

Anti-CCP Antibody Detection Facilitates Early Diagnosis and Prognosis of Rheumatoid Arthritis

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Abstract: Rheumatoid arthritis (RA) is a common systemic autoimmune disease with a prevalence of about 1% worldwide [1]. The American College of Rheumatology (ACR) criteria for the classification of RA [2] are not very well suited to diagnose RA at an early stage of the disease [3, 4], because these criteria rely heavily on the expression of clinical symptoms of RA. In early RA these clinical parameters are often not (yet) manifest. Therefore, a specific and sensitive (serological) marker, which is present very early in the disease, is needed. A good marker should ideally not only indicate the development of the disease, but also be able to predict its erosive or non-erosive progression. The serological parameter that meets these requirements for a good and useful marker for early RA is the anti-citrullinated protein antibody. The sensitivity of this antibody is comparable to that of the rheumatoid factor (RF) (approximately 80%), but its specificity is much higher, about 98%. Several assays have been developed to detect this class of autoantibodies, which are termed anti-CCP because the most sensitive test is based upon cyclic citrullinated peptides. This review will discuss the potential of this autoantibody system for the diagnosis and prognosis of RA.

Keywords: Anti-CCP antibodies; diagnosis; prognosis; rheumatoid arthritis; serological marker.

RHEUMATOID ARTHRITIS

RA is characterized by chronic inflammation of the synovial joints, which leads to joint swelling, progressive joint erosions and eventually to disability. Current therapies for RA are mainly anti-inflammatory strategies and so far no therapeutics have been developed that cure the disease. Nevertheless, these therapies can slow down the extent of swelling and erosive damage (reviewed in [5]). Insights gained over the last years suggest that aggressive therapy given early in the disease has the greatest therapeutic potential [6, 7]. Importantly, rheumatologists need to be able to target the use of potentially toxic and expensive drugs to those patients where the benefits clearly outweigh the risks [8]. It is therefore crucial to have a reliable and specific test to identify the RA patients prior to the occurrence of joint damage. The only serological marker included in the ACR criteria is RF. Although RF antibodies can be detected in the majority of RA patients, they are not very specific for RA [9, 10]. Because of their early presence and high specificity, anti-CCP antibodies represent a superior marker for the diagnosis and prognosis of RA, as evidenced by the data summarized below.

RA-ASSOCIATED AUTOANTIBODIES

Throughout the last decades several autoantibody systems have been described that are associated with RA.

RF, the oldest and most widely known of these autoantibodies, is directed to the Fc part of IgG molecules [11, 12]. RF can be detected in up to 80% of RA patients, but these antibodies are found also in several other diseases as well as in healthy controls (especially elderly: 10-30% [13]), lowering its specificity for RA.

Several other RA-associated antibody systems have also been described (reviewed in [14]). Based on a lack of sensitivity or specificity most of these systems have never made it as an acknowledged serological test for RA.

RA-SPECIFIC AUTOANTIBODIES

The autoantibody system with the greatest clinical potential for RA are the antibodies directed to citrulline-containing epitopes. Citrulline is a nonstandard amino acid, as it is not incorporated into proteins during protein synthesis. It can, however, be generated via post-translational modification of arginine residues by peptidylarginine deiminase (PAD; EC 3.5.3.15, reviewed in [15, 16]) enzymes. Conversion of arginine into citrulline involves the replacement of an amine group by an oxygen atom in the side chain of this amino acid, and is associated with the loss of a positive charge (at neutral pH). Although this conversion results in a relatively small chemical alteration of the protein involved, the reactivity of autoantibodies reactive with citrulline-containing epitopes seems to be critically dependent on the presence of a citrulline residue [17].

The history of anti-citrullinated protein antibodies started exactly four decades ago when the APF (anti-perinuclear

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factor) antibodies were described [18]. These antibodies, as well as the independently described anti-keratin antibodies (AKA) [19], can be detected in about half of the RA patients with high specificity. Because these assays are laborious and inconvenient immunofluorescence tests, which hampers inter-laboratory standardization, they never became mainstream diagnostic tests. With the discovery that filaggrin (a naturally citrullinated protein from epidermis) is the common antigen targeted by both the APF and the AKA [20, 21] new assays for the detection of the RA-specific antibodies could be developed using isolated filaggrin as the antigen [22]. The reactivity of the antibodies was shown to be completely dependent on the presence of citrulline residues in the targeted antigen [17, 23]. With this knowledge several studies have attempted to improve the sensitivity of the anti-filaggrin antibody (AFA) assay by using *in vitro* citrullinated recombinant filaggrin as an antigen. AFA could be detected in about 60% of RA patients [24, 25]. A problem with these assays is, however, that it is difficult to prepare filaggrin with a reproducible citrulline content, making standardization of these tests difficult. In addition, it has been shown that in sera from RA and non-RA individuals (natural) autoantibodies to non-citrullinated filaggrin exist [25]. This may render false positive results.

