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REVIEW

Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is considered a systemic autoimmune disease with the main characteristic of persistent joint inflammation that results in joint damage and loss of function. Numerous studies have shown that substantial irreversible damage occurs within the first two years, as evidenced by the maximal rate of erosive joint disease that appears early on.[1,2] There is growing evidence that therapeutic intervention early in the disease course of RA leads to earlier disease control and less joint damage.[3-7] Moreover, in the last years there has been a rapid development of powerful therapeutic agents for RA.[8] Rheumatoid arthritis should be considered a medical emergency that requires prompt diagnosis and appropriate treatment.[7,9] On the other hand, many early arthritis patients not diagnosed as RA have self-limiting disease.[10] Since treatment of early arthritis with disease-modifying antirheumatic drugs (DMARDs) is only justified when the cost-benefit ratio is favourable, it is mandatory to be able to differentiate between RA and other forms of arthritis early after symptom onset.[7,11] Therefore, diagnostic criteria for RA that are maximally accurate and at the same time usable in clinical practice are needed. In this context it would be extremely helpful to have a simple serological marker that is highly specific for RA, present early in disease and prognostic as to whether the disease will be erosive or not. The search for such an ideal serological marker of RA that could be included in the diagnostic criteria has been going on for decades, but only recently appears to have yielded results, as will be discussed below.
In this review we will focus on diagnostic criteria for early RA and the possible role herein of specific autoantibody activities.

EARLY ARTHRITIS

The term early arthritis is often used in literature but is not well defined. From a practical clinical point of view early arthritis could be defined as arthritis newly presented to a clinician that poses a diagnostic, prognostic and therapeutic challenge. Early arthritis patients constitute a very heterogeneous group of patients, both as to their clinical presentation and their outcome. Depending on the way these patients are selected, about one third of the patients have a disease that may ultimately be classified as RA, one third will have another classifiable inflammatory disorder and one third of the patients remain unclassified.[10]

ACR 1987 CLASSIFICATION CRITERIA FOR RA

As diagnostic criteria

The 1987 American College of Rheumatology (ACR; formerly, American Rheumatism Association) classification criteria for RA are shown in *table 1*. In clinical practice these criteria are often used as a diagnostic tool for RA. However, these criteria were developed in a population of selected RA and non-RA patients as a means of classifying RA, not as a way to diagnose RA.[12] This probably explains the poor diagnostic performance of the ACR criteria in early arthritis.

As a gold standard

A problem to be dealt with in the diagnostic research of RA is the lack of an independent gold standard for the disease. In most studies the disease classification according to the ACR criteria has been used as the gold standard. A draw-

Table 1
The American College of Rheumatology 1987-revised criteria for the classification of rheumatoid arthritis (traditional format)

1	Morning stiffness of at least one hour before maximal improvement
2	Arthritis of three or more joint areas
3	Arthritis of hand joints
4	Symmetric arthritis
5	Rheumatoid nodules
6	Rheumatoid factor (RF) positivity
7	Radiographic changes on hand and wrist radiographs (erosions or decalcification)

For classification purposes, a patient will be said to have rheumatoid arthritis if he/she has satisfied at least four of these seven criteria. Criteria one to four must have been present for at least six weeks.

back of this gold standard is that it is dependent on the diagnostic tests that are evaluated. This leads to circularity and overestimation of the diagnostic properties of these tests. Another drawback is that one third of the patients with persistent arthritis do not fulfil any of the international classification criteria. For the clinician it is unclear how these unclassifiable forms of persistent arthritis should be treated.
Defining the gold standard of RA in terms of arthritis outcome prevents the occurrence of circularity.[13] Moreover, predicting the outcome of arthritis is more relevant for therapeutic decision-making than predicting whether arthritis will ever satisfy a set of classification criteria. Clinicians now have several powerful drugs at their disposal that will improve outcome when applied at an early stage of the disease but also have high toxicity profiles or are expensive. Treatment with these drugs is only justified when the cost-benefit ratios for individual patients are favourable. Both for the patient and the clinician confronted with early arthritis, the knowledge of arthritis outcome is therefore indispensable for their choice of management strategies.

