ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Value of Anti-Cyclic Citrullinated Peptide and Rheumatoid Factor Antibodies in relation to Rheumatoid Arthritis: Association with Osteoporosis

Youssef M. Mosaad[1], Enas M. Hammad[2], Iman Fawzy[3], Samy Marouf[1], Mohammad Al-Harrass [1], Adel Abdelsalam[4], Mohamed S. Abdelgawad [4], Mohamed M. El Rakhawyi[5]

1- Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
2-Rheumatology & Rehabilitation Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
3- Laboratory Medicine Department, Mansoura Fever Hospital, Ministry of Health, Mansoura, Egypt
4-Internal Medicine Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
5- Radiology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Manuscript Info

Manuscript History:

Received: 15 June 2015
Final Accepted: 26 July 2015
Published Online: August 2015

Key words:

Anti-CCP, Rheumatoid arthritis, Erosion, Osteoporosis, Severity

Corresponding Author

Youssef M. Mosaad

Abstract

Objective: To study the role of anti-Cyclic Citrullinated Peptide antibody (anti-CCP) and Rheumatoid Factor (RF) in Rheumatoid Arthritis (RA) and to evaluate their relation to the development of erosion, osteoporosis, disease activity, disease severity and functional disability. Methods: Anti-CCP, RF and CRP assays were done for 128 patients diagnosed with RA, in addition to 151 healthy and 30 osteoporotic postmenopausal female controls. Assessment measures for disease Activity, disease severity, erosions, osteoporosis and functional disability were done for RA patients. ***Result***: The titer and seropositivity of the studied serologic markers (Anti-CCP and RF) have significant association with RA. Osteoporosis complicating RA is positively associated with seropositivity of anti-CCP. While, the seropositivity of anti-CCP antibody is significantly associated with more severe and active disease, the seropositivity of RF is positively associated only with disease severity. The high positive anti-CCP or high positive RF is positively associated with increased disease severity or activity and presence of osteoporosis. Higher titer of anti-CCP is a significant risk to the development of erosion and OP in RA patients either in crude or adjusted models. ***Conclusion:*** Due to its higher specificity, anti-CCP is useful serologic marker for diagnosis of RA as compared to RF and can predict disease activity. Anti-CCP antibodies identify a subset of patients who are more likely to accrue more joint damage, more severe disease course and to develop osteoporosis.

Copy Right, IJAR, 2015,. All rights reserved

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease characterized by chronic joint inflammation and extra-articular manifestations. The disease leads to progressive destruction of articular cartilage and ankylosis of the joints that eventually leading to permanent disability without early therapeutic interventions. Although the cause of RA is unknown, autoimmunity plays a pivotal role in both its chronicity and progression (Lee and Weinblatt 2001, Zintzaras et al. 2008, Block et al. 2012).

RA afflicts up to 1% of the world's population, affecting females to a greater extent (3–5 fold) than males (van Venrooij et al. 2008). Diagnosis of RA was based on the American College of Rheumatology (ACR) criteria (Arnett et al. 1988). Rheumatoid factor (RF), an antibody directed against the constant region of IgG, was the only serological marker included in ACR criteria. RF has moderate sensitivity and do not detect early asymptomatic disease. In the same time, it lacks clinical specificity due do its positivity in many rheumatic (other than RA), infectious diseases and even in healthy individuals especially elderly (Van Venrooij et al. 2002, Mikuls et al. 2004, Wiik et al. 2010).

Anti-cyclic citrullinated peptide (anti-CCP) antibodies are IgG autoantibodies which recognize citrullinated peptides and offer improved specificity in early diagnosis of RA compared to RF (Wiik et al. 2010). Recently anti-CCP testing has become substantial part of ACR-EULAR classification criteria for RA (Aletaha et al. 2010).

RA is defined by its ability to induce bone and cartilage destruction. Erosive RA is associated with pain, disability, deformity and early mortality (Pincus et al. 1984). In addition, bone destruction in RA is associated with systemic osteoporosis and susceptibility to fragility fractures because both phenomena fundamentally reflect high inflammatory disease activity (Sambroook 2000). Recent data support that even minimal subclinical inflammation can lead to bone loss and increased fracture risk (Schett et al. 2006). Osteoporosis and related fragility fractures are one of the most common complications in patients with RA and affect quality of life dramatically in these patients. Several factors such as race, BMI, dietary calcium and vitamin D intake, duration and activity of RA, grade of disability, use of corticosteroids and DMARDs and menopausal status have been studied for their influence on bone loss in RA (Ranganathan 2009, Mosaad et al 2014).

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 (Manivelavan and Vijayasamundeeswari 2012). The serological parameter that meets these requirements is anti-CCP antibody. The citrulline residues are essential part of the antigenic determinants recognized by the RA antibodies. So anti-CCP testing is particularly useful in the diagnosis of RA with high specificity present early in the disease process (Sambroook, 2000 and Block et al., 2012) and ability to identify patients who are likely to have severe disease and irreversible damage (Van Venrooij et al. 2002 , Shankar et al 2006, Niewold et al. 2007).

There is evidence that CCP-assays provide comparable performance with that of RF (Coenen et al. 2007, Nishimura et al 2007, Sun et al 2014). However, analysis of the association between anti-CCP antibody titer and RA activity produced contradictory results (Greiner et al. 2005, van Gaalen et al. 2005). Therefore, we planned this work to study the role of anti-CCP and RF antibodies in RA and to evaluate their relation to the disease activity, disease severity, development of osteoporosis, presence of erosion and functional disability.

2. Material and methods:

2.1. Patients and healthy controls

This case control study on a cohort of Egyptian population included patients with RA and two control groups of healthy individuals and postmenopausal osteoporosis females. A total of 128 patients with RA (103 females and 25 males) with mean age of 46.91± 11.73 diagnosed according to the American College of Rheumatology / European League against Rheumatism collaborative initiative for RA (Aletaha et al 2010) were consecutively recruited into the study between February 2012 and January 2013 with disease duration of 10.33± 7.01 years. Patients were recruited from the outpatient's clinic of Rheumatology and Rehabilitation Department and Rheumatology and Immunology Unit of Internal Medicine Department, Mansoura University Hospitals, Egypt. All patients underwent clinical evaluation by a rheumatologist and data about demographics and disease parameters were collected.

Disease activity was evaluated using disease activity score 28 (DAS28-CRP) that measures 28 tender and swollen joints, degree of pain by visual analogue scales (VAS), patient's and doctor's global assessment of disease activity using VAS and inflammatory markers such as C-reactive protein (CRP) (van Riel , Schumacher 2001). DAS-28 as described by Prevoo et al. 1995 defines the level of RA activity as follows; ≥ 5.1 indicate high disease activity; between 5.1 and 3.2 indicates moderate disease activity; between 3.2 and 2.6 indicates low disease activity; and < 2.6 indicates clinical remission. Assessment of severity was performed using rheumatoid arthritis medical records based index of severity (RARBIS) based on potential indicators including radiological and laboratory

results; surgeries; extra-articular manifestations; clinical and functional status; and medications (Cabral et al., 2005, Ting et al., 2008).

All patients underwent radiographs of the hands and feet. X-rays were scored by an expert radiologist using the Steinbrocker method 1949. Antero Posterior radiographs of hand (including wrist) and feet were taken to assess the erosive changes, and cervical spine in flexion and extension views for instability sublaxation. All RA patients underwent bone densitometry scanning using dual energy x-ray absorpotometry (DEXA). BMD of lumbar 2-4 vertebrae was assessed and T scores determined to differentiate between cases with normal bone mineral density (BMD) and those with reduced BMD.

