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The role of the shared epitope in arthralgia with anti-cyclic citrullinated peptide antibodies (anti-CCP), and its effect on anti-CCP levels

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ABSTRACT

Objectives: Patients presenting with both arthralgia and antibodies to cyclic citrullinated peptide (anti-CCP) have an increased risk of developing rheumatoid arthritis (RA). To further characterise this patient group and shed more light on its relationship with clinically manifest early arthritis and established RA, an immunogenetic and serological analysis was performed.

Methods: In a group of 111 patients with anti-CCP-positive arthralgia, anti-CCP levels and shared epitope (SE) status were determined. Data were compared with 125 and 128 patients with anti-CCP-positive early arthritis and established RA respectively.

Results: In patients with anti-CCP-positive arthralgia, the frequency of SE allele positivity is significantly lower when compared with anti-CCP-positive early arthritis and established RA (58% vs 80%, and 58% vs 92%, respectively, both p<0.001). Median anti-CCP levels were higher in the group of patients with SE-positive arthralgia compared with the group of patients with SE-negative arthralgia (p = 0.02). Median anti-CCP levels were similar in the groups of patients with SE-positive arthralgia and arthritis.

Conclusions: The lower frequency of SE positivity in patients with arthralgia compared with patients with RA indicates that, compared with patients who were SE positive, patients who were SE negative as a group go through a longer arthralgia phase, or alternatively have a lower risk for transition from anti-CCP positive arthralgia to RA. Furthermore, the present results suggest that in this early stage the effect of the SE on disease risk may be mediated through higher anti-CCP levels.

Antibodies to cyclic citrullinated peptide (anti-CCP) often precede the development of rheumatoid arthritis (RA) and can therefore be used to detect those at risk for the development of RA.1–3 Another major risk factor for RA is the shared epitope (SE) at the HLA-DRB1 locus.4–6 The SE hypothesis postulates that highly conserved amino acid sequences bordering the peptide-binding groove of the HLA-DRB1 molecule are involved in the pathogenesis of RA, for example, by enabling the presentation of arthritogenic peptides to T cells.7 In support of the latter concept it has been shown that the DRB1*0401 peptide-binding groove allows for a high-affinity interaction with citrullinated peptides, resulting in efficient antigen presentation.8 In a study analysing preclinical blood samples of patients with RA, the highest risk for RA was associated with the presence of both anti-CCP and SE.7 Recently, it was suggested that the increased risk for RA in SE-positive undifferentiated arthritis is in fact not due to the SE, but to anti-CCP positivity.9 In the latter study, the presence of SE alleles was associated with significantly higher levels of anti-CCP antibodies, suggesting that the SE alleles act as classic immune response genes. Therefore, the effect of the SE on RA development may be mediated through anti-CCP levels.

Patients presenting with both arthralgia and anti-CCP probably confer a high risk for the development of arthritis; however, this patient group has not been extensively studied. We assessed the frequency of SE and levels of anti-CCP in these patients, and compared this with early and established RA.

PATIENTS AND METHODS

Study population

The frequency of SE positivity in anti-CCP antibody positive unrelated Dutch Caucasians aged 18 or older was measured in three cohorts: patients with arthralgia (possible preclinical RA); patients with early arthritis; and patients with RA with established disease starting anti-tumour necrosis factor treatment.

Between September 2004 and 2007, 190 patients with anti-CCP- or IgM-RF-positive arthralgia were recruited at rheumatology clinics in Amsterdam (the Netherlands). Patients with arthralgias who were referred by their general practitioner were seen by a rheumatologist at our outpatient rheumatology clinic. In the absence of arthritis, patients who were anti-CCP positive (n = 147) were referred for inclusion in the present study, which was a mean 2 months after the first visit to the outpatient clinic. At the first study visit, a trained medical doctor (WB) and a senior rheumatologist (DS) independently scored for the absence of arthritis in all joints at physical examination. The senior rheumatologist was blinded for the reported joint complaints and the anti-CCP status. If clear, absence of arthritis was seen by both doctors (n = 125), and anti-CCP positivity was confirmed in the baseline serum sample (at least 1 month after the initial positive sample; n = 115), patients were included. Patients previously or at presentation treated with a disease-modifying antirheumatic drug and patients in whom history and chart review revealed past arthritis were
Concise report

excluded (n = 4). In total, 111 patients with anti-CCP-positive arthralgia were eligible for analysis. (See supplementary table 1 for criteria for anti-CCP-positive arthralgia.)

