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, ºCIGES, Faculty of Medicine, Universidad de la Frontera, Temuco, Chile; Center for Innovative Therapy, UCSD, Division of Rheumatology, Allergy and Immunology, La Jolla, California, USA.

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 general 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 filagrin 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 America, and 2 in Asia. These 27 studies are the sources of data for this systematic review.

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

<table>
<thead>
<tr>
<th>Author (ref. no.)</th>
<th>Reference</th>
<th>Country</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alessandri (8)</td>
<td>Ann Rheum Dis 2004</td>
<td>Italy</td>
<td>#4</td>
</tr>
<tr>
<td>Berglin (12)</td>
<td>Arthritis Research 2004</td>
<td>Sweden</td>
<td>#3</td>
</tr>
<tr>
<td>Bobbio-Pallavicini (13)</td>
<td>Arthritis Research 2004</td>
<td>Italy</td>
<td>#4</td>
</tr>
<tr>
<td>Bogliolo (14)</td>
<td>J Rheumatol 2005</td>
<td>Italy</td>
<td>#2</td>
</tr>
<tr>
<td>Bombardieri (15)</td>
<td>Arthritis Research 2004</td>
<td>Italy</td>
<td>#1</td>
</tr>
<tr>
<td>Choi (17)</td>
<td>J Korean Med Sci 2005</td>
<td>Korea</td>
<td>#1</td>
</tr>
<tr>
<td>Correa (18)</td>
<td>Biomedica 2004</td>
<td>Colombia/Argentina</td>
<td>#1, #6</td>
</tr>
<tr>
<td>De Ricke (19)</td>
<td>Ann Rheum Dis 2004</td>
<td>Belgium</td>
<td>#1, #5, #6</td>
</tr>
<tr>
<td>Dubucquoi (20)</td>
<td>Ann Rheum Dis 2004</td>
<td>France</td>
<td>#1</td>
</tr>
<tr>
<td>Ferucci (21)</td>
<td>Arthritis Rheum 2005</td>
<td>USA</td>
<td>#2</td>
</tr>
<tr>
<td>Forsslind (22)</td>
<td>Ann Rheum Dis 2004</td>
<td>Sweden</td>
<td>#5</td>
</tr>
<tr>
<td>Gottenberg (23)</td>
<td>Ann Rheum Dis 2005</td>
<td>France</td>
<td>#2</td>
</tr>
<tr>
<td>Grootenboer-Mignot (24)</td>
<td>Scand J Rheumatol 2004</td>
<td>France</td>
<td>#1</td>
</tr>
<tr>
<td>Kasapcopur (25)</td>
<td>Ann Rheum Dis 2004</td>
<td>Turkey</td>
<td>#2</td>
</tr>
<tr>
<td>Lee (26)</td>
<td>Ann Rheum Dis 2003</td>
<td>USA</td>
<td>#1</td>
</tr>
<tr>
<td>Lopez-Hoyos (27)</td>
<td>Rheumatology 2004</td>
<td>Spain</td>
<td>#1</td>
</tr>
<tr>
<td>Low (28)</td>
<td>J Rheumatol 2004</td>
<td>USA</td>
<td>#2</td>
</tr>
<tr>
<td>Mikuls (31)</td>
<td>Arthritis Rheum 2004</td>
<td>USA</td>
<td>#4</td>
</tr>
<tr>
<td>Nielen (33)</td>
<td>Ann Rheum Dis 2005</td>
<td>The Netherlands</td>
<td>#1, #5</td>
</tr>
<tr>
<td>Pinheiro (34)</td>
<td>Ann Intern Med 2003</td>
<td>Brasil</td>
<td>#1</td>
</tr>
<tr>
<td>Ratapaa-Dahlqvist (35)</td>
<td>Arthritis Rheum 2003</td>
<td>Sweden</td>
<td>#3</td>
</tr>
<tr>
<td>Suzuki (38)</td>
<td>Scand J Rheumatol 2003</td>
<td>Japan</td>
<td>#1</td>
</tr>
<tr>
<td>Vallbracht (39)</td>
<td>Ann Rheum Dis 2004</td>
<td>Germany</td>
<td>#1</td>
</tr>
<tr>
<td>Van Gaalen (40)</td>
<td>Arthritis Rheum 2004</td>
<td>The Netherlands</td>
<td>#3</td>
</tr>
<tr>
<td>Van Gaalen (41)</td>
<td>Ann Rheum Dis 2005</td>
<td>The Netherlands</td>
<td>#1, #5</td>
</tr>
<tr>
<td>Van Noord (42)</td>
<td>Ann Rheum Dis 2005</td>
<td>The Netherlands</td>
<td>#2</td>
</tr>
<tr>
<td>Vander Cruysen (44)</td>
<td>Ann Rheum Dis 2005</td>
<td>Belgium</td>
<td>#2</td>
</tr>
</tbody>
</table>
In 1998, Schellekens (5) demonstrated results of the first ELISA anti-CCP test synthesized as a phosphorylated precursor that reacts with 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 deiminase. 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>
<thead>
<tr>
<th>Author</th>
<th>Manufacturer</th>
<th>Cut-off for a positive test</th>
<th>Patients</th>
<th>Sensitivity (95% CI)</th>
<th>Controls</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombardieri</td>
<td>2</td>
<td>&gt; 5 U</td>
