JKAU: Med. Sci., **Vol. 19** No. 3, pp: 3-20 (2012 A.D. / 1433 A.H.)
DOI: 10.4197/Med. 19-3.1

Disease Activity and Anti-Cyclic Citrullinated Peptide (Anti-CCP) Antibody in Saudi RF-Negative Rheumatoid Arthritis Patients

Mohammad-Ayman A. Safi, PhD-UK**, Dhia Alhaj Houssien**[1]**,** PhD-UK**,** and **David L. Scott**[2]**,** MD-UK

Department of Medical Microbiology, Faculty of Medicine, King Abdulaziz University, [1]*Dr. Dhiya Centre for Rheumatism and Physiotherapy and Acupuncture, Jeddah, Saudi Arabia*
[2]*Kings College Hospital, London, UK*
aymansafi4@gmail.com

Abstract. An investigation of disease activity and prevalence of anti-cyclic citrullinated peptide in 200 Saudi Rheumatoid Factor-negative rheumatoid arthritis patients; 164 females and 36 males; with a mean age of 45.14 years (SD = 12.54). 97% were receiving disease modifying therapy; as single (77%) and as combination treatment (20%). Hydroxychloroquine was the most used drug (92 patients). Prevalence of anti-cyclic citrullinated peptide was 61/200 (30.5%). Disease activity scores for 28 joints were low (< 3.2) in 2 (1%) patients, moderate (3.2-5.1) in 38 (19%), and high (> 5.1) in 160 (80%). No significant differences ($p > 0.05$) between positive and negative anti-cyclic citrullinated peptide patients for Disease Activity Score for 28 Joints, morning stiffness, C-reactive protein, disease duration, and presence of mixed diseases. A substantial number (30.5%) of rheumatoid factor negative patients are anti-cyclic citrullinated peptide positive – therefore anti-cyclic citrullinated peptide antibodies are a useful diagnostic tool for rheumatoid arthritis in RF-ve rheumatoid arthritis patients from Saudi Arabia. In conclusion, there is no difference in the severity or the treatment of rheumatoid arthritis patients with RF–ve/CCP+ve compared to RF-ve/CCP-ve patients. Therefore, anti-cyclic citrullinated peptide test is not useful in predicting disease activity and deciding on management in these patients.

Keywords: Anticyclic citrullinated peptide antibodies, Rheumatoid arthritis, Negative Rheumatoid factor.

Correspondence & reprint request to: Dr. Mohammed-Ayman A. Safi
P.O. Box 80205, Jeddah 21589, Saudi Arabia
Accepted for publication: 05 February 2012. Received: 16 October 2011.

Introduction

Rheumatoid arthritis (RA) found in about one percent of the population, making it one of the most common autoimmune diseases in the United States. It can have a serious impact on a patient's quality of life; thus, early intervention is a key to minimizing the damaging effects of the disease[1].

In 1987, the American College of Rheumatology (ACR) established seven criteria for the diagnosis of rheumatoid arthritis (RA), four of which must be fulfilled for diagnosis. Of which, the only criterion based upon laboratory testing is the detection of abnormal amounts of serum rheumatoid factor (RF)[2]. Testing for RF in the diagnosis of RA has been performed for over fifty years. Unfortunately, the RF test does not yield a high specificity for RA[1]. Nevertheless, RF test became the primary laboratory test used in the diagnosis of RA[3].

Rheumatoid factor (RF) seropositivity is found in about 50- 80% of patients with RA[4]. However, individuals with other autoimmune diseases, with infectious diseases, and even healthy persons, particularly elderly individuals may have RF antibodies in relatively high percentages[4-6]. This incomplete sensitivity and specificity of RF tests for RA limits their diagnostic usefulness[7].

Since the discovery of RF, autoantibodies that are more specific have been found in the sera of patients with RA. However, many of these autoantibodies have been either less sensitive or technically inconvenient for routine use[8]. The autoantibodies that showed lower sensitivity for diagnosis of RA than the RF include heavy chain binding protein (BiP)[9,10], a nuclear antigen with a molecular weight of 33 kD (RA33[11], glucose-6-phosphate isomerase[12-14], and anti-filaggrin[15,16].

Other autoantibodies are either not very specific or their antigens still to be characterized. The RA33 autoantibody[17] are not very specific for RA, since they are also found in about one-third of systemic lupus erythematosus (SLE) and on mixed connective tissue disease (MCTD) patients[4]. The anti-calpastatin has the same reason as reported by Blass *et al.*[18]. The p68 autoantibodies were described for RA[19] but the antigen needs further characterization[20].

Interestingly, antibodies to perinuclear granules in the cytoplasm of human buccal cells (antiperinuclear factor (APF)) and antibodies to

cytokeratin of stratum corneum of rat esophagus epithelium (antikeratin antibodies (AKA)) were described in 1964 and 1979, respectively. Both were found to be highly specific for RA[15,21] and were grouped into a family of antibodies called anti-filaggrin autoantibodies (AFA)[22]. Antibodies to Sa antigen were first described using the serum of an RA patient whose name began with 'Sa'[23,24]. They were also detected in sera of patients with RA and are specific for RA[23-26]. Researchers discovered that APF, AKA, and anti Sa antibodies target citrullinated proteins[27,28].