Postmenopausal Osteoporosis (OP) group: 30 women (mean age 59.12± 11.97) with postmenopausal OP not suffering from RA were enrolled as a disease control group at the time of diagnosis of osteoporosis and before receiving bone protection therapy. Diagnosis of OP was based on measurement of bone mineral density using DEXA scan for lumbar spine (L2– 4). According to the WHO 1994 [42], osteoporosis is a score of < -2.5.

The healthy control group consisted of 151 unrelated healthy subjects with mean age 40 ± 15.83 without RA or risk factors of osteoporosis were recruited from blood bank donors. Written informed consent was obtained from patients and controls after approving the study protocol by Local Ethical Committee.

Exclusion criteria: Patients with other metabolic bone disease, endocrinal disorders, other autoimmune diseases, hepatic and renal disorders, malignancy and those taking medication known to affect bone metabolism such as bisphosphonates, or vitamin D were excluded from the study. Six patients were excluded from the study due to missing data.

Blood samples were obtained from all groups for determination of serum calcium, phosphorous, alkaline phosphatase (Coobas Integra 400 plus, Roche Diagnostic, Germany) and Parathormone (electro-chemiluminescent immunoassay, Elecsys 2010, Roche Diagnostic, Germany). Immunoturbidimetric assay was used to quantify RF and CRP using Turbox-plus analyzer (Orion Diagnostica, Espoo, Finland). Anti-CCP antibodies were analyzed by third generation ELISA (CCP3 IgG, INOVA QUANTA LiteTM, Sandiego, USA). According to manufacturer, serum is considered positive when the titer is ≥10 mg/ L for CRP and ≥25 IU/ml for RF. RF grading is; 25-50 IU/ml = borderline positive, 50 – 100 IU/ml= positive and ≥100 IU/ml= strongly elevated.

Anti-CCP Antibody Assay

Anti-CCP antibodies were analyzed by third generation ELISA (CCP3 IgG, INOVA QUANTA LiteTM, Sandiego, USA). The wells were coated with highly purified synthetic cyclic citrullinated peptide. One hundred µl of the prediluted positive, negative controls, calibrators and the diluted patient samples (1:101 dilution of each patient sample by adding 5µL of sample to 500 µl of HRP Sample Diluent) was added to the wells and incubated for 30 minutes at room temperature. After 3 cycles of wash, 100 µl of conjugate was added to each well and incubated for 30 minutes at room temperature. Another round of washing was done, followed by addition of 100 µl of the chromogen. After incubation for 30 minutes in the dark at room temperature, 100 µl of the stopping solution was added. Finally, the absorbance (OD) of each well was read at 450 with a reference wavelength at 620 nm within one hour of stopping the reaction. The intensity of the color develops was proportional to the concentration of autoantibody in the sample. According to the manufacturer's recommendation, the cut off value of the assay was 20 units/ ml. Values <20 U= negative; 20 -39 U=weak positive; 40- 59 U=moderate positive, and ≥60 U=strong positive.

2.3. Statistical Analysis

The statistical analysis of data was done by using excel (Microsoft office 2010 program) and SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 20. Kolmogorov-Smirnov test was done to test the normality of data distribution. Qualitative data were presented as frequency and percentage. Chi square and Fisher exact tests were used to compare groups. Quantitative data were presented as mean and standard deviation. For comparison between two groups; student t-test and Mann-whitney test (for non parametric data) were used. Logistic or linear regression analysis was performed to identify independent factors that might jointly have a significant influence on RA progression. N.B: p is significant if <0.05 at confidence interval 95%.

3. Results:

Demographic and baseline characteristics of RA patients are summarized in Table 1. Comparisons of anti-CCP and RF antibody titer and seropositivity in RA patients, healthy controls and OP females were done. While the patients with RA had a higher titer and increased prevalence of seropositivity of the studied markers, the healthy controls and OP females showed a lower titer and increased prevalence of seronegativity and the differences in titers and seropositivity were statistically significant ($P<0.001$). Serum calcium was significantly lower and alkaline phosphatase was significantly higher in RA when compared to control subjects Table 2

In the same time, the titer and seropositivity of anti-CCP and RF antibodies in RA patients were analyzed in relation to the presence or absence of osteoporosis. A statistical significant association was found only between OP complicating RA and seropositivity of anti-CCP antibodies ($P=0.03$). However, the higher titer of RF showed marginal significant association with the presence of OP in RA patients ($P=0.051$). No significant differences were found regarding serum calcium, phosphorus, alkaline phosphatase and parathormone (PTH) in RA patients with and without OP. Table 2

The seropositivity of anti-CCP and RF were analyzed in relation to demographic and baseline characteristics of RA patients. The seropositivity of anti-CCP antibody was significantly associated with older patient age ($P=0.038$), higher CRP titer ($P=0.007$) and higher score of; RABRIS ($P=0.001$); DAS28 CRP ($P=0.002$) and DEXA ($P=0.033$). On the other hand, the seropositivity of RF was positively associated only with higher RABRIS score ($P<0.001$). No associations were found between the seropositivity of markers and serum calcium, phosphorous, alkaline phosphatase, PTH and steroid requirements. Table 3

We classified the seropositivity of anti CCP and RF of the present study according to the 2010 Rheumatoid Arthritis Classification Criteria of the ACR into negative RF and negative anti-CCP, low positive RF or low positive anti-CCP and high positive RF or high positive anti-CCP. The demographic and baseline characteristics of RA patients were compared in relation to the three subgroups to evaluate the role of seropositivity and its grades in relation to the presence of erosion, development of osteoporosis, disease severity, disease activity and functional disability. In all comparisons, the seropositivity and higher grade of seropositivity of both markers (either compared to seronegativity or low seropositivity) showed significant association ($P<0.05$) with higher CRP titer and higher scores of; RABRIS (disease severity); DAS28-CRP (disease activity), DEXA (osteoporosis) and lower level of serum phosphorous. No associations were found for functional disability, the presence of erosion, steroids requirement and serum levels of calcium, alkaline phosphatase and PTH ($P>0.05$). Table 4

Analysis of association of the titer / seropositivity of anti-CCP and RF antibodies in crude and adjusted models (adjusted for age, gender and steroid requirement) with the presence of erosion, disease severity, presence of osteoporosis and functional disability in RA patients was presented in table 5. Higher anti-CCP titer had a significant risk to the development of erosion and OP in RA patients either in crude or adjusted models ($P=0.038$, 0.022, 0.029, and 0.032 respectively). In the same time, the titer and seropositivity of both anti-CCP and RF were associated with severe RA in either crude or adjusted models ($P<0.05$). On the other hand, anti-CCP and RF titer and seropositivity either alone or combined had no association with the functional disability ($P>0.05$).

ROC analysis was conducted to identify the optimal anti-CCP and RF serum levels for potential prediction of development of OP within RA patients. From this curve, the anti-CCP best cut-off value was 77 U/mL, with a sensitivity of 94.9% and a specificity of 20.2%. The area under the curve (AUC) was 0.624, 95% CI=0.527-0.720, $p=0.049$. Although the test is significant, its AUC had poor discriminatory power. The RF best cut off was 50 U/mL with a sensitivity of 89.7% and specificity of 19.1%, AUC was 0.502, 95% CI=0.391-0.594, $p=0.892$. Then the test is statistically not better than making the diagnostic decision based on pure chance, which has an AUC of 0.5. Figure 1

Anti-CCP antibody is more specific than RF (95% versus 88.7%) with better positive /negative predictive values (PPV and NPV) and higher accuracy (90.3% versus 86.4%) than RF for diagnosis of RA. However, both serologic markers had nearly similar sensitivity (84%). Combination of both tests improved the sensitivity (93.8%), NPV (88.9%) and accuracy (91.8%). Anti-CCP, RF and their combination had appropriate performance characteristics in the diagnosis of RA. However, they had good sensitivities but low specificities in discrimination of erosions and osteoporotic lesions in patients with RA. Table 6

