

# Diagnostic Value of Antibodies Against a Modified Citrullinated Vimentin in Egyptian Patients with Rheumatoid Arthritis

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## Abstract

**Objective:** To investigate the sensitivity and specificity of sero-positivity to antibodies against modified citrullinated vimentin antibodies (anti-MCV) in comparison with anti-CCP2- in rheumatoid arthritis (RA) among Egyptians, considering the possible correlation to demographic and disease related features in the study group.

**Patients and methods:** This study included forty patients with Rheumatoid arthritis (RA) and thirty matching healthy controls. Patients' assessment measures involved the disease activity score (DAS-28), visual analogue scale (VAS) and health assessment questionnaire (HAQ). Thirty healthy subjects matched for age and sex served as a control group. Blood samples were obtained from patients and controls for erythrocyte sedimentation rate (ESR), C reactive protein (CRP), rheumatoid factor (RF). Anti-CCP2 and anti-MCV were determined using ELISA technique.

**Results:** Estimated serum levels of anti-CCP2 and anti-MCV were significantly higher in patients compared to controls ( $p < 0.001$ ). Serum levels of anti-MCV didn't show any significant variations with age, disease duration, duration of morning stiffness, number of swollen and tender joints, HAQ or ESR in patients with RA, yet serum levels of anti-MCV correlated significantly with DAS28, VAS and CRP ( $p < 0.05$ ). Anti-CCP2 correlated significantly with DAS28, VAS and CRP and ANA ( $p < 0.05$ ). Serum levels of anti-MCV and anti-CCP2 showed a consistently significant correlation with each other ( $r = 0.483$ ;  $p < 0.001$ ). Statistical analysis showed that anti-MCV had diagnostic specificity, sensitivity of 93.3%, 75.5%, respectively, while anti-CCP2 specificity, sensitivity of 98.1%, 85%, respectively.

**Conclusion:** Serum anti-MCV as well as the anti-CCP-2 assay perform comparably well in the diagnosis of RA. In the high-specificity range, however, the anti-CCP2 assay appears to be superior to the anti-MCV test.

**Keywords:** Anti-cyclic citrullinated peptide (anti-CCP2); Anti-citrullinated vimentin antibody (anti-CMV); Rheumatoid arthritis (RA)

## Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune, systemic disease, which primarily involves the joints, leading to inflammation, swelling, pain, stiffness, with inevitable progressive functional deterioration [1]. Considering the aggressive nature of the disease process with the significant residual disability, the International Societies of Rheumatology potentially recommended early aggressive treatment for tight control of the inflammatory process aiming to prevent joint destruction and preserve function [2].

The last two decades witnessed an advent in the molecular diagnostics of autoimmune diseases specifically RA with expansion of the spectrum of disease specific autoantibodies. Several different autoantibodies with varying sensitivity and specificity have been found in the serum of patients with RA [3], rheumatoid factor (RF) has been identified for long as the only autoantibody specifically related to RA and subsequently was included in the 1987 American College of Rheumatology (ACR) classification criteria for diagnosing established RA [4]. However, testing for RF alone wasn't enough as it is detectable in 70-80% of RA patients. It might also be present in other rheumatic disorders, viral infections, malignancies, drugs as well as in healthy individuals [5]. In the recent ACR/EULAR 2010 criteria for classifying patients with early arthritis as having RA we had another group of disease specific autoantibodies added to the criteria for establishing early diagnosis these were antibodies to cyclic citrullinated peptides which were lately proven to be highly specific to rheumatoid arthritis. Antibodies recognizing citrullinated proteins/peptides, especially the

peptide mixture designated cyclic citrullinated peptides (CCP), have reasonable sensitivity and high specificity for RA and are increasingly being used in the evaluation of patients with RA [6,7]. It is found to be highly specific for early diagnosis (97%) as well as prognosis of RA regarding radiographic progression and erosive disease [8].

Vimentin is an intermediate filament that is widely expressed by mesenchymal cells and macrophages and easy to detect in the synovium. Citrullination and molecular modification of this protein develops in macrophages undergoing apoptosis, increasing the antigenicity of such intra-cellular protein which gets uncovered and expressed on the apoptotic cell surface together with the defective clearance of the apoptotic end products such antigenic self-protein remains in the circulation stimulating autoantibody production with autoimmunity [9]. Recently, anti-mutated citrullinated vimentin (anti-MCV) antibodies have been recommended to be better diagnostic marker for early arthritis [10].

