
The use of second generation anti-CCP antibody (anti-CCP2) testing in rheumatoid arthritis – A systematic review

J.P. Riedemann¹, S. Muñoz², A. Kavanaugh³

¹Department of Rheumatology and Clinical Epidemiology, ²CIGES, Faculty of Medicine, Universidad de la Frontera, Temuco, Chile; Center for Innovative Therapy, UCSD, Division of Rheumatology, Allergy and Immunology, La Jolla, California, USA.

J. Pablo Riedemann, MD, MSc, Associate Professor of Rheumatology and Clinical Epidemiology; Sergio Muñoz, PhD, Professor of Biostatistics; Arthur Kavanaugh, MD, Professor of Medicine.

Please address correspondence to Prof. J. Pablo Riedemann, MD.
E-mail: riedeman@ufro.cl

Clin Exp Rheumatol 2005; 23 (Suppl. 39): S69-S76.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2005.

Key words: Rheumatoid arthritis, diagnosis, anti-CCP2 antibodies, systematic review.

ABSTRACT

Objective: To evaluate the diagnostic properties and predictive value of the second generation of anti-CCP antibodies (anti-CCP2) in rheumatoid arthritis (RA) patients.

Methods: A systematic review of the published literature between January 2002 and June 2005 was performed. Data were extracted regarding the sensitivity and specificity of anti-CCP2 antibodies in making an accurate diagnosis of RA, predicting future development of RA, and predicting future radiological damage in RA patients. In addition, the prevalence of CCP2 antibodies in patients with other rheumatic diseases was examined.

Results: Among 38 studies initially identified, 27 provided information on the use of anti-CCP2 testing. Diagnostic properties were assessed in 13 studies; reported sensitivities ranged from 14.4% to 96%, and specificities from 88.9% to 100%. Odds ratios (OR) for the future development of RA varied from 15.9 among previously healthy individuals to 37.8 among a group of patients with undifferentiated arthritis. Several studies suggested that the presence of anti-CCP2 antibodies is highly predictive of current radiographic damage and further damage progression.

Conclusions: Anti-CCP2 has a low sensitivity to be used as a screening test. However, a positive test is highly specific for RA. In addition, anti-CCP2 appears to be highly predictive of the future development of RA in both normal individuals and patients with undifferentiated arthritis. Finally, the presence of anti-CCP2 antibodies appears to predict radiographic damage and progression among patients with RA.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease that affects approximately 0.5–1% of the gen-

eral population (1). It is generally progressive and affects many joints. In patients who do not respond to therapy, RA can cause significant functional disability and loss of quality of life (2). This poor prognosis has led to an emphasis on early diagnosis and aggressive treatment (3). Unfortunately, early diagnosis is difficult in many patients. For example, in many cases of early RA, the ACR classification criteria may not be met.

Over the past few years, several new autoantibodies have been described in patients with RA, and their clinical value has been assessed. Most, such as antiperinuclear factor antibodies (APF), antikeratin antibodies (AKA), and anti-RA33, have not been successfully incorporated into routine clinical practice (4). A new group of autoantibodies that has generated particular interest are the anti-cyclic citrullinated peptide antibodies (CCP). First described by Schellekens (5), the anti-CCP test has generated great interest in the past years.

In recent years a second generation of anti-CCP antibody tests, known as anti-CCP2, have been developed, which may have better performance characteristics than the first generation of tests. The number of publications on anti-CCP2 antibodies is growing exponentially, and a balanced presentation of the characteristics, merits and drawbacks of this test would appear of value for the practicing clinical rheumatologist. This essay presents a critical systematic review of published studies concerning the diagnostic usefulness of the anti-CCP2 antibodies.

Methods

A systematic review of published literature following the methods of evidence-based medicine was performed.

Literature review

A search was conducted using electronic databases (MEDLINE and EM-

BASE), restricted to English and Spanish language articles. Since the term "CCP2 antibodies" has not yet been defined as a MeSH term, free text search was conducted using the following combination:

CCP or CCP2 or anti-citrullinated fibrin antibodies or anti-cyclic citrullinated peptide antibodies or autoantibodies to cyclic citrullinated peptide

In addition, references of the papers initially detected were hand-searched to identify additional relevant reports. Finally, as a quality control, a manual hand search of all reports published during 2004 in the *Annals of Rheumatic Diseases* was performed (no additional publications were detected).

Paper review

The scope of the review was restricted only to those studies in which CCP2 antibodies have been used. According to information provided by the manufacturers (6), kits for the determination of anti-CCP2 antibodies were introduced in early 2002. For some time afterwards, some overlap existed with both anti-CCP and anti-CCP2 tests being available. Thus, this systematic literature review was restricted to papers published between January 2002 and June 2005, in which it was clearly specified that a CCP2 test was used.

As general methodology, an adaptation of the methods proposed by an ACR subcommittee to evaluate the utility of immunologic laboratory testing in rheumatic diseases was used, with special emphasis on the diagnostic aspects (7). The key matters that were to be addressed are:

- 1) Definition of the test;
- 2) Background (historic and methodological considerations);
- 3) Clinical use of the test as a diagnostic tool:
 - Prevalence of a positive test among different patient populations (diseases and country of origin) and normals;
 - Diagnostic test properties (sensitivity, specificity, positive and negative predictive values [PPV and NPV]);
 - Test titer in different populations;
 - Effect of the titer on the performance characteristics of the test;

- Comparison among different manufacturers;
- Clinical use of the test (to whom, what other test might be needed).
- 4) Future areas of research necessary to help define optimal use of the test.