ANTI-CYCLIC CITRULLINATED PEPTIDE AUTOANTIBODIES: DIAGNOSTIC POTENTIAL

A major breakthrough came from the development of an ELISA that used a synthetic citrullinated peptide derived from filaggrin [17]. This procedure greatly simplified the detection of anti-citrullinated protein antibodies and allowed for better standardization. The use of a cyclic filaggrin-derived peptide, in which the citrulline-containing epitope is optimally exposed, resulted in an enhanced sensitivity [26]. Using this first generation CCP test (CCP1) a sensitivity of 68% could be reached with a satisfying high specificity for RA of 97-98% [26]. Studies using the CCP1 test have been reviewed by van Boekel *et al.* [14].

To improve the sensitivity of the CCP test, several dedicated libraries of citrulline-containing peptides were screened with a pool of RA sera. This screening finally led to the development of the second generation CCP test

(CCP2) that displays sensitivities comparable to that of RF (approximately 80%) but with superior specificity (98%) [9]. Over the last few years many independent studies have confirmed the diagnostic performance of the CCP2 test (reviewed by Vossenaar *et al.* [27]). The results of these studies are summarized in Table 1.

The high specificity of anti-CCP can be valuable in distinguishing RA from other diseases which are clinically very similar to RA in its early stages and in which RF positivity is relatively frequently observed. Examples of such disorders are systemic lupus erythematosus associated with erosive arthritis [28] and hepatitis C virus (HCV) associated arthritis [29]. In two recent studies [30, 31], none [30] and 7% [31] of patients with HCV-associated arthritis patients tested positive for anti-CCP2 antibody, while, respectively, 38% [30] and 76% [31] of these patients were RF positive.

From all reported studies it is clear that anti-CCP antibodies enable clinicians to more effectively diagnose RA patients, which eventually will lead to a reduction of overtreatment.

ANTI-CCP AUTOANTIBODIES AS PREDICTIVE MARKERS FOR DISEASE

Next to being sensitive and specific, good markers of disease should be detectable as early in the disease process as possible. In healthy individuals the occurrence of anti-CCP antibodies is less than 1%. When a random population attending a rheumatology clinic was tested for anti-CCP, about 2-5% of the patients tested positive, but did not appear to have RA (our unpublished observations). Two recent studies [32, 33] have provided convincing evidence that such supposedly "false positive" individuals might actually be in the process of developing RA (Table 2).

Rantapää-Dahlqvist and collaborators [32] analyzed blood samples from 83 blood donors who subsequently developed RA. They found that anti-CCP2 antibodies could be detected in some patients 10 years before appearance of the first clinical symptoms. The mean percentage of anti-CCP2 positive individuals (25% were positive more than 1.5 years before onset of the first symptoms) increased sharply to 52% in the last 1.5 years before manifestation of the first

Table 1. Diagnostic Performance of CCP2 Test

Study	Reference	RA patients	Controls	Sensitivity (%)	Specificity (%)
Van Venrooij	[9]	390	904	82	98
Vasishtha	[41]	682	1189	79	97
Pinheiro	[42]	150	unknown	80	98
Lee	[43]	103	146	66	90
Suzuki	[44]	549	528	88	89
Dubucquoi	[45]	140	131	65	96
Grootenboer-Mignot	[46]	265	91	63	91
Vallbracht	[47]	295	420	64	97

Table 2. Predictive Value of Anti-CCP Antibodies

Study	Reference	Test	Cohort	RA patients	PPV ¹ (%)	NPV ¹ (%)	Remarks
Rantapää	[32]	CCP2	former blood donors	83	82	86	Frequency as well as titers of anti-CCP increase to the onset of RA Anti-CCP detectable up to 10 years before clinical manifestation
Nielen	[33]	CCP1	former blood donors	79	97	74	Increasing anti-CCP prevalence over time before appearance of symptoms Anti-CCP detectable up to 10 years before clinical manifestation
Van Gaalen	[34]	CCP2	undifferentiated arthritis (UA) (n = 318)	127	93	75	83% of UA patients that were CCP-positive at baseline fulfill ACR criteria for RA after 1 year follow-up (93% after 3 years)
Vittecoq	[25]	CCP2	early arthritis (n = 314)	176	96	36	90% of early arthritis patients that were CCP-positive at baseline were classified as RA at the end of 1 year follow-up

¹PPV: positive predictive value; NPV: negative predictive value

symptoms of the disease. RFs were also detectable in the pre-disease serum samples, although at lower frequencies than anti-CCP antibodies. More than 70% of the patients were anti-CCP2 positive at their first visit to the rheumatology clinic [32].

