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Diagnostic Utility of Anti-Citrullinated Protein Antibody and its Comparison with Rheumatoid Factor in Rheumatoid Arthritis

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ABSTRACT

Objective: To assess the diagnostic utility of anti-citrullinated protein antibody (anti-CCP) in rheumatoid arthritis (RA) and compare it with rheumatoid factor (RF).

Study Design: Analytical study.

Place and Duration of Study: Section of Chemical Pathology, Department of Pathology and Microbiology and Medicine, the Aga Khan University, Karachi, from January to May 2010.

Methodology: A review of medical records of patients presenting to the clinics with complaints of muscular or joint pains and who were tested for their serum anti-CCP was done. Inclusion criteria were presence of clinical synovitis in at least one joint and an absence of alternative diagnosis. Patients with arthralgia alone or with missing acute phase reactants information were excluded. Scoring and classification of RA was done using the 2010 RA Classification Criteria by the American College of Rheumatology (ACR).

Results: Out of the 98 charts reviewed, ACR criteria showed 54 cases with RA. The mean age of the group was 46 ± 15 years, 82.7% being females. High titers of anti-CCP corresponded with the ACR scores. The sensitivity and specificity of anti-CCP and RF reactivity for the diagnosis of RA were 54.7% and 95.5% versus 59.3% and 88.4% respectively.

Conclusion: Anti-CCP is useful for the diagnosis of RA due to its higher specificity as compared to RF and can predict disease severity.

Key words: Rheumatoid arthritis. Anti-CCP. Rheumatoid factor.

INTRODUCTION

The estimated worldwide prevalence of RA is 1 – 2% that increases with age, approaching 5% in women over age 55.¹ In a study done on adults from northern Pakistan, a high overall burden of rheumatic diseases has been shown, and the prevalence of RA was calculated to be 5.5 per 1000.² A recent report from a rheumatology clinic in Karachi showed 12.9% of adult patients to be suffering from RA and of these 3.5% had extra-articular complications.³ A survey of joint symptoms among 4232 adults, evenly distributed between poor and affluent areas of Karachi, Pakistan reported the prevalence of RA as 0.9 and 1.98 per thousand in the two areas, respectively.⁴

The focus of recent research was on the development of markers for early diagnosis of RA to prevent absolute crippling deformity of joints and extra articular complications involving lungs, pericardium and the sclera.⁵ Since the discovery of RF by Waaler in 1940, its role in the pathogenesis of RA and its diagnostic utility has been widely investigated. Changes in serum RF levels are used as indicators of disease activity, and in

monitoring responses to therapy, especially to biological disease modifiers.⁶⁻¹⁰ However, the RF test is not always discriminative. High serum levels of RF have also been reported in chronic viral infections such as infection from *hepatitis C virus* and bacterial endocarditis.^{11,12}

Antibodies to cyclin citrullinated antigen (anti-CCP) bind to antigenic determinants that contain the amino acid citrulline, a reaction catalyzed by the enzyme peptidyl arginine deiminase that involves post-transcriptional modification of arginine residues. These antibodies have been shown to be the same as antikeratin and anti-filaggrin which have been described to play a significant role in the pathogenesis of RA.¹⁴

This study was conducted to compare the utility of anti-CCP and RF in the diagnosis of RA in patients presenting with pain to medical clinics at the Aga Khan University Hospital (AKUH), Karachi.

METHODOLOGY

This cross-sectional analytical study was undertaken at the Section of Chemical Pathology, Department of Pathology and Microbiology and the Bone and Rheumatology Clinic, Department of Medicine at the AKUH, Karachi. A review of medical records of patients presenting to the clinics of medicine, metabolic bone diseases and rheumatology AKUH with complaints of muscular or joint pains and who were tested for their serum anti-CCP levels from 1st January 2010 to 31st May 2010 at the Clinical Laboratory of AKUH was performed.

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A preformed questionnaire comprising of demographic and clinical details about symptoms and signs of the patients were recorded along with the duration of the symptoms, values of erythrocyte sedimentation rate (ESR) and the serum levels of C-reactive protein (CRP), RF and anti-CCP. The study was approved by ethical review committee of AKUH for publication.

