Rheumatoid arthritis is a systemic, chronic, inflammatory autoimmune disease characterised by joint inflammation that often leads to joint destruction. Rheumatoid arthritis is the most common inflammatory joint disease, affecting 1–2% of the world population. So far, the diagnosis of this disease has been based primarily on clinical manifestations. However, especially during the first few months of the disease, the 1987 revised criteria of the American College of Rheumatology (ACR)\(^1\) are rarely met. The highly variable and unpredictable course of the disease suggests the need for highly sensitive and specific diagnostic tests. Serological support is limited and mainly based on the presence of rheumatoid factors (RF).\(^2\) The specificity and sensitivity of RF have been improved by the development of enzyme linked immunosorbent assays (ELISA), which permits the detection and quantitative measurement of RF in various immunoglobulin classes (IgG-RF, IgA-RF, and IgM-RF), which can be detected in up to 70–80% of patients with rheumatoid arthritis.\(^3\) However, RF are not very specific for this disease and can also be detected in other rheumatic disorders, infections, and in apparently healthy individuals.\(^4\) As the current therapeutic strategies in rheumatoid arthritis recommend increasingly aggressive regimens early in the course of the disease, diagnostic tests with high specificity are desirable for choosing the optimal treatment.

During recent years various circulating non-RF antibodies have been discovered and reported to be of potential diagnostic and clinical value.\(^5\)–\(^10\) However, most of these autoantibodies—including antinuclear antibodies (ANA), antiperinuclear factor antibodies (APF), antikeratin antibodies (AKA), and anti-RA33—could not be shown to have adequate specificity for supporting clinical and therapeutic decisions. Of special interest, however, were the anti-cyclic citrullinated peptide antibodies (CCP), first described by Schellekens\(^11\) and van Jaarsveld and coworkers.\(^12\) CCP proved to be very specific in the diagnosis of rheumatoid arthritis,\(^13\)–\(^16\) and recent studies have shown that APF and AKA both bind specifically to substrates containing the modified amino acid citrulline.\(^17\) The CCP ELISA is based on highly purified synthetic peptides containing modified arginine residues (citrulline) serving as antigen. We conducted a prospective study to evaluate the additional diagnostic and clinical value of the second generation of CCP antibodies (CCP2) compared with RF isotypes. We evaluated the sensitivity and specificity of all the tests undertaken, and the agreement of all methods with disease activity, radiologically visible joint destruction, and disease duration. We also investigated the presence of CCP in RF negative patients with rheumatoid arthritis.

**METHODS**

**Assays**

**RF isotypes**

The RF isotypes for IgG, IgA, and IgM were measured by ELISA. The assay has been described in detail by Jonsson et al.\(^19\) We used a commercially available test system, Aeskulisa RF-AGM (Aesku.lab Diagnostika, Wendelsheim, Germany). Results were expressed in U/ml. The ELISAs were considered positive at values greater than 15 U/ml. This cut-off value was applied to all RF isotypes. For statistical

**Abbreviations:** ACR, American College of Rheumatology; AKA, antikeratin antibodies; APF, antiperinuclear factor; AUC, area under the curve; CCP, anti-cyclic citrullinated peptide; ELISA, enzyme linked immunosorbent assay; RF, rheumatoid factor; ROC, receiver operating characteristic curve; VAS, visual analogue scale
analysis the results were analysed as continuous and dichotomous variables.

Anti-cyclic citrullinated peptide antibodies
The CCP ELISA was carried out according to the manufacturer’s instructions. We used the commercially available second generation test CCP2 (Immunoscan RA–Mark 2, Euro-Diagnostica BV, Arnhem, Netherlands). Results were expressed in arbitrary units. The samples were considered positive if the antibody titre was greater than 25 arbitrary units. For statistical analysis the results were recorded as continuous and dichotomous variables.

Patients and controls
We studied 561 patients with suspected rheumatic diseases who attended the hospital between January 1997 and January 2003, and 154 healthy controls. The study population comprised 206 male (28.8%) and 509 female patients (71.2%) with a mean (SD) age of 56.8 (18.1) years. The study population included 295 patients with clinically proven rheumatoid arthritis (41.3%) according to the 1987 revised ACR criteria for the disease.1 To analyse the sensitivity and specificity of the tests, we used as controls pooled data from 163 patients with degenerative or other inflammatory joint diseases (22.8%), including psoriatic arthritis, reactive arthritis crystal arthropathy, osteoarthritis, and spondylarthropathy, 103 patients with connective tissue disease or vasculitis (14.4%), and 154 healthy individuals (21.5%) (table 1).

