Clinical utility of the anti-CCP assay in patients with rheumatic diseases

D M Lee, P H Schur

OBJECTIVES: To determine the frequency of antibodies to cyclic citrullinated peptides (CCP) in a group of patients with a diversity of rheumatic diseases.

METHODS: 249 consecutive sera from an arthritis clinic sent for rheumatology testing were selected for testing with the anti-CCP2 assays and for the presence of rheumatoid factor (RF). Patient charts were reviewed for demographic information, clinical diagnosis, radiographic information, and other laboratory data.

RESULTS: The sensitivity and specificity of anti-CCP reactivity for the diagnosis of rheumatoid arthritis (RA) were 66.0% and 90.4%, respectively. This compared with the sensitivity and specificity of RF for RA at 71.6% and 80.3%. Furthermore, 10/29 (34%) RF− patients with RA demonstrated reactivity to CCP. The presence of either anti-CCP or RF increased testing sensitivity for diagnosis of RA to 81.4%; the presence of both RF and anti-CCP demonstrated a testing specificity similar to that of anti-CCP reactivity alone for the diagnosis of RA (91.1%).

CONCLUSIONS: The detection of anti-CCP is useful for the diagnosis of RA, in fact even more so than RF, because of its higher specificity.
METHODS

Serum samples

Two hundred and forty nine unique consecutive serum samples sent from the BWH Arthritis Center to the BWH Clinical Immunology Laboratory for rheumatology testing were selected for further analysis. Patient charts were reviewed for demographic information, clinical diagnosis, radiographic information, and other laboratory data. Rheumatic diagnoses were established by diagnosis of the attending rheumatologist and/or by review of laboratory, radiological, and clinic notes, applying ACR classification criteria. In this cohort, 226 patients had inflammatory disease (RA, n=103; systemic lupus erythematosus (SLE), n=39; psoriatic arthritis (PsA), n=21; juvenile rheumatoid arthritis (JRA), n=21; “inflammatory arthritis”, n=26; spondylitis, n=11; other, n=5) and 23 patients had non-inflammatory disease (osteoarthritis (OA), n=10; fibromyalgia, n=10; mechanical pain, n=2, arthralgia, n=1). One hundred and ninety seven (79%) of these patients were female with a wide variation in age (18–86 years) (table 1).

Radiographic analysis

Radiographic identification of joint erosions was investigated in the subsets of patients diagnosed with RA, PsA, JRA, and inflammatory arthritis. Joint radiographs were available for 129/171 patients. All radiographic diagnoses were abstracted from formal interpretation by an attending radiologist.

Data measurement and analysis

CCP measurement: anti-CCP activity was determined by an enzyme linked immunosorbent assay (ELISA) using a commercial anti-CCP2 assay provided by the Axis-Shield Corp. Rheumatoid factor measurement: total RF was determined by nephelometry on 214 of the 249 patients in this study; 35 samples contained insufficient volume to measure RF. Receiver operating characteristic (ROC) curves were generated by the method of Metz. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated as described. Best fit curves were generated by using non-linear regression calculations.

Table 1 Patient demographics by diagnosis group

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Number of patients</th>
<th>Age, years Mean (range)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>103</td>
<td>55.4 (24–86)</td>
<td>87 (84%)</td>
</tr>
<tr>
<td>JRA</td>
<td>21</td>
<td>30.9 (15–50)</td>
<td>18 (86%)</td>
</tr>
<tr>
<td>PsA</td>
<td>21</td>
<td>44.6 (24–70)</td>
<td>15 (71%)</td>
</tr>
<tr>
<td>Spondylitis</td>
<td>11</td>
<td>39.0 (26–54)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td>Inflammatory arthritis</td>
<td>26</td>
<td>46.2 (16–77)</td>
<td>15 (58%)</td>
</tr>
<tr>
<td>SLE</td>
<td>39</td>
<td>37.7 (18–61)</td>
<td>36 (92%)</td>
</tr>
<tr>
<td>Non-inflammatory</td>
<td>23</td>
<td>49.6 (19–82)</td>
<td>19 (83%)</td>
</tr>
<tr>
<td>Other inflammatory condition</td>
<td>5</td>
<td>59.2 (49–80)</td>
<td>3 (60%)</td>
</tr>
</tbody>
</table>

