

## EXTENDED REPORT

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

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**Objective:** To assess the additional diagnostic and clinical value of the second test generation of anti-cyclic citrullinated peptide antibodies (CCP2) compared with rheumatoid factor isotypes (IgG-RF, IgA-RF, IgM-RF) in patients with rheumatoid arthritis.

**Methods:** This was a prospective study on 715 patients: rheumatoid arthritis (n=295), degenerative or other inflammatory joint disease (n=163), connective tissue disease or vasculitis (n=103), and healthy controls (n=154). Sera from each subject were tested for CCP2 and RF isotypes by enzyme linked immunosorbent assay (ELISA). Agreement with clinical indices such as disease activity, joint destruction, disease duration, and other laboratory tests was assessed. Sensitivity and specificity of the tests were evaluated taking the clinical diagnosis as the gold standard.

**Results:** Highest sensitivity was found for IgM-RF (66.4%) and CCP (64.4%). Highest specificity was achieved by CCP (97.1%) and IgG-RF (91.0%). In rheumatoid patients with high disease activity or severe joint damage, CCP was more often present (81.4% and 83.6%) than all RF isotypes. Of special diagnostic value was the detection of positive CCP in 34.5% of all patients with rheumatoid arthritis when all measured RF isotypes (IgG-RF, IgA-RF, and IgM-RF) were negative.

**Conclusions:** As a screening method for rheumatoid arthritis the IgM-RF and the CCP assays are superior to other RF isotypes. Positivity in the highly specific CCP ELISA supports the diagnosis of rheumatoid arthritis. CCP proved to be a powerful diagnostic tool, especially in ambiguous cases or RF negative patients with rheumatoid arthritis.

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)<sup>1</sup> 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).<sup>2</sup> 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.<sup>3</sup> However, RF are not very specific for this disease and can also be detected in other rheumatic disorders, infections, and in apparently healthy individuals.<sup>2, 4–5</sup> 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.<sup>6–10</sup> 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<sup>11</sup> and van Jaarsveld and coworkers.<sup>12</sup> CCP proved to be very specific in the diagnosis of rheumatoid arthritis,<sup>13–16</sup> and recent studies have shown that APF and AKA both bind specifically to substrates containing the modified amino acid citrulline.<sup>6, 17</sup> 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.*<sup>18</sup> 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.<sup>1</sup> 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  $-20^{\circ}\text{C}$  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),<sup>19</sup> and acute phase reactant value (C reactive protein).<sup>20</sup> 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 observer, unaware of the clinical and laboratory data, using the method described by Larsen *et al.*<sup>21</sup> A Larsen score of 0–1 was defined as minimal radiological changes. Larsen scores of

2–3 and 4–5 represent moderate and severe radiological changes, respectively.

### Statistics

Correlations between the tests used and disease activity, radiologically visible joint destruction, and disease duration were determined by Spearman's rank correlation. The  $\chi^2$  test was used to examine the significance of differences in distributions of categorical variables between the different groups. The non-parametric Mann-Whitney U test was used for comparison of continuous variables across different groups. A probability (p) value of  $<0.05$  was considered statistically significant. The sensitivity and specificity for each assay was determined with respect to the gold standard. In addition, receiver operating characteristic (ROC) analysis was carried out to compare test characteristics independently of predefined cut off points across different tests. All statistical analyses were done with SPSS 11.0.1 statistical software (SPSS Inc, Chicago, Illinois, USA).

## RESULTS

### Patient characteristics

In all, 715 patients were entered into the study. Sera from 202 patients (28.3%) were CCP positive, 167 (23.4%) IgG-RF positive, 199 (27.8%) IgA-RF positive, and 271 (37.9%) IgM-RF positive by ELISA. The demographic data and the individual test results are shown in table 1. Patients with rheumatoid arthritis had a higher frequency of RF and anti-cyclic citrullinated antibodies than the control groups. More detailed characteristics of the patients with rheumatoid arthritis, including their clinical characteristics, are shown in table 2.

### 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-, IgA-, 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 1** Patient characteristics

	RA (n = 295; 41.3%)	Degen/inflam joint disease (n = 163; 22.8%)	Conn tiss disease/vasculitis (n = 103; 14.4%)	Healthy controls (n = 154; 21.5%)	Total (n = 715)
Female	237 (80.3%)	107 (65.6%)	81 (78.6%)	84 (54.5%)	509 (71.2%)
Age (mean (SD)) (years)	62.4 (14.7)	56.1 (18.4)	51.6 (19.1)	50.0 (19.7)	56.8 (18.1)
IgG-RF positive	129 (43.7%)	11 (6.7%)	20 (19.4%)	7 (4.5%)	167 (23.4%)
IgA-RF positive	150 (50.8%)	16 (9.8%)	26 (25.2%)	7 (4.5%)	199 (27.8%)
IgM-RF positive	196 (66.4%)	20 (12.3%)	44 (42.7%)	11 (7.1%)	271 (37.9%)
CCP positive	190 (64.4%)	4 (2.5%)	7 (6.8%)	1 (0.6%)	202 (28.3%)

CCP, anti-cyclic citrullinated peptide antibodies; Conn tiss, connective tissue; Degen, degenerative; Ig, immunoglobulin; inflam, inflammatory; RA, rheumatoid arthritis; RF, rheumatoid factor.