The sensitivity and specificity of anti-CCP and RF results of the present work were compared to findings from different studies [30-41]. There were significant differences between different studies as regard the sensitivity of anti-CCP and RF antibodies as well as specificity of RF ($P=$ 0.001, 0.003, 0.015 respectively). However, in

terms of specificity of anti-CCP antibodies, no significant differences among those findings were observed (P=0.259). Table 7

Table 1: Demographic and baseline characteristics of RA patients

			RA patients (n=128)
Age (years) (M ± SD)			46.91± 11.73
Gender : Male / Female			25/103
Disease duration years (M ± SD)			10.33 ± 7.01
Disease onset (M ± SD)			36.65 ± 11.97
Erosion /No erosion			27/101
DEXA (M ± SD)			-1.56 ± 1.82
DEXA grade Normal (>-1) Osteopenia (-1 to – 2.5) Osteoporosis (‹-2.5)			 42 47 39
Osteoporosis (yes/ no)			39/89
RARBIS (M ± SD)			6.88±1.88
Family history (Yes/no)			42/86
Rheumatoid factor IU/ML (M ± SD)			126.31 ± 93.52
Positive /Negative Rheumatoid factor			107/21
Anti-CCP IU/ML (M ± SD)			201.77 ± 121.4
Positive / Negative Anti-CCP			108/20
CRP mg/L (M ± SD)			29.82 ± 33.25
DAS28 (M ± SD)			5.33 ± 1.25
DAS28 grade (number of RA patients) High activity Moderate Low Remission			 70 51 4 3
Calcium (M ± SD)			8.92±0.71
Phosphorous (M ± SD)			4.05±0.49
Alkaline phosphatase (M ± SD)			83.95±23.41
Parathormone (M ± SD)			28.95±16.27
Calcium/Phosphorus ratio			2.22±0.275
Consanguinity (yes/no)			38/90
Medications	DMARDs	Methotrexate	56 (43.8%)
		Hydroxychloroquine	67 (52.3%)
		Leflunomide	68 (53.1%)
		Sulfasalazine	13 (10.2%)
	Steroids		64 (50%)

DMARDs, Disease-modifying antirheumatic drugs.

Table (2): Comparison of Anti-CCP and RF antibodies in Rheumatoid Arthritis patients versus healthy Controls and Osteoporosis Postmenopausal females

Marker	**Control (N=151)**	**RA without OP (N=89)**	**RA with OP (N=39)**	**Post menopausal OP (N=30)**	***P value***

Anti-CCP Titer (M±SD)	6.68±5.53	185.52±125.17	222.92±110.75	7.59±3.58	P1<0.001* P2=0.261 P3<0.001*
Anti-CCP Negative / Positive (N/%)	144 / 7 (95.4 /4.6)	18 / 71 (20.2/79.8)	2 / 37 (5.1 /94.9)	30 /0.0 (100/0.0)	P1<0.001* P2=0.03* P3<0.001*
RF Titer (M±SD)	10.36±7.01	117.18±94.57	133.98±77.67	14.09±6.34	P1<0.001* P2=0.051 P3<0.001*
RF Negative / Positive (N/%)	134 / 17 (88.7/11.3)	17 /72 (19.1/80.9)	4 / 35 (10.3/89.7)	29 / 1.0 (97.7/3.3)	P1<0.001* P2=0.214 P3<0.001*
Calcium (mg/dL) (M ± SD)	9.12±0.572	8.87±0.799	8.99±0.546	7.95±0.899	P1=0.004* P2=0.678 P3<0.001*
Phosphorous (mg/dL) (M ± SD)	4±0.434	4.06±0.542	4.04±0.422	4.29±0.554	P1=0.343 P2=0.786 P3=0.084
Calcium/Phosphorus (M ± SD)	2.3±0.234	2.215±0.302	2.24±0.226	1.89±0.407	P1=0.045* P2=0.684 P3<0.001*
Alkaline phosphatase (U/L) (M ± SD)	76.52±15.3	85.48±25.93	81.60±18.458	66.37±11.55	P1=0.043* P2=0.925 P3<0.001*
Parathormone (pg/mL) (M ± SD)	27.09±13.8	28.65±16.655	29.34±15.808	18.60±7.802	P1=0.617 P2=0.681 P3=0.001*

Anti-CCP= anti-cyclic citrullinated peptide, RF= Rheumatoid Factor, RA= rheumatoid arthritis, OP= Osteoporosis, *significant P value‹0.05. P1: comparison between control and total RA patients, P2: comparison between RA with and without OP, p3: comparison between RA with OP and post menopausal OP.

Table (3): Anti-CCP and RF in relation to demographic and baseline characteristics of RA patients

	Anti-CCP		**P Value**	**RF**		**P Value**
	Negative (n=20)	**Positive (n=108)**		**Negative (n=21)**	**Positive (n=107)**	
Age years (M ± SD)	42.15±11.842	47.79±10.874	**0.038***	43.0±8.485	47.67±11.505	0.080
Gender: Male / Female; N (%)	7/13 (35/65)	18/90 (16.7/83.3)	0.070	6/15 (28.6/71.4)	19/88 (17.8/82.2)	0.245
Disease duration years (M ± SD)	9.40±5.744	10.65±7.097	0.457	10.29±7.184	10.49±6.840	0.903
Disease onset (M ± SD)	33.03±12.587	37.32±11.792	0.142	32.50±10.734	37.27±12.185	0.097
CRP mg/L (M ± SD)	16.90±14.757	32.21±35.171	**0.007***	30.10±39.552	29.77±32.091	0.967
Erosion N (%)	1(5)	24(22.2)	0.12	3(14.3)	22(20.5)	0.76

DEXA (M ± SD)	- 0.81±1.378	-1.71±1.865	**0.023***	-1.181±1.440	-1.64±1.885	0.29
DEXA grade Normal Osteopenia OP	11(55) 7 (35) 2 (10)	31 (28.7) 40 (37) 37 (34.3)	**0.033***	10 (47.6) 7 (33.3) 4 (19)	32 (29.9) 40 (37.4) 35 (32.7)	0.24
RABRIS (M± SD)	6.25±1.803	7.73±1.757	**0.001***	6.0±1.643	7.79±1.725	**<0.001***
DAS28 CRP (M ± SD)	4.55±1.430	5.47±1.170	**0.002***	4.95±1.480	5.41±1.197	0.128
DAS28 grades N (%) High Moderate Low Remission	 7 (35) 9 (45) 2 (10) 2 (10)	 63 (58.3) 42 (38.9) 2 (1.9) 1.0 (0. 9)	0.64	 10 (47.6) 8 (38.1) 1 (4.8) 2 (9.5)	 60 (56.1) 43 (40.2) 3 (2.8) 1.0 (0. 9)	0.636
Calcium (mg/dL) (M ± SD)	8.91±0.640	8.92±0.730	0.520	9.03±0.562	8.89±0.741	0.710
Phosphorous (mg/dL) (M ± SD)	4.14±0.522	4.04±0.495	0.737	4.01±0.366	4.06±0.521	0.359
Calcium/Phosphorus (M ± SD)	2.179±0.246	2.234±0.284	0.343	2.268±0.219	2.218±0.285	0.453
Alkaline phosphatase (U/L**)** (M ± SD)	86.75±27.617	83.52±22.654	0.602	78.95±013.70	85.02±024.79	0.788
Parathormone (pg/mL) (M ± SD)	24.95±15.557	29.64±16.378	0.892	27.30±12.530	29.23±16.954	0.236
Steroid requirement; N (%)	9 (45)	55 (50.9)	0.626	12 (57.1)	52 (48.6)	0.474

Anti-CCP= anti-cyclic citrullinated peptide, RF= Rheumatoid Factor, RA= rheumatoid arthritis, OP= Osteoporosis, *significant P value‹0.05.

