CONCISE REPORT

A comparison of the diagnostic accuracy and prognostic value of the first and second anti-cyclic citrullinated peptides (CCP1 and CCP2) autoantibody tests for rheumatoid arthritis

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Objectives: To compare the diagnostic performance and prognostic value of the anti-cyclic citrullinated peptide (CCP1) and anti-CCP2 autoantibody tests in a clinical setting.
Methods: Anti-CCP1 and anti-CCP2 antibody tests were performed on the same serum samples obtained from 467 patients with early arthritis from the Leiden Arthritis Cohort. The sensitivity, specificity, positive predictive value, and negative predictive value for discriminating between rheumatoid arthritis (RA) and non-RA at 1 year's follow up were calculated for both tests. Results were graphically presented using receiver operating characteristic curves. Progression of radiological joint damage was assessed over 4 years in patients with RA and used to assess the prognostics values of the CCP tests.
Results: At a similar specificity the CCP2 test had a higher sensitivity than the CCP1 test. Both tests identified a subgroup of patients with RA with an increased rate of joint damage progression. The anti-CCP2 test identified more patients with an increased rate of joint damage progression than the anti-CCP1 test, and in multiple regression analysis CCP2 was the better predictor of joint damage.
Conclusions: The CCP2 test had better diagnostic and prognostic ability than the CCP1 test.

Anti-citrulline antibodies (or more correctly anti-citrullinated protein autoantibodies) are together with rheumatoid factors and C reactive protein the main clinically useful biological markers for rheumatoid arthritis (RA).[1–3]

Anti-citrulline antibodies can be detected using enzyme linked immunosorbent assays (ELISAs) containing cyclic citrullinated peptides (CCPs). The first generation of ELISAs for anti-CCP (CCP1) contains citrullinated peptides derived from human filaggrin. To improve the CCP1 test, epitopes that mimic true conformational epitopes were selected from libraries of citrullinated peptides and were used to construct the second generation anti-CCP assay (CCP2). Recent studies report a higher sensitivity for the CCP2 assay than for the CCP1 test.[4] However, as sensitivity and specificity differ according to the background of the patient population studied, results from studies investigating either CCP1 or CCP2 test are difficult to compare. This is relevant because although the CCP2 test is, at present, the only anti-citrulline antibody test which is commercially available, leading clinics and laboratories continue to use the CCP1 test as an in-house ELISA.[5 6] We set out to evaluate the diagnostic and prognostic features of an in-house CCP1 ELISA and the commercially available CCP2 ELISA in a clinical setting: the Leiden Early Arthritis inception cohort.

PATIENTS AND METHODS

Patients

Anti-CCP2 and anti-CCP1 autoantibodies were measured in serum obtained at baseline from 467 consecutive patients with recent onset arthritis enrolled in the Leiden Early Arthritis Cohort.[7] Before study entry all patients gave their written consent. At the first visit a standard diagnostic investigation was carried out, consisting of patient history, physical and laboratory examinations, and radiographs of hands and feet. Definite diagnoses were made at 1 year of follow up according to international classification criteria or using standard rheumatology textbooks. RA was defined according to the 1987 American College of Rheumatology criteria as the "gold standard".[8] When a diagnosis could not be made, the condition was classified as undifferentiated arthritis.

CCP1 and CCP2 ELISA

The CCP1 ELISA was performed as previously described.[9] The previously determined standard cut off value (92 U) was defined as the value at which the accuracy (sensitivity plus specificity divided by 2) was the highest.[9]

The anti-CCP2 antibody ELISA (Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands) was performed according to the manufacturer's instructions with the cut off point at 25 U as recommended.

Radiographic progression

Radiographs of hands and feet at baseline, at 6 months, and at years 1, 2, 3, and 4 were available for 91 of the 153 patients with RA used in this study.[10] Radiographic damage was scored using the modified Sharp/van der Heijde method[11] in two sessions by one experienced rheumatologist. All patients had at least three radiographs scored.

