Synovial fluid anti-citrulline-containing peptide antibody and its role in the diagnosis of rheumatoid arthritis

Ali I. Fouda[a], Ibrahim Rageh[b], Nashwa I. Hashaad[a], Sabreen Hamza[a]

Departments of [a]Rheumatology, Rehabilitation & Physical Medicine, [b]Clinical Pathology, Faculty of Medicine, Benha University, Benha, Egypt

Correspondence to Nashwa I. Hashaad, MD, Department of Rheumatology, Rehabilitation & Physical Medicine, Faculty of Medicine, Benha University, Benha, 13511, Egypt;
Tel: + 20 132 722 080; fax: +20 133 272 3803;
e-mail: zeinahashaad @yahoo.com

Objective
This study aimed to measure the level of anti-citrulline-containing peptide (anti-CCP) antibody (ab) in the synovial fluid (SF) of rheumatoid arthritis (RA) patients to study its relations with anti-CCP ab serum level, RA disease activity, and severity parameters as well as to explore its diagnostic value in RA.

Patients and methods
This study was conducted on 60 RA and 60 osteoarthritis (OA) patients. Patients were subjected to thorough history taking and full clinical examination including locomotor system examination. The Health Assessment Questionnaire was used for the assessment of functional capacity. Complete laboratory investigations including a complete blood count, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, serum and SF anti-CCP ab levels were carried out. The Sharp score was calculated for the assessment of RA severity in posteroanterior plain radiographic views of both hands and feet.

Results
The mean SF anti-CCP ab level was highly significantly increased ($P=0.0001$) in RA patients (133.93±41.3 ng/l) in comparison with OA patients (5±13.2 ng/l). There were statistically significant correlations of SF anti-CCP ab level with rheumatoid factor ($P=0.049$), serum anti-CCP ab ($P=0.0001$), and Sharp score ($P=0.037$), whereas other clinical and laboratory parameters did not show any significant correlation ($P>0.05$). The specificity of SF anti-CCP ab and serum anti-CCP ab of RA patients was 100%. Besides, the sensitivity of serum anti-CCP ab was 90%, whereas the sensitivity of SF anti-CCP ab was 83.3%. The positive predictive value of both serum and SF anti-CCP abs was 100% in RA patients and their negative predictive value was 90.9% in the serum of RA patients and 85.7% in the SF. The cutoff value of anti-CCP ab was 65.4 ng/l in the serum and 63.35 ng/l in the SF.

Conclusion
SF anti-CCP ab is significantly increased in RA compared with OA and could be considered an appreciated diagnostic marker having a major role in the identification of RA patients not fulfilling the criteria for the RA disease diagnosis. Its relation to RA disease severity could not be established.

Introduction

Rheumatoid arthritis (RA) is a chronic multisystem inflammatory disease characterized mainly by inflammation of the synovial lining. The inflammation in the RA joint is associated with and driven by inflammatory cell infiltration, synovial lining hyperproliferation, and excessive proinflammatory mediator production [1]. RA has varied clinical symptoms that extend from significant morning stiffness, mild arthralgia to severe inflammatory damage and even loss of function. Despite that, articular manifestations are the fundamental symptoms; there is a wide scale of extra-articular manifestations [2]. The diagnosis of RA depends on clinical symptoms, laboratory investigations, and imaging. The rheumatoid factor (RF) of the immunoglobulin M isotype is the most routinely used RA laboratory marker in practice. Recently, several autoantibodies were reported with diagnosis, activity, and/or prognosis of RA [3]. An anti-citrulline-containing peptide (anti-CCP) antibody (ab) has been recently recognized as a potential diagnostic and prognostic marker in RA. In nature, citrulline is an unusual amino acid resulting from an enzymatically modified arginine residue, which was detected in certain proteins from humans. The detection of anti-CCP antibodies in RA is highly specific and sensitive [4]. Determination of anti-CCP ab in the serum of RA patients can distinguish among other arthropathies. In addition, it can represent a prognostic marker that predicts erosive, persistent, and

more aggressive synovitis. In early RA, the assessment of anti-CCP ab is extremely useful when anti-CCP ab positivity precede clinical symptoms by years [5]. This study aimed to investigate the diagnostic value of synovial fluid (SF) anti-CCP ab in the management of RA and to explore its relation with serum level as well as clinical and laboratory parameters of RA.