Table (4): Combined Anti-CCP and RF in relation to demographic and baseline characteristics of RA patients.

		Negative Anti-CCP and RF (n=8)	**Positive Anti-CCP and RF (n=96)**	**Low positive anti-CCP or low positive RF (n=33)**	**High positive anti-CCP or high positive RF (n=87)**	***P*[1]**	***P*[2]**	***P*[3]**	***P*[4]**
Age years (M ± SD)		40.38±10.04	48.09±11.24	42±12.278	47.61±11.03	0.06	0.9	0.15	0.2
Gender; N (%)	Males Female	3 (37.5) 5 (62.5)	16 (16.7) 80 (83.3)	8 (24.2) 25 (75.8)	14 (16.1) 73 (83.9)	0.15	0.6	0.15	0.3
Disease onset age (M ± SD)		31.33±12.972	37.60±12.078	35.83±10.187	36.93±12.045	0.16	0.6	0.28	0.8
Disease duration years (M ± SD)		9.750±7.8876	10.594±7.0913	6.67±4.083	10.68±6.984	0.74	0.2	0.8	0.1
CRP mg/L (M ± SD)		13.25±9.823	30.86±33.221	20.30±19.155	34.95±37.441	0.14	0.3	0.1	**0.006***
Erosion		1 (12.5)	22 (22.9)	3 (9.1)	21 (24.1)	0.68	1.0	0.67	0.07
DEX	(M ± SD)	-0,763±1,0084	-1,743±1,8824	-1,445±2,1316	-1,694±1,7368	0.15	0.3	0.14	0.51
	Normal	5 (62.5)	27 (28.1)	14 (42.4)	23 (26.4)	0.06	0.5	**0.035***	0.15

A	**Osteopenia**	3 (37.5)	36 (37.5)	12 (36.4)	32 (36.8)				
	Osteoporosis	0 (0)	33 (34.4)	7 (21.2)	32 (36.8)				
DAS28 CRP (M ± SD)		3.91±1.29	5.46±1.16	4.86±1.00	5.49±1.17	**<0.001***	0.4	**0.001***	0.2
DAS28 grades; N (%)									
Remission		2 (25)	1 (1)	0 (0)	1 (1.1)				
Low		1 (12.5)	2 (2.1)	1 (3)	2 (2.3)	**0.005***	**0.02***	**0.008***	0.94
Moderate		3 (37.5)	37 (38.5)	14 (42.4)	34(39.1)				
High		2 (25)	56 (58.3)	18 (54.5)	50 (57.5)				
RABRIS (M± SD)		5.25±1.66	7.90±1.70	6±1.549	7.78±1.72	**<0.001***	0.85	**<0.001***	**0.015***
MHAQ		7.75±7.61	11.31±6.7	10.17±8.9	11.19±6.7	0.15	0.74	0.24	0.72
Calcium (mg/dL) (M ± SD)		8.96±0.68	8.89±0.75	8.86±0.89	8.94±0.64	0.888	0.847	0.754	0.861
Phosphorous (mg/dL) (M ± SD)		4.06±0.30	4.05±0.50	4.24±0.62	3.98±0.44	0.665	0.433	0.365	**0.020***
Calcium/Phosphorus (M ± SD)		2.214±0.2	2.225±0.28	2.127±0.3	2.266±0.2	0.765	0.396	0.389	**0.009***
Alkaline phosphatase (U/L) (M ± SD)		78.13±12.08	84.48±23.72	83.65±22.67	84.72±24.56	0.696	0.798	0.745	0.870
Parathormone (pg/mL) (M ± SD)		21.10±6.43	29.59±16.70	27.16±18.48	30.33±15.85	0.291	0.923	0.145	0.136
Steroid requirement; N (%)		5 (62.5)	48 (50)	15 (45.5)	44 (50.6)	0.716	0.445	0.716	0.487

Anti-CCP= anti-cyclic citrullinated peptide, RF= Rheumatoid Factor, RA= rheumatoid arthritis, p1:comparison between both negative RA and anti-CCP vs both positive; p2:comparison between low positive RF or low positive anti-CCP vs both negative; p3:comparison between high positive RF or high positive anti-CCP vs both negative; p4:comparison between high positive RF or high positive anti-CCP vs low positive RF or low positive anti-CCP; *significant P value‹0.05.

Table (5):Analysis of risk of erosion, osteoporosis, severity, and functional activity according to anti-CCP and RF titer and seropositivity in RA patients.

Erosion	**Crude model[a]**		**Adjusted model[a]**	
	P	**OR (95% CI)**	***P***	**OR (95% CI)**
RF	0.068	1.004 (0.99-1.009)	0.059	1.005 (1.00 -1.010)
Anti-CCP	**0.038***	1.004 (0.99-1.009)	**0.022***	1.005 (1.00- 1.010)
RF and anti-CCP	0.093	1.002 (0.98-1.003)	0.051	1.004 (0.996-1.009)
low positive RF or low positive anti-CCP	0.466	1.492 (0.509-4.37)	0.26	1.873 (0.629-5.579)
high positive RF or high positive anti-CCP	0.447	0.319 (0.017-6.05)	0.43	1.603 (0.490-5.245)
Osteoporosis				
RF	0.104	1.007 (0.562-1.012)	0.113	1.006 (0.671-1.011)
Anti-CCP	**0.029***	1.003 (1.001-1.007)	**0.032***	1.003 (1.001-1.016)
RF and anti-CCP	0.058	4.259 (0.951-2.068)	0.152	1.137 (0.656-5.013)
low positive RF or low positive anti-CCP	0.355	1.111 (0.433-2.284)	0.192	1.824 (0.595-3.407)
high positive RF or high positive anti-CCP	**0.041***	2.202 (1.034-4.687)	0.070	2.094 (0.940-4.663)

Severity						
	P	**β**	**SE**	***P***	**β**	**SE**
RF	**<0.001***	0.006	0.002	**0.001***	0.006	0.002
Anti-CCP	**<0.001***	0.005	0.001	**0.001***	0.005	0.001
RF and anti-CCP	**<0.001**	2.655	0.630	**<0.001**	2.522	0.662
low positive RF or low	**<0.001**	0.154	0.827	**0.001**	1.958	0.560

positive anti-CCP						
high positive RF or high positive anti-CCP	**<0.001**	0.967	0.253	**0.001**	0.934	0.261
Functional disability						
	P	**β**	**SE**	***P***	**β**	**SE**
RF	0.078	0.012	0.007	0.122	0.011	0.007
Anti-CCP	0.506	0.003	0.005	0.745	0.002	0.005
RF and anti-CCP	0.169	3.454	2.496	0.340	1.505	1.553
low positive RF or low positive anti-CCP	0.742	1.321	3.949	0.749	1.417	4.335
high positive RF or high positive anti-CCP	0.248	1.170	1.009	0.527	0.653	1.028

a Logistic regression models were was used for risk of erosion and osteoporosis; linear regression models were used for risk of severity and functional disability. Covariates in adjusted models include age, gender, disease duration, and steroid use. *Significant P value. β, regression coefficient; SE, standard error.