Table 2 Sensitivity and specificity of anti-CCP and RF for presence of rheumatoid arthritis (RA). CCP2 (n=249); RF (n=214)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCP</td>
<td>66.0</td>
<td>90.4</td>
<td>82.9</td>
<td>79.0</td>
</tr>
<tr>
<td>RF</td>
<td>71.6</td>
<td>80.3</td>
<td>76.8</td>
<td>75.2</td>
</tr>
<tr>
<td>CCP or RF</td>
<td>81.4</td>
<td>79.5</td>
<td>78.3</td>
<td>82.4</td>
</tr>
<tr>
<td>CCP and RF</td>
<td>56.9</td>
<td>91.1</td>
<td>85.3</td>
<td>69.9</td>
</tr>
</tbody>
</table>

Table 3 Comparison of anti-CCP and RF reactivity

<table>
<thead>
<tr>
<th></th>
<th>Patients with RA</th>
<th>Other patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>CCP (+)</td>
<td>(n=68)</td>
<td>(n=14)</td>
</tr>
<tr>
<td>RF +</td>
<td>58 (85)</td>
<td>10/11* (91)</td>
</tr>
<tr>
<td>RF –</td>
<td>10 (15)</td>
<td>1/11* (9)</td>
</tr>
<tr>
<td>CCP (-)</td>
<td>(n=35)</td>
<td>(n=132)</td>
</tr>
<tr>
<td>RF +</td>
<td>15/34* (44)</td>
<td>12/101* (12)</td>
</tr>
<tr>
<td>RF –</td>
<td>19/34* (56)</td>
<td>89/101* (88)</td>
</tr>
</tbody>
</table>

*RF analysis performed on 214 of 249 samples.
RESULTS
CCP correlation with RA
In this cohort of 249 patients dominated by rheumatic disease (table 1), 82/249 samples tested positive for anti-CCP activity at >5 units reactivity. Of these 82 patients, 68 had RA. This translates into a sensitivity and specificity of anti-CCP reactivity for the diagnosis of RA of 66.0% and 90.4%, respectively (table 2). In the RA cohort, 58/68 (85%) CCP+ patients were also RF+. These tests also had independent reactivity in a significant subset of patients: 10/29 (34%) patients with RA who were RF− showed reactivity to CCP and 15/34 (44%) CCP− patients with RA showed reactivity to RF (table 3).

To determine the diagnostic characteristics of the anti-CCP and RF assays in our rheumatic disease cohort we determined both the relation between sensitivity and specificity at different cut off values (displayed graphically in our ROC plots (fig 1)) and the positive and negative predictive values of these assays (table 2). These analyses confirmed the optimal cut off value for CCP (anti-CCP >5 units). There were two apparent inflection points in the RF analysis, one at RF>10 (our cut off value) and another at the higher cut off value of RF>22.

We also examined the utility of combining the RF and anti-CCP diagnostic tests at optimal test performance values. Allowing the presence of either autoantibody (either RF or anti-CCP) increased the sensitivity for detecting RA to 81.4% (table 2) without substantially altering the specificity for RA (79.5%) from that of RF alone. Conversely, requiring the presence of both autoantibodies (RF and anti-CCP positivity) decreased the sensitivity for diagnosis of RA to 56.9% without demonstrating a substantial increase in specificity (91.1%) relative to that of anti-CCP reactivity alone (90.4%).

CCP reactivity in rheumatic disease subsets
Although the specificity of anti-CCP for RA in our cohort was 90.4%, we sought to delineate the presence of anti-CCP activity in other rheumatic conditions. Of the 14 anti-CCP+ patients without RA in this cohort, 13 had another inflammatory disease (JRA, n=6; inflammatory arthritis, n=3; other, n=4) and only one had a non-inflammatory disease (fibromyalgia); most “false positives” were accounted for by the JRA subset of patients (fig 2). With the exception of the JRA cohort, there was virtually no anti-CCP reactivity in serum from patients with PsA (2/21), SLE (1/39), spondylitic variants (0/11), or inflammatory arthritis (3/26) (fig 2). It should be noted that our JRA cohort comprised adults (average age 31) with longstanding disease (average disease duration 21 years) and high prevalence of erosions (79%).

Correlation of RF and CCP reactivity
Knowing there existed a substantial correspondence of reactivity between the RF and CCP assays, we sought to determine if levels of reactivity correlated between these tests. In a comparison of levels of anti-CCP and RF activity, we found no substantial correlation (R=0.34) (fig 3).