Patients and methods

Sixty patients with RA diagnosed according to the 2010 classification criteria of the American College of Rheumatology/European League against Rheumatism for RA [6] attending the Rheumatology, Rehabilitation and Physical Medicine Outpatients Clinic and Inpatients Department of Benha University Hospitals were included in this study. Sixty patients with knee osteoarthritis (OA) diagnosed according to the criteria of American College of Rheumatology Subcommittee on OA [7] were selected as a control group. Patients suffering from autoimmune diseases, hematological diseases, and metabolic diseases or those having a history of a previous injection or joint trauma were excluded from this study. Benha Faculty of Medicine review board approval for this study was obtained, and informed written consent was obtained from each patient.

Clinical assessment, radiological studies, and blood tests

All patients were subjected to the following: complete history taking, full clinical and musculoskeletal examination, assessment of RA disease activity using the Disease Activity Score 28 (DAS-28) [8], and assessment of functional status using the Health Assessment Questionnaire (HAQ). Plain radiograph (posteroanterior view) of both hands and feet was obtained and the modified Sharp score was applied [9]. Complete laboratory investigations including complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF, liver function tests, and synovial and serum anti-CCP ab were carried out.

Measurement of synovial fluid anti-citrulline-containing peptide antibody

Five milliliter of SF drawn from each RA and OA patient through aspiration of knee effusions under complete aseptic precautions was collected and stored at –20°C until the time of analysis. Anti-CCP ab was determined using enzyme-linked immunosorbent assay (WKEA MED Supplies Comp, Chang chun.) following the manufacturer's instructions. The kit assays Human anti-CCP level in the sample. The microtiter plate wells are layered with purified antigen. When sera containing anti-CCP were added to the wells, another enzyme-labeled antigen was added to each well producing antigen–ab–enzyme–antigen complex. After washing, the substrate was added producing blue color. The reaction is completed by the addition of sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of anti-CCP in the samples was then determined by comparing the optical density of the samples to the standard curve.

Statistical analysis

Data of this study were coded and entered using the SPSS version 22 (SPSS Inc., Chicago, Illinois, USA) on an IBM compatible computer. The data were summarized using mean and SD for the quantitative variables and percentage for qualitative variables. Comparisons between groups were made using the χ^2-test for qualitative variable and nonparametric Mann–Whitney U-test for quantitative data. Correlations were made to show the relation between quantitative variables. A P-value less than 0.05 was considered as statistically significant. Receiver operating characteristic curve was plotted to show the validity of anti-CCP in distinguishing patients with RA.

Results

RA patients were distributed as 50 (83.3%) female and 10 (16.7%) male patients. Their ages ranged from 20 to 59 years with a mean of 36.16±11.75 years. Their disease durations had a mean of 4.59±3.42 years. Morning stiffness duration had a mean of 63.3± 51.6 min. As regards laboratory investigations of RA patients, first-hour ESR had a mean of 40.6 ±14.5 mm/first hour, CRP had a mean of 11.06±7.7 mg/ml, hemoglobin (Hb) concentration had a mean of 10.02±1.4 g/dl, white blood cells had a mean of 5.80±1.64×10³/ml, and platelets had a mean of 332±112.323×10³/ml. As regards RF titer, there were 42 (70%) seropositive patients and 18 (30%) seronegative patients. RF titer had a mean of 44.4±36.47 U/ml. As regards the titer of serum anti-CCP ab, it had a mean of 133.93±41.3 ng/l, whereas synovial anti-CCP level had a mean of 133.93±41.3 ng/l (Table 1).

As regards the functional capacity, there were 20 (33.3%) RA patients classified as grade 1, 26 (43.3%) RA patients classified as grade 2, and 14 (23.3%) patients classified as grade 3 (Fig. 1).

Table 2 shows that the mean serum anti-CCP ab level was statistically highly significantly increased

(P=0.0001) in RA patients in comparison with OA patients (137.1±45.3 vs. 48.4±12.4 ng/l, respectively). The mean SF anti-CCP ab was statistically highly significantly increased (P=0.0001) in RA in comparison with OA (133.93±41.3 vs. 51.1± 13.2 ng/l, respectively).

Table 3 shows that there were statistically significant correlations of serum anti-CCP ab with RF (P=0.047) and Sharp score (P=0.034), whereas other clinical and laboratory parameters did not show any significant correlations (P>0.05).

