

# Autoimmunity to cyclic citrullinated peptide in rheumatoid arthritis

Manole COJOCARU, MD, PhD<sup>a</sup>, Inimioara Mihaela COJOCARU MD, PhD<sup>b</sup>,  
Isabela SILOSI MD, PhD<sup>c</sup>

<sup>a</sup>Department of Physiology, Faculty of Medicine,  
"Titu Maiorescu" University, Bucharest, Romania

"Dr. Ion Stoia" Center for Rheumatic Diseases, Bucharest, Romania

<sup>b</sup>Clinic of Neurology, "Carol Davila" University of Medicine and Pharmacy,  
"Colentina" Clinical Hospital, Bucharest, Romania

<sup>c</sup>Department of Immunology, Faculty of Medicine,  
University of Medicine and Pharmacy, Craiova, Romania

## ABSTRACT

*Rheumatoid arthritis (RA) is a common autoimmune disease characterized by persistent inflammation of joints with progressive destruction of cartilage and bone. In recent years, a number of novel autoantibodies have been described in RA. In particular new anti-cyclic citrullinated peptide (anti-CCP) antibodies are the most remarkable because high specificity in RA patients, which may be able to serve as an early diagnostic marker and a prognostic factor of joint destruction. Compared to rheumatoid factors (RF), antibodies against CCP are more closely associated with RA and are therefore more suitable for diagnostics. With a new serological test RA can be reliably diagnosed in 70-80% of cases. The pathogenetic role of the anti-CCP antibodies is not known. However, B-cells from synovial fluid of RA cells are able to spontaneously secrete anti-CCP antibodies. This article reviews and discusses the nature of target autoantigens as well as clinical and possible etiopathogenic significance of anti-citrullinated antibodies in RA.*

**Key words:** anti-cyclic citrullinated peptide (CCP) antibodies, citrullinated protein, rheumatoid arthritis

**T**he autoantibody test gives significant information for diagnosis of autoimmune diseases Rheumatoid arthritis (RA) has a significant genetic predisposition and an association with the HLA complex, in particular the HLA-DRBI shared epitope (SE) alleles. There is a notable

association between SE and anti-CCP antibodies positivity (1).

The diagnosis of RA depends primarily on the clinical manifestations of the disease. Rheumatoid arthritis is one of the most common systemic autoimmune diseases characterized by chronic and erosive polyarthritis by abnormal

*Address for correspondence:*

Manole Cojocaru, MD, PhD, "Titu Maiorescu" University, 67A Gheorghe Petrascu Street, District 3, Zip Code 031593, Bucharest, Romania  
email address: manole\_cojocaru@yahoo.com

growth of synovial tissue or pannus, and it causes irreversible joint disability. The chronic nature of this disease results in multiple joint inflammation with subsequent destruction of joint and cartilage and erosion of bone.

Approximately 0,5-1% of the world population is affected, and twice as many women as men get the disease. Patients do not always show typical symptoms and signs at their early stage, and are often difficult to be diagnosed since they may not fulfill the classification criteria for RA.

The only serological test included in the American College of Rheumatology (ACR) revised classification criteria is rheumatoid factor (RF) which is not specific for RA (2,3).

The exact pathogenesis of RA remains largely elusive, however, cumulative evidence has suggested that T-cell-mediated autoimmune responses play a crucial role in the pathogenesis of RA. It is well accepted that CD4+ T cells play a critical role in the pathogenesis of several autoimmune diseases including RA.

Diagnosis is made on a clinical basis, supported by the determination of RF. RA is also categorized among systemic autoimmune diseases because of the presence of RF, autoantibodies against the Fc portion of IgG, and other autoantibodies. RF can be in the IgM, IgG and IgA immunoglobulin classes. RF has been clinically utilized as the only serologic marker of RA so far. However, the sensitivity of RF is 60-80% in RA, and the specificity is rather low since. Therefore, despite the fact that RF is adopted into the criteria for classification of RA, its diagnostic value is unsatisfactory especially in the early disease (4). Until recently, the only serological test routinely used was the determination of the presence of RF in the serum. The presence of IgM RF is a hallmark of RA, found in approximately 50-90% of the patients (1,2).

RF is also detected widely and frequently in many other conditions including various connective tissue diseases, chronic liver diseases and infectious diseases, and even in a few healthy people (3-5).