Initial studies using citrullinated peptide as substrate demonstrated a sensitivity of 76% and a specificity of 96% for RA[27]. Subsequently, a modified assay was developed using cyclic citrullinated peptide (CCP)[29]. The first generation anti-CCP (CCP1) assay proved to be as sensitive as the RF test with a much higher specificity[30]. Its specificity for RA (96%) was better than that previously reported in the RF test for RA (48-92%)[29,31-34].

A second-generation anti-CCP (CCP2) assay was soon developed that employed other citrullinated peptides and yielded a better sensitivity (75%-80%) than the CCP1 assay[30]. Whereas its specificity ranges between 90% and 99%[1,8,35-38]. A correlation of anti-CCP antibodies with radiographic joint damage has also been reported[39-42]. Anti-CCP assay was also found to have a higher disease predictability[43,44] and prognostic value[38,45] than the RF test, while RF appears to be a better marker for patient response to treatment than anti-CCP[46]. Anti-CCP can be detected at an early stage, even before onset of clinical symptoms of RA[44].

Recently, a third-generation anti-CCP (CCP3) assay was developed to demonstrate a greater sensitivity than that of the CCP2 assay[47,48].

Most studies on the diagnostic utility of the anti-CCP test in RA have been done in industrialized countries on subjects of European ancestry[49], with only a few done in the developing world: Egyptians[50], Iranians[51], Thai population[52], Indians[53], and black South Africans[54]. These antibodies, to the best of our knowledge, have never been studied in Saudi Arabia. This study investigated the prevalence of anti-CCP in Saudi Rf-negative RA patients seen in a private rheumatology center in Jeddah, Saudi Arabia and examined their relationship to disease activity.

Materials and Methods

Over a period of two years (2/2009 - 2/2011), demographic and clinical data were collected at the first visit from RA patients attended to Dr. Dhiya Centre for Rheumatism & Physiotherapy and Acupuncture, Jeddah, Saudi Arabia. All patients met the American College of Rheumatology (ACR) 1978 classification criteria for RA[55]. Demographic data included age, sex and ethnicity. Clinical data included disease duration (dd) by years; disease-modifying anti-rheumatic drugs (DMARDs) use and date of first use. Physician's global assessment (PGA) as 100 mm on scale; a 28-joint counts for tender joints (TJ) and for swelling joints (SJ); early morning stiffness (EMS) in minutes. In addition to laboratory data, which included erythrocyte sedimentation rate (ESR, mm/h), C-reactive protein (CRP), RF, hemoglobin (Hb) and platelets (PLT). For financial reasons, the policy was to order anti-CCP test only for those who were RF negative. Anti-CCP was measured by Electrochemiluminescence (Elecsys analyzer from Roche), which is a CE approved second-generation assay for anti CCP (anti-CCP2), and was considered positive when the concentrations were ≥ 3 IU/ml according to the manufacturer instructions. Hence, IgM rheumatoid factor measured by latex agglutination. Disease activity was assessed using the 28-joint disease activity score (DAS28)[56], which was calculated according to TJ, SJ, ESR and PGA. Presence of other diseases and family history were also documented.

The collected data were part of standard clinical review, thus ethics approval and informed consent were not obtained.

Statistical Analysis

The data were analyzed using statistical package for social science (SPSS) software version 10.

Results

Demographic, clinical and laboratory features of RA patients are shown in Table 1. Two hundred RF-negative RA patients who were females 164(82%), males 36(18%); mean age 45.14 years (SD 12.54) were studied. All patients were Saudi who met the American College of Rheumatology 1987 diagnostic criteria for RA. Sixty-three patients (31.5%) had disease duration (dd) ≤ 1 year, while 137 (68.5%) had disease durations >1 year, with a mean of 4.98 years (SD = 5.13). Most

Table 1. Demographic and clinical characteristics of Saudi RF negative patients (n=200).

Variable	Mean (SD) or Number (%)
Age	45.14 years (SD 12.54) years
Sex (F/M)	164 (82%)/36(18%)
Disease Duration	4.98 (5.13) years
Early RA (1 year)	63 (31.5%)
Morning Stiffness	63.6 (38.24) minutes
Tender Joint Count	13.5 (5)
Swollen Joint Count	2 (1.46)
Patient Global Assessment	63.2 (10.5) mm
Anti-CCP Positivity	61 (30.5%) in the cohort. 22/63 (34.92%) in the early RA group. 47/164 (28.6%) in F, 14/36 (38.8%) in M with insignificant difference ($p > 0.05$). 48/144 (33.33%) if no MixD, reduced to 11/49 (22.44%) if with MixD; with significant reduction ($p < 0.05$)
Hydroxychloroquine sulphate (Plaque)	92 (46%) 64 (32%) single+28 (14%) combination
Sulfasalazine	76 (38%) 61 (30.5%) single+15 (7.5%) combination
Methotrexate	51 (25.5%) 26(13%) single + 25 (12.5%) combination
Glucocorticoids(prednisolone)	19(9.5%) 2 (1%) single +17 (8.5%) combination
Leflunomide (Arava)	4 (2%) 1 (0.5%) single+ 3 (1.5%) combination
Adalimumab (HUMIRA)	3 (1.5%) combination
Remicade	1 (0.5%) combination
Rituximab (MabThera)	1 (0.5%) combination
Imuran	1 (0.5%) combination
Combination DMARDs	40 (20%)
No DMARDs treatment	6 (3%)
High disease activity (DAS28 > 5.1)	160/200 (80%)
DAS28	5.69 (0.87)