Table 4 shows that there were statistically significant correlations of synovial anti-CCP ab level with RF (P=0.049) and Sharp score (P=0.037), whereas other clinical and laboratory parameters did not detect any statistically significant correlation (P>0.05).
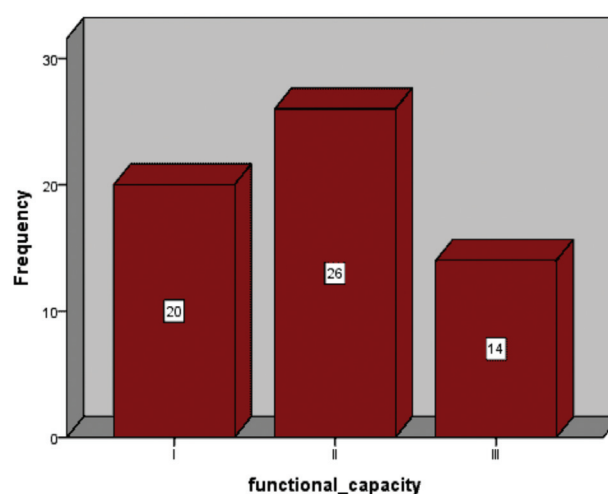
Figure 2 shows that there was a statistically significant correlation between serum and synovial anti-CCP ab levels.

Table 5 shows that the specificity of anti-CCP ab in the serum and SF of RA patients was 100%. Besides, the sensitivity of anti-CCP ab in the serum of RA patients was 90%, whereas the sensitivity of synovial anti-CCP ab was 83.3%. The positive predictive value of both serum and SF anti-CCP ab in RA patients was 100% each, whereas their negative predictive value in the serum of RA patients was 90.9 and 85.7% in the SF (Fig. 3).

Discussion

RA is an autoimmune disease of unknown origin characterized by chronic joint inflammation leading to destruction of the bone and cartilage, reduction of functional capacity, and increased mortality [10]. Over one-quarter of RA patients do not meet the RA classification criteria, which renders the diagnoses at the early stage difficult and deteriorates accurate medical treatment. Using the

Table 1 Different clinical and laboratory parameters of rheumatoid arthritis patients

Parameters	Mean±SD
Age (years)	36.16±11.75
Disease duration (years)	4.59±3.42
Morning stiffness (min)	63.3±51.6
VAS (cm)	4.83±1.96
DAS-28	4.4±0.91
SJC	5.5±2.6
TJC	8.2±3.1
Sharp score	194.7±35.7
ESR (mm/h)	40.6±14.5
CRP (mg/l)	11.06±7.7
Hb (g)	10.02±1.4
WBCs ($\times 10^3$)	5.803.7±1.645.5
Platelets ($\times 10^3$)	332.000±112.323
Serum RF (IU)	44.4±36.47
Serum anti-CCP ab (ng/l)	173.1±45.3
Synovial anti-CCP ab (ng/l)	133.93±41.3
ALT	33±13.5
AST	39±11.3

Ab, antibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCP, citrulline-containing peptide; CRP, C-reactive protein; DAS-28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale; WBCs, white blood cells.

Figure 1

The functional capacity grading of rheumatoid arthritis patients.

Table 2 Anti-citrulline-containing peptide antibody synovial and serum levels in rheumatoid arthritis patients and osteoarthritis patients

	RA patients (n=60)		OA patients (n=60)		P-value
	N (%)	Mean±SD	N (%)	Mean±SD	
Synovial anti-CCP ab					
Positive	50 (83.3)	133.93±41.3	0 (0)	51.1±13.2	0.0001*
Negative	10 (16.7)		60 (100)		
Serum anti-CCP ab					
Positive	54 (90)	137.1±45.3	0 (0)	48.4±12.4	0.0001*
Negative	6 (10)		60 (100)		

Ab, antibody; CCP, citrulline-containing peptide; OA, osteoarthritis; RA, rheumatoid arthritis. *P<0.001 is highly significant.

