Anti-citrullinated peptide antibodies may occur in patients with psoriatic arthritis


Background: Anti-cyclic citrullinated peptide (anti-CCP) antibodies are considered highly specific markers of rheumatoid arthritis. Despite the high specificity of the test, anti-CCP antibodies have also been observed in psoriatic arthritis.

Objective: To determine the frequency of anti-CCP antibodies in psoriatic arthritis and to describe the clinical characteristics of such patients.

Methods: Serum samples from 192 patients with psoriatic arthritis were analysed for anti-CCP antibodies. A previously defined cut off point was applied at a specificity level of ≥98.5% (42 U/ml). Antibodies against pepA and pepB (two synthetic citrullinated peptides) were determined on samples containing anti-CCP antibodies by line immune assay. The swollen joint count and the numbers of affected joints (present or past) were recorded. Clinical features were noted and if available radiographs of hands and feet were scored for erosions. Rheumatoid factor was determined in all samples.

Results: Anti-CCP antibodies were found in 15 patients (7.8%); 13 of 15 anti-CCP2 positive samples were also positive for anti-pepA or pepB antibodies. The prevalence of anti-CCP antibodies was higher than expected in view of the highly specific cut off applied in the test. Detailed analysis of the clinical and radiological features makes it improbable that the high prevalence of anti-CCP antibodies resulted solely from concomitant psoriasis and rheumatoid arthritis or from misclassification.

Conclusions: Anti-CCP antibodies may be present in patients with psoriatic arthritis. Although some of the present cohort could have had psoriasis with concomitant rheumatoid arthritis, a proportion at least had the typical characteristics of psoriatic arthritis as the primary diagnosis.

Psoriatic arthritis is a type of inflammatory joint disease in which axial or peripheral arthritis is associated with psoriasis. The picture of psoriatic arthritis is broad and comprises oligoarticular or polyarticular peripheral arthritis and axial involvement. Psoriatic arthritis shares features of spondyloarthopathies and rheumatoid arthritis. Evolution from oligoarticular disease to polyarticular disease has been described. McGonagle et al suggested features of psoriatic arthritis that are helpful in distinguishing it from rheumatoid arthritis. These comprise the following: asymmetrical oligoarticular disease predominantly of the lower limbs, distal interphalangeal joint (DIP) involvement, enthesisitis, dactylitis, typical radiological features (pencil-in-cup phenomenon, ankylosis of small hand or feet joints, arthritis mutilans (fig 1)), radiological sacroilitis, and inflammatory low back pain. The broad spectrum of the disease makes it difficult to develop good criteria for diagnosis and classification. Although different criteria have been suggested, none are widely accepted. At present an international multicentre validation of diagnostic criteria is under way.

Anti-cyclic citrullinated peptide (CCP) antibodies are antibodies against synthetic citrullinated peptides and are specific markers of rheumatoid arthritis. They belong to a group of anti-citrullinated protein/peptide antibodies (ACPA). Rheumatoid sera were found to contain antibodies that were detected by indirect immunofluorescence in rat oesophagus (the so called anti-keratin antibodies) or in human buccal mucosal cells (the so called anti-perinuclear factor), and on western blotting of human epidermis (anti-filaggrin antibodies). This reactivity was found to be critically dependent on the presence of epitopes containing the amino acid citrulline. Based on this knowledge, other assays were developed using synthetic citrullinated peptides such as CCP, peptide A (pepA), and peptide B (pepB). The anti-CCP enzyme linked immunosorbent assay (ELISA) is a more widely available test, based on citrulline containing peptides derived from filaggrin and later improved by screening dedicated peptide libraries.

Despite the described high specificity of the anti-CCP test, we and others have identified patients with psoriatic arthritis who are positive for anti-CCP antibodies. Based on these observations, we analysed a large group of patients diagnosed by their treating rheumatologist as having psoriatic arthritis, based on the presence of psoriasis with arthritis. We have assessed the prevalence of anti-CCP antibodies in these cases and described in detail their clinical and radiological characteristics in order to determine whether they can be considered genuine cases of psoriatic arthritis.