Results

Between January 2002 and June 2005, a total of 38 papers about the clinical use of anti-CCP antibodies have been published (8-45). In the description of the methods, 24 of the investigators specified that their study involved a second generation or anti-CCP2 test. In 12 publications the type of anti-CCP test (i.e. CCP or CCP2) was not specified, and in 2 studies investigators used their own reagents to measure anti-CCP antibodies. The principal authors of the 12 papers who did not specify the type of test evaluated were contacted via e-mail; 11 responded, indicating that 3 had included anti-CCP2. Therefore, 27 of the 36 studies that were performed using commercially available

anti-CCP tests and were published since 2002 involved anti-CCP2 kits. These 27 studies are the sources of data for this systematic review.

The 27 reports in which an anti-CCP2 test was used addressed different aspects of its potential clinical applications including (some publications investigated more than one topic):

- 1) Diagnostic performance: 13 publications (15, 17-20, 24, 26, 27, 33, 34, 38, 39, 41),
- 2) Prevalence and use in other rheumatic diseases: 7 publications (14, 21, 23, 25, 28, 42, 44),
- 3) Use as predictor of future development of RA: 3 publications (12, 35, 40),
- 4) Association with x-ray damage: 4 papers (19, 22, 33, 41),
- 5) Association with changes due to treatment: 3 publication (8, 13, 31),
- 6) Association with RA clinical manifestations: 2 papers (18, 19).

Of these 27 studies, 19 were performed in Europe, 4 in the USA, 2 in Latin

Table I. Reports in which an anti-CCP2 test was used, addressing different aspects of its potential clinical applications

Author (ref. no.)	Reference	Country	Topic
Alessandri (8)	<i>Ann Rheum Dis</i> 2004	Italy	#4
Berglin (12)	<i>Arthritis Research</i> 2004	Sweden	#3
Bobbio-Pallavicini (13)	<i>Arthritis Research</i> 2004	Italy	#4
Bogliolo (14)	<i>J Rheumatol</i> 2005	Italy	#2
Bombardieri (15)	<i>Arthritis Research</i> 2004	Italy	#1
Choi (17)	<i>J Korean Med Sci</i> 2005	Korea	#1
Correa (18)	<i>Biomedica</i> 2004	Colombia/Argentina	#1, #6
De Ricke (19)	<i>Ann Rheum Dis</i> 2004	Belgium	#1, #5, #6
Dubucquoi (20)	<i>Ann Rheum Dis</i> 2004	France	#1
Ferucci (21)	<i>Arthritis Rheum</i> 2005	USA	#2
Forslind (22)	<i>Ann Rheum Dis</i> 2004	Sweden	#5
Gottenberg (23)	<i>Ann Rheum Dis</i> 2005	France	#2
Grootenboer-Mignot (24)	<i>Scand J Rheumatol</i> 2004	France	#1
Kasapcopur (25)	<i>Ann Rheum Dis</i> 2004	Turkey	#2
Lee (26)	<i>Ann Rheum Dis</i> 2003	USA	#1
Lopez-Hoyos (27)	<i>Rheumatology</i> 2004	Spain	#1
Low (28)	<i>J Rheumatol</i> 2004	USA	#2
Mikuls (31)	<i>Arthritis Rheum</i> 2004	USA	#4
Nielen (33)	<i>Ann Rheum Dis</i> 2005	The Netherlands	#1, #5
Pinheiro (34)	<i>Ann Intern Med</i> 2003	Brasil	#1
Ratapaa-Dahlqvist (35)	<i>Arthritis Rheum</i> 2003	Sweden	#3
Suzuki (38)	<i>Scand J Rheumatol</i> 2003	Japan	#1
Vallbracht (39)	<i>Ann Rheum Dis</i> 2004	Germany	#1
Van Gaalen (40)	<i>Arthritis Rheum</i> 2004	The Netherlands	#3
Van Gaalen (41)	<i>Ann Rheum Dis</i> 2005	The Netherlands	#1, #5
Van Noord (42)	<i>Ann Rheum Dis</i> 2005	The Netherlands	#2
Vander Cruyssen (44)	<i>Ann Rheum Dis</i> 2005	Belgium	#2

America, 1 in Japan and 1 in Korea. The first author, journal and year of publication, place of the study and scope of the paper are summarized in Table I. The present review will address topics 1 to 4 listed above.

Definition of the test

Anti-CCP corresponds to a microtitre based enzyme immunoassay for the *in vitro* detection of antibodies in human serum or plasma that react with synthetic peptides containing citrullinate residues.

An antibody system directed against a protein component of the keratohyaline granules in the cytoplasm of buccal mucosa cells was first described in 1964, referred to as anti-perinuclear factor (APF) (46). Despite reasonable sensitivity and specificity, the test never achieved widespread use perhaps owing to technical difficulties.

In 1979, a new group of RA-specific antibodies was described. They were referred to as antikeratin antibodies (AKA). These antibodies react and stain keratin-like structures in the cornified layer of esophagus cryostat section. Different studies have shown that both APF and AKA react with the same antigen moiety, the protein filaggrin (47).

Filaggrin (filament-aggregatin protein) is produced during the late stages of epithelial cell differentiation. It is first synthesized as a phosphorylated precursor protein (profilaggrin), which is partly dephosphorylated and then cleaved in 10-12 filaggrin subunits during differentiation of epithelial cells. At the conclusion of the process, about 20% of the arginine residues are converted into citrulline by action of the enzyme peptidylarginine deaminase.