Clinical evaluation
The patients were evaluated by clinical examination and laboratory tests. The final clinical diagnosis according to the ACR criteria served as the gold standard for the diagnosis of rheumatoid arthritis. The examiner was blinded to the CCP results at the time of diagnosis. Blood samples were obtained at first clinical presentation and stored at −20°C until assayed. Disease activity of patients with rheumatoid arthritis was assessed at their first visit according to the ACR activity score, including tender joint count (maximum 68), swollen joint count (maximum 66), patient’s assessment of pain (VAS), patient’s global assessment of disease activity (VAS), physician’s global assessment of disease activity (VAS), patient’s assessment of physical function (HAQ),17 and acute phase reactant value (C reactive protein).18 An ACR score of <35 was defined as low disease activity, between 35 and 65 as moderate, and >65 as high.

Radiological evaluation
In the patients with rheumatoid arthritis, radiographic examination of the hands and feet was done at first presentation. All radiographs were scored by an experienced physician’s global assessment of disease activity (VAS), patient’s global assessment of disease activity (VAS), physician’s global assessment of disease activity (VAS), patient’s assessment of physical function (HAQ),17 and acute phase reactant value (C reactive protein).18 An ACR score of <35 was defined as low disease activity, between 35 and 65 as moderate, and >65 as high.

In the patients with rheumatoid arthritis, radiographic examination of the hands and feet was done at first presentation. All radiographs were scored by an experienced physician’s global assessment of disease activity (VAS), patient’s global assessment of disease activity (VAS), physician’s global assessment of disease activity (VAS), patient’s assessment of physical function (HAQ),17 and acute phase reactant value (C reactive protein).18 An ACR score of <35 was defined as low disease activity, between 35 and 65 as moderate, and >65 as high.

In the patients with rheumatoid arthritis, radiographic examination of the hands and feet was done at first presentation. All radiographs were scored by an experienced physician’s global assessment of disease activity (VAS), patient’s global assessment of disease activity (VAS), physician’s global assessment of disease activity (VAS), patient’s assessment of physical function (HAQ),17 and acute phase reactant value (C reactive protein).18 An ACR score of <35 was defined as low disease activity, between 35 and 65 as moderate, and >65 as high.

Diagnostic value of CCP and RF isotypes
The highest sensitivity for detecting rheumatoid arthritis was obtained by IgM-RF ELISA (66.4%) and CCP ELISA (64.4%). The best specificity was achieved by CCP ELISA (97.1%) (table 3). Sensitivity for the diagnosis of rheumatoid arthritis could be further increased by a combination of the CCP and RF tests. The combined use of CCP and all RF isotypes (IgG-, IgM-, and IgM-RF) resulted in a respectably high sensitivity of 80.7% (table 3). Furthermore in our study cohort, seven of the CCP positive patients in the “vasculitis/mixed connective tissue disease” group presented with an articular manifestation (non-erosive arthritis). Perhaps CCP2 has the potential to detect a so far undetected overlap syndrome in these patients.

For further comparisons of the diagnostic value of each assay, we undertook an ROC (receiver operating characteristic) analysis and calculated the area under the curve (AUC). The ROC analysis displays the pairs of sensitivity and specificity for different cut off points of CCP, IgG-RF, IgA-RF, and IgM-RF concentrations. The AUC was best for CCP, at 0.84. The values for IgM-RF, IgA-RF, and IgG-RF were 0.83, 0.78, and 0.74, respectively (fig 1). It could clearly be