Table 3 Correlations of serum anti-citrulline-containing peptide antibody with clinical and laboratory parameters of rheumatoid arthritis patients

Variables	R	P-value
Age	−0.17	0.36
Disease duration	0.07	0.68
Morning stiffness	0.11	0.54
VAS	0.27	0.14
DAS-28	0.18	0.34
ESR	0.15	0.07
CRP	0.51	0.08
Hb	0.27	0.14
WBCs	−0.16	0.57
Platelets	0.26	0.15
RF	−0.17	0.049
Synovial anti-CCP ab	0.99	0.0001
Sharp score	0.65	0.034

Ab, antibody; CCP, citrulline-containing peptide; CRP, C-reactive protein; DAS-28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; RF, rheumatoid factor; VAS, visual analogue scale; WBCs, white blood cells.
$P>0.05$=nonsignificant. $P<0.05$=significant. $P<0.001$=highly significant.
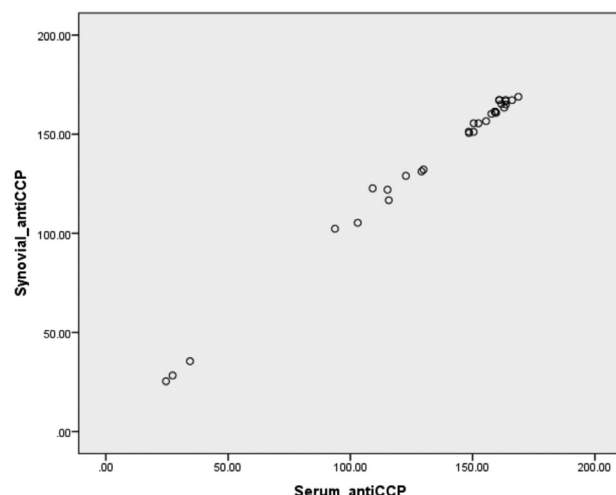
Table 4 Correlations of synovial anti-citrulline-containing peptide antibody with clinical and laboratory parameters of rheumatoid arthritis patients

Variables	R	P-value
Age	−0.08	0.67
Disease duration	0.07	0.68
Morning stiffness	0.10	0.59
VAS	0.27	0.14
DAS-28	0.17	0.35
ESR	0.46	0.056
CRP	0.63	0.66
Hb	0.27	0.13
WBCs	0.11	0.54
Platelets	0.28	0.12
RF	0.07	0.7
Serum anti-CCP ab	0.99	0.0001
Sharp score	0.62	0.037

Ab, antibody; CCP, citrulline-containing peptide; CRP, C-reactive protein; DAS-28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; RF, rheumatoid factor; VAS, visual analogue scale; WBCs, white blood cells.
$P>0.05$=nonsignificant. $P<0.05$=significant. $P<0.001$=highly significant.

CCP variants to detect autoantibody to those peptides is highly specific and predictive for RA, but with poor sensitivity [11]. In anti-CCP ab-positive RA patients, B cells from the SF, but not peripheral blood B cells, spontaneously produce anti-CCP ab, and hence activation of an antigen-driven B cells specific for citrullinated proteins exists in the synovial tissue at the site of inflammation in RA [12]. Determination of anti-CCP in the serum can distinguish RA among other arthropathies, and it can represent a prognostic marker that predicts

Figure 2

Correlation between serum and synovial anti-citrulline-containing peptide (anti-CCP) antibody in rheumatoid arthritis patients.

Table 5 Specificity and sensitivity of serum and synovial anti-citrulline-containing peptide antibody in the diagnosis of rheumatoid arthritis

Variables	Serum anti-CCP ab	Synovial anti-CCP ab
AUC	0.907	0.828
Sensitivity (%)	90	83.3
PPV (%)	100	100
NPV (%)	90.9	85.7
P-value	0.0001[*]	0.0001[*]
Cutoff	65.4	63.35

AUC, area under curve; CCP, citrulline-containing peptide; NPP, positive predictive value; PPV, positive predictive value.
$P>0.05$=nonsignificant. $P<0.05$=significant. [*]$P<0.001$=highly significant.

Figure 3
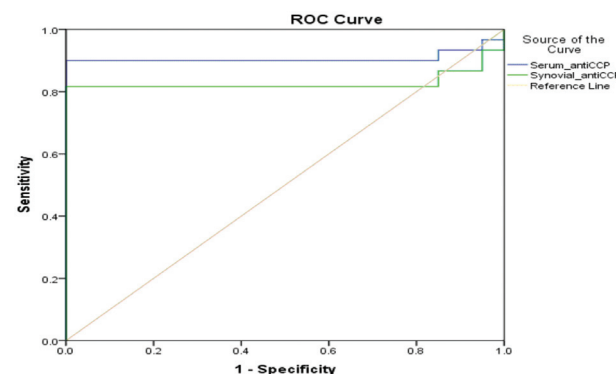
Receiver operating characteristic curve for the detection of specificity and sensitivity of anti-citrulline-containing peptide (anti-CCP) antibody in the diagnosis of rheumatoid arthritis.

erosive, persistent, more aggressive synovitis. In early RA, the assessment of anti-CCP is extremely useful when anti-CCP positivity may precede clinical symptoms by years [13].

