

The role of Rheumatoid Factor and Anti-Cyclic Citrullinated Peptide Antibody in Diagnosis of Rheumatoid Arthritis

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Abstract

Background: Rheumatoid Factor (RF) occurs in 70- 90% of patients with established rheumatoid arthritis (RA). Anti-cyclic citrullinated peptide antibody (Anti-CCP) detection may be used to confirm the diagnosis of RA in patients with uncharacterized chronic inflammatory arthritis. This study is an attempt to evaluate the role of RF and anti-CCP in the diagnosis and prognosis of rheumatoid arthritis.

Methods: Blood samples were obtained from 55 patients with established RA and 55 healthy controls. Anti-CCP and RF were measured by ELISA and nephelometry. The patient's demographics, disease duration, DMARDs usage, ESR and radiographic abnormalities were recorded.

Results: Fifty five RA patients and 55 healthy age and sex matched controls were enrolled. Odd ratio (OR) was 87.42 (CI=11.2-680.1) for RF and 27.48 (6/08-124/09) for Anti- CCP. Five patients had used more than 3 DMARDs; all of them were RF positive and 4 were also anti-CCP positive. Eleven patients had erosions in their radiographs; 10 of them were seropositive for RF and 9 positive for anti-CCP.

Conclusion: Our study suggests that despite lack of specificity, RF continues to be a central part of the definition of RA due to favorable sensitivity profile and the combined use of RF. Also, anti-CCP is a more powerful diagnostic and prognostic tool.

Keywords: Anti-CCP; Rheumatoid arthritis; Rheumatoid factor

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory arthritis with the potential to cause significant morbidity and mortality. The prevalence of RA is 1% in the general population with a predilection for women.¹ Autoimmune diseases, such as RA are believed to develop as a result of dysregulation of the immune system, leading ultimately to RA, clinical features of which are inflammation and destruction of several joints.² The etiology of RA has been suggested to be an interaction between genetic and environmental

factors. To date, it has not been possible to identify individuals at early stages of this dysregulation (i.e. before presentation of clinically obvious polyarthrititis). If methods were available to predict the future development of RA, a better understanding of the events triggering the disease would be achieved, thereby creating the possibility of developing and testing preventive measures and of instituting therapy at earlier stages of disease development than is currently practiced.³

Rheumatoid Factor (RF) is one of the criteria proposed by American College of Rheumatology (ACR) for diagnosis of RA. RF occurs in 70- 90% of patients with established RA but population-based studies have shown much lower rates of RF positivity and it may be present in non-rheumatic conditions such as infections and even healthy individuals.¹

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Cyclic Citrullinated Peptide (CCP) antibodies have been described as highly specific for RA. In several studies, the diagnostic sensitivity and specificity of anti-CCP vary from 50- 82%^{4,6} and 92.6-100%, respectively.^{4,6,8} Because of low specificity of RF and low sensitivity of anti-CCP, it is necessary to conduct more studies on these tests. The accuracy of diagnosis is important even in cases with uncharacterized chronic inflammatory arthritis; for example, a patient with lupus is treated with sulfasalazine or psoriasis arthritis with hydroxychloroquine.¹

There are some studies about association of RF and anti-CCP in rheumatoid arthritis. Because of the frequency of RF and anti-CCP, antibody increases significantly over time.^{9,12} These studies were done on nested samples or the samples obtained years before the disease onset.^{1,9-11} This study was designed to evaluate the simultaneous presence and association of these antibodies in patients with RA.

Materials and Methods

In this case control study, 55 patients with RA 1987 ACR from Rheumatology Outpatient Clinic in Sari, Northern Iran and 55 healthy sex and age matched individuals without any inflammatory disease or arthritis (according to history, physical examination and normal ESR) were compared. The patients and controls were recruited from June to September 2008. The clinical diagnosis was made by an attending rheumatologist according to the 1987 ACR revised criteria. The study was approved by Ethics Committee of Mazandaran University of Medical Sciences.

The patients with RA were further evaluated by age, duration of disease, presence of radiographic abnormalities (erosions) and use of disease modifying drugs (DMARDs). RF and anti-CCP were measured in all of the patients and controls.