In a similar type of study, Nielen and coworkers [33] measured anti-CCP1 and IgM-RF levels in serial blood samples of 72 blood donors that later developed RA. Anti-CCP positivity could be observed up to 14 years before the first clinical symptoms and 41% of the patients were anti-CCP1 positive at presentation to the clinician. For IgM-RF the corresponding parameters were 10 years and 28% positivity, respectively. Thus, anti-CCP detected more positive subjects and longer before the appearance of clinical symptoms compared to IgM-RF [33].

ANTI-CCP AUTOANTIBODIES ARE ALSO PREDICTIVE FOR THE OUTCOME OF EARLY ARTHRITIS PATIENTS

Observations described by van Gaalen *et al.* show that anti-CCP positivity can be used to identify a subset of patients with undifferentiated arthritis (UA) that in two to three years will progress into RA [34]. In this study 318 out of 936 patients attending an early arthritis clinic could not be diagnosed as having RA at first presentation and were thus classified as UA. After 3 years of follow-up 40% of the UA patients could be clinically classified as suffering from RA. Of the UA patients that were negative for anti-CCP2 at baseline, 25% developed RA in 3 years. By contrast, 93% of the UA patients with a positive anti-CCP2 test at baseline, developed RA within 3 years (79% after 1 year), representing an odds ratio (OR) of 38. A recent study of Vittecoq and coworkers studying 314 early arthritis patients gave very similar results [25]. In this study, 90% of the patients that were CCP2 positive at baseline were classified as established RA patients within 12 months of follow-up [25]. The conclusion from these studies is that anti-CCP antibodies are present early in disease (Table 2), and that their presence enables accurate prediction of the development of RA.

PROGNOSTIC ABILITY OF ANTI-CCP

It has been known for some time that IgM-RF antibodies are able to predict joint erosions in RA patients. Several studies performed with the relatively insensitive first generation CCP1 test also showed that the presence of anti-CCP may predict erosive disease (reviewed in ref. [27]). Similar results have recently been obtained using the CCP2 test. Forslind and colleagues [35] assessed anti-CCP2 in 379 early RA patients and measured radiological joint damage and disease progression after two years follow-up. They found that the presence of anti-CCP2 at baseline was associated with significantly higher Larsen scores both at baseline and at endpoint compared to RF and other disease parameters. In another study, Kastbom and coworkers [36] followed 242 patients with recent-onset RA for three years. Their results showed that anti-CCP2 had similar diagnostic sensitivity as RF in early RA, but was superior as a predictor of the disease course over three years.

All these studies indicate that the presence of anti-CCP antibodies may predict erosive disease (Table 3). However, the notion that anti-CCP antibody is present preferentially in patients with erosive disease does not imply that all patients with anti-CCP belong to this clinical subgroup. How can one be sure that (erosive) RA is developing in a CCP-positive individual without obvious clinical complaints? Because RA is most likely a multi-factorial disease, future investigations will focus on combination models to identify individuals who have the highest probability of developing RA. Arguably such an "RA passport" (see Table 4) should contain **serological data** (IgM-RF, OR of about 2 and anti-CCP2, OR of about 25-38), **genetic data** (e.g. HLA-DR4, OR of about 2) and several **clinical parameters** as suggested by Visser and coworkers [37]. As a first attempt to develop such an RA passport Berglin and coworkers [38] studied the ability of a combination of genetic and serological factors to predict future development of RA in individuals without any signs of joint disease. The highest predictive value was reached by a combination of HLA-DR4 Shared Epitope (SE) positivity and anti-CCP2 positivity (OR 67) [38]. The prognostic value of this combination was reported in a recent