Scoring for RA was performed using the 2010 Rheumatoid Arthritis Classification Criteria by American College of Rheumatology (ACR) for each patient.¹³ The criterion was applied only to eligible patients, in whom the presence of obvious clinical synovitis in at least 1 joint was present and there was an absence of alternative diagnosis. Patients with mere arthralgia were excluded. Criteria had four categories which were included in the proforma as joint involvement, serology, acute phase reactants and duration of symptoms.

To classify a patient as having or not having definite RA, a history of symptom duration, a thorough joint evaluation, and at least one serologic test (RF or anti-CCP) and one acute-phase response measure (ESR or CRP) were evaluated.

Levels of anti-CCP < 17 U/ml were considered normal, 17-51 U/ml as low-level positive and values > 51 U/ml as high-level positive. The levels of RF < 14 U/ml were considered normal, 14 – 42 U/ml as low-level positive and values > 42 U/ml as high-level positive.

As per ACR criteria, an individual patient may meet the definition of RA without the need of all tests. However, for the purposes of research, documentation of each domain was done. A cut-off of ≥ 6 was used to classify the patients as RA.

Serum anti-CCP was assayed (using a second generation anti-CCP assay kit) by electrochemiluminescence on automated analyzer, Cobas 601 (Roche Diagnostics, USA). Quality controls provided by the manufacturer (Elecsys Preci Control anti-CCP 1 and 2) were run with every batch analyzed. The within-run CVs were 3.1% and 2.5% at concentrations of 16.9 U/ml and 356 U/ml. The measuring range of the assay was 7 – 500 U/ml. Samples with anti-CCP concentrations above the measuring range were diluted 1:2 to 1:5 manually.

Serum RF was analyzed by photometry on Hitachi (Roche Diagnostics, USA). The system was closely monitored by routine practice of running both high and low controls with every batch analyzed. The within-run CV was 1.48% at concentration 18.1 IU/ml and 0.46 at 55.2 IU/ml. The day-to-day precision was 4.66% and 2.48% at concentrations 17.5 IU/ml and 57.7 IU/ml respectively.

Erythrocyte sedimentation rate was measured using the Westergren method. The system for ESR was monitored by two point calibration. The laboratory also successfully

participated biannually in external quality control system by College of American Pathologists (CAP) for ESR. Values of ESR greater than 20 and 15 mm/hour were considered abnormal for females and males respectively.

Serum CRP was analyzed on Synchron CX 9 (Beckman, USA). Low, normal and high controls were run with each batch. The within-run and total CVs were 5% and 7% respectively. It was also closely monitored by biannual participation in external proficiency program of CAP. Values of CRP greater than 1 mg/dl were considered abnormal.

Statistical Package for Social Sciences (SPSS) version 19 was used for statistical analysis of the data. A p-value < 0.05 was considered significant throughout. Median and interquartile range (IQR) for quantitative variables was computed. Receiver operator curves (ROC) were generated and areas under the curves (AUC) were calculated. The AUC was used as an index in evaluating the inherent capacity of the two antibodies to discriminate between “positive” and “negative” RA cases. The sensitivity, specificity, positive predictive and negative predictive values were calculated for both RF and anti-CCP. An association between the scores and anti-CCP levels was analyzed using Spearman's correlation. Kolmogorov test was applied for checking the normality of data distribution (p value < 0.05). Mann Whitney U-test was applied to compare anti-CCP levels between RA positive and negative groups.

RESULTS

During the study period, 98 patients presented with symptoms of muscular and joint pains in ambulatory care and investigated for anti-CCP at Clinical Laboratory of AKUH. The mean age of the patients was 46.5 ± 15 years. It consisted of 81 females (82.7%) and 17 males (17.3%). Out of the total, 53 patients were classified as RA (ACR score ≥ 6) and 45 as RA negative (ACR score < 6) based on ACR criteria. A summary of the patients' clinical symptomatology that was collected from the questionnaire is presented in Table I. Joint pain, swelling and stiffness were the predominant symptoms. The disease chiefly affected the small joints of the hands, though almost all the joints in the body were affected to some extent. Number of joint involvement is reflected in higher ACR scores.

The median anti-CCP levels in 98 cases was 3.5 IU/ml (IQR = 3.5 – 500). Out of the total, 31 patients had anti-CCP levels > 17 IU/ml (median 374 U/ml, IQR = 19.3 – 500 IU/ml), 21 of these individuals had RA on the basis of ACR score. Levels of anti-CCP were within normal reference range in 67 patients (median 3.5 U/ml IQR = 3.5 – 12.7 IU/ml). The ROC analysis revealed AUC of 0.76 for anti-CCP as depicted in Figure 1 (p < 0.01).