In our study, the mean serum level and mean synovial level of anti-CCP ab in RA were correlated and both have showed a statistically highly significant difference compared with OA patients ($P=0.0001$). These results are consistent with the findings of Spadaro *et al.* [14], who documented lower levels of anti-CCP ab in the SF of OA and psoriatic arthritis compared with RA patients. This work explored a statistically highly significant correlation between serum anti-CCP ab level and synovial anti-CCP ab level ($P=0.001$) that emphasized the results of Caspi *et al.* [15] in their study on 29 RA patients, 20 psoriatic arthritis patients, and 19 OA patients.

In this study, there were statistically nonsignificant correlations of both serum and synovial anti-CCP ab with ESR ($P=0.07$) and CRP ($P=0.08$) in RA patients, which supported the results of Landmann *et al.* [16], who concluded that serum anti-CCP ab measurement is not useful for monitoring the disease activity in RA. This is in disagreement with the findings of Forslind *et al.* [17], who reported that serum anti-CCP abs positively correlated with higher ESR, CRP, swollen joint count, and worse physician global assessment ratings. Furthermore, we were on the contrary with Münevver *et al.* [18] who found a significant correlation between DAS 28 and serum anti-CCP ab. In this work, there were statistically insignificant correlations of both serum anti-CCP abs ($P=0.09$) and synovial fluid anti-CCP abs ($P=0.87$) with functional capacity in RA patients. In this work, there were statistically nonsignificant correlations of both serum anti-CCP abs ($P=0.09$) and SF anti-CCP abs ($P=0.87$) with functional capacity in RA patients. Therefore, we agreed with Aridoğan *et al.* [19], who could not find any significant association between RA activity (DAS-28) and HAQ or serum anti-CCP ab. Moreover, our results were similar to those of Porto *et al.* [20], who did not establish an association between anti-CCP and HAQ but we contrasted Nehir *et al.* [21] who found an association between serum anti-CCP ab and both of HAQ and morning stiffness. Although, there was statistically significant correlation of RF titer with serum anti-CCP abs ($P=0.047$ in agreement with Masooleh *et al.* [22] we found insignificant correlation of sf anti-CCP and RF ($P=0.7$) accorded to Liu *et al.* [11] who found the same result and stated that ESR, CRP and RF levels changed after treatment but anti-CCP level didn't change.

We also disclosed statistically significant correlations of Sharp score for radiological severity with serum anti-CCP ab ($P=0.024$) and SF anti-CCP ab ($P=0.031$) in RA patients. This is in agreement with the findings of Kim *et al.* [23], who found a significant correlation between anti-CCP ab and radiological joint score. Li *et al.* [24] documented that bone erosions at the wrist joint examined by means of MRI have a positive correlation with serum anti-CCP ab level. Furthermore, Guler *et al.* [25] observed more erosions in RA patients with positive anti-CCP ab. We found statistically nonsignificant correlations of both serum and SF anti-CCP abs with Hb concentration ($P=0.14$), white blood cells ($P=0.57$), and platelets ($P=0.15$).

We agree with Laura *et al.* [26], who concluded that there were no relations between serum anti-CCP ab and any extra-articular manifestations in RA patients. This work revealed that statistically nonsignificant correlations were found between disease duration and both serum ($P=0.767$) and SF anti-CCP ab ($P=0.966$). This finding is in accordance with the finding of Kastbom *et al.* [27], who detected that anti-CCP ab did not correlate with disease duration, HAQ, CRP, and Hb, but they differed when they found an association of serum anti-CCP abs with high DAS-28 and long duration of morning stiffness. We found statistically nonsignificant correlations of both serum and SF anti-CCP abs and the duration of morning stiffness ($P=0.54$). This is contradictory to the findings of Samanci *et al.* [28], who found an association between both HAQ and morning stiffness with serum anti-CCP ab.