METHODS

Patients and characteristics
We included patients with skin or nail psoriasis who also had spondylitis or peripheral arthritis, and in whom serum samples had been sent to the laboratory of the department of rheumatology for routine work up. Spare serum was available from 192 patients. The study was approved by the local ethics committee. Patient and disease characteristics of all 192 patients included in the study are given in table 1.

Abbreviations: ACPA, anti-citrullinated protein/peptide antibodies; CCP, cyclic citrullinated peptide; DIP, distal interphalangeal joint; ESSG, European Spondyloarthropathy Study Group; pepA, peptide A; pepB, peptide B; RF, rheumatoid factor; RXJC, radiological joint count; SJC, swollen joint count

The clinical and radiological characteristics of the patients were obtained by retrospective analysis of the clinical records and review of the radiographs. A swollen joint count (SJC) was calculated at the sampling date: 74 joints were taken into account and included shoulders, elbows, wrists, metacarpophalangeal joints, temporomandibular joints, acromioclavicular joints, sternoclavicular joints, knees, ankles, tarsus, metatarsophalangeal joints, and all interphalangeal joints, counted as distinct units. Based on radiographs, the number of erosive or destructed hand or foot joints was calculated, taking into account the wrist, all metacarpophalangeal joints, the tarsus, all metatarsophalangeal joints, and all interphalangeal joints as distinct units (radiological joint count (RXJC), maximum 60). Enthesopathy, inflammatory back pain, and radiological sacroilitis were defined according to the ESSG criteria for spondyloarthropathy, briefly summarised as follows:

- **enthesopathy**: past or present spontaneous pain or tenderness on examination of the site of the insertion of the Achilles tendon or plantar fascia;
- **inflammatory back pain**: history of or present symptoms of spinal pain in the back or neck meeting at least four of the following criteria: onset before age 45, insidious onset, improvement with exercise, association with morning stiffness, and of at least three months’ duration;
- **radiological sacroilitis**: bilateral grade 2 or more or unilateral grade 3 or more.

Dactylitis was defined according to the definitions by Moll and Wright as follows: interphalangeal involvement with flexor tendon sheath effusion; pencil-in-cup phenomenon; arthritis mutilans. Ankylosis of small hand or foot joints was considered a typical radiological feature of psoriatic arthritis.

### Anti-CCP antibodies

Anti-CCP antibodies were detected by a commercially available ELISA containing synthetic peptides (Immunoscan rheumatoid arthritis, mark 2, Eurodiagnostica, Arnhem, Netherlands). The ELISA was carried out according to the manufacturer’s instructions. Briefly, serum samples were diluted 1/50 with dilution buffer and incubated for one hour at 37°C. After removing the liquid and washing three times with rinsing buffer, the conjugate solution (peroxidase conjugated anti-human IgG antibodies) was added into each well and incubated for one hour at 37°C. After three washing steps with rinsing buffer, the substrate solution (tetramethyl benzidine) was added and incubated for 30 minutes at room temperature. The stop solution (sulphuric acid, 0.5 mol/l) was added and the absorbance values were read immediately at 450 nm.

### Rheumatoid factor (RF)

RF was determined by the latex fixation method. A suspension of uniform polystyrene particles sensitised in glycine buffer with heat altered human IgG (BD Diagnostic Systems, Sparks, Maryland, USA) was incubated with progressive dilutions of human sera in microtitre wells. After incubation, the plates were inspected for observable agglutination. The dilution titre present in the last well showing agglutination was recorded.

### Calculation of cut off points

In an independent cohort of patients with inflammatory joint symptoms (containing only four patients with psoriatic arthritis), we previously defined cut off points for anti-CCP antibodies and RF as a function of a preset specificity level. For anti-CCP antibodies a cut off of 1280 corresponded to a specificity of 98.5% (98.6%; 95% confidence interval (CI), 95.8 to 99.8). For RF, a cut off of 160 corresponded to a specificity of 98.6% (95%; 95% CI, 91.7 to 99.3). An RF titre of >1280 corresponded to a specificity of >98.5% (98.6%; 95% CI, 95.8 to 99.8), comparable with the specificity of the anti-CCP test.

