 67.9% (106/156) were RF positive, both presenting significant difference in relation to the control group \( P < 0.0001 \) as well as to the relatives \( P < 0.0001 \). The frequency of anti-CCP2 was significantly increased in relatives of RA patients (80.3% versus 75% in patients). The frequency of RF was also increased in relatives of RA patients (75% versus 67.9% in patients).

**Table 1**

Demographic data of RA patients, relatives and control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients, ( n = 156 )</th>
<th>Relatives, ( n = 200 )</th>
<th>First-degree relatives, ( n = 188 )</th>
<th>Second-degree relatives, ( n = 12 )</th>
<th>Control group, ( n = 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), years</td>
<td>51.3 (24–84)</td>
<td>36.7 (7–91)</td>
<td>37.3 (7–91)</td>
<td>27.8 (8–63)</td>
<td>47.6 (23–81)</td>
</tr>
<tr>
<td>Female, ( n/N ) (%)</td>
<td>136/156 (87.2)</td>
<td>122/200 (61)</td>
<td>113/188 (60.1)</td>
<td>8/12 (66.7)</td>
<td>82/100 (82)</td>
</tr>
<tr>
<td>Male, ( n/N ) (%)</td>
<td>20/156 (12.8)</td>
<td>78/200 (39)</td>
<td>75/188 (39.9)</td>
<td>4/12 (33.3)</td>
<td>18/100 (18)</td>
</tr>
</tbody>
</table>
patients (5.5%; 11/200) when compared with healthy individuals (1%; 1/100; \( P = 0.050 \)), whereas no significant difference was observed for RF (8%; 16/200 vs 6%; 6/100; \( P = 0.69 \), respectively).

Among the 156 RA patients, 60.9% (95/156) presented concomitant positivity to anti-CCP2 and RF, 7% (11/156) presented positivity only to RF and 14.1% (22/156) were only anti-CCP2 positive. A significant correlation between anti-CCP2 levels and RF titres was observed (\( r = 0.43; P < 0.0001; \) Spearman’s test).

The distribution of the detected levels of anti-CCP2 antibodies in all the individuals showed that in RA patients, positive anti-CCP2 ranged from 21 to 254 U [140.4 (75.7)] and in relatives it ranged from 20 to 234 U [115.6 (84.2); \( P = 0.30 \)].

Regarding the concentration of antibodies, 76.9% (90/117) of the anti-CCP2-positive patients presented strong reactions, 8.5% (10/117) moderate and 14.5% (17/117) weak positive reaction. Among the anti-CCP2-positive relatives, 54.5% (6/11), 9.1% (1/11) and 36.4% (4/11), respectively, presented strong, moderate and weak reactions. There was no significant difference comparing both groups.

Demographic characteristics of relatives and patients
The anti-CCP2- and IgM-RF-positive relatives had a mean age of 39.4 (15.9) years (16–65 years) and 39.7 (13.7) years (8–70 years), respectively. Among those relatives with positivity to anti-CCP2/RF the mean age was 39.7 (9.3) years (25–46 years). The mean age of RA patients with anti-CCP2 positivity was 52.6 (12.4) years, with RF positivity 51.5 (11.7) years (24–80 years) and with concomitant anti-CCP2/RF 52.1 (11.8) years (24–80 years). A significant decrease in the age of relatives in relation to patients was observed for positive anti-CCP2 (\( P = 0.0081 \)), RF (\( P < 0.001 \)) and for both concomitantly (\( P = 0.0125 \)).

In relation to gender, 74.3% (101/136) of female RA patients were anti-CCP2 positive, 66.2% (90/136) were RF positive and 58.8% (80/136) were positive to both anti-CCP2 and RF. In male individuals, 80% (16/20) presented positivity for either anti-CCP2 or RF and 75% (15/20) were concomitantly positive to CCP2/RF. A significant difference in the frequency of anti-CCP2, RF and anti-CCP2/RF was observed among women (\( P = 0.026 \)), but no differences were detected when comparing female vs male RA patients, in spite of the latter showing higher values.