Some studies showed that anti-MCV antibody is more sensitive compared to other antibodies against citrulline-containing epitopes

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for RA diagnosis, and that anti-MCV antibody was present even earlier in the course of RA than anti-CCP, and correlated with DAS and therefore was a better marker of early RA [11]. The presence of anti-MCV antibodies at disease onset is associated with a more severe disease course, measured as higher level of inflammatory activity. The sensitivity and specificity of anti MCV was comparable to those of anti-CCP in RA [12-15].

The objective of this work was to investigate the impact of seropositivity to antibodies against modified citrullinated vimentin antibodies (anti-MCV) in comparison with anti-CCP2 in terms of their respective sensitivity and specificity in rheumatoid arthritis (RA) among Egyptians with special consideration of their possible correlation to different demographic and disease related features in the study group.

## Patients and Methods

This case control study was conducted on RA patients attending the outpatient clinic of Rheumatology and Rehabilitation Department, Sohag University Hospital, in the period between May 2012 and September 2012. Forty patients (26 females and 14 males) fulfilling the 1987 American College of Rheumatology (ACR) criteria for a diagnosis of RA with a mean age of  $46.95 \pm 11.60$  years were studied, thirty healthy age and sex matched control subjects (18 females, 12 males) were included for comparative assessment of the investigated serological disease markers. The mean age of the control subjects was  $43.7 \pm 9.9$  years. Informed consent was obtained from patients and controls participating in the study. The age, sex, disease duration, Body Mass Index (BMI) ( $\text{kg}/\text{m}^2$ ), as well as swollen and tender joint counts of the RA patients were recorded. Duration of morning stiffness, Visual Analog Scale of pain (VAS) [16] disease activity index 28 (DAS28) [17], health assessment questionnaire (HAQ), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were used to assess disease activity. Disease Activity Score 28 (DAS28) was used as a validated index of RA activity. The DAS 28 score is composed of four measuring parameters: 28 tender (TJC28) and swollen joint counts (SJC28), ESR, and patient global health assessment (VAS). Patients were grouped according to DAS28 scores as having high disease activity ( $\text{DAS28} > 5.1$ ) or moderate disease activity ( $3.2 < \text{DAS28} < 5.1$ ), and mild disease activity ( $2.6 < \text{DAS28} < 3.2$ ).

## Laboratory methods

Six mL of peripheral venous blood were withdrawn aseptically from each patient and from each control subject. Two mL blood were left to clot for 15 minutes then centrifuged and sera were put into aliquots and stored at  $-20^\circ\text{C}$  until assayed for anti-MCV and anti CCP2 antibodies for both patients and controls. The remaining 3 ml were used for other investigations done to patients:

- Complete blood picture CBC was performed on The CELL-DYN 3700 automated hematology analyzer.
- Renal and liver function tests were performed on Autoanalyzer Bechman Synchron cx5 system.
- Measurement of ESR by the Westergren method.
- Serum CRP concentrations were determined by immunonephelometry methods on a Turbox nephelometer (Orion Diagnostica, Finland). The titer of 6 mg/l were considered positive for CRP.
- Rheumatoid factor IgM isotype was analyzed using the ELISA kit for RF IgM quantitation (Orgentec Diagnostika GmbH, Germany)

according to the manufacturer's instructions. The titer of 20 IU/ml was regarded as positive [18].

f) Measurement of anti-nuclear antibody (ANA) was detected by the Fluro-kit supplied by DiaSorine Catalog No 1740 based on indirect immunofluorescent for the screening and titration ANA.

g) Anti-CCP2 antibodies were detected by enzyme-linked immunosorbent assay Kit supplies by INOVA Diagnostics Cat. NO 570139 is a semiquantitative enzyme-linked immunosorbent assay for the detection of IgG anti-CCP2 Cyclic Citrullinated Peptide 2, Lebanon) antibodies in patient sera. The antigen is bound to the surface of a microwell plate, allowing any CCP2 IgG antibodies present to bind to the immobilized antigen. A second incubation allows the enzyme labeled antihuman IgG to bind to any patient antibodies that have been attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops spectro-photometrically. A titer above 20 units was considered as positive.