In 1998, Schellekens (5) demonstrated that citrulline was a major constituent of antigenic determinants recognized by antibodies present in RA sera. In 2000, the same authors published the results of the first ELISA anti-CCP test study, in which they evaluated sera of patients with RA, non RA rheumatic diseases (RD), and some infectious diseases, reporting excellent specificity (98%) and reasonable sensitivity (68%) (48).

Further laboratory work has been performed, and a new generation of highly purified synthetic peptides, containing cyclic citrullinated residues called CCP2, was introduced at the beginning of 2002. This new synthetic peptide is used in the commercial test that is currently available. The anti-CCP2 test is now available primarily from 3 different manufacturers:

- 1) Euro-Diagnostica – The Netherlands (<http://www.eurodiagnostica.com>),
- 2) Axis-Shield – UK (<http://www.axis-shield.com>), and
- 3) Inova Diagnostics – USA (<http://www.inovadx.com>).

1. Clinical use of anti-CCP2 as a diagnostic test

Thirteen publications have addressed the usefulness of CCP2 in the diagnosis of RA (15, 17-20, 24, 26, 27, 33, 34, 38, 39, 41).

Patients

Most studies included RA patients defined according the ACR 1987 criteria. The characteristics of patients included in study populations are described in Table II. Five of the 13 publications included in their sample both patients with early and established RA (18, 20, 24, 38, 39). Four publications studied only established RA, patients with median disease duration of 5 to 14.6 years (15, 17, 19, 34). One publication studied samples of blood donated before clinical diagnosis of RA (33). In one publication, elderly-onset RA and classical RA patients were studied (27). One recent report evaluated CCP2 in a cohort of early arthritis patients (41), and in one report no data were provided about the type of RA patients studied (26), only that the patients had a mean age of 55.4 years.

The female/male rate of the study population was described in 9 reports; the percentage of female patients in those studies range from 55% to 84.9%. The average age was reported in 9 studies, and ranged from 45.9 to 64 years. The average disease duration was from 5 to 14.6 years in established RA patients, and 0.4 years for the early RA patients. Of the 13 papers in which anti-CCP2 was evaluated as a diagnostic test, the manufacturer was Euro-Diagnostica in

6 reports, Axis-Shield in 5 reports, and Inova Diagnostics in 1 report; in 1 report the manufacturer was not specified.

The cut-off point to define a positive test varied from >3.8 IU to 50 IU; 2 studies did not specify the cut-off point used.

Sensitivity and specificity

The diagnostic properties of the anti-CCP2 test evaluated in each report are also presented in Table II. Results varied depending upon the specific characteristics of the RA patients studied.

The sensitivity of the anti-CCP2 test reported for established RA patients varied from 64.4% (39) to 96% (18). In early RA patients, the sensitivity varied from 14.4% (39) to 83.5% (38). In the report of elderly onset RA patients, the reported sensitivity was 64.7% (27). It was not possible to make a summary estimate or weighted average of the sensitivity of anti-CCP2 testing, due to the substantial heterogeneity among individual studies.

Patients included in control groups to assess the specificity of the CCP2 test also had different characteristics. In 6 studies, the control group included both patients with rheumatic diseases and normal individuals (17, 18, 20, 27, 38, 39). In 5 studies, patients with various rheumatic diseases, including inflammatory and non-inflammatory conditions, but no normal individuals, were studied (19, 26, 38, 39, 41). In 3 studies, the type of rheumatic diseases included in the control group were only inflammatory conditions (18, 20, 24). In 3 studies, the control group consisted only of patients with only one specific condition, including patients with hepatitis C virus infection (HCV) (15), patients with polymyalgia rheumatica (PMR) (27), and patients with undifferentiated arthritis (33). Among healthy individuals included as part of the control groups, in most studies the prevalence of anti-CCP2 antibodies ranged from 0 to 0.6%. One study including only 10 healthy controls reported a 10% prevalence (18).

The specificity values reported ranged from 88.9% (38) to 100% (27) (Table II).

Table II. The diagnostic properties of the anti-CCP2 test evaluated in each report.