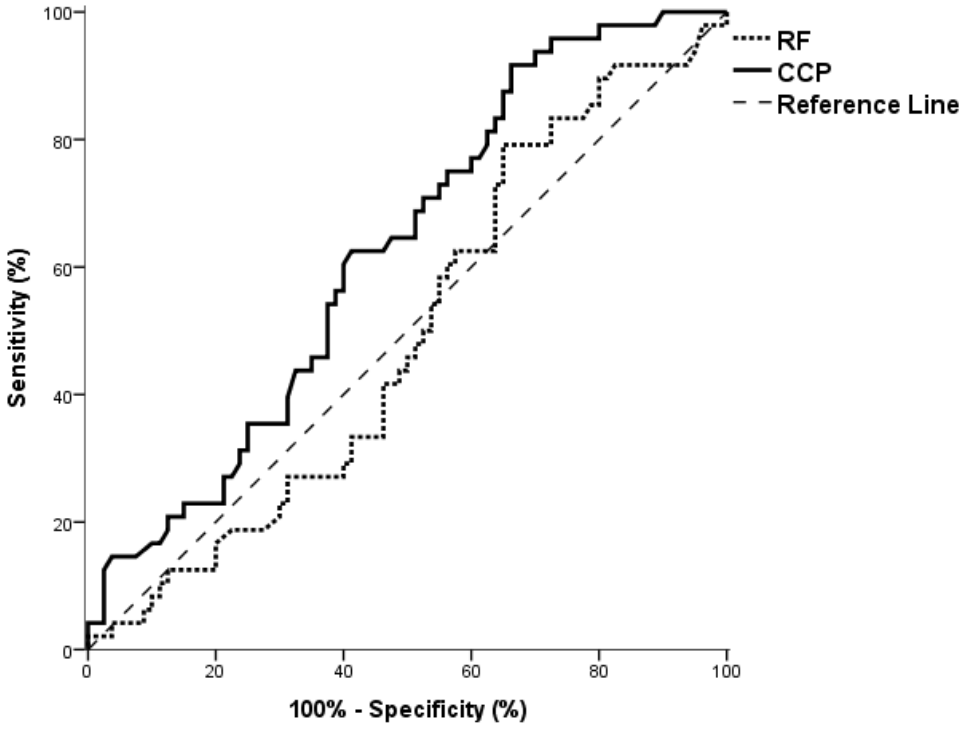

Figure (1): A receiver operating curve (ROC) analysis of anti-CCP levels for the prediction of OP within RA patients. Anti-CCP Area under the curve (AUC)=0.624, Standard error (SE)=0.049, *p*=0.020, 95% CI=0.527-0.720; the best cut off was 77 U/mL with sensitivity

=94.9%, specificity=20.2%. RF AUC=0.502, SE=0.052, *p*=0.892, 95% CI=0.391-0.594; the best cut off was 50 U/mL with sensitivity =89.7% and specificity=19.1%.

Table (6): Performance characteristics of anti-CCP and RF in the diagnosis, erosion and osteoporosis in RA patients.

		Anti-CCP	RF	RF and Anti-CCP
Diagnosis	**Sensitivity (%)**	84.4	83.6	93.8
	Specificity (%)	95.4	88.7	90.1
	PPV (%)	93.9	86.3	88.9
	NPV (%)	87.8	86.5	94.4
	Accuracy (%)	90.3	86.4	91.8
Erosion	**Sensitivity (%)**	96	88	95.7
	Specificity (%)	18.5	17.5	8.7
	PPV (%)	22.2	20.6	22.9
	NPV (%)	95	85.7	87.5
	Accuracy (%)	33.6	31.3	27.9
Osteoporotic complications	**Sensitivity (%)**	94.9	89.7	100
	Specificity (%)	20.2	19.1	11.3
	PPV (%)	34.3	32.7	34.4
	NPV (%)	90	81.0	100
	Accuracy (%)	43	40.6	39.4

Table (7): Comparison of sensitivity and specificity of anti-CCP and RF from different countries.

Reference	Country	Year	First author	Sensitivity		Specificity	
				Anti CCP	RF	Anti CCP	RF
Current	**Egypt**	2015	Mosaad	84.4	83.6	95.4	88.7
29	**Tunisia**	2008	Sghiri	72.4	65.9	96.1	74.4
30	**Kenya**	2014	Amayo	62.5	50.0	83.9	90.3
31	**Oman**	2012	Al-Shukaili	52	57	97	94
32	**Malaysia**	2010	Maraina	71	80	94.8	74.5
33	**Malaysia**	2013	Abdul Wahab	35	43	100	85
34	**Korea**	2005	Kim	81.6	96.4	95.5	75.5
35	**China**	2011	Liao	78.9	67.4	95.7	84.3
36	**Taiwan**	2008	Lin	82.1	80	88	62.7
37	**Turkey**	2009	Mutlu	60.2	67.8	98.8	91.6
38	**Netherlands**	2009	van der Linden	50	47.7	88.4	86.1
39	**Switzerland**	2002	Bas	68	75	96	74
40	**USA**	2003	Lee	66	71.6	90.4	80.3
	P value			**0.001***	**0.003***	0.259	**0.015***

4. Discussion

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease of unclear etiology that is manifested in by a progressive and destructive polyarthritis in association with serological evidence of autoreactivity. It is characterized by chronic pain and joint destruction that usually progresses from distal to more proximal joints. The progression of this disease can be slowed down with adequate medical control; however, this condition remains as one of the most important cause of inability and disability if not properly treated (Kourilovitch et al 2014).

Anti-CCP and RF are recognized biomarkers associated with RA that are incorporated in the current ACR classification guidance for RA diagnosis (Aletaha et al., 2010). The two biomarkers are promising early diagnostic tests with the potential to support early, aggressive intervention using newer RA treatment options (Taylor et al., 2011).

The present study shows that the titer and seropositivity of the studied serologic markers (Anti-CCP and RF) have significant association with RA (P‹0.001). However the anti-CCP antibody is more specific (95.4%) than RF (89%) for diagnosis of RA and both have similar sensitivity (84%). Combination of both tests improved the sensitivity (94%), NPV, accuracy and decreased the specificity (90%). Therefore, anti-CCP, RF and their combination had appropriate performance characteristics in the diagnosis of RA.

Anti-CCP antibodies, the most important members of the family of ACPAs so far, are specific diagnostic and prognostic markers in RA (Vincent et al 2005). The determination of anti-CCP helps to distinguish RA from other arthropathies (Schellekens et al 2000). Nishimura et al 2007 meta-analysis on 37 studies of anti-CCP antibody and 50 studies of RF to investigate the diagnostic accuracy of anti-CCP antibody and RF for RA demonstrated that the pooled sensitivity, specificity, positive and negative likelihood ratios for anti-CCP antibody were 67%, 95%, 12.46, and 0.36, respectively. For IgM RF, the values were 69%, 85%, 4.86 and 0.38 respectively. Likelihood ratios among IgM RF, IgG RF, and IgA RF seemed to be similar. Results from studies of patients with early rheumatoid arthritis were similar to those from all studies. Three of 4 studies found that risk for radiographic progression was greater with anti-CCP antibody positivity than with IgM RF positivity. They concluded that anti-CCP antibodies are more specific than RF for diagnosing rheumatoid arthritis and may better predict erosive disease.

The most recent meta-analysis was carried out by Sun et al 2014 on 24 studies that conformed to their inclusion criteria to investigate the diagnostic accuracy of combined tests of anti-CCP antibody and RF for rheumatoid arthritis. They reported the summary estimates for anti-CCP antibody and RF positivity (both serum markers had to be positive) in the diagnosis of RA were 57% sensitivity and 96% specificity and the pooled data for anti-CCP antibody or RF positivity (one serum marker had to be positive) were 78% sensitivity and 82% specificity and concluded that both anti-CCP antibody and RF positivity are useful for ruling in the diagnosis of RA, and positivity combined improves the probability of true positivity in the diagnosis. Anti-CCP antibody or RF positivity shows low specificity and should be integrated with other examinations to make a final diagnosis.