h) Determination of Anti-MCV antibodies were measured using ELISA kits (provided by ORGENTEC Diagnostica GmbH, Mainz, Germany) according to the manufacturer's instructions [19]. Serum samples were diluted 1:100 and incubated on MCV coated microtiter wells for 30 minutes at room temperature. Plates were washed three times and incubated with peroxidase-labeled anti-human IgG-conjugate for 15 minutes. Then substrate was incubated for 15 minutes after additional washing. Color development was stopped with 1 M HCl solution, and the optical density was measured. Results are expressed in U/ml using a simple point-to-point curve-fitting method. Values of 20.0 U/ml or greater were considered to be abnormal according to manufacturer's recommendations.

## Statistics

The results were analyzed by IBM-SPSS (version 19). Results were given as means and standard deviations. Student's t-test for continuous variables was used to examine the significance of differences between RA and control groups. P-value less than 0.05 was regarded as significant. The correlation between anti-MCV, anti-CCP2 levels and age, disease duration, duration of morning stiffness, swollen and tender joint counts, VAS, DAS28, HAQ, ESR, CRP and BMI was analyzed by Pearson correlation analyses. Spearman rank correlation was used for nonparametric correlations, receiver operator characteristic curve (ROC) was drawn to find out the best cut-off value of anti-MCV in diagnosing RA. P value  $> 0.05$  was considered insignificant,  $P < 0.05$  was significant and  $P < 0.001$  was highly significant.

## Results

The demographic and clinical features of the study group are displayed in Table 1. Analysis of the calculated DAS28 score showed 100% of the patients to lie in the range of mild to moderate disease activity ( $\text{DAS} 28 < 5.1$ ). Laboratory and serological assessment showed a mean serum Anti-CCP2 in patients with RA ( $89.2 \pm 11.3$  U/ml) which was significantly higher ( $p < 0.001$ ) than controls ( $14.8 \pm 3.21$  u/mL). The anti-MCV levels ( $114.45 \pm 16.57$  U/ml) were also significantly higher ( $p < 0.001$ ) in RA patients compared to healthy control subjects ( $9.05 \pm 3.6$  U/ml) (Table 1, Figures 1 and 2).

Results revealed that the serum levels of anti-MCV correlated positively yet insignificantly with the age of the patients, the duration of the disease, the number of tender joints and the ESR ( $P > 0.05$ ) and

| Characteristics (mean ± SD) | RA patients (40) | Control (30) | p value  |
|-----------------------------|------------------|--------------|----------|
| Age (years)                 | 46.95 ± 11.60    | 43.7 ± 9.9   | 0.542    |
| Sex (F/M)                   | 26/14            | 18/12        | 0.532    |
| Disease duration (years)    | 4.57 ± 2.59      | -            |          |
| Morning stiffness (min)     | 18.84 ± 9.04     | -            |          |
| VAS (0–10)                  | 4.62 ± 1.40      | -            |          |
| Number of swollen joints    | 2.32 ± 1.18      | -            |          |
| Number of tender joints     | 3.89 ± 2.08      | -            |          |
| DAS28 score                 | 3.79 ± 0.66      | -            |          |
| HAQ score                   | 11.49 ± 4.15     | -            |          |
| ESR (mm/1st hr)             | 42.65 ± 8.59     | -            |          |
| Rheumatoid factor (%)       | 46.0 ± 9.8       | -            |          |
| CRP (mg/l)                  | 18.65 ± 9.59     | -            |          |
| Body mass index (BMI)       | 14.15 ± 11.48    | 27.85 ± 2.62 |          |
| Anti-CCP2 (U/ml)            | 89.2 ± 11.3      | 14.8 ± 3.21  | <0.001** |
| Anti-MCV (U/mL)             | 114.45 ± 16.57   | 9.05 ± 3.67  | <0.001** |