Table 3. Prognostic Value of Anti-CCP Antibodies

Study	Reference	Test	Cohort	CCP-positive at baseline (%)	Follow-up period (years)	Remarks
Van Jaarsveld	[48]	CCP1	249 early RA	52	3	Prognostic value in addition to RF in predicting joint damage, but mainly for mild disease
Kroot	[49]	CCP1	273 early RA	66	6	Predicts development of severe radiographic joint damage
Visser	[37]	CCP1	524 early arthritis	ns ¹	2	Important criterion in discrimination between persistent and self-limiting arthritis, and between erosive and non-erosive arthritis
Jansen	[50]	CCP1	282 early RA	32	2	Correlates with radiographic progression (OR 21)
Meyer	[51]	CCP1	145 early RA	57	5	Good prediction of radiographic joint damage (OR 2.5)
Vencovsky	[52]	CCP1	104 early RA	42	2	Prognostic marker for erosive disease
Bas	[53]	CCP1	27 early arthritis	30	8	Correlates with RA disease severity
Sarau	[54]	CCP1	243 early RA	47	2 - 4	Good predictor for erosive disease
Forslind	[35]	CCP2	379 early RA	55	2	Predicts radiographic joint damage (OR 3.6) and progression (OR 2.9)
Kastbom	[36]	CCP2	242 early RA	64	3	Predicts disease activity
Van Gaalen	[39]	CCP2	268 early RA	53	4	In combination with HLA class II RA susceptibility alleles predictor of more severe disease progression
Lindqvist	[40]	CCP2	183 early RA	80	5 - 10	Correlation with more severe joint damage

¹ns: not specified

study demonstrating that more severe disease progression is found in RA patients with both anti-CCP antibodies and SE alleles [39]. Similar data were recently published by Lindqvist and collaborators [40], who demonstrated that the combination of factors reflecting different aspects of the disease process, i.e. inflammation markers, a marker of cartilage turnover, autoantibodies (a.o. anti-CCP) and shared epitope yielded additive prognostic information. For this cohort of 176 patients with early RA, anti-CCP was, together with the inflammation marker C-reactive protein (CRP), the only significant predictor of joint damage in hands and feet after 10 years [40]. At this time it is clear that given its high predictive and prognostic ability, the measurement of anti-CCP2 antibody adds an important parameter in such an RA passport.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Taken together, the data discussed in the present review indicate that anti-CCP represents a superior serological marker for RA. Anti-CCP is (i) highly specific for the

disease, (ii) able to distinguish RA from other arthritides that mimic RA, (iii) present in the majority of patients (good sensitivity), (iv) detectable very early in the disease, and (v) helpful in predicting disease outcome. Its prognostic potential may aid the rheumatologist in reaching decisions on the most optimal treatment strategies. Moreover, anti-CCP can be detected with a reproducible and easily performed ELISA, the CCP2 test, which is important from the perspective of laboratory management.

The observations that anti-CCP autoantibodies often can be detected years before the disease becomes manifest, suggest that the initial trigger for the development of RA may occur long before the appearance of symptoms. Monitoring anti-CCP activity in individuals that may have an increased risk for the development of RA, e.g. based upon genetic factors, may eventually allow earlier treatment of anti-CCP positive individuals in which the antibody titers are increasing. As a consequence, the lag time between first visit to the rheumatology clinic and start of therapeutic intervention may be importantly reduced and joint erosion may already be inhibited at the very early stages (Fig. 1).

Table 4. RA Passport: Factors Predicting the Future Development of RA

	Factor	Odds ratio	Reference
Genetic factors	HLA-DR4	~2	[38]
	PAD4 haplotypes ¹	~2	[55, 56]
Serological factors	IgM-RF	~2	[32, 34, 38]
	Anti-CCP2	25-38	[32, 34, 38]
Clinical parameters	E.g. morning stiffness	~2	[37]

¹May be restricted to certain geographical populations [57].