Osteoporosis complicating RA is positively associated with seropositivity of anti-CCP (P=0.03) and marginally with the high titer of RF (P=0.051). In the same time, anti-CCP seropositivity is associated with high DEXA scores and grades (P=0.023, 0.033 respectively). In addition, the high positive anti-CCP or high positive RF is positivity associated with high DEXA grades (osteoporosis) (P=0.035). However, anti-CCP and RF alone or combined had good sensitivities (94, 89, and 100% respectively) and low specificities (20, 19, and 11% respectively) in discrimination of osteoporotic lesions in patients with RA. No associations are found between the serum levels of calcium, phosphorous, alkaline phosphatase, parathormone and osteoporosis complicating RA when compared with RA patients without OP.

Osteoporosis (OP) is common in patients with inflammatory arthritis. In inflammatory arthritis, OP is caused by a dysregulation of normal bone homeostasis, in which there is an increase in osteoclast activity leading to accelerated bone turnover. This process is mediated by a chronic inflammatory state, in which cytokines and other pro-inflammatory proteins increase bone resorption and slow bone formation. Changes in cortical bone insidiously begin in the periarticular regions and over time and with prolonged systemic inflammation can be see diffusely throughout the skeleton (Redlich et al 2012, Clayton Hochberg 2013, Haugeberg et al 2014). OP in patients with RA is likely mediated by several mechanisms: a generalized pro-inflammatory state, high frequency of glucocorticoid use, relatively low levels of physical activity, as well as the classic risk factors for OP (Ibanez et al 2010, Clayton, Hochberg 2013).

While the risk factors for OP of increasing age, low BMI, and being post-menopausal are widely accepted, there are little data regarding autoantibodies in RA and their contribution to bone loss (Clayton, Hochberg 2013). It was observed that human anticitrullinated protein antibodies (ACPA), when transferred to mice, induce osteoclastogenesis by binding to osteoclasts (Harre et al 2012). Kleyer et al. 2013 studied 15 adults who were seropositive for ACPA and had no clinical signs of arthritis and compared them with 15 seronegative controls with regard to metacarpophalangeal (MCP) joint BMD and cortical thickness and porosity measured using micro-

computed tomography (micro-CT). They found that the ACPA positive individuals had significantly lower BMD and a reduction in cortical thickness in the MCP joints. Porous cortical bone, or pre-erosive areas, was significantly more abundant in the ACPA positive persons. This suggests that the bone changes of RA which are associated with both OP and erosions can occur prior to clinical manifestations of disease in those with ACPA (Clayton, Hochberg 2013).

While, the seropositivity of anti-CCP antibody is significantly associated with high titer of inflammatory marker CRP, more disease severity and activity, the seropositivity of RF is positively associated with disease severity. The high positive anti-CCP or high positive RF is positivity associated with increased inflammatory marker CRP and increased disease severity or activity. Higher titer of anti-CCP is a significant risk to the development of erosion in RA patients either in crude or adjusted models. No association was found for the studied serologic markers and functional disability.

Early reports indicated that anti-CCP seropositivity in RA is a risk factor for the development of erosive disease and more than 60% of the patients with erosive disease had anti-CCP antibodies with a positive predictive value comparable to RF positivity (Schellekens et al 2000). Some studies suggested that RF IgM seropositivity as a better marker of erosion development than anti-CCP antibodies, although positive anti-CCP test was still a risk factor for erosions in these reports (Kroot et al 2000, Bas et al 2002). In early RA, anti-CCP seropositive was found in more than 50% of the patients if they had erosions compared to 22% in non-erosive disease. In addition, most of the RF-negative patients with erosions were reported to be anti-CCP-positive (Vencovsky et al 2003). Anti-CCP antibodies have been confirmed to be a strong predictor of radiological progression and erosion (Bas et al 2003, Meyer et al 2003, Vencovsky et al 2003, van der Helm-van Mil, Inanc et al 2007). Patients positive for both RF and anti-CCP antibodies had a higher prevalence of erosions as compared to patients positive for only one antibody or negative for both. In seronegative RA (RF absent), anti-CCP antibodies were seen in over 50 % of patients and were associated with a higher incidence of erosive disease (Shankar et al 2006).

A number of studies have investigated the association between RF and anti-CCP autoantibodies and RA disease activity. Some debate about relationship between autoantibodies and disease activity still remains (Choe et al 2013). Many reports have demonstrated that anti-CCP-positive RA patients have higher disease activity and have disease that is more progressive with respect to joint destruction compared to anti-CCP-negative RA patients (Forslind et al 2004, del Val del Amo et al 2006, Inanc et al 2007,Shidara et al 2008). Also, previous studies have reported associations between RF and anti-CCP antibody levels, and diverse disease activity markers and indices including ESR, CRP level, swollen and tender joint count, and DAS28. The serum RF titer was significantly associated with DAS28, CDAI, and SDAI in multivariate analysis (Chao et al 2013). The raise in CRP occurred more common in anti-CCP positive than anti-CCP negative and positive anti-CCP with high CRP level were shown to be the only significant predictor related to joint destructions (Lindqvsit et al 2005, Shidara et al 2012, Abdul Wahab et al 2013).

However, other studies have reported no association between anti-CCP antibody and RF as indicators of disease activity such as ESR, CRP, and active joint counts, although a good correlation with joint damage was shown (Eberhardt et al 1990, Ateş et al 2007, Serdaroğlu et al 2008, Chao et al 2013). In a comparative study, anti-CCP had higher positive predictive value for erosive RA than RF, CRP, erythrocyte sedimentation rate (ESR) (Shovman et al 2005, Chao et al 2013).

The sensitivity and specificity of anti-CCP and RF of the present work on Egyptian population was compared with other reported values from other studies on different ethnic population. There was a significant difference in the sensitivity of the anti-CCP antibodies (P=0.001) and RF (P=0.003) among different studies. However, regarding specificity no significant differences among studies were found for Anti-CCP (P=0.259) and significant differences were found for RF (P=0.015). This difference can be attributed to the variation in the techniques (ELISA versus nephelometery), generation of ELISA kit (second versus third generation), number of patients and controls, stage of disease (early versus late, active versus remission, severe versus mild), disease related complications such as erosion, osteoporosis and finally genetic and ethnic differences.

RA is diagnosed based on a set of clinical, serological and radiological criteria (Conrad et al 2010). In many early cases of RA, clinical symptoms are milder and nonspecific; hence patients will not fulfill ACR

classification criteria for RA. Therefore, the detection of a disease-specific autoantibody like anti-CCP could be of great diagnostic and therapeutic importance. Early diagnosis and immediate, effective therapy are crucial in order to prevent joint deterioration, functional disability and unfavorable disease outcome (van Boekel et al 2002, Farid et al 2013).

Therefore it can be concluded that due to its higher specificity, anti-CCP is useful serologic marker for diagnosis of RA when compared to RF and can predict disease activity. Anti-CCP antibodies identify a subset of patients who are more likely to accrue more joint damage, more severe disease course and to develop osteoporosis.