Correlation of anti-CCP activity and radiographic joint erosions
We assessed the correlation between anti-CCP activity and radiographic erosions for patients with radiographs in both the RA subset and the entire anti-CCP(+) groups of patients (table 4, fig 4). In the entire cohort with radiographs, 63% of patients with erosions demonstrated serum anti-CCP reactivity while 65% of patients without erosions lacked anti-CCP reactivity. For the RA patient subset, 72% of those with anti-CCP activity displayed evidence of radiographic erosions. However, a substantial fraction of patients with RA without erosions also demonstrated anti-CCP reactivity (53%).
Extending our analysis to include JRA, PsA, and inflammatory arthritis we found no correlation between anti-CCP reactivity and radiographic joint destruction for inflammatory arthritis (0/6) and PsA (1/5) (Table 4). In the 19 patients with JRA with radiographs, although only 40% of those with erosions demonstrated anti-CCP activity, all patients with anti-CCP reactivity demonstrated erosions.

**DISCUSSION**

Historically, the use of RF as a diagnostic tool for RA has been and remains problematic. After an initially serendipitous recognition that antibodies to IgG were often found in high titre in patients with RA, the sensitised sheep cell (SSC) assay was developed. This assay, cumbersome to perform, was positive in about 60% of patients with RA and infrequently in normal subjects or patients with other rheumatic diseases, and acquired the designation “rheumatoid factor” (RF). This test soon helped to classify patients into “seropositive v seronegative” arthritis. However, shortcomings of the SSC assay led to the development of an assay dependent upon RF anti-Ig activity agglutinating IgG coated latex particles—the latex fixation assay. The latex fixation assay, easier to perform and more reproducible than the SSC assay, increased the sensitivity for RA to about 70–90% in most series. Unfortunately, the latex fixation assay lacks specificity, being positive in many patients with various chronic disease states. Subsequently, a modified fixation assay was developed using CCP. This assay detected IgG anti-CCP and remains problematic. After an initially serendipitous recognition that antibodies to CCP in 68% of patients with RA. Although it had a somewhat lower sensitivity than the RF test, the specificity of anti-CCP for RA in that population was 96%—better than that previously reported in the RF test for RA (48–92%). This represented a great clinical diagnostic improvement.

Subsequent studies have confirmed the highly specific nature of anti-CCP activity in patients with RA and correlated the presence of anti-CCP with erosive disease. Further, inclusion of anti-CCP activity in disease models predicting persistent and erosive disease significantly improved the performance of these models.

Our experience with the anti-CCP assay in 249 patients with rheumatic diseases indicates a sensitivity and specificity for RA of 66% and 90.4% (not in comparison with normal subjects but in comparison with patients with other rheumatic diseases). This high sensitivity and specificity in our hands confirms the initial experience of others. In addition, we observed a low frequency of anti-CCP in other rheumatic diseases including SLE, inflammatory arthritis, PsA, spondylitic variants, OA, and fibromyalgia. Of particular interest was the fact that only 3/26 patients with “inflammatory arthritis” (clinically felt to be distinct from RA) were positive. Whether this lack of reactivity is of prognostic value, as noted by Visser et al, will be of interest in continuing analysis.

Another interesting observation was in JRA: 6/21 had anti-CCP reactivity. This cohort of adult patients with JRA, with an average duration of disease of 21 years (range 6–36), was the only group outside of RA that had a significantly increased frequency of anti-CCP. In JRA, all anti-CCP+ patients were RF+ and had erosive disease, although the majority of patients with erosive disease had no anti-CCP reactivity. Whether these findings portend common pathogenic mechanisms within these subsets of patients with anti-CCP reactivity remains an interesting speculation.

Where does this leave us with respect to finding a laboratory test specific and sensitive for the diagnosis of RA? In our cohort, designed to model “real life” clinical use of this assay, anti-CCP certainly brings us closer than we were with RF, particularly from the vantage of specificity. The low “false positive” rate in inflammatory arthritis groups other than RA (excluding chronic JRA) significantly increases the usefulness of anti-CCP. From a practical perspective, it would be useful to perform the RF and anti-CCP assays concurrently. In our hands, performing both assays and allowing a positive result in either assay (either RF or anti-CCP) confers higher sensitivity for RA (81.4%). Furthermore, both RF and anti-CCP are moderately strongly associated with articular erosions, suggesting that they reflect in some way the severity and progression of RA. Therefore we conclude that detection of anti-CCP is very useful for the diagnosis of RA, in fact even more so than RF, because of its higher specificity. Preliminary observations also suggest that the combination of testing for both RF and anti-CCP may be even more useful.

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REFERENCES