The clinical and genetic associations of anti-cyclic citrullinated peptide antibodies in psoriatic arthritis

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Objectives. Antibodies recognizing a cyclic citrullinated peptide (anti-CCP) are highly specific for rheumatoid arthritis (RA) but their role in psoriatic arthritis (PsA) remains unclear. The aim of this study was therefore to investigate the prevalence of anti-CCP antibodies in PsA and assess their clinical and genetic associations.

Methods. One hundred and twenty-six patients with PsA, 40 patients with seropositive RA and 40 controls were tested for the presence of anti-CCP antibodies, rheumatoid factor (RF) and the HLA-DRB1 shared epitope. Clinical and radiological data were collected prospectively on all patients and compared between anti-CCP-positive and -negative patients.

Results. Seven (5.6%) patients with PsA were positive for anti-CCP antibodies compared with 0% of controls and 97% of patients with seropositive RA. The presence of anti-CCP antibodies in PsA was significantly associated with the HLA-DRB1 shared epitope ($P < 0.005$), erosive disease ($P < 0.05$), number of swollen joints ($P < 0.02$) and DMARD use ($P < 0.05$).

Conclusions. Overall, the increased prevalence of anti-CCP antibodies in this PsA population failed to reach statistical significance. However, when present, they were a marker of disease severity and had RA-linked MHC class II associations. Further studies are needed in a larger population of patients with PsA and appropriate controls to confirm any true association that may be present.

KEY WORDS: Psoriatic arthritis, Anti-citrullinated peptide antibodies, Shared epitope.

Antibodies recognizing cyclic citrullinated peptides (anti-CCP) are highly specific for rheumatoid arthritis (RA) [1]. These antibodies were originally described as ‘antiperinuclear factor’ [2] and subsequently as anti-keratin and anti-filaggrin antibodies [3]. More recently, the major antigenic determinants for these antibodies have been confirmed as peptides containing the post-translationally modified amino acid citrulline [1, 4]. An enzyme-linked immunosorbent assay (ELISA) has been developed to detect these antibodies by employing a cyclic citrullinated peptide (CCP) [5]. The sensitivity of the test has been further increased following the development of a second-generation (CCP2) ELISA.

Several studies have examined the specificity and sensitivity of the anti-CCP ELISA for RA [5]. The antibodies are rarely present in other rheumatological conditions, such as Sjögren’s syndrome [6], SLE [7] or juvenile idiopathic arthritis (JIA) [8, 9]. Using the CCP2 ELISA, only 0–1% of healthy controls and 2–5% of disease controls have anti-CCP antibodies compared with up to 98% of patients with RA [10–12]. More recent studies have suggested that the specificity for RA is not quite so optimistic [13]. Anti-CCP antibodies are present early in the disease process and may even pre-date the onset of RA by many years [14–18]. The presence of anti-CCP antibodies in RA is predictive of the development of erosive disease [5, 16, 19, 20]. This effect seems to be greatest in those patients who are seronegative for RF [17].

The prevalence and prognostic value of anti-CCP antibodies in psoriatic arthritis (PsA) is not known. The CCP ELISA was originally tested in a small number of patients with both psoriasis and PsA, all of whom were negative [5]. One study reported an increased prevalence of anti-perinuclear antibodies (APF) detected by indirect immunofluorescence in a small number of patients with PsA [21]. Recently, two studies have reported a possible increased prevalence of anti-CCP antibodies in PsA of 7.8% [22] and 15.7% [23].

Patients with PsA present with a variety of clinical phenotypes, including oligoarthritis, spondyloarthritis and polyarthritis [24]. In some circumstances, the polyarthritis can be difficult to distinguish from RA. Anti-CCP antibodies may be useful in establishing the correct diagnosis. In addition, they may be an indicator of prognosis and disease severity in PsA, as they are in RA. The aim of this study was therefore to determine the prevalence and associations of anti-CCP antibodies in patients with PsA.