Author	Manufacturer [†]	Cut-off for a positive test	Patients	Sensitivity (95% CI)	Controls	Specificity (95% CI)
Bombardieri 2004	2	> 5 U	30 RA patients DD1* = 10 years	76.66%	39 HCV patients (8 with articular involvement)	100%
Choi 2005	2	> 3.8 IU	324 RA patients	72.8%	142 OA, 24 FM, 20 SLE, 16 Behcet's, 15 IRA, 34 other RDS, 286 healthy controls	92%
Correa 2004	3	> 30 U	79 RA patients (total) 69 established RA DD2 = 6.5 years, (SD ± 6.2 years) 10 early RA	94% 96% 80%	56 EAA, 25 SLE, 50 Sjögren's, 10 healthy	92%
De Rycke 2004	1	> 25 U	118 RA patients DD1 = 5 years (range 0.12–44 yrs.)	74.4% (67.7–83.2)	OA 25, PMR 25, SLE 17, PSA 9, other 70	97.3% (93.8–99.1)
Dubucquoi 2004	2	No data	140 RA patients (total) DD = 75 yrs. > 2 years 65 pts. < 2 years 21 pts. < 6 months	64.3% 77.3% 49.2% 47.6%	33 healthy, 47 Sjögren's, 51 SLE	96.9%
Grotenboer 2004	1	> 25 U	93 established RA 172 early RA	68.8% 59.9%	21 PsA, 26 primary SS, 44 PMRs	91.2%
Lee 2003	2	> 5 U	103 RA patients - no data on DD Mean age 55.4 years	66%	21 IRA, 21 PsA, 11 spondylitis, 26 inflammatory arthritis, 39 SLE, 23 non-inflammatory, 5 other inflammatory conditions	90.4%
Lopez-Hoyos 2004	1	> 50 U	57 EORA 41 classical RA	64.7% 92.7%	49 PMRs, 24 healthy	100%
Nielen 2005	1	> 25 U	258 early RA DD: 0.4 years (0.3 to 0.7)**	57.8%	121 Undifferentiated arthritis DD: 0.4 years (0.3 to 0.6)**	94.2%
Pinheiro 2003	No data	No data	150 RA patients DD: "long duration"	80%	50 controls No description	98%
Suzuki 2003	2	> 4.5 U	549 RA DD1 = 9.4 years (range 6 weeks to 56 years) 91 early RA 166 RF (-) RA patients	87.6% 83.5% 69.3%	56 SLE, 35 SSs, 30 Sjögren's, 24 PM/DM, 16 MCTD, 15 vasculitis, 15 OA, 15 other rheumatic diseases, 320 normal individuals	88.9%
Vallbracht 2004	1	> 25 U	295 RA patients DD2 = 8.3 years, (SD ± 10.1) 97 early RA (DD < 1 year) 87 all RF (-) RA patients 99 IgM RF (-) RA patients	64.4% 14.4% 34.5% 38.4%	163 Deg/inflam joint diseases 103 CTDs/Vasculitis 154 healthy controls	97.1%
Van Gaalen 2005	1	> 25 U	476 early arthritis cohort 153 RA	53.6%	107 Undifferentiated arthritis (UA), 38 crystal, 29 SPA 28 PsA, 25 MCTD, 25 OA, 16 ReAr, 46 others	93.6% (considering only UA)
						95.9% (considering all controls)

[†]Test manufacturer: 1 = Euro-Diagnostica, The Netherlands; 2 = Axis-Shield, UK; 3 = Inova Diagnostic, USA,
* DD = disease duration; 1: median; 2: mean ** median (interquartile range).

Table III.

Author	Test *	Patients	Sensitivity	Controls	Specificity
Dubucquoi	1, 2, 3	46 RA	1 = 85% 2 = 82% 3 = 85%	22 CTDs	1 = 90.9% 2 = 90.9% 3 = 95.5%
Author Low	Test* 2 and 3	Patients 66 JIA	Prevalence of anti-CCP2 2 = RF (+) polyarthritis = 75% RF (-) polyarthritis/oligoarthritis = 0% 3 = RF (+) polyarthritis = 90% RF (-) polyarthritis/oligoarthritis = 25%	Controls 9 adult RA 34 adult and child SLE 25 Healthy	Prevalence 2 = RA = 0% SLE = 0% Healthy = 0% 3 = RA = 56% SLE = 24% Healthy = 0%

*Test manufacturer: 1 = Eurodiagnostica; 2) Axis-Shield; 3) INOVA.

Prevalence and use of anti-CCP2 in other rheumatic diseases

Seven studies have evaluated the prevalence of anti-CCP2 in other rheumatic disease. Two studies involved patients with PsA (14, 44), with reported prevalences of positive anti-CCP2 antibodies of 15.7% and 7.8% respectively. Two studies involved patients with Sjögren's syndrome and the prevalence of positive results was 7.5% (23) and 1.2% (42). Two other publications reported prevalence between 2% (25) and 90% in patients with juvenile idiopathic arthritis; the variability may have been accounted for by the type of arthritis (28). Finally, in one study patients with juvenile arthritis were assessed and the prevalence was 5.65% (21).

Comparison of test results using different manufacturers

Two studies tested the same serum samples using kits from different manufacturers. One report concerning the usefulness of anti-CCP2 as a diagnostic test (20), compared the results from the

3 test manufacturers in 46 patients with RA and 22 patients with connective tissue damage (CTD) as control. The sensitivity and specificity results obtained from the 3 different providers were very similar (Table III). A recent study compared the performance of kits from different providers in 66 patients with juvenile inflammatory arthritis (JIA) and 68 control subjects (28); most control subjects were CCP2 negative with the Axis-Shield test, but only healthy controls were negative with the INOVA test (Table III).

Anti-CCP2 as a predictor of future development of RA

Three studies have addressed the value of anti-CCP2 antibodies as a potential predictor of the future development of RA (12, 35, 40) (Table IV). Ratapaa-Dahlqvist *et al.* (35) designed a nested case control study using 2 Swedish cohorts. They identified 83 RA patients, who had donated blood samples prior to the development of RA, and compared these cases with randomly

selected controls matched for age, sex, date of sampling and residential area. The overall sensitivity of tests for anti-CCP2 antibodies performed prior to the development of arthritis among the samples of RA patients was 33.7%; specificity was 98.2%.

In a subanalysis of the same cohort, Berglin *et al.* analyzed the presence of the shared epitope, anti-CCP2 antibodies' and tests for rheumatoid factor (RF) in the same group of individuals who subsequently developed RA. Because they were interested in the value of the shared epitope, only 59 RA patients who had blood samples available for DNA analysis were considered. In this group, the sensitivity of anti-CCP2 antibodies as predictor of future development of RA was 37%, with a specificity of 98%. In a logistic regression analysis, anti-CCP2 antibodies had the highest predictive value, with an odds ratio (OR) of 15.9 (12).

Van Gaalen *et al.* studied a cohort of 936 consecutive patients with recent-onset arthritis. Of the original 936 pa-

Table IV.