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
<th>RA (n = 295; 41.3%)</th>
<th>Degen/inflamm joint disease (n = 163; 22.8%)</th>
<th>Conn tiss disease/vasculitis (n = 103; 14.4%)</th>
<th>Healthy controls (n = 154; 21.5%)</th>
<th>Total (n = 715)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>237 (80.3%)</td>
<td>107 (65.6%)</td>
<td>81 (78.6%)</td>
<td>84 (54.5%)</td>
<td>509 (71.2%)</td>
</tr>
<tr>
<td>Age (mean (SD) (years)</td>
<td>62.4 (14.7)</td>
<td>56.1 (18.4)</td>
<td>51.6 (19.1)</td>
<td>50.0 (19.7)</td>
<td>56.8 (18.1)</td>
</tr>
<tr>
<td>IgG-RF positive</td>
<td>129 (43.7%)</td>
<td>11 (6.7%)</td>
<td>20 (19.4%)</td>
<td>7 (4.5%)</td>
<td>167 (23.4%)</td>
</tr>
<tr>
<td>IgA-RF positive</td>
<td>150 (50.8%)</td>
<td>16 (9.8%)</td>
<td>44 (42.7%)</td>
<td>7 (4.5%)</td>
<td>199 (27.8%)</td>
</tr>
<tr>
<td>IgM-RF positive</td>
<td>196 (66.4%)</td>
<td>20 (12.3%)</td>
<td>11 (7.1%)</td>
<td>271 (37.9%)</td>
<td>271 (37.9%)</td>
</tr>
</tbody>
</table>

CCP, anti-cyclic citrullinated peptide antibodies; Conn tiss, connective tissue; Degen, degenerative; Ig, immunoglobulin; inflam, inflammatory; RA, rheumatoid arthritis; RF, rheumatoid factor.
shown that CCP ELISA provided the best combination of sensitivity and specificity for detecting rheumatoid arthritis.

We also analysed the benefit of single or combined use of all four antibody assays. We found an impressive additional diagnostic value of CCP compared with the single use of RF isotypes alone. In 30.8% of the 295 rheumatoid patients investigated, all four antibodies were positive. However, in 87 patients (29.5%) with clinically defined rheumatoid arthritis, the conventionally used RF isotypes (IgG-RF, IgA-RF, and IgM-RF) were all negative. In 30 (34.5%) of these 87 patients with negative RF isotypes, CCP was still positive. If only the IgM-RF was used as a single RF test (most laboratories only measure RF, and not the RF isotypes), as many as 99 patients (33.6%) remained undetected. In IgM-RF negative rheumatoid patients, CCP was still positive in 38 (38.4%) of these 99 patients (table 4).

Investigating all 295 rheumatoid patients (not only the 87 RF negative patients), 12.9% (38/295) were IgM-RF negative but CCP positive. If all three RF isotypes (IgG-RF, IgA-RF, and IgM-RF) were negative, CCP was still positive in 10.2% (30/295) (table 4). The diagnostic advantage of CCP in RF negative patients was even more convincing in the early course of the disease, where up to 14.4% (14 of 97) of the rheumatoid patients with a disease duration of less than one year were CCP positive but tested negative for all RF isotypes (IgG-RF, IgA-RF, and IgM-RF), compared with 5.1% (3/59) and 9.4% (13/139) in rheumatoid patients with a disease duration of 1–5 or >5 years, respectively (table 5).

**Table 2** Clinical characteristics of patients with rheumatoid arthritis (n=295)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Values (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean disease duration (years)</td>
<td>8.3 (10.1)</td>
</tr>
<tr>
<td>&lt;1 year*</td>
<td>97 (32.9%)</td>
</tr>
<tr>
<td>1–5 years*</td>
<td>59 (20.0%)</td>
</tr>
<tr>
<td>&gt;5 years*</td>
<td>139 (47.1%)</td>
</tr>
<tr>
<td>ACR disease activity score</td>
<td>44.0 (27.5)</td>
</tr>
<tr>
<td>Patient’s assessment of pain (10 cm VAS)</td>
<td>4.8 (2.6)</td>
</tr>
<tr>
<td>Physician’s global assessment of disease activity (10 cm VAS)</td>
<td>4.3 (2.1)</td>
</tr>
<tr>
<td>C reactive protein (mg/l)</td>
<td>30.4 (44.5)</td>
</tr>
<tr>
<td>HAQ</td>
<td>9.7 (6.0)</td>
</tr>
<tr>
<td>C reactive protein (mg/l)</td>
<td>30.4 (44.5)</td>
</tr>
<tr>
<td>ACR &lt;35*</td>
<td>130 (44.1%)</td>
</tr>
<tr>
<td>ACR &gt;65*</td>
<td>59 (20.0%)</td>
</tr>
<tr>
<td>Larsen score*</td>
<td>2.3 (1.5)</td>
</tr>
<tr>
<td>Larsen 0–1</td>
<td>109 (36.9%)</td>
</tr>
<tr>
<td>Larsen 2–3</td>
<td>115 (39.0%)</td>
</tr>
<tr>
<td>Larsen 4–5</td>
<td>71 (24.1%)</td>
</tr>
</tbody>
</table>

Values are mean (SD) or *n (%).