Median anti-CCP levels showed a rising trend with increasing ACR scores. The levels of anti-CCP were positively correlated with their ACR scores using the Spearman's correlation whereby 'r' was calculated as 0.58 (p < 0.01). Mann Whitney U-test showed that patients with RA (ACR score ≥ 6) had significantly higher anti-CCP levels as compared to those without RA (ACR score < 6) (p < 0.001).

Fifty eight individuals out of the 98 were tested for both auto-antibodies, anti-CCP and RF as a diagnostic workup. Out of them, 48 were females (82.8%) and 10 were males (17.3%). Their mean age was 46 ± 15 years. There were 32 patients who had a score ≥ 6 (RA positive). Amongst them 87.5% were females. Their anti-CCP and RF titers have been recorded in Table II.

Table I: Symptomatology of patients presenting with pain to medical clinics of the Aga Khan University Hospital, Karachi, Pakistan (n=98).

Clinical symptoms	Results n (%)
Joint pain	93.9 (92)
Joint swelling	52.04 (51)
Joint stiffness	63.27 (62)
Fever	16.3 (16)
Unilateral shoulder joint involvement	09.1 (9)
Bilateral shoulder joint involvement	31.6 (31)
Unilateral elbow involvement	08.2 (8)
Bilateral elbow involvement	06.1 (6)
Hip joint involvement	16.3 (16)
Unilateral knee involvement	03.06 (3)
Bilateral knee involvement	41.8 (41)
Ankle involvement	20.4 (20)
Small joints involvement	46.9 (46)

Table II: Comparison of variables of ACR classification among RA positive versus RA negative patients (n = 58).

Variable in ACR classification	RA positive ACR Score ≥ 6* n = 32	RA negative ACR Score < 6* n = 26
RF IU/ml (IQR)	21.2 (3.5-329.4)	7.8 (3.5-23.5)
Anti-CCP IU/ml (IQR)	43.1 (3.5-500)	3.5 (3.5-500)
Haemoglobin in gm/dl (IQR)	11.5 (7.2-14.4)	12.8 (8.6-15.3)
Joint pain in % (n)	100 (30)	89.28 (25)
Joint swelling in % (n)	77.8 (20)	29.6 (8)
Joint stiffness in % (n)	48.4 (14)	22.2 (6)
Patients having a joint involvement score > 2 in % (n)	64.5 (20)	44.4 (12)
Patients with raised acute phase reactants in % (n)	63.3 (18)	48.1 (13)

*2010 Rheumatoid Arthritis Classification Criteria by American College of Rheumatology [28]. ACR = American College of Rheumatology; RA = rheumatoid arthritis; anti-CCP = antibodies to cyclin citrullinated antigen.

Table III: Diagnostic utility of anti-CCP (n = 98) and RF (n = 58) with ACR scores ≥ 6 (RA positive) and ACR score < 6 (RA negative).

Auto Antibodies (n)	Cutoffs (IU/ml)	RA positive ACR score ≥ 6*	RA negative ACR score < 6*	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	Area under the curve
anti-CCP (98)	≥ 17	29	02	54.7	95.5	93.5	64.1	0.75
	< 17	24	43					
RF (58)	≥ 14	19	03	59.3	88.4	86.3	63.8	0.74
	< 14	13	23					

* 2010 Rheumatoid Arthritis Classification Criteria by American College of Rheumatology (ACR) [28]. anti-CCP = antibodies to cyclin citrullinated antigen; RF = rheumatoid factor; ACR = American College of Rheumatology; RA = rheumatoid arthritis; anti-CCP = antibodies to cyclin citrullinated antigen.

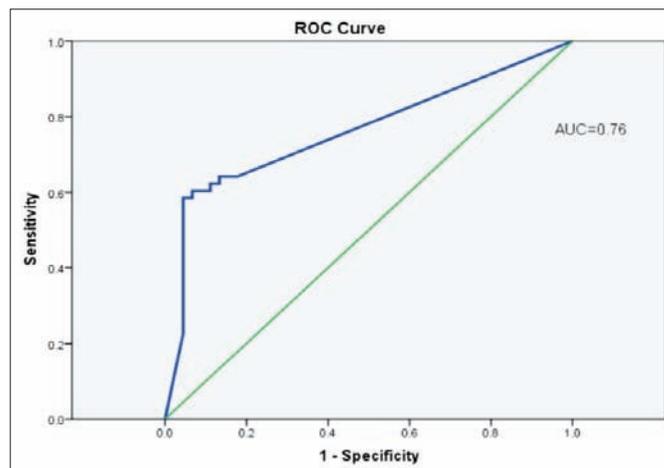


Figure 1: Receiver-operating characteristic curve of anti-CCP levels.