AUTOANTIBODIES IN RA

In systemic autoimmune diseases many autoantibodies directed to ubiquitously expressed antigens are made, and they often show restriction with respect to the autoimmune disease in which they occur. Two examples are:
1) Sm (the 'Smith' autoantigen) is a complex of eight proteins associated with a number of small RNAs present in the nucleus of every eukaryotic cell, including yeast. Nevertheless, it is targeted by autoantibodies almost exclusively produced by SLE patients.
2) The Jo-1 autoantigen is identical to His-tRNA synthetase which is an essential cofactor in the synthesis of proteins. It is therefore present in every eukaryotic cell. Notwithstanding that, autoantibodies to Jo-1 are very typical for myositis.
RA is diagnosed primarily on clinical manifestations and serological support has, up to now, been restricted to the determination of (IgM) rheumatoid factor (RF). However, this antibody, directed to the Fc part of IgG, is not specific for RA because it also occurs in many inflammatory diseases as well as in (elderly) healthy individuals. Most other published autoantibody systems in RA were also shown to occur in more than one rheumatic disease, and thus are not specific for RA.[14] Recently, however, a novel and very specific autoantibody system for RA has been described. It was found that patients with RA develop antibodies to, as yet undefined, proteins containing modified (citrullinated) arginine residues. It has been shown convincingly that the citrulline residues are essential parts of the antigenic determinants recognised by the RA autoantibodies.[15,16] The citrulline moiety in the antigen is so important that essentially every citrullinated peptide or protein will be recognised by autoantibodies in RA sera, albeit with different sensitivities and specificities.

Citrullination: what, where and when

Citrullination, or deimination, is an enzyme-catalysed process in which the positively charged NH_2-group of the amino acid arginine (Arg) is hydrolysed to a neutral oxygen group (*figure 1*). It is this oxygen group of peptidyl citrulline that is specifically recognised by autoantibodies in RA.[14-17] Database searches reveal the existence of four human peptidylarginine deiminases (PAD enzymes), but not much is known about their substrate specificity, their cellular localisation, and how and when these enzymes become activated. What we do know is that these enzymes have a tissue-specific distribution and that, in mice, they are stimulated by female sex hormones.[18]

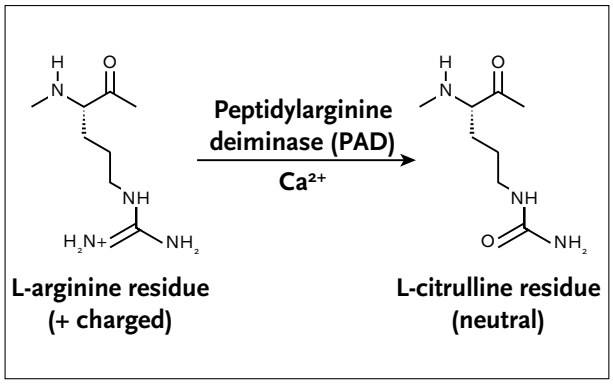

Figure 1
Deimination of peptidyl arginine to peptidyl citrulline by peptidylarginine deiminases

There are only a few citrullinated proteins known to occur in healthy mammalian cells. It is unlikely that one of these (for example myelin basic protein, filaggrin or trychohyalin) would be the citrullinated RA-specific autoantigen, since none of these proteins can be detected in, for example, synovial tissue. However, it appears that citrullinated proteins can be generated during the final stages of the lifecycle of some cells. For example, filaggrin becomes citrullinated during late differentiation of epidermal cells.[19] Vimentin and histones are citrullinated during programmed cell death (apoptosis) of macrophages and HL-60 cells, respectively, and fibrin is citrullinated in inflamed joint tissue.[20-22] Especially the presence of citrullinated fibrin in the inflamed joint is interesting, since it has been shown that the inflamed synovial tissue is the local site where the anticitrullinated protein antibodies are produced. First of all, Masson-Bessière and co-workers found that the titres of IgG antibodies directed to citrullinated protein were several times higher in the pannus tissue than in synovial fluid or serum.[23] Secondly, the titres of such antibodies in synovial fluid are also significantly higher than in paired serum samples (E. Vossenaar, unpublished data). Thirdly, B cells from the synovial fluid of RA patients with anticitrullinated protein antibodies spontaneously produce these antibodies, while peripheral blood B cells or B cells from seronegative RA patients do not.[24] These results not only suggest an antigen-driven maturation of anticitrullinated protein-specific B cells at the site of inflammation in RA, but also indicate that the production of these antibodies is a local process occurring in the inflamed synovium.