### Anti-pepA and anti-pepB antibodies

To confirm ACPA reactivity, anti-pepA and pepB antibodies were determined on all samples showing anti-CCP reactivity by a line immune assay (prototype of INNO-LIA™ rheumatoid arthritis, Innogenetics, Gent, Belgium), as described previously. This test detects antibodies against two synthetic citrullinated peptides, pepA and pepB. Previous studies indicated that the sensitivity and specificity of anti-pepA antibodies were, respectively, 63.4% and >98.5% (100; 95% CI, 98.0 to 100), and the sensitivity and specificity of anti-pepB antibodies were 54% and >98.5% (99.3; 95% CI, 96.8 to 100). Briefly, serum samples were diluted 1/100 and incubated with the strip for one hour at room temperature.
### Table 2 Clinical features of patients with anti-CCP antibodies

<table>
<thead>
<tr>
<th>Sex, age (y)</th>
<th>Dis dur</th>
<th>Anti-CCP [U/ml]</th>
<th>Anti-pepA or pepB reactivity</th>
<th>RF (titre)</th>
<th>SJC</th>
<th>RXJC</th>
<th>Asym oligo dis</th>
<th>Features helpful in distinguishing PsA from RA</th>
<th>Family history</th>
<th>DMARD*</th>
<th>Psoriasis subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>F, 39</td>
<td>15</td>
<td>54</td>
<td>PepA+PepB</td>
<td>160</td>
<td>6</td>
<td>19</td>
<td>No(1)</td>
<td>Enthesitis, dactylitis, inflammatory low back pain, DIP involvement</td>
<td>Infliximab + MTX + SASP</td>
<td>Nail involvement</td>
<td></td>
</tr>
<tr>
<td>M, 24</td>
<td>5</td>
<td>54</td>
<td>Neg</td>
<td>320</td>
<td>10</td>
<td>17</td>
<td>No(1)</td>
<td>DIP involvement</td>
<td>Psoriasis, IBD</td>
<td>MTX + Open</td>
<td>Nail involvement</td>
</tr>
<tr>
<td>M, 60</td>
<td>6</td>
<td>72</td>
<td>PepA+PepB</td>
<td>1280</td>
<td>4</td>
<td>3</td>
<td>No(1)</td>
<td>DIP involvement, dactylitis, sacroiliitis, ankylosis</td>
<td>MTX + CS</td>
<td>MTX</td>
<td>Vulgarnis and nail involvement</td>
</tr>
<tr>
<td>M, 68</td>
<td>17</td>
<td>93</td>
<td>Neg</td>
<td>640</td>
<td>10</td>
<td>34</td>
<td>No(1)</td>
<td>DIP involvement, dactylitis, sacroiliitis, ankylosis</td>
<td>MTX + MTX</td>
<td>MTX</td>
<td>Vulgarnis and nail involvement</td>
</tr>
<tr>
<td>M, 67</td>
<td>20</td>
<td>353</td>
<td>PepA</td>
<td>2560</td>
<td>2</td>
<td>38</td>
<td>No(1)</td>
<td>Enthesitis, dactylitis, sacroiliitis, ankylosis</td>
<td>MTX</td>
<td>MTX</td>
<td>Vulgarnis and nail involvement</td>
</tr>
<tr>
<td>F, 50</td>
<td>5</td>
<td>500</td>
<td>PepA</td>
<td>80</td>
<td>0</td>
<td>NA</td>
<td>No(1)</td>
<td>Enthesitis, dactylitis, inflammatory low back pain</td>
<td>Infliximab + MTX</td>
<td>Vulgarnis</td>
<td></td>
</tr>
<tr>
<td>M, 37</td>
<td>6</td>
<td>1100</td>
<td>PepA+PepB</td>
<td>160</td>
<td>6</td>
<td>1</td>
<td>No(1)</td>
<td>Psoriasis, PsA</td>
<td>MTX + SASP</td>
<td>Vulgarnis and nail involvement</td>
<td></td>
</tr>
<tr>
<td>M, 54</td>
<td>11</td>
<td>1113</td>
<td>PepA</td>
<td>80</td>
<td>0</td>
<td>1</td>
<td>No(1)</td>
<td>Psoriasis, PsA</td>
<td>MTX + SASP</td>
<td>Vulgarnis and nail involvement</td>
<td></td>
</tr>
<tr>
<td>M, 68</td>
<td>6</td>
<td>1375</td>
<td>PepA</td>
<td>160</td>
<td>4</td>
<td>0</td>
<td>No(1)</td>
<td>Psoriasis</td>
<td>MTX + SASP</td>
<td>Vulgarnis and nail involvement</td>
<td></td>
</tr>
<tr>
<td>F, 48</td>
<td>9</td>
<td>1456</td>
<td>Pep B</td>
<td>320</td>
<td>2</td>
<td>14</td>
<td>No(1)</td>
<td>Enthesitis</td>
<td>MTX + SASP</td>
<td>Vulgarnis and nail involvement</td>
<td></td>
</tr>
<tr>
<td>F, 31</td>
<td>1</td>
<td>&gt;1600</td>
<td>PepA+PepB</td>
<td>640</td>
<td>5</td>
<td>0</td>
<td>No(1)</td>
<td>Enthesitis</td>
<td>MTX + SASP</td>
<td>Vulgarnis and nail involvement</td>
<td></td>
</tr>
<tr>
<td>F, 51</td>
<td>8</td>
<td>&gt;1600</td>
<td>PepA</td>
<td>1280</td>
<td>1</td>
<td>0</td>
<td>No(1)</td>
<td>Dactylitis, enthesitis</td>
<td>MTX</td>
<td>MTX</td>
<td>Vulgarnis and nail involvement</td>
</tr>
<tr>
<td>M, 46</td>
<td>1</td>
<td>1600</td>
<td>PepA+PepB</td>
<td>320</td>
<td>0</td>
<td>0</td>
<td>Yes</td>
<td>Enthesitis</td>
<td>MTX</td>
<td>MTX</td>
<td>Vulgarnis and nail involvement</td>
</tr>
<tr>
<td>M, 63</td>
<td>5</td>
<td>&gt;1600</td>
<td>PepA+PepB</td>
<td>1280</td>
<td>9</td>
<td>7</td>
<td>No(1)</td>
<td>DIP involvement, dactylitis</td>
<td>MTX</td>
<td>MTX</td>
<td>Vulgarnis and nail involvement</td>
</tr>
<tr>
<td>M, 69</td>
<td>6</td>
<td>&gt;1600</td>
<td>PepA+PepB</td>
<td>160</td>
<td>0</td>
<td>NA</td>
<td>No(2)</td>
<td>MTX, gold</td>
<td>MTX</td>
<td>Vulgarnis and nail involvement</td>
<td></td>
</tr>
</tbody>
</table>