Statistical analysis

The ability of the anti-CCP1 and anti-CCP2 tests to discriminate between RA and other forms of inflammatory arthropathy was studied. Receiver operating characteristic (ROC) curves were used to determine the optimal cut off point for the CCP1 and CCP2 ELISA in this cohort. As these cut off points differed only marginally from the previously determined cut off point for CCP1 or the recommended

Abbreviations: AUC, area under the curve; CCP, cyclic citrullinated peptide; CI, confidence interval; ELISA, enzyme linked immunosorbent assay; ROC, receiver operating characteristic; RA, rheumatoid arthritis

Table 1 Baseline characteristics at 1 year of 467 of patients with arthritis enrolled in the Early Arthritis Cohort

Characteristic	Value
Age (years), median (range)	49 (16–93)
Female (%)	55
Symptom duration at baseline (months), median (range)	3 (0–24)
IgM rheumatoid factor positive (%)	23
Anti-CCP1 antibody positive (%)	16
Anti-CCP2 antibody positive (%)	20
Swollen joints (n), median (range)	2 (1–14)
Erosions in hands and/or feet (%)	11

manufacturer's cut off point for CCP2, these values were used in the analysis.

In the analysis of rate of joint damage, to correct for radiographs missing, the course of radiographic progression for each patient was fitted using simple linear regression. Differences between groups of interest were tested with a two tailed Mann-Whitney U test. Differences between proportions were tested for their statistical significance using the χ^2 test. For all tests, p values $\leqslant 0.05$ were considered significant.

RESULTS

In this study 467 patients with arthritis who presented at the outpatient clinic of the Leiden University Medical Centre were analysed. Table 1 summarises the baseline characteristic of these patients.

Figure 1 shows the relationship between the sensitivity and specificity of the two anti-CCP tests for different cut off values in ROC curves when testing for a diagnosis of rheumatoid arthritis (RA) at 1 year. In the interval ranging from specificity of 1% to 99% the CCP2 test is more sensitive than the CCP1 test, and accordingly the area under the curve (AUC) for CCP2 is significantly higher than for CCP1: AUC 0.78 (standard error (SE) 0.03) and 0.71 (SE 0.03), respectively.

When the previously optimised cut off point for CCP1 (92 U/l) and the cut off point for CCP2 as recommended by the manufacturer (25 U/l) were used, antibodies against anti-CCP1 were detected in 65/153 (42%) patients diagnosed with RA at 1 year and anti-CCP2 antibodies were found in 82/153 (54%) patients with RA (table 2). In total, 85 patients with RA had at least one anti-CCP antibody, with the majority, 62/85 (73%), having both anti-CCP1 and anti-CCP2 antibodies. Twenty of 85 (24%) anti-CCP positive patients with RA had just anti-CCP2 antibodies and 3/85 (4%) anti-CCP positive patients with RA only had anti-CCP1 antibodies.

The sensitivity of the CCP2 test of 54% was significantly higher than the sensitivity of the CCP1 test of 42% (p = 0.05). At these sensitivities, the specificity for CCP1 of 97% was slightly higher than the specificity for CCP2 of 96%; this was not significant (p = 0.8).

The degree of joint damage, assessed by radiographic imaging, is an important long term outcome of RA. As the presence of anti-citrulline antibodies is an important marker of severe joint damage progression, we evaluated the ability of the CCP1 and CCP2 test to predict joint damage assessed using the Sharp/van der Heijde scoring system.

Over 4 years patients with RA without anti-citrulline antibodies (CCP1−/CCP2−) had a significantly lower rate of joint damage (mean (SD) 1.6 (3.1) Sharp points per year) than RA patients with anti-citrulline antibodies (CCP1−/CCP2+ mean (SD) 7.3 (4.6) points; p = 0.003 and CCP1+/CCP2+ 6.3 (10.3) points; p<0.0001) (data not shown). RA patients with anti-citrulline antibodies had a similar rate of joint damage progression irrespective of the fact that they had both CCP1 and CCP2 antibodies (CCP1+/CCP2+) or just
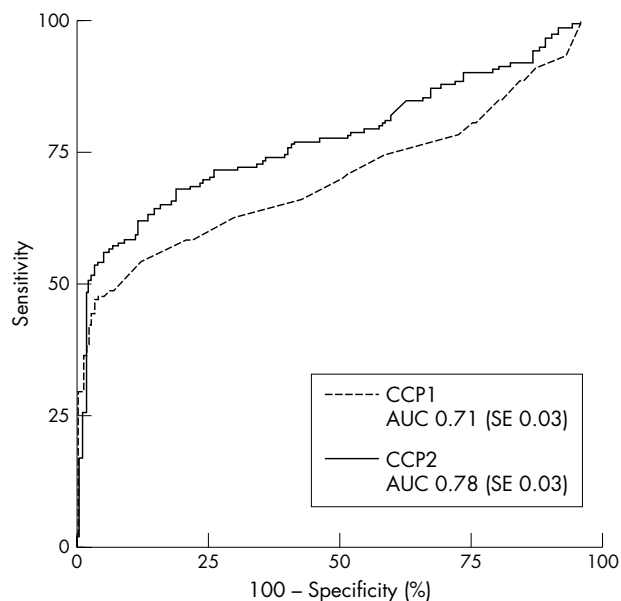