RF was measured, using nephelometry (Pars Azmon Iran) and levels above 15 units regarded as positive. Anti-CCP antibodies were measured, using IgG ELISA (Aeskulisa, Germany) and a cut off above 18 units was taken as positive based on the manufacturers'

recommendation. Coefficient of variation (CV) was 2% for RF and 1.5%.for anti-CCP. Statistical analysis was performed, using SPSS statistical software, version 16 (Chicago, IL, USA) for estimation of odd ratio (OR) for association of RF and anti-CCP with RA along with 95% confidence intervals (CI). The group means were compared by t-test.

Results

There were 50 females and 5 males in both groups. The mean age of the patients was 44.5±9.4 years and that of the controls was 45.9±9.3 years (*p*=0.976). The mean ESR was 31.5±24.4 in the patients and 26.1±9.8 in the controls.

In RA patients, the mean duration of the disease was 5.52±0/79 years. 11 patients (20%) had erosions in radiographs and 5 (8%) used more than 3 DMARDs including prednisolone, methotrexate, sulfasalazine, hydroxychloroquin and cyclosporine. In the RA group, 34 patients (61/8%) were found to be RF positive compared with 28 (50.9%) who were anti-CCP positive, and 21 (38%) patients had both antibodies. One sample in the control group (1.8%) was RF positive and two other samples (3.6%) were anti-CCP positive. There was no sample in the controls with both positive RF and anti-CCP positive (Table 1).

Table 1: Comparison of anti-CCP and RF reactivity

	RA n=55	Controls n=55
anti-CCP+	n=28	n=2
RF+	21	0
RF-	7	2
anti-CCP-	n=27	n=53
RF+	13	1
RF-	14	52

The odd ratio (OR) was 87.42 (CI=11.2-68.1) for RF and 27.48 (6.08-124/09) for anti-CCP. The characteristics of the patients with or without these antibodies are shown in Table 2. Five patients used more 3 DMARDs; all of them were RF positive and 4 of

Table 2: Comparison of characteristic of patients with or without RF and anti-CCP

	RF+	RF-	P value	anti-CCP+	anti-CCP-	P value
Number of cases	34	21		28	27	
Mean age	48.3	43.5	0.775	45.7	47.2	0.815
Mean duration of disease	6.9	3.2	0.054	7.2	3.9	0.003
Mean ESR	32.7	29.6	0.864	32.7	30.2	0.811

them were also anti-CCP positive.

Eleven patients had erosions in radiographs, 10 were seropositive for RF, and 9 for anti-CCP.

Discussion

There is growing evidence that therapeutic interventions early in the course of RA lead to earlier disease control, less joint damage and a better prognosis. It is necessary to differentiate between RA and other forms of arthritis early after symptom development.⁶ The presence of positive anti-CCP antibodies could imply more aggressive disease and they may provide clinicians with an additional tool to identify RA patients at high risk for aggressive disease, who may be found even within traditional low risk seronegative groups.

In our study, odd ratio (OR) was 87.42 (CI=11.2-680.1) for RF and 27.48 (6/08-124/09) for anti-CCP. In the Berglin *et al.* study, OR was 14.9 for IgM RF+SE (shared epitope) versus 66.8 for anti-CCP+SE. In this study, OR for anti-CCP alone for developing RA was 25.1 but OR for RF alone was not reported.³ In the Dahlqvist *et al.* study, OR for IgM RF and anti-CCP were 14.6 and 31.4, respectively 1.5 years before the clinical presentation of the disease but in samples gotten earlier these were 4.1 and 29.6, respectively.⁹ In the Nielen's study, OR for anti-CCP in the new onset RA was 10.6.⁸ In these studies, the samples were gotten years before disease presentation but in our study we worked on patients suffering from RA for several years and found that there was a significant difference between seropositive (RF+ or anti-CCP+) and seronegative patients in disease duration. The accuracy of diagnosis is important even in old cases, because sometimes a patient with lupus or psoriasis is treated as RA with an anti RA agent for several years.¹

This study showed that IgM RF had more association with RA than with anti-CCP, perhaps because RF depends on the time interval till disease presentation.^{9,12} Our data indicated that RF and anti-CCP were

more highly associated with a higher prevalence of bone erosion and more DMARDs therapy. In another study, there was no significant difference in the number of DMARDs between seropositive and seronegative patients¹³ but the difference for radiographic signs of joint damage was significant.^{7,13,14}

In our study, 7 RF negative RA patients (33.3%) were anti-CCP positive. This study suggests that in establishing the diagnosis of RA, especially in ambiguous or rheumatoid factor negative cases, anti-CCP is very helpful and could be an additional diagnostic marker for RA, as shown in other studies.⁶ There was no sample in the control group with both RF and anti-CCP.