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

Mean disease duration (years)	8.3 (10.1)
<1 year*	97 (32.9%)
1–5 years*	59 (20.0%)
>5 years*	139 (47.1%)
ACR disease activity score	44.0 (27.5)
Patient's assessment of pain (10 cm VAS)	4.8 (2.6)
Patient's global assessment of disease activity (10 cm VAS)	4.3 (2.1)
Physician's global assessment of disease activity (10 cm VAS)	4.0 (2.3)
Swollen joint count	6.0 (7.1)
Tender joint count	12.2 (11.3)
HAQ	9.7 (6.0)
C reactive protein (mg/l)	30.4 (44.5)
ACR <35*	130 (44.1%)
ACR 35–65*	106 (35.9%)
ACR >65*	59 (20.0%)
Larsen score <sup>21</sup>	2.3 (1.5)
Larsen 0–1	109 (36.9%)
Larsen 2–3	115 (39.0%)
Larsen 4–5	71 (24.1%)

Values are mean (SD) or \*n (%).  
ACR, American College of Rheumatology; HAQ, health assessment questionnaire; VAS, visual analogue scale.

shown that CCP ELISA provided the best combination of sensitivity and specificity for detecting rheumatoid arthritis.

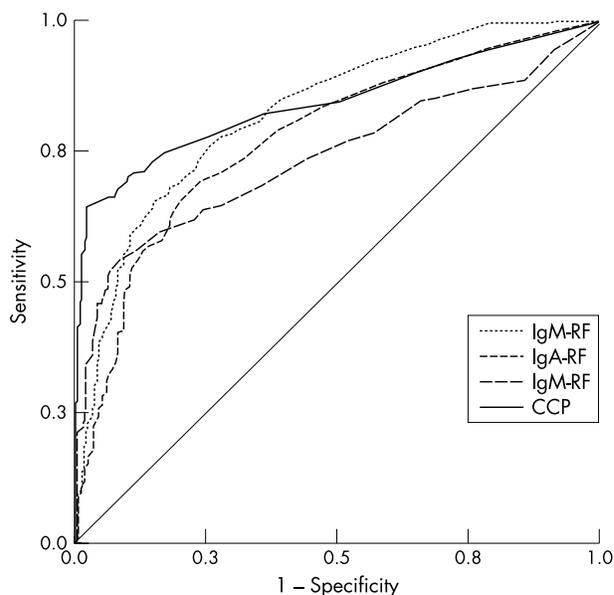
We also analysed the benefit of single or combined use of all four antibody assays. We found an impressive additional diagnostic value of CCP compared with the single use of RF isotypes alone. In 30.8% of the 295 rheumatoid patients investigated, all four antibodies were positive. However, in 87 patients (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 with rheumatoid arthritis (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 3** Sensitivity and specificity of RF isotypes and CCP: single tests and test combinations

Assays	Sensitivity (%)	Specificity (%)
IgG-RF	43.7	91.0
IgA-RF	50.9	88.3
IgM-RF	66.4	82.1
CCP2	64.4	97.1
IgG-/IgA- or IgM-RF	70.5	74.5
IgG-/IgA-/IgM-RF or CCP2	80.7	73.3
IgG-RF or CCP2	72.9	89.0
IgA-RF or CCP2	73.6	86.4
IgM-RF or CCP2	79.3	80.7

CCP2, second generation anti-cyclic citrullinated peptide antibodies; Ig, immunoglobulin; RF, rheumatoid factor.



**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.

**CCP as a marker for disease activity**

A low disease activity was reported in 130 rheumatoid patients (44.1%). Moderate or high activity was present in 106 patients (35.9%) and 59 patients (20.0%), respectively. The percentage of CCP positive rheumatoid patients was significantly higher in those with high disease activity (81.4%), v 58.5% with low disease activity (p<0.05) (table 6). In rheumatoid patients with high disease activity, CCP showed a higher sensitivity than all RF isotypes (table 6). The proportion of rheumatoid patients with negative RF isotypes but positive CCP results was much greater in patients with high disease activity than in those with low disease activity, at 17.0% (10/59) v 8.5% (11/130) (table 5). Despite this obvious diagnostic advantage of CCP, the best correlation of all the tests performed with disease activity was achieved by IgG-RF (r = 0.281, p<0.01).