of the patients, 194 (97%) received disease modifying therapy – DMARDs, including Hydroxychloroquine sulphate (Plaque), (92 patients (64 of them as single treatment)), sulfasalazine (75(61)), methotrexate (52(26)), prednisolone (19(2)), Leflunomide (Arava) (4(1)) and Adalimumab (HUMIRA) (3 as combination); Remicade, Rituximab (MabThera) and Imuran (1 as combination). Combination treatment by DMARDs was used in 40 (20%) patients. Frequency of the patients within the age groups showed an ascending pattern with a peak at 40-49 years, which then descends (Fig 1). Fifty (25%) RA patients were

associated with other diseases (Table 2). Prevalence of the anti-CCP antibodies in the patient cohort was 61/200 (30.5%): 47/164 (28%) in females and 14/36 (38.88 %) in males (Table 3). Disease activity which was assessed by DAS28 scores were low (< 3.2) in 2 (1%) RA patients, moderate (3.2–5.1) in 38 (19%), and high (> 5.1) in 160 (80%) (Table 4). Distribution of anti-CCP antibodies results between the DAS28 groups is also shown in Table 4. Linear Regression Analysis (Stepwards Model) of different factors related to anti-CCP positivity showed significant correlation with methotrexate ($p = 0.000$) and prednisolone ($p = 0.001$), but not with other used DMARDs, DAS28, ESR values (Table 5). Linear regression analysis (Stepwards Model) of factors related to DAS28, DMARDs and other factors illustrated a statistical significance ($p < 0.05$) for TJ, SJ, ESR, PGA and age. However, exclusion for the following, sex, CRP, TJ minus SJ, family history, dd, EMS, early RA, and CCP positivity. In addition to the presence of mix diseases**,** presence of treatment combination, type of treatment combination, Hydroxychloroquine sulphate (Plaque), steroids, sulfasalazine**,** methotrexate, leflunomide (Arava), Remicade, HUMIRA, MabThera and Imuran (Table 6). High DAS28 (> 5.1) was present among 80% of the patients with a peak at the age group of 40-49 years (Table 7).

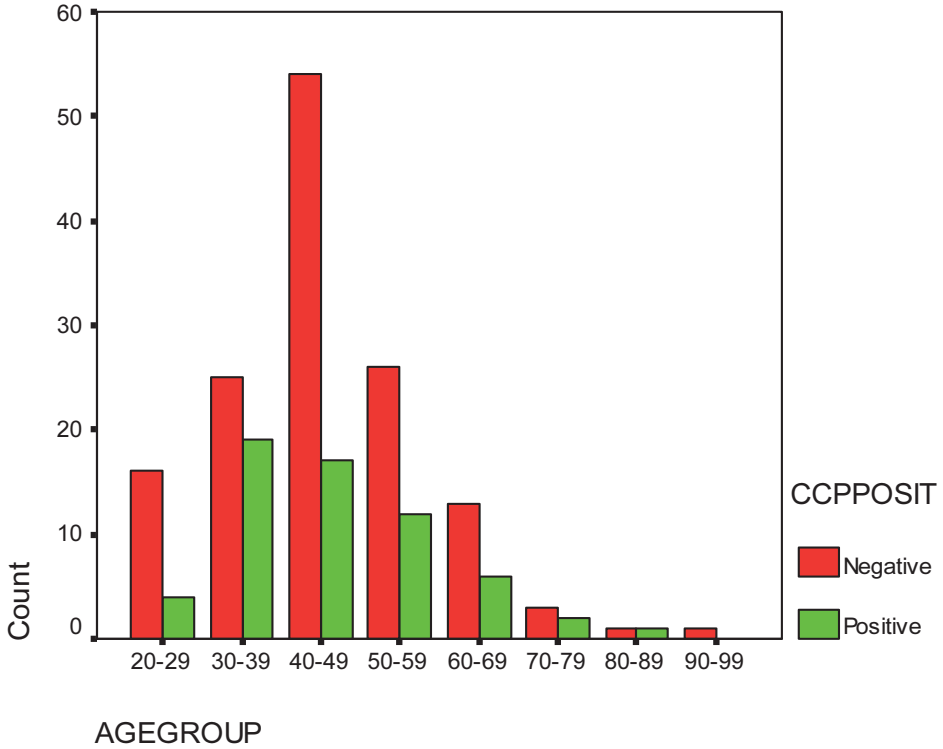

Fig. 1. Frequency of age groups with the distribution of anti-CCP antibodies.

Table 2. Association of RA with other diseases.

	Frequency	Percent
No Association	150	75%
Hypothyroidism	4	2%
Hypertension, Thyroid: Hypothyroidism	1	0.5%
Hypertension,	8	4.%
Diabetes Mellitus ,Hypertension	11	5.5%
Diabetes Mellitus	15	7.5%
Asthma	1	0.5%
Osteoporosis	1	0.5%
Systemic Lupus Erythematosus (SLE)	1	0.5%
Tuberculosis (TB)	1	0.5%
Tempo Mandibular Joint (TMJ)	2	1%
Lung Affect	1	0.5%
Gout	1	0.5%
Thyroid	3	1.5%
Total	200	100.0

Table 3. Prevalence of anti-CCP antibodies in the cohort and in males and females.

		Female	Male	Total
anti-CCP	Negative	117 (58.55%) [71.35%]	22 (11%) [81.12%]	139 (69.5%)
	Positive	47 (23.5%) [28.65%]	14 (7 %) [38.88%]	61 (30.5%)
Total		**164 (82%) [100%]**	**36 (18%) [100%]**	**200 (100%)**

p = .229

Table 4. Distribution of frequency of anti-CCP antibodies between DAS28 groups.