It has been known for many years that anti-perinuclear autoantibodies, also called anti-keratin (AKA), are found in people with RA. A circular peptide containing citrulline called cyclic citrullinated peptide (CCP) was found to be better at discriminating RA patients from other patients than either the perinuclear antibody test or the test for RF. In recent years, the intro-

duction of anti-cyclic citrullinated peptide (anti-CCP) antibodies showed some promise as a diagnostic tool in RA. These antibodies are detected by enzyme linked immunosorbent assay (ELISA), technique where a synthetic CCP is used as a substrate. Individuals with RA frequently have autoantibodies to CCP derived from filaggrin or fibrin. Citrulline can be formed by the posttranslational enzymatic conversion of arginin residues, catalyzed by peptidylarginin deiminase.. The sensitivity of the anti-CCP test ranges between 64 and 74% whereas the specificity ranges between 90 and 99% (6)

Together with RF, anti-CCP antibodies increases the ability to diagnose patients with early RA. The rest might help to predict which patients will develop persistent disease with evidence of radiologic lesions (7).

In recent studies, it has been demonstrated that RA patients produce not only RF but also a variety of other autoantibodies. For this reason, development of the anti-cyclic citrullinated peptide (anti-CCP) antibody assay, a highly disease-specific serological marker for RA, has been a great step forward for the rheumatologist and the clinical laboratory. Anti-CCP antibodies are especially noteworthy because of their high high specificity. Assaying for the presence of anti-CCP antibodies may therefore be useful for diagnosing RA, and the deposition of these immunocomplexes in the articular synovium might be involved in pathogenesis (8,9).

There are many studies that anti-CCP antibodies may serve as a powerful serologic marker for early diagnosis of RA and prognostic prediction of joint destruction. Anti-CCP antibodies are locally produced in RA joints, and citrullinated proteins (most are fibrins) are localized in RA synovial tissue. This finding strongly suggests a possibility that local citrullination of intraarticular proteins might be the initial event leading to autoantibody production in RA (4).

Rheumatoid arthritis is a multifunctional autoimmune disease with a complex genetic background. Genetic factors such as HLA and a gene polymorphism of the citrullinating enzyme, PADI, (that might express more stable mRNA and cause over-citrullination of proteins) might be associated with the breakage of self-tolerance and induction of autoimmunity against citrullinated proteins. Genetic factors such as a gene polymorphism of the citrullinating enzyme, PADI, might be associated with the

breakage of self-tolerance and induction of autoimmunity against citrullinated proteins (10,11).

It is important for disease management to diagnose and treat people with RA as early as possible (14).

Recently it was discovered that these antibodies recognize an epitope that contains the deiminated form of arginine called citrulline. Additional, RA patients with anti-CCP may progress to a more severe disease than those who do not have anti-CCP (5,15,16).

Anti-CCP antibodies were detected in 68% of RA patients. Although the sensitivity was decreased to 48% in early RA cases, still the high specificity was maintained at 96% (17).

Studies performed on RA patients have shown that the specificity of anti-CCP is excellent greater than 90%. However, its sensitivity has been variable with values from 56-80% in established RA (3). Perhaps the most useful role of anti-CCP testing is the early classification of RA, so that early institution of the newer therapies could be undertaken to achieve remission. In early RA patients with symptoms less than two years, anti-CCP has been found in 41-81% (5).

Primary Sjögren's syndrome patients may present with arthritis with a prevalence of RF of about 60%. Anti-CCP will be helpful in this situation as its prevalence in Sjögren's syndrome is about 3% (7).

In SLE, arthritis is common and RF positivity is about 20%. If the arthritis is of the typical non-erosive type, anti-CCP prevalence is 0.5%. In a small subset group of patients with SLE and erosive arthritis, anti-CCP prevalence was 20%. In published literature, 23% of patients with DM/PM are positive for anti-CCP antibodies, 7-39% with active tuberculosis, while only about 2% of healthy blood donors are positive. In juvenile idiopathic arthritis, anti-CCP is found in the subgroup with IgM-RF positive polyarthritis. No definite advantage of anti-CCP over IgM-RF testing has been found (17).

A subset of psoriatic arthritis patients may present with RA-like arthritis. In this subset, anti-CCP is detected in 12%. Compared to the entire group of psoriatic arthritis patients, anti-CCP conveys a risk of erosive disease with an odds ratio (OR) of 9.8 (18).

In a study of 318 unclassified arthritis patients who were followed up for three years,

127 patients were subsequently classified as RA. The presence of anti-CCP initially conveyed a risk of RA with an OR of 37.8 (13).