RF: Rheumatoid Factor; MS: Morning Stiffness; VAS: Visual Analog Scale of pain; DAS28: Disease activity for 28 joint indices score; HAQ: Health Assessment Questionnaire; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; BMI: Body Mass Index; Anti-CCP2: Antibodies against Cyclic Citrullinated Peptide; Anti-MCV: Antibodies against Mutated Citrullinated Vimentin  
\*\*Highly significant

**Table 1:** The demographic and clinical features of the RA patients and controls.

| Parameters               | Anti-CCP2 |         | Anti-MCV |         |
|--------------------------|-----------|---------|----------|---------|
|                          | r         | p-value | r        | p-value |
| Age                      | 0.264     | 0.231   | 0.359    | 0.65    |
| onset of RA              | 0.658     | 0.455   | 0.236    | 0.742   |
| Disease Duration         | 0.633     | 0.333   | 0.302    | 0.561   |
| Number of tender joints  | 0.222     | 0.432   | 0.301    | 0.543   |
| Number of swollen joints | 0.651     | 0.652   | -0.053   | 0.344   |
| VAS                      | 0.561     | 0.003** | 0.747    | 0.005** |
| MS                       | 0.233     | 0.645   | -0.341   | 0.123   |
| DAS28                    | 0.351     | 0.001** | 0.802    | 0.001** |
| HAQ                      | 0.321     | 0.356   | -0.29    | 0.06    |
| ESR                      | 0.452     | 0.442   | 0.029    | 0.367   |
| CRP                      | 0.568     | 0.031*  | 0.084    | 0.021*  |
| RF                       | 0.529     | 0.032*  | 0.560    | 0.312   |
| ANA                      | 0.333     | 0.051*  | 0.651    | 0.231   |
| Anti-CCP2 (U/ml)         | -         | -       | 0.483    | 0.001** |

Pearson correlation coefficients between Anti CCP2, Anti MCV R, RF in RA patients.

RF: Rheumatoid Factor; MS: Morning Stiffness; VAS: Visual Analog Scale of Pain; DAS28: Disease activity for 28 joint indices score; HAQ: Health Assessment Questionnaire; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; BMI: Body Mass Index. Anti-CCP2: Antibodies against Cyclic Citrullinated Peptide; Anti-MCV: Antibodies against Mutated Citrullinated Vimentin  
\*Significant \*\*Highly significant

**Table 2:** Correlations between serums Anti CCP2, Anti MCV and patients' characteristics.

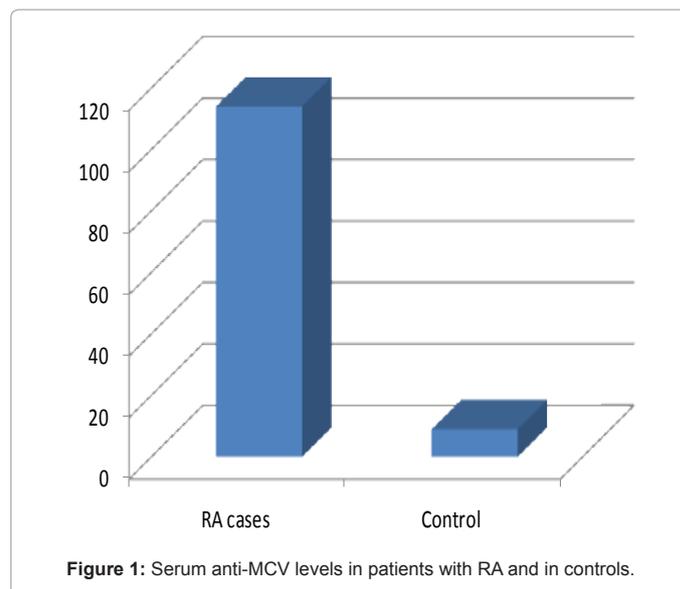
correlated negatively with the number of swollen joints ( $r=-0.053$ ,  $P>0.05$ ) in RA patients. The anti-MCV level significantly correlated with CRP, VAS and DAS 28 ( $p<0.05$ ). Similarly, the serum levels of anti-CCP2 correlated positively but insignificantly with age, duration of the disease, number of tender joints ( $p<0.5$ ) and significantly with VAS, DAS28, CRP, RF and ANA ( $p<0.05$ ). There was a significantly positive correlation between the levels of anti-MCV and anti-CCP2 (correlation coefficient=0.483;  $p<0.001$ ) eliciting a consistently synchronous increase in the levels of both antibodies within individual patient (Table 2).