Our study suggests that the combined use of RF and anti-CCP is a more powerful diagnostic and prognostic tool and would allow the clinician to choose more powerful DMARDs early in the course of the disease, even when clinical judgment might not yet indicate the need for such drugs. The use of combination of RF and anti-CCP could be better than testing for either antibody alone in order to exclude the diagnosis of RA. Despite a well documented lack of specificity, RF continues to be a central part of the definition of RA, primarily because of favorable sensitivity profile. These factors' association with clinical signs of joint erosions suggests their potential usefulness as markers for prognosis.

This study had some limitations. For example, the patients had a long duration of disease. For better estimation of the prognosis, further studies in this regard and a longitudinal follow up are recommended.

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References

- 1 NG KP, Austin P, Ameratunga R, McQueen F. Role of anti cyclic citrullinated peptide 2 assay in long-standing rheumatoid arthritis. *APLAR J Rheumatol* 2006;**9**:211-5. [doi: 10.1111/j.1479-8077.2006.00203.x]
- 2 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315-24. [3358796] [doi:10.1002/art.1780310302]
- 3 Berglin E, Padyukov L, Sundin U, Hallmans G, Stenlund H, Van Venrooij WJ, Klareskog L, Dahlqvist SR.

- A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. *Arthritis Res Ther* 2004;**6**:R303-8. [15225365] [doi:10.1186/ar1187]
- 4 Serdaroğlu M, Cakirbay H, Değer O, Cengiz S, Kul S. The association of anti-CCP antibodies with disease activity in rheumatoid arthritis. *Rheumatol Int* 2008;**28**:965-70. [18418600] [doi:10.1007/s00296-008-0570-3]
 - 5 Kastbom A, Strandberg G, Lindroos A, Skogh T. Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). *Ann Rheum Dis* 2004;**63**:1085-9. [15308517] [doi:10.1136/ard.2003.016808]
 - 6 Vallbracht I, Rieber J, Oppermann M, Förger F, Siebert U, Helmke K. Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. *Ann Rheum Dis* 2004;**63**:1079-84. [15308516] [doi:10.1136/ard.2003.019877]
 - 7 Agrawal S, Misra R, Aggarwal A. Autoantibodies in rheumatoid arthritis: association with severity of disease in established RA. *Clin Rheumatol* 2007;**26**:201-4. [16572283] [doi:10.1007/s10067-006-0275-5]
 - 8 Nielen MM, van der Horst AR, van Schaardenburg D, van der Horst-Bruinsma IE, van de Stadt RJ, Aarden L, Dijkmans BA, Hamann D. Antibodies to citrullinated human fibrinogen (ACF) have diagnostic and prognostic value in early arthritis. *Ann Rheum Dis* 2005;**64**:1199-204. [15640269] [doi:10.1136/ard.2004.029389]
 - 9 Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, Sundin U, van Venrooij WJ. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:2741-9. [14558078] [doi:10.1002/art.11223]
 - 10 Goldbach-Mansky R, Lee J, McCoy A, Hoxworth J, Yarboro C, Smolen JS, Steiner G, Rosen A, Zhang C, Ménard HA, Zhou ZJ, Palosuo T, Van Venrooij WJ, Wilder RL, Klippel JH, Schumacher HR Jr, El-Gabalawy HS. Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. *Arthritis Res* 2000;**2**:236-43. [11056669] [doi:10.1186/ar93]
 - 11 Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Vandenbroucke JP, Dijkmans BA. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;**50**:380-6. [14872479] [doi:10.1002/art.20018]
 - 12 Aho K, Palosuo T, Raunio V, Puska P, Aromaa A, Salonen JT. When does rheumatoid disease start? *Arthritis Rheum* 1985;**28**:485-9. [4004958] [doi:10.1002/art.1780280503]
 - 13 Bas S, Genevay S, Meyer O, Gabay C. Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. *Rheumatology (Oxford)* 2003;**42**:677-80. [12709545] [doi:10.1093/rheumatology/keg184]
 - 14 Guler H, Turhanoglu AD, Ozer B, Ozer C, Balci A. The relationship between anti-cyclic citrullinated peptide and bone mineral density and radiographic damage in patients with rheumatoid arthritis. *Scand J Rheumatol* 2008;**37**:337-42. [18609264] [doi:10.1080/03009740801998812]