Anti-CCP antibodies

In principle, every citrullinated protein or peptide can be used in serological tests to detect anticitrullinated protein antibodies. So far, only citrullinated filaggrin has been used to detect the so-called antifilaggrin antibodies (AFA).[25] In our first attempts to find suitable substrates for RA autoantibodies we developed a number of linear peptides containing one citrulline residue. These citrullinated peptides were specifically recognised by the RA autoantibodies and, more important, their arginine-containing counterparts were not. However, most peptides reacted with only 30 to 45% of the RA sera, although more than 75% of RA sera reacted with at least one of the nine peptides tested.[15] We tested several parameters to increase the sensitivity of the test, and found that the most successful optimisation was to make the peptides cyclic. Our cyclic citrullinated peptides (CCP) have a three-dimensional design that is optimally structured for recognition of the antigenic group by the heterogeneous population of RA autoantibodies. By using a single CCP as antigen in an ELISA test, we could increase the sensitivity of the assay to about 68%, with a specificity of more than 97%.[26]

Recent selections from dedicated peptide libraries yielded novel peptides with improved recognition properties. Using such peptides, the sensitivity of the test can be increased to at least 80%, with a specificity of >98% (see CCP2 test, *table 2*).[27]

Table 2

Sensitivity and specificity of the anti-CCP2 test compared with the IgM-RF test

	CCP2			IGM-RF		
	N	POS	%	N	POS	%
RA (chronic)	390	320	82	390	312	80
Healthy individuals	95	1	1	95	1	1
Various connective tissue diseases[a]	299	9	3	264	40	15
Osteoarthritis	29	0	0	27	1	4
Reactive arthritis	40	1	3	40	4	10
Various inflammatory disease[b]	113	1	1	113	2	2
Various viral infections[c]	117	0	0	106	13	12
Various bacterial infections[d]	118	1	1	118	11	9
Various parasitic infections[e]	93	2	2	93	20	22
	809	14	2	761	91	12

[a] = including systemic lupus erythematosus, scleroderma, primary Sjögren's syndrome, vasculitis, [b] = including Crohn's disease, colitis ulcerosa, [c] = including Epstein-Barr, Parvovirus B19, [d] = including Treponema pallidum (syphilis), Chlamydia trachomatis, Legionella, Borrelia, Yersinia, Salmonella, Streptococcus pyogenes, Mycobacterium tuberculosis, [e] = including , Toxoplasma, Plasmodium falciparum (malaria), Leishmania, Schistosoma, Trypanosoma cruzi.

RECENT STUDIES USING THE ANTI-CCP SYSTEM

A simple, specific and quantitative ELISA test using a single cyclic citrullinated peptide (cfc1-cyc2) as immunosorbent has been developed and released on the market as the anti-CCP1 test (Immunoscan RA).[28] The studies performed with this test allow the following conclusions to be made.

Anti-CCP antibodies are extremely specific for RA

Various groups of researchers testing different cohorts of RA patients reached a specificity varying between 96 and 99%.[26,27-33] The anti-CCP test is thus clearly more specific that the RF test (see also *table 2*). The extreme specificity of the anti-CCP antibody system will be a great help in the early diagnosis and earlier treatment of this disease.

Sensitivity of anti-CCP and RF test is comparable

The first generation anti-CCP test had a sensitivity of 60 to 68%, somewhat lower than the RF test (70 to 75%). The

second generation CCP2 test[27,28] uses other citrullinated peptides that raises the sensitivity to 75 to 80% (*table 2*). It has appeared from various, as yet unpublished, studies of different cohorts of patients that the sensitivity of the CCP2 test is very comparable with that of the IgM-RF test.[27] In both the CCP1 and the CCP2 test about 35 to 40% of the RF-negative patients scored positively for anti-CCP.