Patients and methods

Patient recruitment

Patients with PsA ($n = 126$) were recruited from a specialist clinic at the Royal National Hospital for Rheumatic Diseases (RNHRD) in Bath, UK. All patients had inflammatory arthritis in association with psoriasis. The presence of RF was not regarded as an exclusion criterion if classical clinical and radiological features of PsA were present. Patients attending the clinic for their routine follow-up were invited to take part in the study from sequential clinics over a period of 6 months. The patients had established disease, with a mean disease duration of 14 yr (range 7–56 yr). The patients were divided into three clinical subgroups: polyarthritis (at least four joints involved); oligoarthritis (less than four joints involved); and spondyloarthritis (sacroilitis or syndesmophytes and inflammatory back pain). The assignment of subgroup was based on that seen at the most recent clinical assessment. Clinical information was recorded prospectively on the patients every 12 months, including a modified Ritchie articular index [25], tender and swollen...
joint counts, and psoriasis area severity index (PASI) [26]. Where serial measurements of disease activity were available, a mean of the variable was calculated and used in the subsequent analysis. Radiographs of the hands and feet taken at the most recent clinic visit were scored for the presence or absence of erosions. Radiographs of the sacroiliac joints were reported where available.

A control group (n=40) matched for sex and age was recruited from random blood donors from the Bristol area (20 male, 20 female, mean age 52 yr). A further control group of patients with RA who were seropositive for rheumatoid factor (RF) (n=40) were recruited from a study at the RNHRD in Bath. Full ethical approval was obtained for this study from the Bath Local Ethics Committee. A blood sample was taken from each patient following written informed consent according to the Declaration of Helsinki. Serum was then extracted and frozen until required. DNA was extracted using a standard salting-out procedure and used for the HLA-Cw6, HLA-B27 and HLA-DRB1 typing.

**Anti-CCP antibodies and RF**

All patient and control serum samples were tested for the presence of anti-CCP antibodies using a commercially available ELISA (CCP2; Axis Shield, UK). All samples were tested in duplicate with repetition of eight standards on each plate. The inter- and intra-assay reliability was quantified by means of the intraclass correlation coefficient. The interassay reliability was 0.998 [95% confidence interval (CI) 0.995–0.999] and the intraassay reliability was 0.999 (95% CI 0.997–0.999). The concentration of anti-CCP antibodies present in each sample was calculated directly from the absorbency readings by the software attached to the plate reader (Multiskan Ascent; Labsystems, Finland). A cutoff value of >6 U/ml was used to indicate a positive result. This was adjusted for the study control population, as recommended by the manufacturer based on the mean value +2 S.D. (mean 3.82 U/ml, s.D. 1.17). The samples were also tested in duplicate using a commercial ELISA for RF (Sigma Diagnostics, St Louis, MO, USA).

**HLA-DRB1, HLA-B27 and HLA-Cw6**

The extracted DNA from all PsA patients was tested for the presence of the HLA-DRB1 shared epitope (HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *1001) [27], as previously described [28]. The PsA samples were also tested for the presence of HLA-Cw6 and HLA-B27 using sequence-specific primers [29].

**Statistical analysis**

Direct comparisons between patients and controls were made using the $\chi^2$ test with Fisher’s exact test when necessary. A mean of the disease activity outcome measures (e.g. PASI, tender and swollen joint counts) was calculated from each of the consecutive visits that the score was recorded. These variables were compared using a Mann-Whitney U test. A $P$ value $<0.05$ was considered to represent a significant difference between the groups. Any significant results were also expressed as a relative risk with 95% CI.

**Results**

**Anti-CCP antibodies in PsA, RA and controls**

The number of patients with PsA who were positive for anti-CCP antibodies did not differ significantly from the control population [7/126 (5.6%) vs 0/40 (0%)]. The titres of anti-CCP antibodies found in the seven positive PsA patients ranged from 0.5 to >100 U/ml, with 5/7 patients showing titres of >20 U/ml. All but one of the patients with RA were positive for anti-CCP antibodies, 36/40 (90%) showing a titre of >20 U/ml. RF was positive in 8.7% of patients with PsA, 100% of patients with RA (selected to be seropositive for RF) and 5% of controls (Table 1). It should be noted that the choice of the control population of random blood donors may lead to underestimation of potential false positives in a non-arthritis population and thus overestimation of the specificity of the test. Equally, the use of an RA population selected for positivity for RF may result in overestimation of the sensitivity of the test.

**Rheumatoid factor and anti-CCP antibodies**

Anti-CCP antibodies were not always associated with the presence of RF. In the patients with PsA, it was uncommon for both tests to be positive: only two patients with PsA were positive for both anti-CCP antibodies and RF. Neither of the RF-positive controls was positive for anti-CCP antibodies. Thirty-nine out of 40 patients with seropositive RA were also positive for anti-CCP antibodies.