Figure 1 ROC curves of the first (CCP1) and second (CCP2) generation anti-CCP ELISAs as tested on 467 patients with early arthritis with RA at 1 year as the outcome. The difference between the AUC of the ROC of CCP1 and CCP2 was significant (Z = 2.42). When standard cut off points were used the diagnostic properties were as follows (95% confidence interval (CI)): CCP1: sensitivity 42% (95% CI 35% to 50%), specificity 97% (95% CI 95% to 99%), positive predictive value 89% (95% CI 79% to 94%), and negative predictive value 78% (95% CI 73% to 81%); CCP2: sensitivity 54% (95% CI 46% to 61%), specificity 96% (95% CI 93% to 98%), positive predictive value 86% (95% CI 78% to 92%), and negative predictive value 81% (95% CI 77% to 85%).

Table 2 Diagnosis at 1 year of follow up in 467 patients with arthritis enrolled in the Early Arthritis Cohort associated with the presence of anti-CCP1 and CCP2 antibodies at baseline

Diagnosis	All	Anti-CCP1 positive	Anti-CCP2 positive
Rheumatoid arthritis	153	65	82
Undifferentiated arthritis	107	6	7
Crystal induced arthritis	38	0	0
Spondyloarthropathy	29	0	2
Psoriatic arthritis	28	0	1
Mixed connective tissue disease	25	1	0
Osteoarthritis	25	0	0
Reactive arthritis	16	0	1
Sarcoidosis	12	0	0
Malignancy related	6	0	0
Post-traumatic arthritis	5	0	0
Palindromic rheumatism	4	1	1
Septic arthritis	5	0	0
Lyme arthritis	5	0	0
Systemic lupus erythematosus	4	0	0
Juvenile chronic arthritis	1	0	1
Other	4	0	0
Total	467	73	95

CCP2 antibodies (CCP1−/CCP2+) (p = 0.1). One patient was positive for CCP1 but not for CCP2 (CCP1+/CCP2−) and had a rate of joint damage of 9.8 Sharp points per year, similar to other patients with anti-citrulline antibodies (not shown).

Regression analysis with the presence of HLA-DRB1 shared epitope, IgM rheumatoid factor, CCP1 and CCP2 as the predictors identified CCP2 as the most important predictor of

joint damage progression. After exclusion of CCP2, CCP1 was the most important predictor (not shown).

DISCUSSION

Anti-CCP autoantibodies have become one of the key serological markers of RA. We have compared the two currently used CCP ELISAs in patients with early arthritis. The CCP2 test had better diagnostic properties and prognostic relevance than the CCP1 test. The AUC of the ROC curve for the CCP2 test was significantly greater than the AUC for the CCP1 test. In line with previous studies RA patients with anti-CCP antibodies had a higher rate of joint destruction. The presence of anti-CCP2 antibodies was a better prognostic marker than the presence of anti-CCP1 antibodies. Patients positive for CCP2 but not for CCP1 (CCP2+/CCP1−) had a similar rate of joint destruction to that of patients with CCP2 and CCP1 antibodies (CCP1+/CCP2+), and in regression analysis CCP2 was the most important predictor of joint damage progression.

With their high specificity and reasonable sensitivity for RA, anti-CCP ELISAs are particularly useful in the differential diagnosis of early arthritis. When the CCP2 test is used for this purpose its higher sensitivity clearly makes it more useful than the CCP1 test. However, it is not yet clear if this advantage is retained when testing of CCP antibodies is combined with testing for IgM rheumatoid factor. Moreover, the commercially available CCP2 has been optimised by the manufacturer for use on human blood and does not make use of non-citrullinated control peptides. The test, therefore, cannot be recommended for use on samples other than blood of patients with a normal antibody repertoire.

As a result, in-house CCP1 ELISAs are still used by several leading clinics that have the facilities to devise their own assays. A major advantage of the CCP1 test is that the substrates are in the public domain and therefore costs are easier to control. Moreover, as an in-house ELISA this test uses non-citrullinated control peptides, which has the advantage that the test, in addition to being used on patients' blood, may also be optimised for use on animal samples[5] and human synovial fluid.[12]

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