Author (ref.)	Sample/Design	Follow-up	Sensitivity	Specificity	OR (95% CI)
Ratapaa-Dahlqvist (35)	Nested case-control study 83 RA patients	Retrospective analysis of blood samples collected before disease onset	33.7%	98.2%	No data
Berglin (12)	Same group as above; subset of 59 RA patients	Same as above	37.0%	98.0%	15.9
van Gaalen (40)	936 recent onset arthritis; 318 patients with UA studied	3 years	50.4%	98.4%	37.8 (13.8 – 111.9)

tients, 346 could not be classified with any specific diagnosis after 2 weeks of evaluation. These patients were considered to have undifferentiated arthritis (UA), and were followed for 3 years.. Among 318 of the original 346 available for analysis after 3 years, 40% (127 of 318) had developed RA as defined by ACR criteria. Among 69 UA patients who had anti-CCP2 (+) at baseline, 64 (93%) developed RA (OR 37.8, 95% CI 13.8-111.9) (40).

Possible associations of anti-CCP2 with radiographic damage

The utility of anti-CCP2 antibodies to identify patients who had radiographic progression has been analyzed in 4 reports (19, 22, 33, 41). De Ricke *et al.* reported a higher progression rate among 117 CCP2 (+) patients compared to 63 CCP2 (-) patients ($p = 0.001$) (19), according to a modified Larsen score (from 0 to 160 points) divided by the disease duration in years. Forslin *et al.* (22), reported on 379 patients who had disease durations ≤ 1 year and anti-CCP2 analyses. At baseline, the median Larsen score was 5 (0 to 11) in the 208 CCP2 + patients versus 2 (0 to 10 /25th to 75th centile) among the 171 CCP2 - patients ($p = 0.008$). At the end of the follow up, the Larsen score was 15 (5 to 27) in the CCP2 + patients versus 5 (0 to 14) in the CCP2 - patients ($p = 0.0005$). There was also a higher change score (radiological progression) from baseline to end point of 12 in CCP + patients (4 to 25) versus 4 (0 to 12) in CCP - patients, $p = 0.0005$.

The best predictor of both radiological joint damage and progression, in univariate and multiple analyses, was the Larsen score followed by anti-CCP2.

Nielen *et al.* in a study compared the usefulness of antibodies to citrullinated human fibrinogen vs anti-CCP2 (33), in consecutively gathered 379 early arthritis patients (258 RA and 121 UA) who were followed over 2 years. At the end of the period, they had complete data in 296 (78.1%) patients. Radiological progression was evaluated at 2 years using the Sharp/van der Heijde method. With logistic regression analysis, they identified the anti-CCP2 (+) as the best predictor of x-ray progression

with an OR of 14.8 (95% CI 7.2 to 30.2).

In a recent report, van Gaalen *et al.* (41) compared the diagnostic accuracy and prognostic value of anti-CCP1 vs anti-CCP2 tests in 467 early arthritis patients with a median symptom duration of 3 months. Of those, 153 had RA when evaluated over 4 years. Radiographs of hands and feet were taken at baseline, 6 months, and years 1, 2, 3 and 4, which were available in 91 of the 153 patients. A high rate of joint damage over a period of 4 years was seen in patients who were CCP2 (+) (mean 7.3 points, SD 4.6, $p = 0.003$), compared with those who were negative for anti-CCP2 (mean 1.6 Sharp-points per year; SD 3.1). In regression analysis which included the shared epitope, anti-CCP1, anti-CCP2, and IgM RF, anti-CCP2 antibodies were the most significant predictor of joint damage.

Discussion

The diagnosis of RA may be difficult in early patients who may not have developed typical manifestations of RA. An early definitive diagnosis is desirable for early aggressive treatment. Clinicians have been particularly interested in this new group of anti-CCP2 antibodies, which appear to improve early diagnostic capacities. In the present review, we identified 13 published studies in which anti-CCP2 antibodies were evaluated as a diagnostic test in RA. Important differences were seen in the characteristics of the patients evaluated, as well as the cut points to define a positive test in individual studies. These differences may explain the wide range of sensitivity results reported.

The sensitivity of a diagnostic test (the proportion of true positives in a group of individuals with a certain condition of interest) is a specific property of the test, and should remain constant. However, the calculated sensitivity will vary depending on characteristics of the patients used to evaluate it. If the sample studied includes only more severely affected patients, the sensitivity will probably be higher than in populations with patients who have milder disease, or a group of more heterogeneous pa-

tients. The most fair and accurate estimate of the sensitivity of a diagnostic test will emerge from studies of patients who are truly representative of the population to which the test will be applied in clinical practice.

In this review, the sensitivity of anti-CCP2 antibodies appears reasonably good in studies which evaluated established RA patients, and varied from 64.4% to 96%. However, the sensitivity was as low as 14.4% in early RA or UA, in which clinicians may require more capacity to make a definitive diagnosis. The specificities ranged from 88.9% to 100%. However, the highest specificity (100%) was seen in a study in which the control group is rather small and included only patients with polymyalgia rheumatica and healthy individuals.

Analysis of published studies suggests that is at least debatable whether anti-CCP2 antibodies are a substantial advance as a diagnostic tool. Further studies are required to determine the possible advantages of the test. However, currently it is reasonable to suggest that the anti-CCP2 antibody test should not be used as a screening test to detect RA. One possible exception is psoriatic arthritis, where an important minority of patients have been shown to have anti-CCP2 antibodies in 2 studies.