For direct comparison of the diagnostic values of the anti-MCV and the anti-CCP2 assays, we performed Receiver operator characteristic

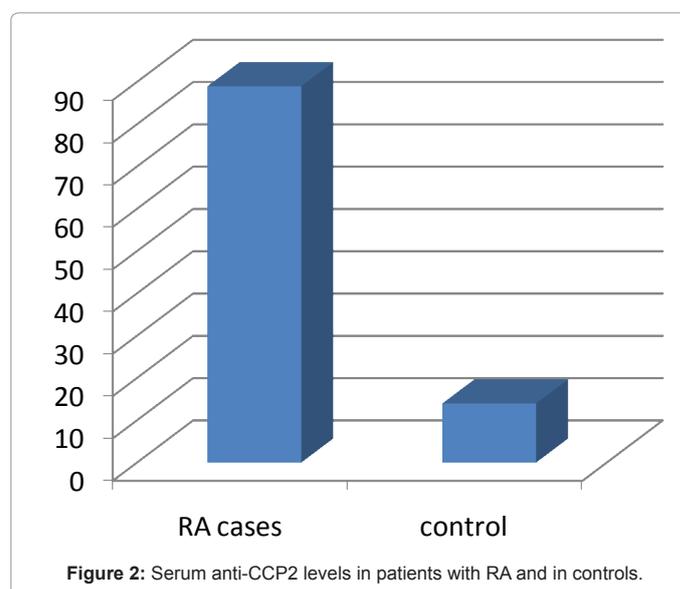
curve ROC analysis. The best cut-off value for the anti-MCV test was found at 21.5 U/mL (Figure 3). The area under the curve (AUC) was 0.893 at the 95% CI, Sensitivity was 75.6% and specificity was 93.3% in diagnosing RA patients, both positive and negative predictive values were (82.8% and 87.4%, respectively). At cut-off value 20 U/mL for the anti-CCP2 test it was found that, the area under the curve was AUC 0.926 at the 95% CI, Sensitivity was 85.71% and specificity was 98.1% in diagnosing RA patients positive and negative predictive values were (86% and 93%, respectively) (Table 3 and Figure 4).

## Discussion

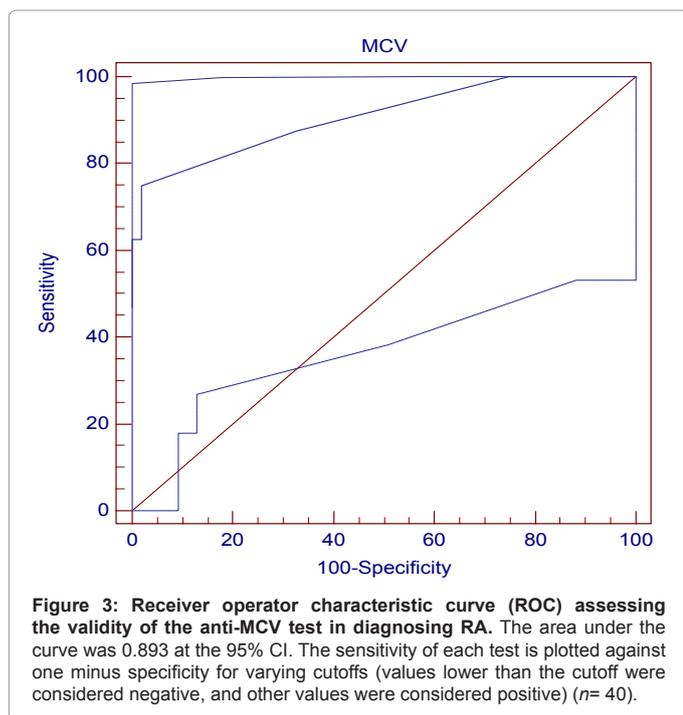
In the latest decades there has been a global intent in the era of biologic therapy to establish a treatment strategy in rheumatoid arthritis that would promote arrest of the disease with induction and maintenance of remission or at least low disease activity. In a way to establish such strategy there are continuous efforts directed towards identifying the potentially pathogenic molecules and autoantibodies involved in the disease process and their contribution to disease patterns,