Anti-CCP antibodies have prognostic value

This is because they are predominantly present in patients with erosive disease. Although only a few studies on the prognostic abilities of these antibodies have been performed so far, the studies by Van Jaarsveld *et al.*,[34] Kroot *et al.*,[31] Visser *et al.*,[35] and Vencovský *et al.*[32] support the idea that RA patients positive for anti-CCP develop significantly more severe radiological damage than anti-CCP-negative patients. Additional studies are necessary to further underline the prognostic ability of this test.

Anti-CCP antibodies are present very early in the disease

In studies of patients from early arthritis clinics[26,31,32,35] as well as cohorts of patients with early synovitis,[30] anti-CCP antibodies were present in 40 to 70% of the cases. In several yet unpublished studies the antibodies were detected up to ten years before the first RA symptoms were noted. These results indicate that citrullination of synovial antigens and the production of antibodies to these citrullinated antigens is initiated very early in disease.

A PREDICTION MODEL FOR PERSISTENT (EROSIVE) ARTHRITIS

In a recent study a clinical model was described for the prediction of three forms of arthritis outcome: self-limiting, persistent non-erosive and persistent erosive arthritis.[35] The prediction model was developed in a cohort of 524 early arthritis (EA) patients derived from the Leiden Early Arthritis Clinic. Outcome was determined at two years. A schematic representation of the study design is shown in *figure 2*. The developed prediction model is shown in *table 3* and consists of seven variables: symptom duration, morning

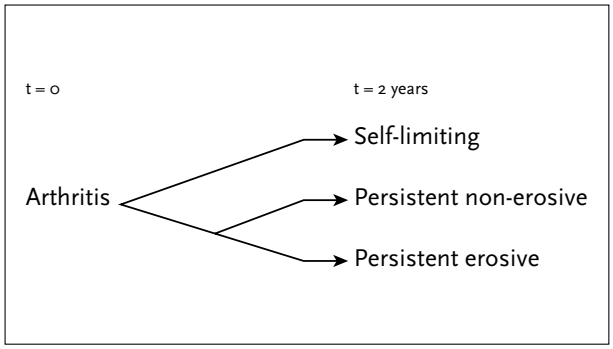

Figure 2
Schematic representation of the study design

The development of diagnostic criteria to discriminate at the first visit between three forms of arthritis outcome recorded at two-year follow-up: self-limiting arthritis, persistent non-erosive arthritis and persistent erosive arthritis.[35]

Table 3
The seven variables of a prediction model for persistent (erosive) arthritis

	PERSISTENT ⟷ SELF-LIMITING		EROSIVE ⟷ NON-EROSIVE GIVEN PERSISTENCE	
	ODDS RATIO	SCORE	ODDS RATIO	SCORE
Symptom duration				
≤6 weeks <6 months	2.49	2	0.96	0
≥6 months	5.49	3	1.44	0
Morning stiffness ≥1 hour	1.96	1	1.96	1
Arthritis ≥3 joint groups	1.73	1	1.73	1
Bilateral compression pain MTPs	1.65	1	3.78	2
IgM RF ≥5 IU	2.99	2	2.99	2
Anti-CCP1 ≥92 IU	4.58	3	4.58	3
Erosions X-rays hands or feet	2.75	2	Infinite	Infinite

Intercept persistent versus self-limiting = -2.31, intercept erosive versus non-erosive given persistence = -2.42, MTPs = metatarsophalangeal joints, RF = rheumatoid factor, CCP = cyclic citrullinated peptide. For each variable two odds ratios and two simplified scores are shown, one for its association with persistent arthritis and one for its association with erosions given arthritis is persistent.[35]