Beyond diagnostic sensitivity and specificity, one must consider possible positive and negative predictive values in a clinical decision. The positive predictive value is the likelihood that an individual with a positive test result actually has the particular disease of interest, whereas the negative predictive value is the likelihood that an individual with a negative test result actually does not have the disease. These characteristics are highly dependant on the clinical scenario in which the test is used. In other words, even with known and fixed sensitivity and specificity for the test, the results will vary depending on the pretest probability.

To illustrate this point, we analyze an example with a sensitivity of 75% and a specificity of 95% (Table V). Results are presented using pre-test odds, positive and negative likelihood ratios and post test odds, as well as predictive val-

ues. If the test is used in a group of patients with a high probability of having the disease (established and typical clinical picture of RA - estimated pre-test probability 80% - pre-test odds = 4), the positive predictive value will be 98.4% and the negative predictive value 48.7%. However, if the probability of having or not having the disease is even (pre-test probability 50% - pre-test odds = 1), a positive test result will determine a positive predictive value of 88.2% and a negative predictive value of 78.3%. A third situation could be if the same test is applied to a population of undifferentiated arthritis (estimated pre-test probability of RA 20% - pre-test odds 0.25), the positive predictive value will be 65.2% and the negative predictive value 93.5%. These differences illustrate that predictive values and the post-test odds are as important as sensitivity and specificity in evaluating performance of a laboratory test in clinical care.

An additional concern may involve the comparability of different available anti-CCP2 tests evaluated in only two reports. In the study of Dubucquoi (20), reagents from 3 manufacturers yielded comparable results in terms of sensitivity and specificity. By contrast, some important differences were observed in the study of Low (28), which compared results of tests performed with the Axis-Shield and the INOVA reagents. In patients with juvenile arthritis, the prevalence of anti-CCP2 in patients with rheumatoid factor positive polyarthritis varied from 75% with the Axis-Shield test to 90% with the INOVA test. In patients with rheumatoid factor negative polyarthritis/oligoarthritis, the prevalence of anti-CCP was 0% with the Axis-Shield tests, versus 25% with the INOVA test. Differences

were also seen in the control groups; all patients were CCP2 negative with the Axis-Shield test with only healthy individuals negative and 56% of RA and 24% of SLE patients positive with the INOVA test, which all were negative with the Axis-Shield tests.

Another issue is the cut off value used to define a positive result. As presented in the results, the cut off varied significantly in various reports of studies conducted with a CCP2 test, even when provided by the same manufacturer. Some standardization would appear desirable.

In addition to diagnostic utility, anti-CCP2 antibodies have been evaluated as predictors of future development of RA and as predictors of radiological damage, using 2 different approaches. Two studies (12,35) evaluated the presence of anti-CCP2 antibodies in blood samples donated prior to the onset of disease in normal individuals. The sensitivity of the anti-CCP2 to identify future RA was estimated at 33.7% to 37%, with a specificity of 98%, an odds ratio of 15.9. In the other study (40), anti-CCP2 identified an odds ratio of future development of RA of 37.8 in patients with undifferentiated arthritis. Thus, a normal individual or a patient with undifferentiated arthritis with a positive anti-CCP2 antibody has a substantial risk of future development of RA.

The 4 individual studies that have addressed radiological progression all agree that the presence of anti-CCP2 antibodies is associated with greater radiographic progression, with an odds ratio of 14.8 (95% CI 7.2 to 30.2). The presence of anti-CCP2 antibodies should be considered as a predictor of future development of RA as well as marker of progressive radiologic progression.

It remains unknown whether titers of anti-CCP2 antibodies are associated with higher risk of radiological progression.

References

1. LEE DM, WEINBLATT ME: Rheumatoid arthritis. *Lancet* 2001; 358: 903-11.
2. WILES NJ, SYMMONS DPM, HARRISON B *et al.*: Estimating the incidence of rheumatoid arthritis: trying to hit a moving target. *Arthritis Rheum* 1999; 42: 1339-46.
3. WEINBLATT ME: Rheumatoid arthritis: More aggressive approach improves outlook. *Cleve Clin J Med* 2004; 71: 409-13.
4. VAN BOEKEL MAM, VOSSENAAR ER, VAN DEN HOOGEN FHJ, VAN VENROOIJ WJ: Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* 2002; 4: 87-93.
5. SCHELLEKENS GA, DE JONG BAW, VAN DEN HOOGEN FHJ, VAN DE PUTTE LBA: Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1998; 101: 273-81.
6. EURO-DIAGNOSTICA B.V.: Beijerinckweg 18, L-6827 BN Arnhem, The Netherlands. <http://www.eurodiagnostica.com>
7. Guidelines for immunologic laboratory testing in rheumatic diseases: An introduction. *Arthritis Rheum* 2002; 47: 429-33.
8. ALESSANDRI C, BOMBARDIERI M, PAPA N *et al.*: Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNF α therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. *Ann Rheum Dis* 2004; 63: 1218-21.
9. AVCIN T, CIMAZ R, FALCINI F *et al.*: Prevalence and clinical significance of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis. *Ann Rheum Dis* 2002; 61: 608-11.
10. BAS S, GENEVAY S, MEYER O, GABAY C: Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. *Rheumatology* 2003; 42: 677-80.
11. BAS S, PERNEGER TV, SEITZ M, TIERCYJM, ROUX-LOMBARD P, GUERNE PA: Diagnostic tests for rheumatoid arthritis: comparison of anti-cyclic citrullinated peptide antibodies, anti-keratin antibodies and IgM rheumatoid factor. *Rheumatology* 2002; 41: 809-14.
12. BERGLIN E, PADYUKOV L, SUNDIN U *et al.*: A combination of autoantibodies to cyclic cit-

Table V.