**Table 3** Sensitivity and specificity of RF isotypes and CCP: single tests and test combinations

<table>
<thead>
<tr>
<th>Assays</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG-RF</td>
<td>43.7</td>
<td>91.0</td>
</tr>
<tr>
<td>IgA-RF</td>
<td>50.9</td>
<td>88.3</td>
</tr>
<tr>
<td>IgM-RF</td>
<td>66.4</td>
<td>82.1</td>
</tr>
<tr>
<td>CCP</td>
<td>73.6</td>
<td>86.4</td>
</tr>
<tr>
<td>IgG-/IgA- or IgM-RF</td>
<td>78.7</td>
<td>80.7</td>
</tr>
<tr>
<td>IgG-/IgA-/IgM-RF</td>
<td>70.5</td>
<td>74.5</td>
</tr>
<tr>
<td>CCP</td>
<td>73.6</td>
<td>86.4</td>
</tr>
</tbody>
</table>

DISCUSSION

The objective of our study was to assess the additional diagnostic and clinical value of CCP compared with RF isotypes in rheumatoid patients. Rheumatoid arthritis is associated with a few more or less specific autoantibodies1,20; however, most of these have failed to demonstrate adequate diagnostic and prognostic value so far. As there is growing evidence that therapeutic intervention early in the course of rheumatoid arthritis leads to earlier disease control, less joint

---

**Figure 1** Receiver operating characteristic curves of the anti-cyclic citrullinated peptide antibodies and rheumatoid factor isotypes. CCP, anti-cyclic citrullinated peptide antibodies; Ig, immunoglobulin; RF, rheumatoid factor.
damage, and a better prognosis.\textsuperscript{35-37} It is mandatory to differentiate between rheumatoid arthritis and other forms of arthritis early after symptom development.\textsuperscript{38} Schellekens and coworkers were the first to report on the diagnostic properties of the rheumatoid arthritis specific autoantibodies CCP, using the first generation test CCP\textsuperscript{1}.\textsuperscript{9,11}

Our study is one of the first to be published using the second generation test, CCP\textsuperscript{2}. The first important finding from our data was that CCP is a highly specific marker in the diagnosis of rheumatoid arthritis. Comparable with the results of other studies using the CCP\textsuperscript{1} assay and of a pre-production test, we found a specificity of 97%.\textsuperscript{13,29} The somewhat lower specificity and higher sensitivity of other studies using the CCP\textsuperscript{2} assay may reflect different cut off levels.\textsuperscript{30,31} The sensitivity of CCP has also been increased during recent years by the second test generation CCP\textsuperscript{2} (Immunoscan RA, Mark 2), now comparable in sensitivity to IgM-RF. As in the results of Lee and Schur,\textsuperscript{30} we found a specificity of 64%. A higher sensitivity of about 80% has been described by other investigators.\textsuperscript{30-34} The lower sensitivity in our study cohort may reflect the presence of a relatively high percentage of early rheumatoid patients and a higher cut off level. By combining the use of all four antibodies (CCP or RF isotypes positive), a sensitivity up to 80.7% can be demonstrated in our study cohort as well.

An additional point of interest in our study cohort was the observation that all the seven CCP positive patients in the "vasculitis/mixed connective tissue disease" group were patients with an articular disease manifestation (non-erosive arthritis). This suggests that these patients may not really have been "false positive" but that the positive CCP results were identifying patients with a so far undetected overlap syndrome.