*Current treatment in bold.

**asym oligo dis**, asymmetrical oligoarticular disease; [No(1)], polyarticular disease; [No(2)], symmetrical oligoarticular disease; CCP, cyclic citrullinated peptide; CS, corticosteroids; CyA, ciclosporine; DIP, distal interphalangeal joint; [Dis dur], disease duration; Dpen, D-penicillamine; IBD, inflammatory bowel disease; MTX, methotrexate; NA, no radiographs available; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; RXJC, radiological erosive or destructive joint count; SASP, salazopyrine; SJC, number of swollen joints; y, years.

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Anti-citrullinated peptide antibodies in psoriatic arthritis

The clinical features of the patients with anti-CCP antibodies are summarised in table 2. There were no major differences between the two diagnostic groups in median disease duration or treatment. Patients with anti-CCP antibodies had a larger number of eroded joints (median 3 and 0, p = 0.032) but no significant difference in the swollen joint count at the date of sampling. There was no difference in the age of sampling.

### RESULTS

#### Anti-citrullinated peptide antibodies and rheumatoid factor

The clinical features of the patients with anti-CCP antibodies are summarised in table 2. Differences between patients with and without anti-CCP antibodies are indicated in table 3. There were no major differences between the two diagnostic groups in median disease duration or treatment. Patients with anti-CCP antibodies had a larger number of eroded joints (median 3 and 0, p = 0.032) but no significant difference in the swollen joint count at the date of sampling.