		DAS28 < 3.2 (Low Disease Activity)	DAS28 = 3.2 -5.1 (Moderate Disease Activity)	DAS28 > 5.1 (High Disease Activity)	Total
Anti-CCP positivity	Negative	0.0 (0.0%)	28 (14 %)	111 (55.5 %)	139(69.5%)
	Positive	2 (1%)	10 (5 %)	49 (24.5 %)	61 (30.5%)
Total		**2 (1%)**	**38 (19 %)**	**160 (80 %)**	**200 (100%)**

p = .674

Table 5. Linear Regression Analysis (Stepwise Model) of Factors Related to anti-CCP positivity.

Variable	Un-standardized Coefficients		Significance
	B	*Std Error*	
Methotrexate	.286	.070	.000
Prednisolone	.368	.104	.001

Dependant variable: anti-CCP positivity.
R^2=14.57%
Excluded variables: ESR value, DSA28, Sex, CRP positivity, TJ, SJ, PGA, family history, dd, EMS, presence of mix diseases, Hydroxychloroquine sulphate (Plaque), sulfasalazine, leflunomide (Arava), bemicade, HUMIRA, MabThera and Imuran.

Table 6. Linear Regression Analysis (Stepwise Model) Of Factors Related To DAS28.

Variable	Un-standardized Coefficients		Significance
	B	*Std Error*	
TJ	0.0765	.007	.000
ESR	00171	.001	.000
PGA	0.0177	.003	.000
SJ	0.0977	.022	.000
Age	-0.005	.002	.016

R^2=84%
Excluded variables: Sex, CRP, TJ minus SJ, family history, dd, EMS, early RA, CCP positivity, presence of mix diseases, presence of treatment combination, Hydroxychloroquine sulphate(Plaque), Steroids, Sulfasalazine, Methotrexate, Leflunomide (Arava), Remicade, HUMIRA, MabThera and Imuran.

Table 7. Distribution of frequency of age groups between DAS28 groups.

Age Groups (Years)	DAS28 Groups			Total
	DAS28 < 3.2 LDA	DAS28 = 3.2 -5.1 MDA	DAS28 > 5.1 HDA	
20-29		6	14	20
30-39	1	5	38	44
40-49		19	52	71
50-59		2	36	38
60-69	1	4	14	19
70-79			5	5
80-89		1	1	2
90-99		1		1
Total	**2 (1%)**	**38 (19%)**	**160 (80%)**	**200 (100%)**

Discussion

The present study included consecutive patients who fulfilled at their first visit to the center the ACR classification criteria for RA and who were RF negative; exempted those who, for some reasons, did not bring their anti-CCP results and the non Saudi patients. Two hundred Saudi RF negative RA patients were involved in the study. All patients have been tested for anti-CCP antibodies by using a second-generation assay (CCP2).

How does CCP2 compare with other generations? CCP2 is a CE approved assay with better sensitivity (75%-80%) than the CCP1 assay[30], whereas its specificity ranges between 90% and 99%[1,8,35-38]. The third-generation anti-CCP (CCP3) assay was found to have the same specificity but greater sensitivity than that of the CCP2 assay[47,48]. Anti-MCV (autoantibodies against mutated citrullinated vimentin), in some publications, has been described as being somewhat more sensitive than the CCP2 test; however, in such cases the anti-MCV test almost always shows a lower specificity as well[57]. In this cohort of patients, the prevalence of the anti-CCP2 antibodies was 30.5% exhibiting a useful diagnostic tool for RA in RF-negative RA patients from Saudi Arabia.

How do our results compare with the reports from other population groups with RF negative RA? Prevalence of 43.2% was reported by Kroot *et al.*[58] from Sweden. However, Sihvonen *et al.*[59], from Finland, and Kastbom *et al.*[37], from Sweden, concluded that 40% of the RF-negative patients were anti-CCP-positive. In black South Africans with early rheumatoid arthritis, the report was 36.4% of the 22 RF negative RA patient were positive for anti-CCP[54]. A percentile of 34.9% in Greek patients, and 34.5% in German patients with RF-negative rheumatoid arthritis were reported by Alexiou *et al.*[60] and Vallbracht *et al.*[61], respectively to have anti-CCP antibodies. Moreover, among a group of Japanese patients, Matsui and coworkers[62] reported that anti-CCP2 was positive in 22% of RF-negative subjects. The authors concluded that anti-CCP2 antibodies are the single most useful test for the overall diagnosis of RA, but that a combination of anti-CCP2 and RF is more useful than either test alone for the diagnosis of very early RA. For the Thai population, anti-CCP2 was positive in 20% of RF-negative subjects[52]. Thus, the authors suggested its usefulness in patients with suspected RA who have had a negative RF test. Anti-CCP1 antibodies

(first generation) and anti-CCP2 antibodies (second generation) were found to have different frequencies in various RA patient cohorts[60]. The authors attributed these differences to the presence of different subsets of anti-CCP antibodies directed against different epitopes in citrulline-containing molecules[27,34]; the influence of HLA alleles so that serum anti-CCP antibody levels were higher in RA patients with the shared HLA-DRB1 (SE) epitope, than in RA patients lacking the SE epitope[41]; the RA treatment (such as anti-TNFα treatment) that has been found to decrease serum anti-CCP antibody levels[63].