The early arthritis group consisted of 125 patients, anti-CCP positive, who were randomly selected from the early arthritis clinic (EAC) of the Jan van Breemen Institute of whom SE status was available (n = 337/–1700). Random selection was performed by the Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, IL, USA). Inclusion and exclusion criteria for this cohort have been described previously. Ninety-seven patients fulfilled the American College of Rheumatology criteria for RA at baseline, and nine additional patients during 2 years of follow-up.

A total of 128 consecutive patients with established RA who were anti-CCP positive were started on anti-tumour necrosis factor treatment at the Jan van Breemen Institute (n = 113) and the Academic Medical Centre, Amsterdam (the Netherlands) (n = 15). All fulfilled the American College of Rheumatology criteria for RA; mean disease duration was 12.6 years.

Laboratory investigations

Anti-CCP levels were determined using the serum samples that were obtained at inclusion by second-generation anti-CCP ELISA (Axis Shield, Dundee, UK). The cut-off level for anti-CCP antibody positivity was set at 5 arbitrary units/ml (AU) (according to the manufacturer’s instructions). Sera reaching 1000 AU/ml were not further diluted.

HLA-DQ typing was performed as described previously. The HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410 and *1001 alleles were taken to contain the SE. HLA-DRB1 SE carrier status (one or two SE copies) was inferred from HLA DQA1-DQB1 haplotypes using strong linkage disequilibrium (LD) with HLA-DRB1 alleles in Caucasians. This HLA-DRB1 typing procedure was validated in 87 established RA patients by sequence-based high resolution typing (Sanquin, Amsterdam, the Netherlands). The technique correctly classified SE carriage in 86 of 87 patients. Independent confirmation in a second cohort of DRB1 and DQ-typed Dutch patients with RA has shown that with DQA1 and DQB1 typing only two of 167 patients would have been incorrectly classified.

Analysis

Frequencies were analysed by Fisher exact test, and odds ratios (ORs) were determined, whereas quantitative differences were analysed using the Mann-Whitney test or t-test where appropriate. One-sided testing (p1) was performed when appropriate.

RESULTS

The 111 patients with arthralgia (mean age 48 (range 22–81) years) were as expected significantly younger on average than both the 125 patients with early arthritis and 128 established RA (mean ages 55 (range 23–76) and 55 (range 24–97) years respectively). The sex distribution did not differ among the three groups (female sex 75, 66 and 73% respectively). Table 1 shows the baseline characteristics of the patients with arthralgia.

Low shared epitope frequency in anti-cyclic citrullinated peptide-positive arthralgia compared with arthritis

Among individuals who were anti-CCP-positive the fraction positive for SE was significantly higher in established RA than in early arthritis (OR = 3.0; 95% confidence interval (95% CI) 1.4–6.4; p = 0.006), and significantly higher in patients with early arthritis than in those with arthralgia (OR = 2.9; 95% CI 1.6–5.2; p < 0.001). Compared with the arthralgia group, the odds ratio of SE positivity for anti-CCP-positive established RA is even higher (OR = 8.7; 95% CI 4.1–18.3; p < 0.001) (fig 1).

Shared epitope carriage is associated with higher anti-cyclic citrullinated peptide levels in patients with arthralgia only

SE carriage is associated with higher anti-CCP levels in patients with anti-CCP-positive arthralgia (median level 69 vs 18 AU, p1 = 0.02, see fig 2). This association is not present in the early arthritis (median level 75 vs 73 AU, p1 = 0.43) and established RA group (median level 91 vs 236 AU, p1 = 0.1).

Patients with shared epitope-negative arthralgia have low anti-cyclic citrullinated peptide levels when compared with patients with arthritis

Anti-CCP levels were higher in established RA when compared with patients with arthralgia (median levels 101 (IQR 31–397) vs 48 (IQR 14–135) AU, p1 < 0.001). Patients with early arthritis who were anti-CCP positive had intermediate anti-CCP levels (75 (IQR 23–162) AU), these levels were higher than in patients with arthralgia (p1 = 0.02) and lower than in established RA (p1 = 0.01).

Anti-CCP levels were similar among the individuals that were SE positive in the three groups (median levels 69, 75 and 91 AU for arthralgia, early arthritis and established RA respectively; see fig 2). However, in individuals that were SE negative, the median anti-CCP levels were lower in the arthralgia group (19 AU) when compared with the early arthritis group (73 AU; p1 = 0.02), the established RA group (236 AU; p1 = 0.002) or the pooled arthritic group (early arthritis and established RA, 82 AU; p1 = 0.005). The median anti-CCP levels in the patients who were SE positive were similar in the arthralgia and the pooled arthritic groups (median levels 69 vs 76 AU, p1 = 0.06).