Pre-test probability	Pre-test odds	Sensitivity	Specificity	LR+ (*)	LR-	PPV(**)	NPV(**)	Post-test odds of a (+) result
80%	4	75%	95%	15	0.263	98.4%	48.7%	60
50%	1	75%	95%	15	0.263	88.2%	78.3%	15
20%	0.25	75%	95%	15	0.263	65.2%	93.5%	3.75

*LR: Likelihood ratio; **PPV: positive predictive value; NPV: negative predictive value

- rullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. *Arthritis Res* 2004; 6: R303-08.
13. BOBBIO-PALLAVICINI F, ALPINI C, CAPO-
RALI R, AVALLE S, BUGATTI S, MONTECUC-
CO C: Autoantibody profile in rheumatoid
arthritis during long-term infliximab treat-
ment. *Arthritis Res* 2004; 6: R264-272.
 14. BOGLIOLO L, ALPINI C, CAPO-
RALI R *et al.*: Antibodies to cyclic citrullinated peptides in
psoriatic arthritis. *J Rheumatol* 2005; 32: 511-
15.
 15. BOMBARDIERI M, ALESSANDRI C, LABBA-
DIA G *et al.*: Role of anti-cyclic citrullinated
peptide antibodies in discriminating patients
with rheumatoid arthritis from patients with
chronic hepatitis C infection-associated poly-
articular involvement. *Arthritis Res* 2004; 6:
R137-141.
 16. CARAMACHI P, BIASI D, TONOLLI E, PIER-
OPAN S *et al.*: Antibodies against cyclic cit-
rullinated peptides in patients affected by
rheumatoid arthritis before and after inflix-
imab treatment. *Rheumatol Int* 2005 February
On Line.
 17. CHOI SW, LIM MK, SHIN DH, PARK JJ, SHIN
SC: Diagnostic performances of anti-cyclic
citrullinated peptides antibody and antifilag-
grin antibody in Korean patients with rheu-
matoid arthritis. *J Korean Med Sci* 2005; 20:
473-8.
 18. CORREA PA, TOBON GJ, CITERA G *et al.*:
Anticuerpos anti-CCP en artritis reumatoidea:
relacion con características clínicas, cito-
quinas Th1/Th2 y HLA-DRB1. *Biomedica* 2004;
24: 140-52.
 19. DE RYCKE L, PEENE I, HOFFMAN IEA *et al.*:
Rheumatoid factor and anti citrullinated pro-
tein antibodies in rheumatoid arthritis: diag-
nostic value, associations with radiological
progression rate, and extra-articular manifes-
tations. *Ann Rheum Dis* 2004; 63: 1587-93.
 20. DUBUCQUOI S, SOLAU-GERVAIS E, LE-
FRANC D *et al.*: Evaluation of anti-citrulli-
nated filaggrin antibodies as hallmarks for the
diagnosis of rheumatic diseases. *Ann Rheum
Dis* 2004; 63: 415-19.
 21. FERUCCI ED, MAJKA DS, PARRISH L *et al.*:
Antibodies against cyclic citrullinated pep-
tide are associated with HLA-DR4 in simplex
and multiplex polyarticular-onset juvenile
rheumatoid arthritis. *Arthritis Rheum* 2005;
52: 239-46.
 22. FORSLIND K, AHLMEN M, EBERHARDT K,
HAFSTROM I, SVENSSON B for the BARFOT
STUDY GROUP: Prediction of radiological out-
come in early rheumatoid arthritis in clinical
practice: role of antibodies to citrullinated
peptides (anti-CCP). *Ann Rheum Dis* 2004;
63: 1090-5.
 23. GOTTENBERG J-E, MIGNOT S, NICAISSE-
ROLLAND P *et al.*: Prevalence of anti-cyclic
citrullinated peptide and anti-keratin antibod-
ies in patients with primary Sjögren's syn-
drome. *Ann Rheum Dis* 2005; 64: 114-17.
 24. GROOTENBOER-MIGNOT S, NICAISE-RO-
LAND P, DELAUNAY C, MEYER O, CHOL-
LET-MARTIN S, LABARRE C: Second gener-
ation anti-cyclic citrullinated peptide (anti-
CCP2) antibodies can replace other filaggrin
antibodies and improve rheumatoid arthritis
diagnosis. *Scand J Rheumatol* 2004; 33: 218-
20.
 25. KASAPCOPUR O, ALTUN S, ASLAN A *et al.*:
Diagnostic accuracy of anti-cyclic citrullinat-
ed peptide antibodies in juvenile idiopathic
arthritis. *Ann Rheum Dis* 2004; 63: 1687-9.
 26. LEE DM, SCHUR PH: Clinical utility of anti-
CCP assay in patients with rheumatic dis-
eases. *Ann Rheum Dis* 2003; 62: 870-4.
 27. LOPEZ-HOYOS M, RUIZ DE ALEGRIA, BLAN-
CO R *et al.*: Clinical utility of anti-CCP anti-
bodies in the differential diagnosis of elderly-
onset rheumatoid arthritis and polymyalgia
rheumatica. *Rheumatology* 2004; 43: 655-
657.
 28. LOW JM, CHAUHAN AK, KIETZ DA, DAUD
U, PEPMEUILLER PH, MOORE TL: Determi-
nation of anti-cyclic citrullinated peptide anti-
bodies in the sera of patients with juvenile
idiopathic arthritis. *J Rheumatol* 2004; 31:
1829-33.
 29. MADDALI S, MANETTI R, MELCHIORRE D
et al.: Anti-cyclic citrullinated Peptide anti-
bodies are highly associated with severe bone