References

1. Abdul Wahab A, Mohammad M, Rahman MM, Mohamed Said MS (2013). Anti-cyclic citrullinated peptide antibody is a good indicator for the diagnosis of rheumatoid arthritis. Pak J Med Sci. May; 29(3):773-7.
2. Aletaha D, Neogi T, Silman AJ, et al. (2010). The 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 69:1580-1588.
3. Al-Shukaili A, Al-Ghafri S, Al-Marhoobi S, Alkaabi J. (2012) Evaluation of Anti-Mutated Citrullinated Vimentin Antibodies, Anti-Cyclic Citrullinated Peptide Antibodies and Rheumatoid Factor in Omani Patients with Rheumatoid Arthritis. International Journal of Rheumatology 2012:285854.
4. Amayo AA, Ayunga AO and Oyoo GO. (2014) Performance characteristics of anti-cyclic citrullinated peptide and rheumatoid factor tests in rheumatoid arthritis and undifferentiated arthritis at Kenyatta National Hospital. East African Journal of Pathology 1: 23-27.
5. Arnett FC, Edworthy SM, Bloch DA, et al. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 31:315-324.
6. Ateş A, Kinikli G, Turgay M, Akay G, Tokgöz G. (2007) Effects of rheumatoid factor isotypes on disease activity and severity in patients with rheumatoid arthritis: a comparative study. Clin Rheumatol 26:538–545
7. Bas S, Genevay S, Meyer O, Gabay C. (2003) Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. Rheumatology (Oxford) 42:677–680
8. Bas S, Perneger TV, Seitz M, Tiercy JM, Roux-Lombard P, Guerne PA. (2002) Diagnostic tests for rheumatoid arthritis: comparison of anti-cyclic citrullinated peptide antibodies, anti-keratin antibodies and IgM rheumatoid factors. Rheumatology (Oxford) 41:809–814
9. Block DR, Jenkins SM, Dalenberga DA, Balsaneka JG, Snydera MR, Saenger AK. (2012) Analytical and clinical comparison of anti-CCP assays with rheumatoid factor for the diagnosis of rheumatoid arthritis. Clin Chim Acta 413:1015-1017.
10. Cabral D; Katz JN; Weinblatt, ME; Ting G; Avorn J and Solomon DH.2005:'Development and assessment of indicators of rheumatoid arthritis severity: results of a Delphi Panel. Arthritis rheum. 2005; 53:61-66.
11. Carbonare L, Arlot ME, Chavassieux PM, Roux JP, Portero NR, Meunier PJ. (2001) Comparison of trabecular bone microarchitecture and remodeling in glucocorticoid-induced and postmenopausal osteoporosis. J Bone Min Res 16: 97-103
12. Choe JY, Bae J, Lee H, Bae SC, Kim SK. (2013) Relation of rheumatoid factor and anti-cyclic citrullinated peptide antibody with disease activity in rheumatoid arthritis: cross-sectional study. Rheumatol Int. Sep; 33(9):2373-9.
13. Clayton ES and Hochberg MC. (2013) Osteoporosis and osteoarthritis, rheumatoid arthritis and spondylarthropathies. Curr Osteoporos Rep. Dec; 11(4):257-62.
14. Coenen D, Verschueren P, Westhovens R, Bossuyt X. (2007) Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. Clin Chem. 53:498–504.
15. Conrad K, Roggenbuck D, Reinhold D, Dorner T. (2010) Profiling of rheumatoid arthritis associated autoantibodies. Autoimmun Rev 9 (6), 431–5.
16. del Val del Amo N, Ibanez Bosch R, Fito Manteca C et al. (2006) Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: relation with disease aggressiveness. Clin Exp Rheumatol 24:281–286
17. Eberhardt KB, Truedsson L, Pettersson H, et al. (1990) Disease activity and joint damage progression in early rheumatoid arthritis: relation to IgG, IgA, and IgM rheumatoid factor. Ann Rheum Dis 49:906–909
18. Farid SSh, Azizi G, Mirshafiey A. (2013) Anti-citrullinated protein antibodies and their clinical utility in rheumatoid arthritis. Int J Rheum Dis. Aug; 16(4):379-86.
19. Forslind K, Ahlmén M, Eberhardt K et al. (2004) Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 63:1090–1095

20. Greiner A, Plischke H, Kellner H, Gruber R. (2005) Association of anti-cyclic citrullinated peptide antibodies, anti-citrullin antibodies, and IgM and IgA rheumatoid factors with serological parameters of disease activity in rheumatoid arthritis. Ann N Y Acad Sci. 1050:295–303.
21. Harre U, Georgess D, Bang H, et al. (2012) Introduction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest. 122:1791–802.
22. Haugeberg G, Helgetveit KB, Førre Ø, Garen T, Sommerseth H, Prøven A. (2014) Generalized bone loss in early rheumatoid arthritis patients followed for ten years in the biologic treatment era. BMC Musculoskelet Disord. Sep 2;15:289
23. Ibanez M, Ortiz AM, Castrejon I, et al. (2010) A rational use of glucocorticoids in patients with early arthritis has a minimal impact on bone mass. Arthritis Res Ther. 12:R50.
24. Inanc N, Dalkilic E, Kamali S et al. (2007) Anti-CCP antibodies in rheumatoid arthritis and psoriatic arthritis. Clin Rheumatol 26:17–23
25. Kim HR, Shin JW, Lee JN. (2005) Evaluation of the usefulness of anti-cyclic citrullinated peptide antibodies measured by an automated enzyme immunoassay. J Lab Med Qual Assur 27:183-188.
26. Kleyer A, Finzel S, Rech J, et al. (2014) Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. Ann Rheum Dis. May; 73(5):854-60.
27. Kourilovitch M, Galarza-Maldonado C, Ortiz-Prado E. (2014) Diagnosis and classification of rheumatoid arthritis. J Autoimmun. 48-49:26-30.
28. Kroot EJA, de Jong BAW, van Leeuwen MA et al. (2000) The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 43:1831–1835
29. Lee D and Schur P. (2003) Clinical utility of the anti-CCP assay in patients with rheumatic diseases. Annals of the Rheumatic Diseases 62(9):870-874.
30. Lee DM and Weinblatt ME. (2001) Rheumatoid arthritis. Lancet. Sep 15; 358(9285):903-11.
31. Liao J, Ip WS, Cheung KY, Wan WM, Cautherley GW, Cai X, Lin X, Renneberg R, Chan CP. (2011) Diagnostic utility of an anti-CCP point-of-care immunotest in Chinese patients with rheumatoid arthritis. Clin Chim Acta. 412:778-81
32. Lin H, Lan J, Der-Yuan Chen D, Chen Y, Huang W, Hsieh T, Hsieh C, Chen H. (2008) The diagnostic value of anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis. Formosan Journal of Rheumatology 22:68-73.
33. Lindqvsit E, Eberhardt K, Bendtzen K. (2005) Prognostic laboratory markers of joint damage in rheumatoid arthritis. Ann Rheum Dis. 64:196–201
34. Mahmoud AA and Ismail MA. (2012) Anemia, Iron Status and Calcium-Phosphorus levels in Rheumatoid Arthritis Patients. Nature and Science, 10(7):110-114.
35. Manivelavan D. and Vijayasamundeeswari CK. (2012) Anti-Cyclic Citrullinated Peptide Antibody: An Early Diagnostic and Prognostic Biomarker of Rheumatoid Arthritis. J Clin Diagn Res. 6:1393-1396.
36. Maraina C. H. C., Nurdayana A. K., Rusni D., Azwany Y. (2010) "Diagnostic value of anti-modified citrullinated vimentin in rheumatoid arthritis," International Journal of Rheumatic Diseases vol. 13, no. 4, pp. 335–339.
37. Meyer O, Labarre C, Dougados M et al. (2003) Anticitrullinated protein/peptide antibody assay in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis 62:120–126
38. Mikuls TR, O'Dell JR, Stoner JA, et al. (2004) Association of rheumatoid arthritis treatment response and disease duration with declines inserum levels of IgM rheumatoid facto and anti-cyclic citrullinated peptide antibody. Arthritis Rheum. 50:3776–3782.
39. Mosaad YM, Hammad EM, Fawzy Z, Abdal Aal IA, Youssef HM, ElSaid TO, Monir R, El-Deek BS. (2014) Vitamin D receptor gene polymorphism as possible risk factor in rheumatoid arthritis and rheumatoid related osteoporosis. Hum Immunol. May; 75(5):452-61.
40. Mutlu N., Bicakcigil M., Tasan D. A., Kaya A., Yavuz S., Ozden A. I. (2009) "Comparative performance analysis of 4 different anti-citrullinated protein assays in the diagnosis of rheumatoid arthritis," Journal of Rheumatology vol. 36, no. 3, pp.491–500.
41. Niewold TB, Harrison MJ, Paget SA. (2007) Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. QJM. 100:193–201.
42. Nishimura K, Sugiyama D, Kogata Y, et al. (2007) Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med. Jun 5; 146(11):797-808.
43. Pincus T, Callahan LF, Sale WG, Brooks AL, Payne LE, Vaughn WK. (1984) Severe functional declines, work disability, and increased mortality in seventy-five rheumatoid arthritis patients studied over nine years. Arthritis Rheum. Arthritis Rheum. 27:864-872.