DISCUSSION

In this first report on HLA typing in patients with anti-CCP-positive arthralgia we observed that the anti-CCP-positive arthralgia group includes a relatively large SE-negative subgroup of patients compared with the anti-CCP-positive early arthritis group and established RA group. However, patients with anti-CCP-positive arthralgia are clearly more often SE positive than Caucasian population healthy controls (58% vs 26–46%). Patients with shared epitope-negative arthralgia have low anti-CCP levels when compared with patients with arthritis.

The current data shed more light on recent seemingly contrasting results on the role of SE and anti-CCP in RA. The presence of both SE and anti-CCP antibodies was associated with the highest risk for the development of RA in a retrospective analysis of patients with preclinical RA, suggesting that the SE independently contributes to the risk for RA. However, a redundant role of the SE in the progression from anti-CCP-positive undifferentiated arthritis to RA was recently reported. Our study reconciles both results as we show that only in anti-CCP-positive arthralgia the SE is associated with higher anti-CCP levels, suggesting that the SE only operates on anti-CCP levels in the early phase up to early arthritis, but not in the later transition from early arthritis to established RA.
This supports the conclusion that the SE does not itself predispose to the development of RA but rather operates through higher anti-CCP levels, not only in the clinical phase but also in the preclinical stages of RA. The presence in the arthralgia group of a relatively large group of SE-negative patients with low anti-CCP levels compared with the arthritis groups suggests that among patients with SE-negative arthralgia development of arthritis is restricted to patients with high anti-CCP levels. Alternatively, this could mean that a large part of the patients with SE-positive RA do not go through a stage of anti-CCP-positive arthralgia but rather simultaneously develop frank arthritis with the onset of arthralgia. The differences in observed anti-CCP levels were probably not due to age effects. In line with previous studies, we did not observe an effect of age on anti-CCP levels in the three patient groups, neither when analysed individually nor when combined. Furthermore, age was similar among the patients with SE-negative and -positive arthralgia (data not shown).

In summary, patients with anti-CCP-positive arthralgia have a relatively low frequency of the SE compared with anti-CCP-positive early and established RA. Furthermore, these patients have relatively low anti-CCP levels. In later stages, the SE is not associated with differences in anti-CCP levels. This correlates with observations reported earlier that the presence of the SE, together with anti-CCP positivity increases the risk for RA, but that the SE does not influence the transition of anti-CCP-positive early arthritis to RA. Together these findings suggest that the SE may influence RA risk through effects on anti-CCP levels. Longitudinal follow-up of arthritis development in patients with arthralgia may further clarify the effect of the SE and anti-CCP (levels) on RA development.

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Competing interests: None.

Table 1 Baseline characteristics of patients with anti-cyclic citrullinated peptide-positive arthralgia*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n = 111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>48 (12)</td>
</tr>
<tr>
<td>Cohorts SE positive, no of cases (%)</td>
<td></td>
</tr>
<tr>
<td>Female sex</td>
<td>98 (73)</td>
</tr>
<tr>
<td>Symptom duration at initial presentation in months, median (IQR)</td>
<td>12 (6–36)</td>
</tr>
<tr>
<td>Number of tender joints reported at baseline visit, median (IQR)</td>
<td>8 (2–14)</td>
</tr>
<tr>
<td>Distribution of tender joints</td>
<td></td>
</tr>
<tr>
<td>Small joints</td>
<td>24 (22%)</td>
</tr>
<tr>
<td>Large joints</td>
<td>24 (22%)</td>
</tr>
<tr>
<td>Small and large joints</td>
<td>63 (57%)</td>
</tr>
<tr>
<td>Symmetric distribution tender joints</td>
<td>76 (69%)</td>
</tr>
<tr>
<td>Localisation of tender joints</td>
<td></td>
</tr>
<tr>
<td>Upper extremities</td>
<td>49 (44%)</td>
</tr>
<tr>
<td>Lower extremities</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>Upper and lower extremities</td>
<td>53 (48%)</td>
</tr>
<tr>
<td>Morning stiffness for more than 1 h</td>
<td>15 (14%)</td>
</tr>
<tr>
<td>Pain on a 100 mm VAS, median (IQR)</td>
<td>28 (7–50)</td>
</tr>
<tr>
<td>Number of tender joints at physical examination, median (IQR)</td>
<td>0 (0–1)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; VAS, visual analogue scale.
*Except were indicated otherwise, values are the number of patients (%).
†Data missing in the remainder of patients.
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