**CCP as a marker for joint damage**

In our study, 109 rheumatoid patients (36.9%) presented with minimal, 115 (39.0%) with moderate, and 71 (24.1%) with severe joint destruction. Again, in patients with severe joint destruction, CCP was more often positive (80.3%) than in patients with minimal joint damage (54.1%) (p<0.05), and showed a higher sensitivity in those with severe joint destruction than all the RF isotypes (table 6). Negative RF isotypes but positive CCP test results were more often found in rheumatoid patients with severe joint destruction than in those with minimal joint destruction: 15.5% (11/71) v 8.3% (9/109) (table 5). Of all the tests done, CCP showed the best correlation with joint damage (r = 0.217, p<0.01).

**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 autoantibodies<sup>3, 22</sup>; 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

**Table 4** Varying combinations of rheumatoid factor and anti-cyclic citrullinated peptide antibodies in 295 patients with rheumatoid arthritis

RF combinations	All	CCP positive	CCP negative
All RA patients	295 (100)	190 (64.4)	105 (35.6)
All three RF isotypes positive	107 (36.3)	91 (85.0)	16 (15.0)
All three RF isotypes negative	87 (29.5)	30 (34.5)	57 (65.5)
At least one RF isotype positive	208 (70.5)	160 (76.9)	48 (23.1)
At least one RF isotype negative	188 (63.7)	99 (52.7)	89 (47.3)
Only IgG-RF negative	166 (56.3)	86 (51.8)	80 (48.2)
Only IgA-RF negative	145 (49.2)	67 (46.2)	78 (53.8)
Only IgM-RF negative	99 (33.6)	38 (38.4)	61 (61.6)
Only IgG-RF positive	2 (1)	2 (100)	0 (0.0)
Only IgA-RF positive	8 (2.7)	4 (50)	4 (50)
Only IgM-RF positive	38 (12.9)	26 (68.4)	12 (31.6)
At least IgG positive	129 (43.7)	104 (80.6)	25 (19.4)
At least IgA positive	150 (50.9)	123 (82.0)	27 (18.0)
At least IgM positive	196 (66.4)	152 (77.6)	44 (22.4)

Values are n (%).

CCP, anti-cyclic citrullinated peptide antibodies; Ig, immunoglobulin; RA, rheumatoid arthritis; RF, rheumatoid factor.

damage, and a better prognosis,<sup>23-27</sup> it is mandatory to differentiate between rheumatoid arthritis and other forms of arthritis early after symptom development.<sup>28</sup> Schellekens and coworkers were the first to report on the diagnostic properties of the rheumatoid arthritis specific autoantibodies CCP, using the first generation test CCP1.<sup>11-13</sup>

Our study is one of the first to be published using the second generation test, CCP2. 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 CCP1 assay and of a pre-production test, we found a specificity of 97%.<sup>13-29</sup> The somewhat lower specificity and higher sensitivity of other studies using the CCP2 assay may reflect different cut off levels.<sup>30-31</sup> The sensitivity of CCP has also been increased during recent years by the second test generation CCP2 (Immunoscan RA, Mark 2), now comparable in sensitivity to IgM-RF. As in the results of Lee and Schur,<sup>30</sup> we found a sensitivity of 64%. A higher sensitivity of about 80% has been described by other investigators.<sup>30-34</sup> 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,<sup>13</sup> as well as by Lee and Schur, using the CCP2 test and nephelometry as the RF test.<sup>30</sup> Most of the recent studies that compared CCP and RF have assessed IgM-RF alone as a single marker.<sup>13-15-35</sup> 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 CCP2 were also analysed in pre-disease serum samples.<sup>36</sup> They showed that CCP and IgA-RF predict the development of rheumatoid

**Table 5** Additional diagnostic value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes, taking into account disease duration, radiological damage, and disease activity

	All	IgG/IgA/IgM-RF negative, CCP positive	IgM-RF negative, CCP positive	IgG-RF negative, CCP positive	IgA-RF negative, CCP positive
All RA patients	295 (100)	30 (10.2)	38 (12.9)	86 (29.2)	67 (22.7)
<1 year*	97 (32.9)	14 (14.4)	15 (15.5)	30 (30.9)	26 (26.8)
1-5 years*	59 (20.0)	3 (5.1)	6 (10.2)	12 (20.3)	7 (11.9)
>5 years*	139 (47.1)	13 (9.4)	17 (12.2)	44 (31.7)	34 (24.5)
Larsen 0-1	109 (36.9)	9 (8.3)	10 (8.3)	30 (27.9)	22 (20.2)
Larsen 2-3	115 (39.0)	10 (8.7)	15 (13.0)	33 (28.7)	23 (20.0)
Larsen 4-5	71 (24.1)	11 (15.5)	13 (18.3)	23 (32.4)	22 (31.0)
ACR <35	130 (44.1)	11 (8.5)	14 (10.8)	41 (31.5)	31 (23.9)
ACR 35-65	106 (35.9)	9 (8.5)	12 (11.3)	28 (26.4)	24 (22.6)
ACR >65	59 (20)	10 (17.0)	12 (20.3)	17 (28.9)	12 (20.3)

Values are n (%).