<td>30 RA patients DD1 = 10 years</td>
<td>76.66%</td>
<td>39 HCV patients, (8 with articular involvement)</td>
<td>100%</td>
</tr>
<tr>
<td>Choi</td>
<td>2</td>
<td>&gt; 3.8 IU</td>
<td>324 RA patients</td>
<td>72.8%</td>
<td>142 OA, 24 F M, 20 SLE, 16 Behcet’s, 15 RA, 34 other RDS, 286 healthy controls</td>
<td>92%</td>
</tr>
<tr>
<td>Correa</td>
<td>3</td>
<td>&gt; 30 U</td>
<td>79 RA patients (total) 69 established RA DD2 = 6.5 years, (SD = 6.2 years) 10 early RA</td>
<td>94% 96%</td>
<td>56 EAA, 25 SLE, 50 Sjögren’s, 10 healthy</td>
<td>92%</td>
</tr>
<tr>
<td>De Rijke</td>
<td>1</td>
<td>&gt; 25 U</td>
<td>118 RA patients DD1 = 5 years (range 0.12-44 yrs.)</td>
<td>74.4% (67.7-83.2)</td>
<td>OA 25, PMR 25, SLE 17, PSA 9, other 70</td>
<td>97.3% (93.8-99.1)</td>
</tr>
<tr>
<td>Dubucquoy</td>
<td>2</td>
<td>No data</td>
<td>140 RA patients (total) DD = 75 yrs. &gt; 2 years 65 yrs. &lt; 2 years 21 yrs. &lt; 6 months</td>
<td>64.3% 77.3% 48.2% 47.6%</td>
<td>33 healthy, 47 Sjögren’s, 51 SLE</td>
<td>96.9%</td>
</tr>
<tr>
<td>Groteboer</td>
<td>1</td>
<td>&gt; 25 U</td>
<td>93 established RA 172 early RA</td>
<td>68.8% 89.9%</td>
<td>21 PsA, 26 primary SS, 44 PMRs</td>
<td>91.2%</td>
</tr>
<tr>
<td>Lee</td>
<td>2</td>
<td>&gt; 5 U</td>
<td>103 RA patients - no data on DD Mean age 25-4 years</td>
<td>60%</td>
<td>21 RA, 21 PsA, 11 spondylitis, 26 inflammatory arthritis, 39 SLE, 23 non-inflammatory, 5 other inflammatory conditions</td>
<td>90.4%</td>
</tr>
<tr>
<td>Lopez-Hoyos</td>
<td>1</td>
<td>&gt; 50 U</td>
<td>57 EORA 41 classical RA</td>
<td>64.7% 92.7%</td>
<td>49 PMRs, 24 healthy</td>
<td>100%</td>
</tr>
<tr>
<td>Nielsen</td>
<td>1</td>
<td>&gt; 25 U</td>
<td>258 early RA DD 0.4 years (0.3 to 0.7)**</td>
<td>57.8%</td>
<td>121 Undifferentiated arthritis DD: 0.4 years (0.3 to 0.6)**</td>
<td>94.2%</td>
</tr>
<tr>
<td>Pinheiro</td>
<td>No data</td>
<td>No data</td>
<td>150 RA patients DD &quot;long duration&quot;</td>
<td>80%</td>
<td>50 controls No description</td>
<td>98%</td>
</tr>
<tr>
<td>Suzuki</td>
<td>2</td>
<td>&gt; 4.5 U</td>
<td>549 RA DD1 = 9.4 years (range 6 weeks to 50 years) 91 early RA 166 RF (+) RA patients</td>
<td>87.6% 88.5% 69.3%</td>
<td>56 SLE, 35 SSs, 30 Sjögren’s, 24 PM/DM, 16 MCTD, 15 vasculitis, 15 OA, 15 other rheumatic diseases, 320 normal individuals</td>
<td>88.9%</td>
</tr>
<tr>
<td>Völlbracht</td>
<td>1</td>
<td>&gt; 25 U</td>
<td>295 RA patients DD = 8.3 years, (SD = 10.1) 97 early RA (DD &lt; 1 year) 87 all RF (+) RA patients 99 mMRF (-) RA patients</td>
<td>64.4% 14.4% 34.5% 38.4%</td>
<td>163 Deg/inflamm joint diseases 118 CTDs Vasculitis 154 healthy controls</td>
<td>97.1%</td>
</tr>
<tr>
<td>Van Gaalen</td>
<td>1</td>
<td>&gt; 25 U</td>
<td>476 early arthritis cohort 153 RA</td>
<td>53.6%</td>
<td>107 Undifferentiated arthritis (UA), 38 crystal, 29 SPA 28 PsA, 25 MCTD, 25 OA, 16 ReAr, 46 others</td>
<td>93.6% (considering only UA) 95.9% (considering all controls)</td>
</tr>
</tbody>
</table>

* Test manufacturer: 1 = Euro-Diagnostica, The Netherlands; 2 = Axis-Shield, UK; 3 = Inova Diagnostic, USA, 4 = Di-91, Germany.

* DD = disease duration: 1: median; 2: mean ** median (interquartile range).
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 patients, 318 with U. studied 3 years later. The sensitivity and specificity of anti-CCP2 antibodies were 50.4% and 98.4% respectively (37.8 (13.8 – 111.9).

Table III.

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

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

Table IV.

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

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Table V.

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

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