The disease activity DAS28 (5.69) in this cohort fits within the ranges (3.1-6.0) among the 25 countries[64]. Although 160 (80%) patients had high DAS28 scores (> 5.1), but there was no significant differences ($p > 0.10$) between positive anti-CCP2 and negative anti-CCP2 neither for the disease activity as judged by the DAS28, nor for its constituent measures, morning stiffness, CRP and disease duration. This indicates that anti-CCP2 have limited value in predicting disease activity in these patients. This is in concordance with the findings of Vanichapuntu and coworkers[52] who found no correlation with DAS 28. Also, in Egyptian patients, Abdel-Nasser *et al.*[50] found no significantly correlations of anti-CCP2 titre with parameters of disease activity, although a correlation was found between anti-CCP2 titre and disease severity (rheumatoid nodules, rheumatoid factor (RF), and radiological damage) and the Health Assessment Questionnaire - Disability Index (HAQ-DI) ($p < 0.05$). Other investigators found that anti-CCP1-positivity[58], anti-CCP2-positivity[40] and anti-CCP[37,60] to be predictors of a severe disease course in RA, as defined either by radiological joint outcome[40,58] or by swollen joint count and increased CRP concentration[37,60]. Among the sixty-three early RA patients (dd ≤ 1 year) we found 22/63 (34.92%) with positive anti-CCP antibodies. This proportion makes anti-CCP antibodies test a valuable predictor of the disease in the early RA patient when RF still cannot be detected. In this respect, we are in concordance with Kastbom *et al.*[37] who concluded that anti-CCP antibody status, in Swedish early RA patients (dd ≤ 1 year), are a valuable predictor of the disease.

Second generation anti-CCP (CCP2) assays yielded a sensitivity of 75%-80%[28] and a specificity of 90%-99%[1,8,35-38]. Recently, in India, Gupta *et al.*[53] used a second-generation anti-CCP assay (CCP2) and found a 85.71% anti-CCP positivity in the RA patients versus 9.8% in the

non-RA group, reflecting a sensitivity of 85% and a specificity of 90.19%. We did not assess the sensitivity and specificity in this baseline cross-sectional analysis. However, assessment of the sensitivity and specificity of anti-CCP assay in Saudi RA patients can be a plan for next study. Presence of mixed diseases was found to lower the prevalence of the anti-CCP positivity, as among the fifty patients that had mixed diseases, only twelve patients (12/50 (24%)) had positive anti-CCP. But this reduction was not statistically significant ($p > 0.1$). Anti-CCP2 positivity was significantly correlated ($p \leq 0.01$) with prednisolone and, methotrexate, indicating a statistically significant distribution of each of these drugs (usage) between positive anti-CCP2 and negative anti-CCP2 groups. However, the prognostic value of the anti-CCP test with respect to response to DMARDs and radiographic progression can be a suggestion for a prospective longitudinal study in Saudi RA patients.

Conclusion and Recommendations

The proportion (30.5%) of RF-negative RA patients, which were anti-CCP positive suggest that anti-CCP2 antibodies had a better diagnostic value than RF for the Saudi RF-negative RA patients, thus, anti-CCP antibodies are a useful diagnostic tool for RA in these patients. This current evidence suggests that anti-CCP antibodies should be measured in RA patients from Saudi Arabia when RF results are negative. However, they have limited value in predicting disease activity in these patients, thus, the treatment decisions cannot be based on the anti-CCP test alone. More studies should be performed in Saudi Arabia, concerning the value of anti-CCP antibodies in RA patients, the sensitivity and specificity of the anti-CCP test in Saudi RA patients, together with its prognostic value with respect to response to DMARDs and radiographic progression.

References

[1] **Zendman AJW, van Venrooij WJ, Pruijn GJM**. Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. *Rheumatology* 2006; **45**(1): 20-25.

[2] **Renaudineau Y, Jamin C, Saraux A, Youinou P**. Rheumatoid factor on a daily basis. *Autoimmunity* 2005; **38**(1): 11-16.

[3] **Sullivan E.** Rheumatoid arthritis: test for anti-CCP antibodies joining RF test as key diagnostic tools. *Lab Med* 2006; **37**(1): 179.

[4] **Smolen JS**. Autoantibodies in rheumatoid arthritis. In: Manual of Biological Markers of Disease. van Venrooij WJ, Maini RN, eds. Dordrecht (The Netherlands): Kluwer Acad Pub, 1996. 1-18.

[5] **Van Schaardenburg D, Lagaay A, Otten H, Breedveld F.** The relation between class-specific serum rheumatoid factors and age in the general population. *Br J Rheumatol* 1993; **32**(7): 546-549.

[6] **Aho K, Palosuo T, Kurki P**. Marker antibodies of rheumatoid arthritis: diagnostic and pathogenetic implications. *Semin Arthritis Rheum* 1994; **23**(6): 379-387.

[7] **Visser H, Gelinck LBS, Kampfraath AH, Breedveld C, Hazes JMW**. Diagnostic and prognostic characteristics of the enzyme linked immunosorbent rheumatoid factor assay in rheumatoid arthritis. *Ann Rheum Dis* 1996; **55**(3): 157–161.

[8] **Lee DM, Schur PH.** Clinical utility of the anti-CCP assay in patients with rheumatic diseases. *Ann Rheum Dis* 2003; **62**(9): 870–874.