stiffness of at least one hour, arthritis of three or more joints, bilateral compression pain of metatarsophalangeal joints (MTPs), RF positivity, anti-CCP1 positivity and the presence of erosions on radiographs of hands or feet. The odds ratios of the variables are shown in *table 3* both for self-limiting versus persistent arthritis and for erosive versus non-erosive arthritis. Application of the model in an individual patient results in three clinically relevant predictive values: one for self-limiting arthritis, one for persistent non-erosive arthritis and one for persistent erosive arthritis. The prediction model is easy to use in clinical practice, for example by using a computer. By indicating which criteria are present and absent in a particular patient, the probabilities of the three forms of arthritis outcome can be simply obtained from the model. In the Leiden cohort the prediction model discriminates very well between the different forms of arthritis outcome.[35] The discriminative ability was expressed as the Area Under the Curve (AUC) of the Receiver Operator Characteristic (ROC). A ROC curve plots the relation between sensitivity on the Y-axis and (1 - specificity) on the X-axis, for different cut-off levels of test positivity. The area under the curve is a measure of the overall discriminative value of the model. A value of 0.5 means no discrimination at all, a value higher than 0.7 is acceptable and a value of 1 is perfect. The ROC AUC of the model for discrimination between self-limiting and persistent arthritis is 0.84 (SE 0.02) and for discrimination between erosive and non-erosive arthritis given persistence is 0.91 (SE 0.02). The discriminative ability of the 1987 ACR classification criteria is significantly lower, with ROC AUCs of 0.78 (SE 0.02) and 0.79 (SE 0.03), for self-limiting versus persistent arthritis and erosive versus non-erosive arthritis given persistence, respectively. It was concluded that the ability of the prediction model to discriminate between three forms of arthritis outcome is excellent and generates clinically relevant predictive values.[35] Before a prediction model is implemented into practice, adequate validation is required.[36] Validation means that the performance of a model is tested in a different patient cohort to the sample used to generate the model. A model can predict outcome well in the patients from which it was derived but may be unreliable elsewhere. At the moment the model is validated in different early arthritis cohorts.

Clinical value of anti-CCP antibodies

The sensitivity of the anti-CCP test for RA (the percentage of RA patients with positive test) is 60 to 88%, depending on the characteristics of the RA population.[26,29,33] The specificity of the test for RA (the percentage of non-RA patients with negative test) is very high: 96 to 99%, depending on the characteristics of the non-RA population.[26-33] However, patients and clinicians confronted with early arthritis need probabilities of the different forms of arthritis outcome to be able to choose management strategies, and it is impossible for the clinician to calculate these probabilities from the sensitivity and specificity of isolated tests. The prediction model for persistent (erosive) arthritis is an important and usable tool for prediction of arthritis outcome. The anti-CCP ELISA independently and significantly contributes to the performance of this prediction model.[35] The overall discriminative ability of the prediction model without anti-CCP test is significantly lower than that of the model with anti-CCP test: for persistent versus self-limiting arthritis: ROC AUC 0.82 (SE 0.02), for erosive versus non-erosive arthritis ROC AUC 0.90 (SE 0.02). Therefore, the anti-CCP test has added value in diagnostic and therapeutic decision-making in early arthritis, as indeed can also be concluded from the study by Vencovski *et al*.[32]

APOPTOSIS, AUTOIMMUNITY AND RA

The question arises why RA patients, and only RA patients, make these anti-CCP antibodies. Why are fibrin and probably other synovial proteins being citrullinated in the inflamed synovium during the disease? Such questions become even more intriguing when one realises that citrullination only occurs in certain specialised cell types (for example, myelin basic protein in glia cells) and in certain types of dying cells. Although the presence of apoptotic cells in synovial tissue is not obvious, it is possible that environmental factors (including pathogenic and inflammatory agents) induce abnormal cell death locally. It is not unlikely that during this process extravascular fibrin, and other synovial proteins, are targeted by activated PAD enzymes. We postulate that such modifications, taking place at local sites in the body, generate unique epitopes to which no effective tolerance exists.[37,38] In susceptible individuals a primary and specific immune response will then develop.

CONCLUSION

We have shown that among the many autoantibodies that can be detected in the serum of an RA patient, the autoantibodies directed to citrullinated antigens have a high potential for clinical use. Anti-CCP antibodies are very specific for the disease and can be detected early in the disease. In unselected early arthritis patients the test has added value in predicting persistent (erosive) arthritis. Further research is needed to show that citrullination of relevant self-proteins induces the production of autoantibodies. Such studies may also shed light on whether anticitrullinated protein antibodies have pathological effects or not.

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