44. Prevoo, M.L., van 't Hof, M.A., Kuper, H.H., van Leeuwen, M.A., van de Putte, L.B. & van Riel, P.L. (1995) Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis and Rheumatism 38, 44.
45. Ranganathan P. (2009) Genetics of bone loss in rheumatoid arthritis—role of vitamin D receptor polymorphisms. Rheumatology (Oxford). 48: 342–346.
46. Redlich K, Smolen JS. (2012) Inflammatory bone loss: pathogenesis and therapeutic intervention. Nat Rev Drug Discov. 11(3):234–50
47. Reid IR and Ibbetson HK: (1987) Evidence for decreased tubular reabsorption of calcium in glucocorticoid-treated asthmatics. Horm Res 27: 200-4
48. Ropes MW, Rossmeisl EC, Bauer W. (1943) Calcium and phosphorous metabolism in rheumatoid arthritis and degenerative joint disease. J Clin Invest. Nov;22(6):785-90.
49. Rubin MR and Bilezikian JP.(2002) The role of parathyroid hormone in the pathogenesis of glucocorticoid-induced osteoporosis: A reexamination of the evidence. J Clin Endocrinol Metab 87: 4033-41.
50. Sambroook PN. (2000) The skeleton in rheumatoid arthritis: common mechanisms for bone erosion and osteoporosis. J Rheumatol. 27:2541–2.
51. Sarkis KS, Salvador MB, Pinheiro MM, Silva RG, Zerbini CA, Martini LA. (2009) Association between osteoporosis and rheumatoid arthritis in women: a cross-sectional study. Sao Paulo Med J. Jul;127(4):216-22.
52. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ. (2000) The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 43:155–163
53. Schett G, Kiechl S, Weger S, et al. (2006) High-sensitivity C-reactive protein and risk of nontraumatic fractures in the Bruneck study. Arch Intern Med. 166:2495-2501.
54. Serdaroğlu M, Cakirbay H, Değer O, Cengiz S, Kul S. (2008) The association of anti-CCP antibodies with disease activity in rheumatoid arthritis. Rheumatol Int 28:965–970
55. Sghiri R., Bouajina E., Bargaoui D. et al. (2008) "Value of antimutated citrullinated vimentin antibodies in diagnosing rheumatoid arthritis," Rheumatology International vol. 29, no. 1, pp. 59–62.
56. Shankar S, Grover R, Handa R. (2006) Role of Anti-Cyclic citrullinated peptide antibodies in erosive disease in patients with rheumatoid arthritis. Indian J Med Res. 689–696.
57. Shankar S, Grover R, Handa R. (2006) Role of anti cyclic citrullinated peptide antibodies in erosive disease in patients with rheumatoid arthritis. Indian J Med Res. Dec; 124(6):689-96.
58. Shidara K, Inoue E, Hoshi D, Sato E, Nakajima A, Momohara S, et al. (2012) Anti-cyclic citrullinated peptide antibody predicts functional disability in patients with rheumatoid arthritis in a large prospective observational cohort in Japan. Rheumatol Int. 32(2):361–366.
59. Shovman O, Gilburd B, Zandman-Goddard G et al. (2005) The diagnostic utility of anti-cyclic citrullinated peptide antibodies, matrix metalloproteinase-3, rheumatoid factor, erythrocyte sedimentation rate, and C-reactive protein in patients with erosive and non-erosive rheumatoid arthritis. Clin Dev Immunol 12 (3), 197–202.
60. Spies CM, Straub RH, Cutolo M, Buttgereit F. (2014) Circadian rhythms in rheumatology--a glucocorticoid perspective. Arthritis Res Ther. Nov 13;16 Suppl 2:S3.
61. Steinbrocker O, Traeger CH, Batterman RC. (1949) Therapeutic criteria in rheumatoid arthritis. J. Am. Med. Assoc. 140, 659–662.
62. Sun J, Zhang Y, Liu L, Liu G. (2014) Diagnostic accuracy of combined tests of anti cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis: a meta-analysis. Clin Exp Rheumatol. Jan-Feb; 32(1):11-21. Epub 2013 Sep 18.
63. Taylor P, Gartemann J, Hsieh J, Creeden J. (2011) A systematic review of serum biomarkers anti-cyclic citrullinated Peptide and rheumatoid factor as tests for rheumatoid arthritis. Autoimmune Dis. 2011:815038.
64. Ting G, Schneeweiss S, Scranton R. et al. (2008) Development of a health care utilisation data-based index for rheumatoid arthritis severity: a preliminary study. Arthritis Res Ther. 10(4):R95.
65. van Boekel MA, Vossenaar ER, van den Hoogen FH, van Venrooij WJ. (2002) Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. Arthritis Res 4 (2), 87–93.
66. van der Helm-van Mil AHM, Verpoort KN, Breedveld FC, Toes REM, Huizinga TWJ.(2005) Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis.Arthritis Res Ther 7:R949-R958.
67. Van Der Linden M. P. M., Van DerWoude D., Ioan-Facsinay A. et al. (2009) "Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis," Arthritis & Rheumatism vol. 60, no. 8, pp. 2232–2241.

68. van Gaalen F, Ioan-Facsinay A, Huizinga TW, Toes RE. (2005) The devil in the details: the emerging role of anticitrulline autoimmunity in rheumatoid arthritis. J Immunol. 175:5575–5580.
69. van Riel PL and Schumacher HR Jr. (2001) How does one assess early rheumatoid arthritis in daily clinical practice? Best Pract Res Clin Rheumatol. Mar; 15(1):67-76.
70. Van Venrooij WJ, Hazes JM, Visser H. (2002) Anti-citrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. Neth J Med. 60:383–388.
71. van Venrooij WJ, van Beers JJ, Pruijn GJ. (2008) Anti-CCP antibody, a marker for the early detection of rheumatoid arthritis. Ann N Y Acad Sci. 1143:268-285.
72. Vencovsky J, Machacek S, Sedova L et al. (2003) Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. Ann Rheum Dis 62:427–430
73. Vincent C, Nogueira L, Clavel C, Sebbag M, Serre G. (2005) Autoantibodies to citrullinated proteins: ACPA. Autoimmunity 38:17–24
74. Walwadkar SD1, Suryakar AN, Katkam RV, Kumbar KM, Ankush RD. (2006) Oxidative stress and calcium-phosphorus levels in Rheumatoid arthritis. Indian J Clin Biochem. Sep;21(2):134-7.
75. Wiik AS, van Venrooij WJ, Pruijn GJ. (2010) All you wanted to know about anti-CCP but were afraid to ask. Autoimmun Rev. 10:90-93.
76. World Health Organization. (1994) WHO Technical Report Series, 843. Geneva, Switzerland: WHO;. Assessment of fracture risk and application to screening for postmenopausal osteoporosis.
77. Zintzaras E, Dahabreh IJ, Giannouli S, Voulgarelis M, Moutsopoulos HM. (2008) Infliximab and methotrexate in the treatment of rheumatoid arthritis: a systematic review and meta-analysis of dosage regimens. Clin Ther. 30:1939-1955.