**Statistical analysis**

The Mann–Whitney U test was used to evaluate differences in categorical data. We computed 95% confidence intervals (CI) for differences between groups. Exact x tests were used to compare distributions around specificities as described by Harper and Reeves. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS v12.0, Chicago, Illinois, USA).
Observed number of patients with anti-CCP antibodies vs expected number given the specificity of the test

In a separate study, we calculated cut off points at a preset specificity level of at least 98.5% (a cut off of 42 U/ml for anti-CCP antibodies). The corresponding specificity and 95% CI were 98.6% (95.8% to 99.8%).

In this cohort of 192 patients with psoriatic arthritis, we found 15 (7.8%) with anti-CCP antibodies. This is more than expected in view of the high specificity of the test, obtained in an independent study. Considering only those 15 patients with at least one feature suggested as helpful in distinguishing psoriatic arthritis from rheumatoid arthritis, there were still more patients with anti-CCP antibodies than expected (10 of 187; 5.3%).

DISCUSSION

In this study, we found that 15 of 192 patients (7.8%) with psoriasis (skin or nail or both) and peripheral or axial arthritis had anti-CCP antibodies. This is more than would be expected in view of the high specificity of the test. PepA or pepB reactivity was confirmed in 13 of these 15 patients, pointing to specific anti-citrullinated peptide reactivity. Although patients with anti-CCP antibodies had a similar disease duration and similar treatment, they tended to have a higher radiological joint count than patients without anti-CCP antibodies. Some also had rheumatoid factor. These data raise the question of whether positivity for anti-CCP antibodies in such patients is caused by the co-occurrence of rheumatoid arthritis and psoriasis. When we excluded the patients who did not have at least one feature helpful in discriminating between psoriatic arthritis and rheumatoid arthritis, and looked at differences in joint involvement, the patient subgroups were too small to reach significance (data not shown). It has been shown that patients who have anti-CCP antibodies and who are also positive for the rheumatoid arthritis associated HLA shared epitope are at increased risk of developing rheumatoid arthritis (odds ratio = 66.8). However, HLA data were not available in the present study.

The differential diagnosis between rheumatoid arthritis (in patients with psoriasis) and psoriatic arthritis may be difficult. Moll and Wright proposed that patients who are positive for rheumatoid factor should be considered to have rheumatoid arthritis, though they admitted that false positivity may occur. As RF is false positive in around 5% of healthy controls and in a larger proportion of patients with chronic diseases, we did not exclude patients with a positive RF test at the standard cut off. Using a cut off at a higher specificity would reduce the number of false positive results. However, in the present study we found that one patient of the four with RF at the 98.5% specific cut off point had a disease picture that was not fully compatible with rheumatoid arthritis, while of the remaining three patients two had dactylitis, enthesitis, and DIP involvement—features that are typically attributed to psoriatic arthritis or spondyloarthritis. Excluding patients who fulfil the ACR criteria for rheumatoid arthritis was not helpful either, as many patients with psoriatic arthritis fulfill those criteria. Applying the ESSG criteria for spondyloarthritis does not include the patients with a symmetrical pattern of arthritis that is indistinguishable from rheumatoid arthritis. A French group suggested a classification system based on weighted clinical, radiological, and HLA criteria. The major drawback of this classification system is the inclusion of HLA data. Also, in our present cohort, some psoriasis patients with dactylitis—which is considered to be one of the most typical manifestations of psoriatic arthritis—did not fulfil those criteria. Mcgonagle et al suggested that one should consider all patients with arthritis and psoriasis or a familial history of psoriasis in combination with clinical or radiographic enthesitis, DIP disease, radiological sacroiliitis, uncommon arthropathies, dactylitis, monoarthritis, or asymmetrical oligoarthritis as having psoriatic arthritis. Patients with polyarticular disease without any of those features and without evidence of enthesitis on magnetic resonance imaging were considered to have rheumatoid arthritis. Using this algorithm, which needs to be validated further, we found that 10 patients showed at least one feature suggested by McGonagle to be helpful in discriminating psoriatic arthritis from rheumatoid arthritis. The number we thus obtain (5.3%) is still higher than the 1.5% expected by the high specificity of the assay, obtained in an independent cohort.