[9] **Bläss S, Specker C, Lakomek HJ, Schneider EM, Schwochau M.** Novel68 kDa autoantigen detected by rheumatoid arthritis specific antibodies. *Ann Rheum Dis* 1995; **54**(5): 355–360.

[10] **Corrigall VM, Bodman-Smith MD, Fife MS, Canas B, Myers LK, Wooley PH, Soh C, Staines NA, Pappin DJC, Berlo SE, van Eden W, van der Zee R, Lanchbury JS and Panayi GS.** The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis. *J Immunol* 2001; **166**(3): 1492–1498.

[11] **Hassfeld W, Steiner G, Hartmuth K, Kolarz G, Scherak O, Graninger W, Thumb N, Smolen JS.** Demonstration of a new antinuclear antibody (anti-RA33) that is highly specific for rheumatoid arthritis. *Arthritis Rheum* 1989; **32**(12): 1515–1520.

[12] **Schaller M, Burton DR, Ditzel HJ.** Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease. *Nat Immunol* 2001; **2**(8): 746–53.

[13] **Kassahn D, Kolb C, Solomon S, Bochtler P, Illges H.** Few human autoimmune sera detect GPI. *Nat Immunol* 2002; **3**(5): 411–413.

[14] **Schubert D, Schmidt M, Zaiss D, Jungblut PR, Kamradt T**. Autoantibodies to GPI and creatine kinase in RA. Nat Immunol 2002; **3**(5): 411-412.

[15] **Nienhuis LF, Mandema EA**. A new serum factor in patients with rheumatoid arthritis. The antiperinuclear factor. *Ann Rheum Dis* 1964; **23**: 302–305.

[16] **van Boekel MA, Vossenaar ER, van den Hoogen FH, van Venrooij WJ.** Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* 2002; **4**(2): 87-93.

[17] **Hassfeld W, Steiner G, Graninger W, Witzmann G, Schweitzer H, Smolen JS.** Autoantibody to the nuclear antigen RA33; a marker for early rheumatoid arthritis. *Br J Rheumatol* 1993; **32**(3): 199–203.

[18] **Bläss S, Haferkamp C, Specker C, Schwochau M, Schneider M, Schneider EM.** Rheumatoid arthritis: autoreactive T cells recognizing a novel 68k autoantigen. *Ann Rheum Dis* 1997; **56**(5): 317-322.

[19] **Lackner KJ, Schlosser U, Lang B, Schmitz G.** Autoantibodies against human calpastatin in rheumatoid arthritis: epitope mapping and analysis of patient sera. *Br J Rheumatol* 1998; **37**(11): 1164-1171.

[20] **Blass S, Meier C, Vohr HW, Schwochau M, Specker C, Burmester GR**. The p68 autoantigen characteristic of rheumatoid arthritis is reactive with carbohydrate epitope specific autoantibodies. *Ann Rheum Dis* 1998; **57**(4): 220–225.

[21] **Young BJJ, Mallya RK, Leslie RDG, Clark CJM, HamblinTJ**. Antikeratin antibodies in rheumatoid arthritis. *Br Med J* 1979; **2**(6182): 97-99.

[22] **Lee AN, Beck CE, Hall M**. Rheumatoid ractor and anti-CCP autoantibodies in rheumatoid arthritis: A review. *Clin Lab Sci* 2008; **21**(1): 15-18.

[23] **Deprés N, Boire G, Lopez-Longo FJ, Me´nard HA**. The Sa system: a novel antigen-antibody system specific for rheumatoid arthritis. *J Rheumatol* 1994, **21**(6): 1027-1033.

[24] **Vossenaar ER, Després N, Lapointe E, van der Heijden A, Lora M, Senshu T, J van Venrooij WJ and Ménard HA.** Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. *Arthritis Res Ther* 2004; **6**(2): R142-R150.

[25] **Hayem G, Chazerain P, Combe B, Elias A, Haim T, Nicaise P, Benali K, Eliaou JF, Kahn MF, Sany J, Meyer O**. Anti-Sa antibody is an accurate diagnostic and prognostic marker in adult rheumatoid arthritis. *J Rheumatol* 1998; **26**(1): 7-13.

[26] **Menard HA, Lapointe E, Rochdi MD, Zhou ZJ**. Insights into rheumatoid arthritis derived from the Sa immune system. *Arthritis Res* 2000; **2**(6): 429-32.

[27] **Schellekens GA, de Jong BAW, van den hoogen FHJ, van de Putte LBA.** Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis specific autoantibodies. *J Clin Invest* 1998; **101**(1): 273-281.

[28] **Vincent C, Nogueira L, Clavel C, Sebbag M, Serre G.** Autoantibodies to citrullinated proteins: ACPA. *Autoimmunity* 2005; **38**(1): 17-24.

[29] **Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ.** The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000; **43**(1): 155-163.

[30] **Van Venrooij WJ, Hazes JM, Visser H.** Anticitrullinated protein/ peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. *Neth J Med* 2002; **60**(10): 383-388.

[31] **Weinblatt ME, Schur PH.** Rheumatoid factor detection by nephelometry. *Arthritis Rheum* 1980; **23**(6): 777-779.

[32] **Saraux A, Berthelot JM, Chalès G, Le Henaff C, Mary JY, Thorel JB, Hoang S, Dueymes M, Allain J, Devauchelle V, Baron D, Le Goff P, Youinou P..**Value of laboratory tests in early prediction of rheumatoid arthritis. *Arthritis Rheum* 2002; **47**(2): 155–165.