**Characteristics of the anti-CCP positive PsA patients**

The sex ratio and age of the patients with PsA who were positive for anti-CCP antibodies were no different from those of the anti-CCP negative patients (Table 2). The distribution of subgroups was similar between the two groups. Of the seven positive patients, four had polyarthritis and three had oligoarthritis. One anti-CCP-positive patient (14%) had spondyloarthritis in combination with polyarthritis. Spondyloarthritis was slightly more frequent in the anti-CCP negative group, where 27/119 (23%) of the patients had clinical or radiological features of spondyloarthritis ($P = \text{not significant}$). Of these, only two patients had pure spondylarthritic; in the remaining patients spondyloarthritic was present in combination with either polyarthritis (19/27) or oligoarthritis (6/27). Patients who were anti-CCP-positive were more likely to be on a disease-modifying anti-rheumatic drug (DMARD) [100 vs 61%, $P < 0.05$; relative risk (RR) 1.6, 95% CI 1.4–1.9] and had a higher median swollen joint count ($P < 0.02$). A summary of the features of the anti-CCP-positive patients is presented in Table 3.

**Anti-CCP antibodies and the HLA-DRB1 shared epitope in PsA**

All seven patients with PsA who were positive for anti-CCP antibodies possessed at least one copy of the HLA-DRB1 shared epitope (three patients were HLA-DRB1*0101-positive and four patients were HLA-DRB1*0401-positive, including one HLA-DRB1*0401 homozygote). This was highly significant compared with the anti-CCP-negative PsA patients (7/7 vs 48/119, $P < 0.005$, RR 2.3, 95% CI 1.9–2.9). A comparison of HLA-DR4 between anti-CCP-positive and -negative PsA patients failed to reach significance (4/7 vs 28/119, $P = \text{not significant}$). HLA-DR1 was
Table 2. Characteristics of anti-CCP-positive and RF-positive patients with PsA

<table>
<thead>
<tr>
<th></th>
<th>Anti-CCP-positive patients</th>
<th>Anti-CCP-negative patients</th>
<th>RF-positive patients</th>
<th>RF-negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=119)</td>
<td>(n=11)</td>
<td>(n=115)</td>
</tr>
<tr>
<td><strong>Female:male</strong></td>
<td>4:3</td>
<td>62:57</td>
<td>6.5</td>
<td>60:55</td>
</tr>
<tr>
<td><strong>Age (yr): median (range)</strong></td>
<td>59 (55–72)</td>
<td>59 (33–87)</td>
<td>64 (47–87)</td>
<td>59 (33–85)</td>
</tr>
<tr>
<td><strong>Polyarthritis</strong></td>
<td>4 (57%)</td>
<td>67 (56%)</td>
<td>7 (64%)</td>
<td>64 (56%)</td>
</tr>
<tr>
<td><strong>Oligoarthritis</strong></td>
<td>3 (43%)</td>
<td>48 (40%)</td>
<td>4 (36%)</td>
<td>47 (41%)</td>
</tr>
<tr>
<td><strong>Spondyloarthritis</strong></td>
<td>1 (14%)</td>
<td>27 (23%)</td>
<td>1 (9%)</td>
<td>27 (23%)</td>
</tr>
<tr>
<td><strong>PASI: median (range)</strong></td>
<td>2.0 (0–7.3)</td>
<td>1.0 (0–13.8)</td>
<td>0.42 (0–6)</td>
<td>1.0 (0–13.8)</td>
</tr>
<tr>
<td><strong>Requirement for DMARD</strong></td>
<td>7 (100%)</td>
<td>73 (61%)</td>
<td>8 (73%)</td>
<td>72 (63%)</td>
</tr>
<tr>
<td><strong>Swollen joints: median (range)</strong></td>
<td>7.5 (3–16)</td>
<td>2.0 (0–17)</td>
<td>5.0 (0–10)</td>
<td>2.2 (0–17)</td>
</tr>
<tr>
<td><strong>Tender joints: median (range)</strong></td>
<td>5.5 (0–17)</td>
<td>4.0 (0–34)</td>
<td>8 (0–33)</td>
<td>4 (0–34)</td>
</tr>
<tr>
<td><strong>Presence of erosions</strong></td>
<td>7 (100%)</td>
<td>68 (61%)</td>
<td>4 (40%)</td>
<td>69 (64%)</td>
</tr>
<tr>
<td><strong>HLA-DR1 shared epitope</strong></td>
<td>4 (57%)</td>
<td>48/119 (43%)</td>
<td>49/107 (46%)</td>
<td>29/107 (27%)</td>
</tr>
<tr>
<td><strong>HLA-DR1</strong></td>
<td>3 (43%)</td>
<td>32/119 (27%)</td>
<td>3/10 (30%)</td>
<td>32/10 (30%)</td>
</tr>
</tbody>
</table>

*P<0.05, RR 1.6 (1.4–1.9).

bP<0.02.

cP<0.05, RR 1.6 (1.4–1.9).

dP<0.005, RR 2.3 (1.9–2.9).