Our work emphasized that in RA the sensitivity of synovial anti-CCP ab was 83.3% and specificity was 100%, whereas the sensitivity of serum anti-CCP ab sensitivity was 90% and its specificity was 100%. These results are in accordance with the findings of Heidari *et al.* [29], who detected SF anti-CCP ab sensitivity of 83.7% and specificity of 95.6%, whereas serum anti-CCP ab sensitivity and its specificity were 84.8 and 94.3%, respectively. At the same time, our results were comparable to the results of Sockalingam *et al.* [30] who found the same sensitivity in Malaysian RA patients. We also agree with Liu *et al.* [11], who found that the sensitivity and specificity of SF anti-CCP ab were 82 and 92%, respectively, in their study on 104 RA patients. However, we disagree with Kastbom *et al.* [27] who found serum anti-CCP sensitivity to be 64%. This discrepancy might be because old methods for anti-CCP ab detection were less sensitive compared with recent ones. Schellekens *et al.* [31] found that serum anti-CCP ab detection with the enzyme-linked immunosorbent assay testing has shown a specificity of 96% in the

sera from participants with early RA and 98% in the sera from patients with established RA, whereas they found that anti-CCP ab sensitivity was 48% in early RA cases and 68% in established RA cases. Noteworthy, Aridoğan *et al.* [19] recorded that the sensitivity and specificity of serum anti-CCP ab were 73 and 100%, respectively, whereas a meta-analysis conducted by Hayashi and Kumagai [32] in Japan revealed that pooled sensitivity and pooled specificity for serum anti-CCP ab were 67 and 95%, respectively.

Conclusion

SF anti-CCP ab could be considered an appreciated diagnostic marker having a major role in the identification of RA patients not fulfilling the criteria for RA disease diagnosis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Roberts CA, Dickinson AK, Taams LS. The interplay between monocytes/macrophages and CD4+ T cell subsets in rheumatoid arthritis. Front Immunol 2015; 6:571–579.

2 Ding J, Eyre S, Worthington J. Genetics of RA susceptibility, what comes next? RMD Open 2015; 1:28–33.

3 Iain B, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011; 365:2205–2219.

4 Kapitany A, Szabo Z, Lakos G, Aleksza M, Végvari A. Associations between serum anti-CCP antibody, rheumatoid factor levels and HLA-DR4 expression in Hungarian patients with rheumatoid arthritis. Isr Med Assoc J 2008; 10:32–36.

5 Shibahara T, Matsushita T, Matsuo R, Fukushima Y, Fukuda K, Sugimori H, et al. Anti-cyclic citrullinated peptide antibody-positive meningoencephalitis in the preclinical period of rheumatoid arthritis. Case Rep Neurol 2016; 8:156–160.

6 Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO III, et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010; 62:2569–2581.

7 Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29:1039–1049.

8 Prevoo ML, Van't Hof MA, Kuper HH, Van Leeuwen MA, Van de Putte LB, Van Riel P. 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; 38:44–48.

9 Van der Heidje D, Dankert T, Neiman DF, Rau R, Boers M. Reliability and sensitivity to change of a simplification of the Sharp/van der Heijde radiological assessment in rheumatoid arthritis. Rheumatology (Oxford) 1999; 38:941–947.

10 Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 2002; 4:265–272.

11 Liu D, Guo M, Yun M, Jiao Y, Zhang J, Luo Y, et al. Diagnostic value of serum and joint fluid anti-CCP (citrulline-containing peptide) antibody for rheumatoid arthritis and its relationship with other inflammatory indicators. Sci Res Essays 2011; 6:2465–2469.

12 Mimori T. Clinical significance of anti-CCP antibodies in rheumatoid arthritis. Int Med 2005; 44:1122–1129.

13 Fernandes-Cerqueira C, Ossipova E, Gunasekera S, Hansson M, Mathsson L, Catrina A, et al. Targeting of anti-citrullinated protein/peptide antibodies in rheumatoid arthritis using peptides mimicking endogenously citrullinated fibrinogen antigens. Arthritis Res Ther 2015; 17:155–200.

14 Spadaro A, Riccieri V, Scrivo R, Alessandri C, Valesini G. Anti-cyclic citrullinated peptide antibody determination in synovial fluid of psoriatic arthritis. Clin Exp Rheumatol 2007; 25:599–604.

15 Caspi D, Anouk M, Golan I, Paran D, Kantman I, Wigler I. Synovial fluid levels of anti-cyclic citrullinated peptide antibodies and IgA rheumatoid factor in rheumatoid arthritis and osteoarthritis. Arthritis Rheum 2006; 55:53–56.