lesions in rheumatoid arthritis anti-CCP and
bone damage in RA. *Autoimmunity* 2004; 37:
495-501.
 30. MEYER O, LABARRE C, DOUGADOS M *et al.*:
Anticitrullinated protein/peptide antibody
assays in early rheumatoid arthritis for pre-
dicting five years radiographic damage. *Ann
Rheum Dis* 2003; 62: 120-6.
 31. MIKULS TR, O'DELL JR, STONER JA *et al.*:
Association of rheumatoid arthritis treatment
response and disease duration with declines
in serum levels of IgM rheumatoid factor and
anti-cyclic citrullinated peptide antibody.
Arthritis Rheum 2004; 50: 3776-82.
 32. NIELEN MMJ, VAN SCHAARDENBURG D,
REESINK HW *et al.*: Specific autoantibodies
precede the symptoms of rheumatoid arthri-
tis. A study of serial measurements in blood
donors. *Arthritis Rheum* 2004; 50: 380-6. (in-
house test).
 33. NIELEN MMJ, VAN DER HORST AR, VAN
SCHAANDENBURG D *et al.*: Antibodies to
citrullinated human fibrinogen (ACF) have
diagnostic and prognostic value in early arth-
ritis. *Ann Rheum Dis* Published online Janu-
ary 2005.
 34. PINHEIRO GC, SCHEIBERG MA, APARECIDA
DA SILVA M: Anti-cyclic citrullinated peptide
antibodies in advanced RA. *Ann Intern Med*
2003; 139: 234-5.
 35. RANTAPAA-DAHLQVIST S, DE JONG BAW,
BERGLIN E *et al.*: Antibodies against cyclic
citrullinated peptide and IgA rheumatoid fac-
tor predict the development of rheumatoid
arthritis. *Arthritis Rheum* 2003; 48: 2741-9.
 36. SALVADOR G, GOMEZ A, VIÑAS O *et al.*:
Prevalence and clinical significance of anti-
cyclic citrullinated peptide and antikeratin an-
tibodies in palindromic rheumatism. An abor-
tive form of rheumatoid arthritis? *Rheum-
atology* 2003; 42: 972-75.
 37. SARAUX A, BERTHELOT JM, DEVAUCH-
ELLE V *et al.*: Value of antibodies to citrulli-
nated-containing peptides for diagnosing ear-
ly rheumatoid arthritis. *J Rheumatol* 2003;
30: 2535-9.
 38. SUZUKI K, SAWADA T, MURAKAMI A *et al.*:
High diagnostic performance of ELISA de-
tection of antibodies to citrullinated antigens
in rheumatoid arthritis. *Scand J Rheumatol*
2003; 32: 197-204.
 39. VALLBRACHT I, RIEBER J, OPPERMAN M,
FORGER F, SIEBERT U, HELMKE K: Diag-
nostic and clinical value of anti-cyclic citrul-
linated peptide antibodies compared with
rheumatoid factor isotypes in rheumatoid
arthritis. *Ann Rheum Dis* 2004; 63: 1079-
1084.
 40. VAN GAALEN FA, LINN-RASKER SP, VEN-
ROOIJ WJ *et al.*: Autoantibodies to cycli cit-
rullinated peptides predict progression to
rheumatoid arthritis in patients with undiffer-
enciated arthritis. A prospective cohort study.
Arthritis Rheum 2004; 50: 709-15.
 41. VAN GAALEN FA, VISSER H, HUIZINGA TW:
A Comparison of the diagnostic accuracy and
prognostic value of the first-and second anti-
cyclic citrullinated peptides autoantibody
(CCP1 and CCP2) test for rheumatoid arthri-
tis. *Ann Rheum Dis* published online 30 Mar
2005
 42. VAN NOORD C, HOOIJKAAS H, VAN DEN
GOORBERGH BCM *et al.*: Diagnostic value of
anti-cyclic citrullinated peptide antibodies to
detect rheumatoid arthritis in patients with
Sjögren's syndrome. *Ann Rheum Dis* 2005;
64: 160-2.
 43. VAN ROSSUM M, VAN SOESBERGEN, DE KORT
et al.: Anti-cyclic citrullinated peptide (anti-
CCP) antibodies in children with juvenile
idiopathic arthritis. *J Rheumatol* 2003; 30:
825-9.
 44. VANDER CRUYSSSEN B, HOFFMAN IEA, ZMI-
ERCZAK HG *et al.*: Anti-citrullinated peptide
antibodies may occur in patients with psori-
atic arthritis. *Ann Rheum Dis* Online 4 Feb
2005.
 45. ZENG X, AIM T, TIAN X *et al.*: Diagnostic val-
ue of anti-cyclic citrullinated peptide anti-
body in patients with rheumatoid arthritis. *J
Rheumatol* 2003; 30: 1451-5.
 46. NIENHUIS RLF, MANDENA EA: A new ser-
um factor in patients with rheumatoid arthri-
tis. The antiperinuclear factor. *Ann Rheum
Dis* 1964; 23: 302-5
 47. HOET RM, VAN VENROOIJ WJ: The antiperin-
uclear factor and antikeratin antibodies in
rheumatoid arthritis. In SMOLEN J, KALDEN
J and MAINIRN (Eds.): *Rheumatoid Arthritis*.
Berlin, Springer Verlag 1992: 299-318.
 48. SCHELLEKENS GA, VISSER H, DE JONG BAW
et al.: The diagnostic properties of rheuma-
toid arthritis antibodies recognizing a cyclic
citrullinated peptide. *Arthritis Rheum* 2000;
43: 155-63.77