Table 3. Features of the anti-CCP-positive patients with PsA

<table>
<thead>
<tr>
<th>Patient</th>
<th>Anti-CCP titre</th>
<th>RF titre</th>
<th>HLA-DRB1</th>
<th>Erosions</th>
<th>Nail involvement</th>
<th>Subgroup</th>
<th>Specific features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>&lt;6</td>
<td>*0101</td>
<td>Yes</td>
<td>Yes</td>
<td>Polyarthritis</td>
<td>Asymmetrical, bloody periostitis, DIP erosion</td>
</tr>
<tr>
<td>2</td>
<td>90.3</td>
<td>39</td>
<td>*0401</td>
<td>Yes</td>
<td>Yes</td>
<td>Polyarthritis</td>
<td>Symmetrical, DIP erosion</td>
</tr>
<tr>
<td>3</td>
<td>48.2</td>
<td>&lt;6</td>
<td>*0401, *0401</td>
<td>Yes</td>
<td>Yes</td>
<td>Polyarthritis and spondyloarthritis</td>
<td>Bilateral sacroiliitis, DIP erosion, severe symmetrical destructive arthritis</td>
</tr>
<tr>
<td>4</td>
<td>&gt;100</td>
<td>&lt;6</td>
<td>*0101</td>
<td>Yes</td>
<td>Yes</td>
<td>Polyarthritis</td>
<td>Symmetrical erosive. No specific radiological features to support PsA</td>
</tr>
<tr>
<td>5</td>
<td>&gt;100</td>
<td>15</td>
<td>*0401</td>
<td>Yes</td>
<td>Yes</td>
<td>Oligoarthritis</td>
<td>Large asymmetrical erosions with fluffy periostitis</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>&lt;6</td>
<td>*0401</td>
<td>Yes</td>
<td>Yes</td>
<td>Oligoarthritis</td>
<td>Asymmetrical, early erosions; DIP with new bone formation</td>
</tr>
<tr>
<td>7</td>
<td>6.5</td>
<td>&lt;6</td>
<td>*0101</td>
<td>Yes</td>
<td>Yes</td>
<td>Oligoarthritis</td>
<td>Marked new bone formation; PIP and DIP with erosion</td>
</tr>
</tbody>
</table>

Characteristics of the RF-positive and RF-negative patients with PsA

RF was more common in the older patients with PsA (Table 4). The sex ratio was the same for RF-positive and RF-negative patients. There were no differences in the subgroup distribution, 4/11 RF-positive patients presenting as oligoarthritis and 1/11 with spondyloarthritis. Unlike anti-CCP antibodies, RF was not associated with the HLA-DRB1 shared epitope (SE) in 6/11 anti-CCP-positive patients. Review of the radiographs by two independent observers concluded that, radiographically, these patients showed more features in common with PsA than RA.

Discussion

Overall, the increased prevalence of anti-CCP antibodies in PsA failed to reach statistical significance. This may have been due to the relatively small population and control sizes used in this study. However, the patients who were positive for anti-CCP antibodies illustrated some interesting clinical and genetic features. There was not a strong correlation between...
the presence of RF and the presence of anti-CCP antibodies in PsA; most patients who were positive for one were negative for the other. This would be in agreement with studies of seronegative RA in which a significant proportion of patients had anti-CCP antibodies. In PsA, RF did not show an association with any clinical, radiological or genetic characteristic. This suggests that RF is simply an incidental finding of no relevance to the outcome of PsA. In contrast, we have demonstrated several interesting associations between anti-CCP antibodies and PsA.

A strong correlation between the presence of the HLA-DRB1 shared epitope and anti-CCP antibodies in PsA has been demonstrated. A similar association has been reported in studies involving patients with RA [16, 17]. We have previously shown that the shared epitope correlates with erosive disease in PsA [28] and may therefore define a subgroup of patients with a more severe prognosis. All seven anti-CCP-positive patients with PsA possessed at least one copy of either HLA-DRB1*0101 or HLA-DRB1*0401. The association did appear to be directly correlated with the shared epitope alleles since HLA-DR4 and HLA-DR1 were not significantly higher overall in the anti-CCP positive group.