Comparison of the diagnostic utility of anti-CCP and RF is shown in Table III. While, RF was more sensitive as compared to anti-CCP, anti-CCP was more specific with a higher positive and negative predictive values. The ROC analysis revealed AUC of 0.74 for RF (p < 0.01). Positivity of either auto-antibody (either RF or anti-CCP) did not increase the sensitivity or specificity for detecting RA.

DISCUSSION

The importance of anti-CCP as a diagnostic and prognostic marker for RA has been elucidated widely in the developed world. Majority of the studies published have assessed the diagnostic properties of anti-CCP in concerned patients with confirmed RA according to the ACR 1987 revised criteria.¹⁴ The 1987 ACR classification criteria for RA have been criticized for their lack of sensitivity in early disease. For the first time in this study, the diagnostic performance of anti-CCP was assessed against the new 2010 ACR classification criteria for RA. This classification system re-defines RA by focusing on features at earlier stages of disease that are associated with persistent and/or erosive disease, rather than defining the disease by its late-stage features.

High anti-CCP titers in the patient population in this study were able to differentiate musculoskeletal pain that originated as a result of RA from other medical conditions that resulted in the same symptomatology.

The greater specificity of anti-CCP than RF has been indicated in many studies in the world but none so far

from Pakistan.^{15,16} In this study of patients who were diagnosed with RA, the greater specificity of anti-CCP over RF was further validated. Specificity of anti-CCP in diagnosing RA is comparable to previously published studies and meta-analysis.¹⁷⁻²⁰ A wide range of anti-CCP sensitivities have been reported by these authors ranging from 41 to 87.6%. In a systemic analysis, on the use of anti-CCP in RA patients, Avouac *et al.* reported a diagnostic sensitivity of 39 – 94%.¹⁸ This wide variability in sensitivity is attributed to varying anti-CCP cutoff value in defining a positive test, differing patient populations, and the use of assays with varying principle by different manufacturers. This was also observed by Garcia-Berrola who revealed differences between three anti-CCP second-generation enzyme linked immunosorbent assay (ELISA).¹⁹

High titers of anti-CCP also corresponded with the severity of symptoms as indicated by higher serum values in patients with more severe disease as judged by the symptoms score in ACR scoring system. In an earlier study, the anti-CCP levels correlated with disease activity parameters in 379 early RA patients where it was assessed at baseline and then radiological joint damage and progression were assessed after 2 years of follow-up. The univariate predictor analysis showed that anti-CCP had the highest significant odds ratio for radiological joint damage.²⁰ In another study, anti-CCP testing was done on 242 RA patients who were followed for 3 years. Anti-CCP antibodies were positively correlated with higher acute phase reactants, swollen joint count, and worse physician global assessment ratings.²¹

Multiple studies have shown that anti-CCP positive early RA patients develop a more erosive disease than those without anti-CCP and it has been validated as a marker of erosive disease in RA.²²⁻²⁴ In the present study, anti-CCP showed a higher specificity, a greater positive and negative predictive values but lower sensitivity compared to RF. All diagnostic parameters were highly significant statistically.

The common medical regimen that is prescribed to combat RA includes non-steroidal anti-inflammatory agents, methotrexate, steroids, sulfonamides and disease modifying anti-rheumatic agents, all of which have serious adverse effects. RF has been shown to be a non-specific marker repeatedly; its use as a confirmatory test for RA should be avoided. RF factor positivity can be confusing in areas like ours where prevalence of hepatitis is high especially when the patients present with arthralgia. This can be avoided by the judicious use of anti-CCP.

CONCLUSION

The specificity is the most valuable aspect of anti-CCP for diagnosing RA in a population where there is a high disease burden of chronic hepatitis. Its low sensitivity

does not allow its use as a screening test but can be used in conjunction with RF. Its induction in the normal protocol in diagnosing RA is necessary. It is, therefore, desirable to popularize its use amongst rheumatologists throughout Pakistan.

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