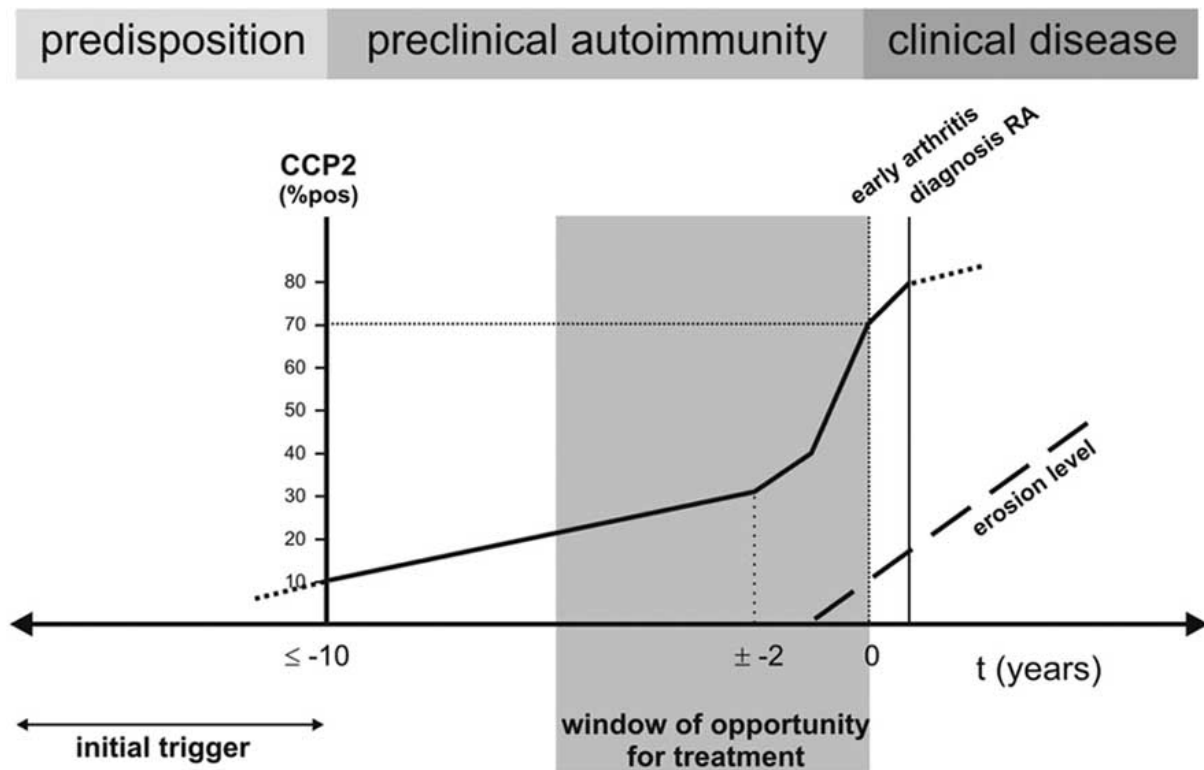


Fig. (1). Presence of anti-CCP2 antibodies precedes the clinical onset of RA by years.

Anti-CCP2 antibodies can be detected years before manifestation of the first symptoms (solid black line), indicating that the initial events that lead to this autoimmune disease may have taken place years before clinical manifestation. The ‘pre-disease activity’ and associated complaints are going up and down until a certain “arthritis threshold” is reached. Only then the patient will pay the first visit to the rheumatology clinic. By that time, about 70% of the patients is anti-CCP2 positive [32]. Nevertheless, in most cases not all clinical parameters of RA are present and it takes on average 6 to 8 months before a definitive diagnosis can be made. This lag time may be significantly reduced when CCP2 is included as a diagnostic criterion.

When a specificity level of 98% is chosen, the sensitivity of the current anti-CCP test (CCP2) will be about 80% for a random population of RA patients (see Table 1). Although this level of sensitivity implies that the test can be successfully applied for the majority of patients, one could ask whether anti-CCP2-negative patients do not contain antibodies to citrullinated epitopes. Because RA is such a complex and diverse disease, reaching 100% sensitivity will be impossible. However, the results of several experiments indicate that the antibody response to citrullinated epitopes is strongly polyclonal (our unpublished observations and Ref.[17]). By increasing the number of citrullinated epitopes that are presented in the CCP test, it should be possible to increase the sensitivity of the assay. Knowledge on the identity of the citrullinated candidate autoantigens could be helpful in designing such additional epitopes. The recent application of microarray and multiplex technologies for autoantibody detection will facilitate the simultaneous detection of autoreactivities to multiple citrullinated epitopes. If the availability of this technology improves, peptide microarrays could become a platform for a “next generation CCP” test. Until that time, the CCP2 ELISA is the best diagnostic test for RA.

Previously, the American College of Rheumatology (ACR) 1987 classification criteria for RA have often been used as a diagnostic tool in patients with recent-onset arthritis. However, these criteria were developed in a

population of patients selected according to their disease status, to classify rather than diagnose RA. Visser and colleagues [37] have developed a diagnostic criteria set for early arthritis that is able to discriminate, at the first visit, between self-limiting, persistent non-erosive, and persistent erosive arthritis. Interestingly, except for the radiographically detected erosions, anti-CCP is the criterion with the highest odds ratio to discriminate between erosive and non-erosive arthritis, substantiating the important role that anti-CCP can play in the early diagnosis of this disease. Further studies with larger patient cohorts will be necessary to confirm these data and to establish possible other correlations that might be helpful to discriminate between subsets of RA patients. These improvements may be of help in designing better tailor-made therapeutic strategies.

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