[33] **Bas S, Perneger TV, Kunzle E, Vischer TL.** Comparative study of different enzyme immunoassays for measurement of IgM and IgA rheumatoid factors. *Ann Rheum Dis* 2002; **61**(6): 505-510.

[34] **Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R.** Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. *Clin Chem* 2001; **47**(6): 1089–1093.

[35] **Suzuki K, Sawada T, Murakami A, Matsui T, Tohma S, Nakazono K, Takemura M, Takasaki Y, Mimori T, Yamamoto K.** High diagnostic performance of ELISA detection of antibodies to citrullinated antigens in rheumatoid arthritis. *Scan J Rheumatol* 2003; **32**(4): 197–204.

[36] **Dubucquoi S, Solau-Gervais E, Lefranc D, Marguerie L, Sibilia J, Goetz J, Dutoit V, Fauchais AL, Hachulla E, Flipo RM, Prin L..** Evaluation of the anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatoid arthritis. *Ann Rheum Dis* 2004; **63**(4): 415-19.

[37] **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**(9): 1085-1089.

[38] **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**(9): 1079–1084.

[39] **De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, Lebeer K, Wyns B, Vincent C, Mielants H, Boullart L, Serre G, Veys EM, De Keyser** Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Ann Rheum Dis* 2004; **63**(12): 1587-1593.

[40] **Forslind K, Ahlmén M, Eberhardt K, Hafström I, Svensson B**; **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**(9): 1090–1095.

[41] **van Gaalen FA, van Aken J, Huizinga TW, Schreuder GM, Breedveld FC, Zanelli E, van Venrooij WJ, Verweij CL, Toes RE, de Vries RR.** Association between HLA class II genes and auto antibodies to cyclic citrullinated peptides (CCPs) influence the severity of rheumatoid arthritis. *Arthritis Rheum* 2004; **50**(7): 2113–2121.

[42] **Rönnelid J, Wick MC, Lampa J, Lindblad S, Nordmark B, Klareskog L, van Vollenhoven RF**. Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. *Ann Rheum Dis* 2005; **64**(12): 1744-1749.

[43] **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**(2): 380-386.

[44] **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**(10): 2741-2749.

[45] **Meyer O, Labarre C, Dougados M, Goupille P, Cantagrel A, Dubois A, Nicaise-Roland P, Sibilia J, Combe B.** Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographie damage. *Ann Rheum Dis* 2003; **62**(2): 120-126.

[46] **De Rycke L, Verhelst X, Kruithof E, Van den Bosch F, Hoffman IE, Veys EM, De Keyser F.**. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis* 2005; **64**(2): 299-302.

[47] **INOVA Diagnostics, Inc.** Third Generation CCP ELISA in Rheumatoid Arthritis Serology. Accessed January 25, 2012. <http://www.inovadx.com/PDF/newletters/NL04.pdf>

[48] **Santiago M, Baron M, Miyachi K, Fritzler MJ, Abu-Hakima M, Leclercq S, Bell M, Hudson M, Mathieu JP, Taillefer S, Jones N, Docherty P, Khraishi M, Markland J, Pope J, Robinson D, Smith D, Sutton E**.. A comparison of the frequency of antibodies to cyclic citrullinated peptides using a third generation anti-CCP assay (CCP3) in systemic sclerosis, primary biliary cirrhosis and rheumatoid arthritis. *Clin Rheumatol* 2008; **27**(1): 77–83.

[49] **Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, Saigo K, Morinobu A, Koshiba M, Kuntz KM, Kamae I, Kumagai S.** Meta-analysis: diagnostic accuracy of anticyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann Intern Med* 2007; **146**(11): 797–808.

[50] **Abdel-Nasser AM, Mahmoud MH, El Mansoury TM, Osman AM** Anti-CCP2 is an adjunct to, not a surrogate for, rheumatoid factor in the diagnosis of rheumatoid arthritis: diagnostic utility of anti-CCP2 antibodies in Egyptian patients with rheumatoid arthritis. *Scand J Rheumatol* 2008; **37**(5): 329-336.

[51] **Aflaky E, Shenavandeh S, Ashraf MJ**. A comparison of performance of anti-cyclic citrullinated peptide 2 and citrullinated protein antibodies in the diagnosis of rheumatoid arthritis in Iranian patients. *Rheumatol Int* 2010; **30**(4): 461-466.

[52] **Vanichapuntu M, Phuekfon P, Suwannalai P, Verasertniyom O, Nantiruj K, Janwityanujit S.** Are anti-citrulline autoantibodies better serum markers for rheumatoid arthritis than rheumatoid factor in Thai population? *Rheumatol Int* 2010; **30**(6): 755-759.

[53] **Gupta R, Thabah MM, Aneja R, Kumar A, Varghese T, Chandrasenan PJ**. Usefulness of anti-CCP antibodies in rheumatic diseases in Indian patients. *Indian J Med Sci* 2009; **63**(3): 92–100.

[54] **Hodkinson B, Meyer P W A, Musenge E, Ally MMT, Wadee AA, Anderson R, Tikly M.** The diagnostic utility of the anti-CCP antibody test is no better than rheumatoid factor in South Africans with early rheumatoid arthritis. *Clin Rheumatol* 2010; **29**(6): 615-618.

[55] **Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG.** The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**(3): 315–324.

[56] **Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL** . Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; **38**(1): 44-48

[57] **van Venrooij WJ, van Beers JJBC, Ger JM, Pruijn GJM.** Anti-CCP antibodies: the past, the present and the future (Review). *Nature Reviews Rheumatology* 2011; **7**(7): 391-398.