Our study showed the additional diagnostic and prognostic value of CCP compared with the different RF isotypes. In particular, in RF negative rheumatoid patients, CCP showed convincing additional diagnostic value. CCP could be detected in up to 38.4% of IgM-RF negative sera of rheumatoid patients. This is of special interest because in many laboratories only IgM-RF and not all RF isotypes are measured routinely. Somewhat lower but comparable results were reported by Sellekens et al, using a preproduction CCP test and IgM-RF assay,\textsuperscript{35} as well as by Lee and Schur, using the CCP\textsuperscript{2} test and nephelometry as the RF test.\textsuperscript{36} Most of the recent studies that compared CCP and RF have assessed IgM-RF alone as a single marker.\textsuperscript{37,38} In the present study, we compared CCP not only with IgM-RF but also with the other RF isotypes IgG-RF and IgA-RF in a large population which reflects a broad spectrum of patients with painful joint disease. We found positive CCP results in 34.5% of patients with rheumatoid arthritis who were negative for IgG-RF, IgA-RF, and IgM-RF. This additional diagnostic value of CCP is even more impressive in the early course of disease, in patients with severe joint destruction, and in patients with very active disease. In the study by Rantapaa-Dalqvist et al, all RF isotypes (IgG-RF, IgA-RF, and IgM-RF) and CCP\textsuperscript{2} were also analysed in pre-disease serum samples.\textsuperscript{39} They showed that CCP and IgA-RF predict the development of rheumatoid arthritis.
Cyclic citrullinated peptide in rheumatoid arthritis

Table 6  Sensitivity of CCP/RF isotypes according to disease duration, radiological damage, and disease activity

<table>
<thead>
<tr>
<th></th>
<th>All RA patients</th>
<th>IgG-RF</th>
<th>IgA-RF</th>
<th>IgM-RF</th>
<th>CCP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>&lt;1 year</strong></td>
<td>295 (100)</td>
<td>129 (43.7)</td>
<td>150 (50.8)</td>
<td>196 (64.4)</td>
<td>190 (64.4)</td>
</tr>
<tr>
<td><strong>1–5 years</strong></td>
<td>97 (32.9)</td>
<td>34 (35.0)</td>
<td>35 (36.1)</td>
<td>55 (56.7)</td>
<td>53 (54.7)</td>
</tr>
<tr>
<td><strong>&gt;5 years</strong></td>
<td>59 (20.0)</td>
<td>31 (52.5)</td>
<td>38 (64.4)</td>
<td>41 (69.5)</td>
<td>40 (67.8)</td>
</tr>
<tr>
<td>Larsen 0–1</td>
<td>139 (47.1)</td>
<td>64 (46.0)</td>
<td>77 (55.4)</td>
<td>100 (71.9)</td>
<td>97 (69.7)</td>
</tr>
<tr>
<td>Larsen 2–3</td>
<td>119 (39.0)</td>
<td>44 (40.4)</td>
<td>50 (45.3)</td>
<td>73 (67.0)</td>
<td>59 (54.1)</td>
</tr>
<tr>
<td>Larsen 4–5</td>
<td>71 (24.1)</td>
<td>34 (47.9)</td>
<td>35 (48.3)</td>
<td>48 (67.6)</td>
<td>57 (80.3)</td>
</tr>
<tr>
<td>ACR &lt; 35</td>
<td>130 (44.1)</td>
<td>48 (36.9)</td>
<td>58 (44.6)</td>
<td>85 (65.4)</td>
<td>76 (58.5)</td>
</tr>
<tr>
<td>ACR 35–65</td>
<td>106 (35.9)</td>
<td>47 (44.3)</td>
<td>50 (47.2)</td>
<td>68 (64.2)</td>
<td>66 (62.3)</td>
</tr>
<tr>
<td>ACR &gt; 65</td>
<td>59 (20)</td>
<td>34 (57.6)</td>
<td>42 (71.2)</td>
<td>43 (72.9)</td>
<td>48 (81.4)</td>
</tr>
</tbody>
</table>

Values are n (%).

REFERENCES


Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis

I Vallbracht, J Rieber, M Oppermann, F Förger, U Siebert and K Helmke

doi: 10.1136/ard.2003.019877

Updated information and services can be found at:
http://ard.bmj.com/content/63/9/1079

These include:

References
This article cites 37 articles, 9 of which you can access for free at:
http://ard.bmj.com/content/63/9/1079#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Connective tissue disease (4253)
- Degenerative joint disease (4641)
- Immunology (including allergy) (5144)
- Musculoskeletal syndromes (4951)
- Rheumatoid arthritis (3258)
- Clinical diagnostic tests (1282)
- Epidemiology (1384)
- Vascularitis (294)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/