**Figure 1:** Serum anti-MCV levels in patients with RA and in controls.



**Figure 2:** Serum anti-CCP2 levels in patients with RA and in controls.



|           | Cut off (U/ml) | Sensitivity % [95% CI] | Specificity % [95% CI] | positive predictive values % [95% CI] | Negative predictive values % [95% CI] |
|-----------|----------------|------------------------|------------------------|---------------------------------------|---------------------------------------|
| Anti-MCV  | 21.5 (U/ml)    | 75.56 (60.5-87.1)      | 93.33 (68.1-99.8)      | 82.24 (78-96.22)                      | 87.4 (69-81.42)                       |
| Anti-CCP2 | 21 (U/ml)      | 85.71 (42.1-99.6)      | 98.11 (89.9-100.0)     | 86 (62.1-89.6)                        | 93 (89.7%-99.6)                       |

**Table 3:** Diagnostic effectiveness of the anti-MCV test and the anti-cyclic citrullinated peptide in RA patients.

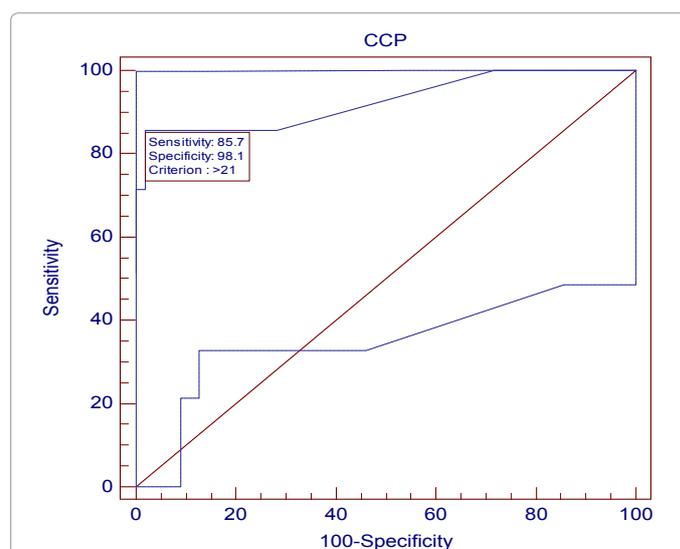
severity, progression and prognosis with treatment with an additional focus on the role of the sensitivity and specificity of new disease specific autoantibodies in establishing early diagnosis that requires early therapeutic intervention. Therapeutic intervention early in the course of RA leads to more efficient disease control, less joint damage, and better prognosis of disease outcome [20]. The identification of a sensitive diagnostic marker which permits early definitive diagnosis of RA is becoming a priority. Several reports have demonstrated high diagnostic value of antibodies directed against citrullinated proteins in the diagnosis of RA. Although the specificity was more than 90% in most studies, the sensitivity of the same antibodies varied between 33% and 87.2%, possibly reflecting diverse genetic backgrounds and/or methodological differences in diverse antigen preparations and detection techniques applied [21]. The variable diagnostic validity of such uprising specific antibodies and their similar still needs to be evaluated in different genetic and ethnic backgrounds to identify their potential pathogenic role and diagnostic significance in different populations with RA.

The current study aimed to investigate the comparable sensitivity and specificity of anti-MCV and anti-CCP in RA in a different ethnic group of RA patients aiming to study the diagnostic value of these two disease related biomarkers and compare them to the available data in other populations. The study showed that the levels of anti-CCP2 were significantly increased in the sera of patients with RA in comparison with the controls (p<0.001), which agrees with what has been reported