[58] **Kroot EJ, de Jong BAW, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, van de Putte LB, van Rijswijk MH, van Venrooij WJ, van Riel PL**. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum* 2000; **43**(8): 1831-1835.

[59] **Sihovonen S, Korpela M, Mustila A, Mustonen J.** The predictive value of rheumatoid factor isotypes, anti-cyclic citrullinated peptide antibodies, and antineutrophil cytoplasmic antibodies for mortality in patients with rheumatoid arthritis. *J Rheum* 2005; **32**(11): 2089-2094.

[60] **Alexiou I, Germenis A, Ziogas A, Theodoridou K, Sakkas LI.** Diagnostic value of anti-cyclic citrullinated peptide antibodies in Greek patients with rheumatoid arthritis. *BMC Musculoskelet Disord* 2007; **8**: 37.

[61] **Vallbracht I, Rieber J, Öppermann M, Förger F, Siebert U, Hemlke K.** Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. *Ann Rheum Dis* 2004; **63**(9): 1079-1084.

[62] **Matsui T, Shimada K, Ozawa N, Hayakawa H, Hagiwara F, Nakayama H, Sugii S, Ozawa Y, Tohma S**. Diagnostic utility of anti-cyclic citrullinated peptide antibodies for very early rheumatoid arthritis. *J Rheumatol* 2006; **33**(12): 2390-2397.

[63] **Alessandri C, Bombardieri M, Papa N, Cinquini M, Magrini L, Tincani A, Valestini G.** Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following

anti-TNFα therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. *Ann Rheum Dis* 2004; **63**(10): 1218-1221.

[64] **Sokka T, Kautiainen H, Pincus T, Toloza S, da Rocha Castelar Pinheiro G, Lazovskis J, Hetland ML, Peets T, Immonen K, Maillefert JF, Drosos AA, Alten R, Pohl C, Rojkovich B, Bresnihan B, Minnock P, Cazzato M, Bombardieri S, Rexhepi S, Rexhepi M, Andersone D, Stropuviene S, Huisman M, Sierakowski S, Karateev D, Skakic V, Naranjo A, Baecklund E, Henrohn D, Gogus F, Badsha H, Mofti A, Taylor P, McClinton C, Yazici Y.** Disparities in rheumatoid arthritis disease activity according to gross domestic product in 25 countries in the QUEST-RA database. *Ann Rheum Dis* 2009; **68**(11): 1666-1672.

نشاط المرض وانتشار أضداد الببتيد الستروليني الحلقي anti-CCP في مرضى التهاب المفاصل الروماتيزمي السعوديين سلبيي عامل آر – إف (RF-ve RA)

محمد أيمن صافي ، وضياء الحاج حسين[١]، وديفيد ل سكوت[٢]

قسم الكائنات الدقيقة الطبية، كلية الطب، جامعة الملك عبدالعزيز

[١]مركز د. ضياء للروماتيزم والعلاج الطبيعي والإبر الصينية

جدة – المملكة العربية السعودية

[٢]مستشفى الكلية الملكية، لندن – المملكة المتحدة

المستخلص. درسنا نشاط المرض وانتشار أضداد الببتيد الستروليني الحلقي anti-CCP في مئتين من مرضى التهاب المفاصل الروماتويدي سلبيي العامل الروماتويدي (آر إف) السعوديين؛ وكانوا ١٦٤ من الإناث و ٣٦ من الذكور؛ مع متوسط العمر ٤٥,١٤ عاما (SD = ١٢,٥٤). ومنهم ٩٧٪ يتلقون العلاج بالأدوية المضادة للرثية المعدلة للمرض (DMARDs)؛ كعلاج واحد (٧٧٪) وكعلاج مزيج (٢٠٪). وكان هيدروكسي كلوروكين الدواء الأكثر استخداما (٩٢ مريضا). وكان انتشار أضداد الببتيد الستروليني الحلقي anti-CCP ٢٠٠/٦١ (٣٠,٥٪). وكانت قيمة نشاط المرض (DAS ٢٨) قيمة منخفضة (< ٣,٢) في ٢ من المرضى (١٪) ، معتدلة (٣,٢–٥,١) في ٣٨ (١٩٪) ، وعالية (> ٥,١) في ١٦٠ (٨٠٪). لم تكن هناك فروق ذات دلالة إحصائية ($p > ٠,٠٥$) بين إيجابيي أضداد CCP والسلبيين لأضداد CCP، ولا من أجل DAS ٢٨، التيبس الصباحي، بروتين سي التفاعلي (CRP)، مدة المرض (dd)، وجود

أمراض مختلفة (مزيج مرضي) عند المريض هناك عدد كبير من المرضى السلبيي RF كانوا إيجابيين لأضداد CCP إيجابية – وبالتالي فإن أضداد CCP هي أداة مفيدة لتشخيص التهاب المفاصل الروماتيزمي في المرضى الذين يعانون التهاب المفاصل الروماتيزمي سلبيي الـRF من المملكة العربية السعودية. من ناحية أخرى ، ليس هناك فرق في شدة أو علاج مرضى التهاب المفاصل الروماتيزمي سلبيي الـ RF-ve/CCP+ve مقارنة مع RF-ve/CCP-ve – وبالتالي فإن اختبار أضداد CCP ليست مفيدة في التنبؤ بنشاط المرض ولا في البت في علاج هؤلاء المرضى.