A recent study found citrullinated proteins in the joints of patients with RA, but not in joints from healthy volunteers (18).

These results lend a theoretical basis for the presence of anti-CCP in RA patients and a possible pathogenic role for these antibodies.

On evaluating in our laboratory the performance of a second-generation enzyme immunoassay for detecting anti-CCP antibodies, directed against the immunogenic target of the AKA, we noticed that some of the foamy AKA sera had very high titers of anti-CCP antibodies.

Anti-CCP antibodies were also associated with erosive disease in RF-negative RA patients. Anti-CCP positivity was a frequent finding in PSA and associated with symmetrical polyarthritis. (19,20)

The second generation of the test was introduced in 2002. Cyclic citrullinated peptide was used as an epitope in order to achieve a better sensitivity. As a result of intensive research during the last 20 years, new therapeutic tools, including biologic agents, have been developed and introduced into the daily practice of treating patients with RA.

Anti-CCP titers have been found to decrease with anti-TNF treatment (21-23)

Recent studies show that joint injury in RA patients progresses within 2 years from onset, and aggressive treatments from the early stage can prevent the following progression of the disease (8,24,25).

All reports of anti-CCP indicate a positive correlation with radiological progression, whereas AKA tend to be independent of the disease severity (26,27).

These findings strongly suggest a possibility that local citrullination of intraarticular proteins might be the initial event leading to autoantibody production in RA (28-30). Further research will be necessary to elucidate the fine mechanism and significance of protein citrullination in etiopathogenesis of RA (31-33).

Because the etiology of RA is unknown, the treatment options remain limited. Implementation of the highly specific anti-CCP test in conjunction with RF would enable reliable early diagnosis in some cases and allow the initiation of aggressive therapy with disease modifying anti-rheumatic drugs (DMARDs). Once viewed

as inexorably progressive, RA has become a potentially treatable disease with the early aggressive use of disease modifying anti-rheumatic drugs DMARDs (3).

In hepatitis C, patients may present with joint pains and a positive RF. Anti-CCP was not seen in a study of 50 patients, in whom RF was positive in 44% (34). □

### CONCLUSION

The RF and the anti-CCP antibodies are complementary in RA. Anti-CCP antibodies can be detected in RA patient serum from early stage of the disease. Anti-CCP is 99% specific for healthy volunteers, 91% specific in rheu-

matic disease controls, and 98% specific in infectious disease controls. This is much better than IgM RF, the autoantibody currently most associated with RA.

Anti-CCP antibodies may be useful as a new serologic marker for RA because of their high specificity in RA.

The emergence of the anti-CCP antibody assay, as a new serological marker for RA, is a significant advance in rheumatological care. The presence of anti-CCP antibodies is linked with early aggressive RA, a greater risk for erosive disease, greater disease activity, and there is a possible association with the shared epitope (anti-CCP antibody titers are positively correlated with the disease activity). □