The presence of anti-CCP antibodies and the shared epitope may be related to the ability of the shared epitope alleles to present citrulline-containing peptides to T cells. Recent molecular modelling has shown that peptides containing citrulline, but not arginine, are bound by HLA-DRB1*0401 molecules [30]. Thus, shared epitope-positive patients may produce anti-CCP antibodies in response to deimination of arginine to citrulline, a process that follows cell injury or death. The antibodies may then play a direct pathogenic role in the development of PsA, although there is some evidence for their role in RA [32]. There are several intracellular and extracellular candidates for the autoantigens that induce the production of anti-CCP antibodies; these include vimentin, histones and fibrin. The polyclonal nature of the antibodies suggests that there are likely to be multiple candidates.

Anti-CCP antibodies were also significantly associated with the presence of radiographic erosions in patients with PsA. All seven anti-CCP-positive patients had erosions of the hands and feet, compared with only 61% of anti-CCP-negative patients. Similar findings have been widely reported in RA, where anti-CCP antibodies predict the future development of erosive disease. The mechanism for the association remains unclear, although it is possible that the antibodies, by sustaining the inflammatory response, are directly involved in joint damage.

Patients with PsA who were positive for anti-CCP antibodies had a higher median number of swollen joints and were more likely to require a DMARD than anti-CCP-negative patients. This would suggest that the antibodies may be a marker for, or contribute to, more severe disease. It might have been predicted that most anti-CCP-positive patients would have polyarthritis. However, equal numbers of anti-CCP positive patients had oligoarthritis and polyarthritis, and one patient had an associated spondyloarthropathy. Therefore, anti-CCP antibodies can be detected throughout the clinical spectrum of PsA and not just in those patients with a phenotype resembling RA.

The key question here is whether these anti-CCP-positive patients truly have severe PsA or whether in fact they represent patients who actually have RA and psoriasis. The associations with erosive disease, shared epitope, DMARD use and a higher number of swollen joints may simply reflect the widely accepted view that RA carries a worse prognosis than PsA. There are, however, several reasons to favour PsA as the more likely diagnosis. Firstly, only two of these patients were also positive for RF and both of these patients had clinical and radiological evidence to support PsA. Secondly, three patients had a true asymmetrical oligoarthritis and one of the polyarthritis patients had associated spondyloarthropathy with radiological sacroiliitis. Most patients (6/7) had radiological features consistent with PsA, such as new bone formation, DIP joint involvement and asymmetry. All patients had confirmed psoriasis of the skin and nail involvement and 50% had a family history of psoriasis, but none had a family history of RA and there was an equal sex incidence.

Even if the definitive diagnosis remained in doubt, the evidence presented here suggests that any patient with PsA who has anti-CCP antibodies falls into a poor prognostic category. The presence of the antibodies may suggest some phenotypic overlap with RA or perhaps potentiates mechanisms leading to more severe disease. We would suggest that if a patient with PsA has anti-CCP antibodies, DMARD therapy should be initiated early in the disease process.

If the process of autoimmune recognition of deiminated proteins is central to the pathogenesis of inflammatory arthritis, then clearly there is potential for new therapeutic targets. Interestingly, anti-CD20 therapy (rituximab) has been shown to reduce circulating levels of anti-CCP antibodies [31]. A better understanding of the immunological and genetic background of individual patients with inflammatory arthritis may allow more appropriate targeting of such therapies. The presence or absence of anti-CCP antibodies may be one factor that influences treatment response.

It remains unclear whether anti-CCP antibodies have any pathogenic role in the development of PsA, although there is some evidence for their role in RA [32]. There are several intracellular and extracellular candidates for the autoantigens that induce the production of anti-CCP antibodies; these include vimentin, histones and fibrin. The polyclonal nature of the antibodies suggests that there are likely to be multiple candidates.

Anti-CCP antibodies cannot really be justified as a prognostic test in a PsA population since such a small number of the more severe patients would be identified. It would probably be better applied to a group of seronegative early arthritis patients to predict those patients most at risk of progressive disease. Thus, there remains a need for a test that will detect the majority of patients who will experience a more severe outcome. PsA is no longer regarded as a benign condition: it carries an increased mortality and considerable morbidity, many patients progressing rapidly to destructive arthropathy. Perhaps clues for a prognostic immunological or genetic marker will come not from studies based on RA but from a better understanding of the immunopathology specific to PsA. The development of such a marker would clearly facilitate more targeted and aggressive disease-modifying therapy for those patients who will truly benefit.
The authors have declared no conflict of interest.

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References