\*Disease duration.

ACR, American College of Rheumatology disease activity score; CCP, anti-cyclic citrullinated peptide antibodies; Ig, immunoglobulin; Larsen, Larsen radiological score<sup>21</sup>; RA, rheumatoid arthritis; RF, rheumatoid factor.

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

	All	IgG-RF	IgA-RF	IgM-RF	CCP
All RA patients	295 (100)	129 (43.7)	150 (50.8)	196 (66.4)	190 (64.4)
<1 year*	97 (32.9)	34 (35.0)	35 (36.1)	55 (56.7)	53 (54.7)
1–5 years*	59 (20.0)	31 (52.5)	38 (64.4)	41 (69.5)	40 (67.8)
>5 years*	139 (47.1)	64 (46.0)	77 (55.4)	100 (71.9)	97 (69.7)
Larsen 0–1	109 (36.9)	44 (40.4)	50 (45.3)	73 (67.0)	59 (54.1)
Larsen 2–3	115 (39.0)	59 (44.3)	65 (56.5)	75 (65.2)	74 (64.3)
Larsen 4–5	71 (24.1)	34 (47.9)	35 (48.3)	48 (67.6)	57 (80.3)
ACR <35	130 (44.1)	48 (36.9)	58 (44.6)	85 (65.4)	76 (58.5)
ACR 35–65	106 (35.9)	47 (44.3)	50 (47.2)	68 (64.2)	66 (62.3)
ACR >65	59 (20)	34 (57.6)	42 (71.2)	43 (72.9)	48 (81.4)

Values are n (%).

\*Disease duration.

ACR, American College of Rheumatology disease activity score; CCP, anti-cyclic citrullinated peptide antibodies; Ig, immunoglobulin; Larsen, Larsen radiological score<sup>21</sup>; RA, rheumatoid arthritis; RF, rheumatoid factor.

arthritis, with CCP having the highest predictive value of all four antibodies.

The value of CCP and RF for predicting the outcome of rheumatoid arthritis has been investigated recently.<sup>9 10 12 14–16</sup> Several groups have found that CCP positive patients develop significantly more severe radiographic joint damage,<sup>12 14 15</sup> though all used the less sensitive CCP1 test. Our study also showed that determination of CCP and RF isotypes, especially IgG-RF and IgA-RF, contributes to the prediction of clinical disease activity and joint damage. The study by Bas *et al*, in which the presence of IgM-RF, IgA-RF, and CCP was investigated, also showed an association of IgA-RF and CCP with clinical signs of disease severity.<sup>37</sup> The high prevalence of CCP in rheumatoid patients with extensive disease activity and severe radiological changes, and even more impressively in rheumatoid patients who are RF negative, suggests that CCP is more useful than the RF isotypes alone in the early prediction of disease outcome and disease activity. Vencovsky *et al* also showed that in patients with erosive rheumatoid arthritis the combination of CCP positivity and IgM-RF negativity was more common than RF positivity combined with CCP negativity.<sup>38</sup> Other investigators, such as Paimela *et al*, have reported that a positive test result of an ELISA based on purified human flaggrin as the antigen failed to predict joint destruction.<sup>39</sup> Vittecoq *et al* claimed that RF was the main factor predicting radiological progression, but they used anti-citrullinated rat flaggrin antibodies rather than the currently available CCP2 assay.<sup>40</sup>

## Conclusions

ELISA for IgM-RF is still most useful as a screening marker in the diagnosis of rheumatoid arthritis. However, the second generation CCP ELISA now has a comparable sensitivity, and in addition it has proved to be the most specific marker for the disease. To establish the diagnosis of rheumatoid arthritis, we therefore recommend the use of the highly specific anti-cyclic citrullinated peptide antibody test. Especially in ambiguous cases or in rheumatoid factor negative patients with suspected rheumatoid arthritis, this test has proved very helpful and could be an additional diagnostic marker for the diagnosis of rheumatoid arthritis. Our study suggests that the combined use of RF isotypes and CCP is the most powerful prognostic and diagnostic tool and has greater value for clinical use than conventional RF tests on their own. This set of diagnostic and prognostic markers would allow the clinician to choose a more powerful disease modifying antirheumatic drug early in the course of disease, even when clinical judgment might not yet indicate the need for such drugs.

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