The prevalence of anti-CCP2 was 7.4% (9/122) and of RF 10.6% (13/122) in the female relatives. Among the male relatives, the positivity to anti-CCP2 and RF was 2.6% (2/78) and 3.8% (3/78), respectively. While 4.1% (5/122) of the female relatives presented concomitant positivity to anti-CCP2/RF, no male relatives presented this feature. Although no difference for anti-CCP2 positivity according to gender was observed in the relatives group, an increased RF positivity and concomitant anti-CCP2/RF was found in females when compared with male relatives (\( P = 0.067 \) and 0.082, respectively).

Among the 188 first-degree relatives, 5.3% (10/188) were anti-CCP2 positive, 8% (15/188) were RF positive and 2.6% (5/188) presented both antibodies. These values did not differ from those of second-degree relatives (\( n = 12 \)). In addition, there was also no statistical difference in the frequency of autoantibodies between tobacco smoker (57/200) and non-tobacco smoker (143/200), relatives of RA patients (anti-CCP2 with \( P = 0.80 \); RF with \( P = 0.56 \)).

Clinical features of anti-CCP- and RF-positive relatives
It was found that 5.5% (11/200) were anti-CCP2 positive, with five of them presenting RF concomitantly. After clinical re-evaluation of these 11 relatives, RA diagnosis was established according to ACR criteria in 2 females, concomitantly positive to anti-CCP2/RF. At the moment, only 3/11 of the anti-CCP-positive relatives presented no joint symptoms suggestive of RA.

The relatives who were with only RF positive (11/200) presented titres ranging from 30 to 1280 IU/ml (median = 60 IU/ml), a mean age of 40.5 (15.6) years and 72.5% (8/11) were women. Eight of these relatives presented joint complaints but none of them had RA diagnosis confirmed.

Discussion
We demonstrated a significant positivity of anti-CCP2 in relatives of RA patients and diagnosed RA in two of them. Our study is pioneering in the clinical assessment of the association of both anti-CCP antibodies and RF in RA patients and their relatives in Southern Brazil.

We observed significant differences in anti-CCP2 positivity between patients, relatives and the control group. Similar results were recently found in a North American native population \[14, 15\]. Interestingly, the relatives of RA patients showed a significant elevation in anti-CCP2 positivity in relation to the control group that did not happen with RF.

The majority of anti-CCP2-positive patients and relatives presented strong titres and anti-CCP2-positive relatives had mean titres similar to the RA patients. The two anti-CCP2-positive relatives who presented the highest titres of the autoantibody had RA diagnosis.

Several studies have shown that female RA patients usually have a more severe course of disease \[16\]; however, these data have been recently questioned \[17\]. The relatives group showed higher seropositivity in women, with a difference tending to significance regarding RF positivity and concomitant CCP2/RF positivity. Two of these young females had RA diagnosis confirmed. Interestingly, the two anti-CCP2-positive males presented lower anti-CCP2 titres compared with women, were RF negative and had no joint complaints.

Although an association between smoking and the development of citrullinated antigens has been demonstrated \[18\], the relationship between tobacco exposure and anti-CCP positivity is still controversial \[19\]. In the present study, we observed no correlation between
anti-CCP2 positivity and tobacco exposure in the relatives evaluated.

At present, two anti-CCP2/RF-positive relatives had RA diagnosis confirmed according to the ACR criteria [13]. It has been shown that the recurrence risk estimates for RA relatives are 4% for siblings, 4.7% for parents or child, 1.9% for second-degree relatives and 1.07% for third-degree relatives and that the sex and age of disease onset of the proband directly affect the familial aggregation of RA [20]. Thus, RA diagnosis in the other relatives with positive anti-CCP2 should not be discarded since characteristics of risk such as the degree of parentage, female sex, high titres of autoantibodies and joint complaints are seen in most of them.

In conclusion, our results emphasize the value of investigating familial aggregation of AID in specific populations due to the great genetic and environmental diversity worldwide. The development of more severe disease related to the familial clustering of RA and the poor prognosis of untreated illness fully justifies efforts aiming at early diagnosis of this disabling disease.

Rheumatology key message

- Anti-CCP2 reveals undiagnosed RA in patients’ relatives from Southern Brazil.

Disclosure statement: The authors have declared no conflicts of interest.

References