EXTENDED REPORT

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

D M Lee, P H Schur

Rheumatoid arthritis (RA) is a common rheumatic disease of uncertain aetiology with a significant level of morbidity. Identification of patients early in the course of disease is crucial to accurately diagnose RA in patients with inflammatory arthritis. The presence of "rheumatoid factor" (RF) was identified in patients with RA over 50 years ago; assays for RF remain one of the American College of Rheumatology (ACR) classification criteria for RA. The RF assay, in its current manifestation, remains suboptimal as a diagnostic test, as it lacks sensitivity (54–88%) and specificity (48–92%). It is frequently present in many other disease states and is found in patients with a variety of rheumatic conditions, including infections, and healthy patients. The incidence increases with age. Although RF significantly predicts worse outcomes, there is substantial room for improvement in predicting disease severity.

The shortcomings of the RF assay have prompted the development of other serological assays for RA. Clinical utility of the anti-CCP assay in patients with RA has been demonstrated. This assay is characterized by high specificity (98%) and sensitivity (81.4%). 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: 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.

Abbreviations: ACR, American College of Rheumatology; AKA, antikeratin antibodies; APF, antiperinuclear factor; CCP, cyclic citrullinated peptides; IF, immunofluorescent; JRA, juvenile rheumatoid arthritis; OA, osteoarthritis; NPV, negative predictive value; PPV, positive predictive value; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; ROC, receiver operating characteristic; SLE, systemic lupus erythematosus; SSC, sensitised sheep cell
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 No (%)</th>
<th>Other patients No (%)</th>
</tr>
</thead>
<tbody>
<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.

Figure 1  ROC curves for anti-CCP (A) and RF (B) assays. Individual datapoints are represented as small squares. A best fit curve was generated by non-linear regression calculation. Arrows mark the cut off values used for this study.

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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). This compared with the sensitivity and specificity of RF for RA at 71.6% and 80.3% (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 test 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 reactivity with 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. Of the patients with RA with erosions, 81% demonstrated anti-CCP reactivity. 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 and 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” and “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 (reviewed by Shmerling and DelBanco, Carson, and Bridges). Although nephelometry, which also detected IgM anti-IgG RF, was technically more reproducible and easier to perform, it did not improve sensitivity (82%) or specificity (92%) for RA relative to latex agglutination.

Concurrently, other autoantibodies have been found in patients with RA who were tested for antinuclear antibodies by the immunofluorescent (IF) technique. These assays are performed both assays and allowing a positive result for 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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