in late studies by Zhu and Feng, 2013 in chinese patients with RA, Sariyildiz et al. 2013 in Turkish patients amongst other multi-ethnic studies [22- 25]. In confirmation to what has been previously reported the study revealed a significantly higher serum anti-MCV antibody level in Egyptian RA patients when compared to healthy controls (p<0.001), supporting the hypothesis that citrullinated vimentin plays an integral role in triggering the inflammatory immune response in RA [25,26]. This antigenic self-protein activates T lymphocytes by binding on HLA-DR4 on the surface of antigen presenting cells and may contribute to certain pathways in the pathogenesis of RA. Several late studies have demonstrated significant elevation in serum anti-MCV in RA patients versus controls which correlated with severity of inflammatory process as evidenced by the associated increase in the inflammatory biomarkers, and evidences of association of anti-mcv with higher incidence of radiographic progression in these patients [22-29], in contrast to this finding, Morbach et al. [29], found no significant difference, a finding which they explained by the fact that vimentin contains 43 arginine residues with 10 citrullination sites experimentally confirmed and anti-MCV antibodies are considered a heterogenous group of antibodies directed against different epitopes on the citrulline molecule [13].

In the present work, anti-MCV titer significantly correlated with DAS-28, VAS and CRP (p<0.05). Such findings are consistent with those reported by Zhu and Feng, 2013, Sariyildiz et al. 2013 and Innala et al. [22-28] who concluded that anti-MCV titres correlated significantly withwith DAS-28, VAS and ESR. Similarly , In a three-year follow-up study of 427 RA patients, Keskin et al. [25] found that patients with active RA had higher anti-MCV titers compared to patients with inactive disease, while, anti-CCP2 titer failed to show this correlation (a non-significant difference in anti-CCP2 titer between patients with active or inactive disease). Similarly the authors found that the mean serum anti-MCV levels correlated with DAS 28, serum CRP levels, serum RF levels, swollen joints number and tender joints number (p=0.001).

Analysis of the diagnostic performance of serum anti MCV antibodies in RA, the ROC curve was plotted. We found that at a cut off value of 21.5 u/ml serum anti MCV antibody had specificity



**Figure 4: Receiver operator characteristic curve (ROC) assessing the validity of the anti-CCP2 test in diagnosing RA.** The area under the curve was 0.923 at the 95% CI. The sensitivity of each test is plotted against one minus specificity for varying cutoffs (values lower than the cutoff were considered negative, and other values were considered positive) (n=40).

and a sensitivity of 93.3% and 75.6% respectively and positive and negative predictive values were (82.8% and 87.4%, respectively). This is consistent with the results of previous reports conducted by Damjanovska et al., Soos et al., and Mathsson et al. [26,29,30]. As the detection of anti-MCV antibodies was shown to provide a sensitivity of 62-84% and specificity of 83-95% for the diagnosis of RA. Poulosom and Charles [10] found a specificity and sensitivity of 87% and 84% respectively, while Dejaco and associates [15] reported values of 90.8% and 69.5% respectively. However, at a cut off value of 25 u/ml, Bang and colleagues [13] reported the specificity and sensitivity to be 88% and 82% respectively. Such variations in the sensitivity and specificity might be attributed to the fact that some of the studies included patients with undifferentiated arthritis and psoriatic arthritis [31].

In the present study, out of 40 patients with established RA, 31 (77.5%) were seropositive for anti-MCV with a sensitivity and specificity of 75.6% and 93.3%, respectively while anti-CCP2 had a diagnostic sensitivity of 85.71% and specificity of 98.1% in the studied patients with RA using the manufacturer's cutoff levels which was consistent with those reported by Dejaco et al. [15].

In conclusion Anti-MCV antibodies can be considered as a promising diagnostic marker in RA patients with high sensitivity and specificity that was fairly consistent in different studies. Autoantibodies to citrullinated proteins are specific for the diagnosis of RA. Antibodies directed against a modified citrullinated vimentin demonstrated comparable overall diagnostic performance in relation to the anti-CCP2. In the high specificity range of both tests, which is clinically the most relevant, the anti-CCP2 appears to be superior to the anti-MCV assay. Serum anti-MCV antibody could be a promising tool in diagnosing RA, show a significant link with disease activity.

## Recommendations

The authors recommend multi-center multi-ethnic studies to provide evidence based meta-analysis clarifying the role of anti-MCV and other anti-citrullinated peptides in RA and additionally assess the potential influence of heterogeneity of race, sex and genetics particularly proteomics and small nuclear proteins and their contribution to the expression and diagnostic value of autoantibodies in RA.

## Conflict of Interest Statement

The authors disclose no conflict of interest.

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