It is possible that some patients with typical psoriatic arthritis and positive anti-CCP or RF antibodies may have psoriatic arthritis patients with concomitant rheumatoid arthritis. This would presuppose a higher prevalence of rheumatoid arthritis in a psoriatic arthritis population than in the general population, in which it is estimated to be 1%. Another possibility is that several psoriatic arthritis patients with anti-CCP antibodies had a disease picture that was not compatible with rheumatoid arthritis.

Our findings show that anti-CCP antibodies are more frequently present in a psoriatic arthritis population than in the populations generally used to assess the specificity of the test, such as healthy controls, patients with different rheumatic diseases, or patients with variant rheumatic complaints. Similarly, the presence of anti-CCP antibodies has also been described in patients with systemic lupus erythematosus and primary Sjögren’s syndrome. These

### Table 3 Comparison between CCP negative and CCP positive patients

<table>
<thead>
<tr>
<th></th>
<th>CCP negative</th>
<th>CCP positive</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median disease duration (range)</td>
<td>7 years (0 to 47)</td>
<td>6 years (1 to 20)</td>
<td>NS</td>
</tr>
<tr>
<td>Use of DMARD or biological agent</td>
<td>149/177</td>
<td>14/15</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SJC</td>
<td>2 (0 to 21)</td>
<td>4 (0 to 10)</td>
<td>NS</td>
</tr>
<tr>
<td>RXJC</td>
<td>0 (0 to 44)</td>
<td>3 (0 to 38)</td>
<td>p = 0.044</td>
</tr>
<tr>
<td>Typical radiographic features*</td>
<td>20/126 (16%)</td>
<td>3/13 (23%)</td>
<td>NS</td>
</tr>
<tr>
<td>Sacroiliitis</td>
<td>40/136 (29%)</td>
<td>2/13 (15%)</td>
<td>NS</td>
</tr>
<tr>
<td>RF positive at 98.5% specific level</td>
<td>0/177</td>
<td>4/15 (27%)</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Persistent oligoarticular disease predominantly of the lower limbs</td>
<td>42/177 (24%)</td>
<td>1/15 (7%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (range) or n (%) unless stated otherwise.

*Pencil-in-cup phenomenon, arthritis mutilans, ankylosis of small hand or foot joints.

CCP, cyclic citrullinated peptide; DMARD, disease modifying antirheumatic drug; RXJC, radiological joint count (number of erosive or destructive joints); SJC, swollen joint count.
findings raise other questions. Thus it could be hypothesised that reactivity for CCP could be a false positive, caused by reactivities to non-specific epitopes in the citrullinated peptide substrate. This phenomenon has been observed in animal models. However, we confirmed real ACPA reactivity with two independent synthetic citrullinated substrates (pepA and pepB), showing the presence of anti-pepA or pepB antibodies in 13 of the 15 psoriatic arthritis patients with anti-CCP antibodies. This supports the view that the observed CCP positivity was caused by genuine anti-citrullinated peptide antibodies.

Another hypothesis could be that those patients with anti-CCP antibodies suffer from an overlap with a preclinical form of rheumatoid arthritis. Indeed, anti-CCP antibodies may be present years before the clinical manifestation of rheumatoid arthritis. Additionally, current DMARD treatment in those patients might prevent the full expression of rheumatoid arthritis. Thus, as suggested before, patients with anti-CCP antibodies require a cautious clinical and radiographic follow up to confirm the absence of future evolution to rheumatoid arthritis.

Conclusions

Our data suggest that anti-CCP antibodies may be present in patients with psoriatic arthritis Although it is possible that some of these patients have rheumatoid arthritis with concomitant psoriasis, in a proportion of them at least there are typical characteristics of psoriatic arthritis. The number of patients with psoriatic arthritis who have anti-CCP antibodies is greater than would be expected in view of the high specificity of the test.

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