16 Landmann T, Kehl G, Bergner R. The continuous measurement of anti-CCP-antibodies does not help to evaluate the disease activity in anti-CCP-antibody-positive patients with rheumatoid arthritis. Clin Rheumatol 2010; 29:1449–1453

17 Forslind K, Ahlmén M, Eberhardt K, Hafström I, Svensson B, BARFOT Study Group. Prediction of radiological outcome in early rheumatoid arthritis in a clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 2004; 63:1090–1095.

18 Münevver Serdaroğlu, Haşim Çakırbay, Orhan Değer, Sevil Cengiz, Sibel Kul. The association of anti-CCP antibodies with disease activity in rheumatoid arthritis. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2493537/ Rheumatol Int 2008; 28:965–970.

19 Aridoğan BC, Kaya S, Savaş S, Cetin ES, Akkuş S, Demirci M. The role of anti-cyclic citrullinated peptide (anti-CCP) antibodies in serologic diagnosis and evaluation of disease activity in rheumatoid arthritis. Mikrobiyol Bul 2008; 42:669–674.

20 Porto LS, Tavares Júnior WC, Costa DA, Lanna CC, Kakehasi AM. Anti-CCP antibodies are not a marker of severity in established rheumatoid arthritis: a magnetic resonance imaging study. Rev Bras Rheumatol 2015; 57:15–22.

21 Nehir Samanci, Ozdem S, Akbas H, Mutlu D, Gultekin M, Arman M, Donmez L. Diagnostic value and clinical significance of anti-CCP in patients with advanced rheumatoid arthritis. J Net Med Assoc 2005; 97:1120–1126.

22 Irandokht Shenavr Masooleh, Asghar Hajiabbasi, Habib Zayeni, Atefeh Ghanbari, Habib Zayeni, Violet Zaker EStegghamat, et al. Anticyclic citrullinated peptide antibody in rheumatoid arthritis: a cross-sectional study in Iran. Turk J Med Sci 2016; 46:1309–1310.

23 Kim DA, Kim TY. RF vs. anti-CCP: competitor or companion? Rheumatol Int 2011; 31:1257–1258.

24 Li T, Bao J, Yin J, Xu HJ. The specificity of anti-cyclic citrullinated peptide antibodies in the diagnosis of rheumatoid arthritis from a large cohort study in the Chinese. Zhonghua Nei Ke Za Zhi 2011; 91:1633–1636.

25 Guler H, Turhanoglu AD, Ozer B, Ozer C, Balci A. The relationship between anti-cyclic citrullinated peptide and bone mineral density and radiographic damage in patients with rheumatoid arthritis. Scand J Rheumatol 2008; 37:337–342.

26 Laura Gonzalez-Lopez, Rocha-Muñoz A, Ponce-Guarneros M, Flores-Chavez A, Salazar-Paramo M, Nava A, et al. Anti-cyclic citrullinated peptide (Anti-CCP) and anti-mutated citrullinated vimentin (anti-MCV) relation with extra-articular manifestations in rheumatoid arthritis. J Immunol Res 2014; 2014:536050.

27 Kastbom A, Strandberg G, Lindroos A, Skogh T. Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). Ann Rheum Dis 2004; 63:1085–1089.

28 Samanci N, Ozdem S, Akbas H, Mutlu D, Gultekin M, Arman M, Donmez L. Diagnostic value and clinical significance of anti-CCP in patients with advanced rheumatoid arthritis. J Net Med Assoc 2005; 97:1120–1126.

29 Heidari B, Heidari P, Hajian-Tilaki K, Firouzjahi AR. The prevalence and diagnostic performance of anti-cyclic citrullinated peptide antibody in rheumatoid arthritis – the predictive and discriminative ability of serum antibody level in recognizing rheumatoid arthritis. Ann Saudi Med 2010; 29:467–470.

30 Sockalingam S, Khuan CS, Sthaneshwar P. Prevalence of anti cyclic citrullinated peptide antibodies in Malaysian rheumatoid arthritis patients and its correlation with disease activity. Int J Rheum Dis 2009; 12:211–215.

31 Schellekens GA, Visser H, De Jong BA, Van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000; 43:155–163.

32 Hayashi N, Kumagai S. Anti-cyclic citrullinated peptide antibodies and rheumatoid arthritis [in Japanese]. Rinsho Byori 2010; 58:466–479.