### REFERENCES

1. de Vries RRP, Huizinga TEJ, Toes REM – Redefining the HLA and RA association: To be or not to be anti-CCP positive. *J Autoimmun Dis* 2005; 25:21-25
2. Nell VK, Machold KP, Stamm TA et al – Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. *Ann Rheum Dis* 2005; 64:1731-1736
3. Schur DH – Anti-cyclic citrullinated peptide antibodies: Diagnostic, predictive and monitoring value in RA. *Int J Adv Rheumatol* 2005; 3:77-83
4. Chalamnuay S, Bridges SL – The role of B cells and autoantibodies in rheumatoid arthritis. *Pathophysiol* 2005; 12:205-216
5. Dahlqvist S, de JongBAW, Berglin E et al – Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predicts the development of rheumatoid arthritis. *Arthritis Rheum* 2003; 48:2741-2749
6. Nijenhuis S, Zendman A, Vossenaar ER et al – Autoantibodies to citrullinated proteins in RA: Clinical performance and biological aspects of an RA-specific marker. *Clin Chim Acta* 2004; 350:17-34
7. van Noordc, Hooijkaas H, van de 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; 64:160-167
8. Galati S, Beauvillain C, Reinier G et al – Comparison and relevance of rheumatoid factors, antikeratin antibodies and anti-cyclic citrullinated peptides antibodies in rheumatoid arthritis. *Ann Biol Clin* 2008; 66(2):157-164
9. Zeng X, Ai M, Tian X et al – Diagnostic value of anti-cyclic citrullinated peptide antibody in patients with rheumatoid arthritis. *J Rheumatol* 2003; 30:1451-1455
10. Bas S, Genevay S, Meyer O et al – Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. *Rheumatol* 2003; 42:677-680
11. Vasishtha A – Diagnosing early-onset rheumatoid arthritis: the role of anti-CCP antibodies. *Am Clin Lab* 2002; 21:34-36
12. Lee DM, Schur PH – Clinical utility of the anti-CCP assay in patients with rheumatic diseases. *Ann Rheum Dis* 2003; 62:870-874
13. van Gallen FA, Rasker SP, van Venrooij WJ et al – Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis. *Arthritis Rheum* 2004; 50:709-715
14. Meyer O, Labarre C, Dougados M et al – Anti-citrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. *Ann Rheum Dis* 2003; 62:120-126
15. van Boekel MAM, Vossenaar ER, van den Hoogen FHJ et al – Auto-antibody systems in rheumatoid arthritis: Specificity, sensitivity and diagnostic value. *Arthritis Res* 2002; 4:87-93
16. Combe B et al – Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. *Arthritis Rheum* 2001; 44:1736-1743
17. Mediwake R, Isenberg DA, Schellekens GA et al – use of anti-citrullinated peptide and anti-RA33 antibodies in distinguishing erosive arthritis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Ann Rheum Dis* 2001; 60:67-68
18. Bogliolo L, Alpine C, Caporali R et al – Antibodies to cyclic citrullinated peptides in psoriatic arthritis. *J Rheumatol* 2005; 32:511-515
19. Inanc N, Dalkilic E, Kamall S et al – Anti-CCP antibodies in rheumatoid arthritis and psoriatic arthritis. *Clin Rheumatol* 2007; 26(1):17-23
20. Riedman JP, Munoz S, Kavanaugh A – The use of second generation anti-CCP antibodies (anti-CCP2) testing in RA – a systematic review. *Clin Exp Rheumatol* 2005; 23:569-576
21. Avouac J, Gossec L, Dougados M – Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatic arthritis: a systematic literature review. *Ann Rheum Dis* 2006; 65:845-851
22. Mimori T – Clinical significance of anti-CCP antibodies in RA. *Internal Med* 2005; 44:1122-1126
23. Nishimura K, Sugiyama D, Kogata Y et al – Meta-analysis diagnostic accuracy of anti-CCP antibody and RF for rheumatoid arthritis. *Ann Int Med* 2007; 146:797-808
24. Suzuki K, Sawada T, Murakami A et al – High diagnostic performance of ELISA detection of antibodies to

- citrullinated antigens in rheumatoid arthritis. *Scand J Rheumatol* 2003; 32:197-204
25. **Pinheiro GC, Scheinberg MA, Aparecida-da Silva M et al** – Anti-cyclic citrullinated peptide antibodies in advanced rheumatoid arthritis. *Ann Intern Med* 2003; 139:234-235
26. **Bizzaro N** – Antibodies to citrullinated peptides: a significant step forward in the early diagnosis of rheumatoid arthritis. *Clin Chem & Lab Med* 2007; 45(2):150-157
27. **Vossenaar ER, Despres N, Lapointe E et al** – Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. *Arthritis Res Ther* 2004; 6: R142-150
28. **van Gaalen FA, Linn-Rasker SP, van Venrooij WJ et al** – Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004; 50:709-715
29. **Rantapää-Dahlqvist S, de Jong BA, Berglin E et al** – Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003; 48:2741-2749
30. **Forslind K, Ahlmen M, Eberhardt K et al** - BARFOT Study Group. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* 2004; 63:1090-1095
31. **Orbach H, Shoenfeld Y** – Anti-cyclic citrullinated peptide antibodies as a diagnostic test for rheumatoid arthritis. *Harefuah* 2003; 142(3):182-5, 239
32. **Finckh A, Liang MH** - Anti-cyclic citrullinated peptide antibodies in the diagnosis of rheumatoid arthritis. Bayes clears the haze. *Ann Intern Med* 2007; 146:816-817
33. **Van Venrooij WJ, Zendman A, Nijenhuis S et al** – Antibodies to cyclic citrullinated peptides are specific for the diagnosis and prognosis of early RA. *Autoimmun Rev* 2006; 6(1):57-41
34. **Wener MH, Hutchinson k, Morishima C et al** – Absence of antibodies to cycle citrullinated peptide in sera of patients with hepatitis C virus infection and cryoglobulinemia. *Arthritis Rheum* 2004; 50:2305-2308

