Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 29 (4), 2022: 184–194.
www.Ejimmunology.org

Antibodies against a mutated citrullinated vimentin in patients with rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disease characterized by autoantibodies against citrullinated antigens. The anti-cyclic citrullinated peptide (Anti-CCP) test is commonly used to diagnose rheumatoid arthritis, whereas the anti-mutated citrullinated vimentin (Anti-MCV) is another anti-citrullinated antibody that reacts with mutated citrullinated vimentin. Therefore, we aimed to assess the diagnostic value of anti-MCV antibodies in RA patients and their relation to disease activity. This study included 60 RA patients and 25 normal controls. The disease activity of RA patients was assessed by disease activity score (DAS-28). ELISA was used to test patients and controls for anti-MCV and anti-CCP. The level of anti-MCV was significantly higher among patients with RA compared to the control group (1.56 ± 0.56 vs. 1.20 ± 0.19 μmol/l; *P*< 0.001). Anti-MCV at cut-off point of > 1.2 μmol/l had 76% sensitivity and 100% specificity, with an overall diagnostic accuracy of 83.2% for diagnosing RA. Regarding early RA diagnosis, anti-MCV at the cut-off point was > 1.2 μmol/l with 70% sensitivity and 100% specificity. For diagnosis of late RA, the cut-off point was > 1.2 μmol/l, with 93.3% sensitivity and 100% specificity, whereas the overall diagnostic accuracy was 96.3%. In this study, patients with positive anti-CCP had a marginally higher level of anti-MCV compared to those with negative anti-CCP (1.64 ± 0.28 vs. 1.48 ± 0.73 μmol/l; *P*= 0.29). We concluded that serum levels of Anti-MCV can be used as a diagnostic test in RA. The increased serum levels of Anti-MCV, demonstrated the importance of Anti-MCV as an independent serum marker in predicting the outcome of RA.

Keywords: Rheumatoid arthritis, Anti-CCP, Anti-MCV.

Date received: 16 June 2022; **accepted:** 24 July 2022

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that causes joint inflammation and eventual joint deterioration, resulting in joint function loss and disability. Joint erosions are present in 25% of RA patients within the first three months of the disease and approximately 75% of patients within the first two years.[1]

Early RA diagnosis can be challenging; nevertheless, with the identification of antibodies against citrullinated protein antigens

(ACPAs) over the last 15 years, there has been significant progress in the pathophysiology of RA. ACPA production is linked to the HLA-DRB1 common epitope, smoking, and periodontitis.[2] ACPAs have been demonstrated to predict joint deterioration[3] and be associated with more severe illnesses and extra-articular symptoms.[4]

The most commonly used commercial assay for detecting ACPAs is the anti-cyclic citrullinated peptide (anti-CCP) test, which utilizes synthetic cyclic citrullinated peptides that mimic RA epitopes.[5] Citrullination is also detected in other autoimmune disorders. Indeed, histone citrullination can result in the release of neutrophil extracellular traps. Moreover, the interaction of citrullinated histones with pathogenic pathogens, complement, and immune complexes can impair tolerance of nuclear autoantigens and promote autoimmunity.[6]

Antibodies to other citrullinated peptides or proteins have been proposed as potential RA diagnostics.[7] Anti-mutated citrullinated vimentin (MCV) antibodies have been suggested as a superior early arthritis diagnostic marker.[8]

Vimentin is an intermediate filament found in the synovium that is widely expressed by mesenchymal cells and macrophages. In apoptotic macrophages, the protein is modified, and antibodies to citrullinated vimentin may elevate if the apoptotic material is not adequately removed.[9] Aim of this work was to evaluate the diagnostic value of anti-MCV antibodies by the enzyme linked immunoassay (ELISA) in patients with RA.

Subjects and Methods

The current study included 60 RA patients and 25 normal controls. Patients were selected from the Rheumatology, Rehabilitation, and Physical Medicine Department, Assiut University Hospital, Assiut, Egypt. The study protocol was reviewed and approved by the Medical Ethics Committee of the Faculty of Medicine, Assiut University , Assiut, Egypt (approval date: 12/9/2017).Written informed consent was obtained from all patients and control subjects who participated in this study after being informed about the nature of the study.

All patients were diagnosed according to the American College of Rheumatology (ACR) European League Against Rheumatism (EULAR) ACR/EULAR RA classification criteria of 2010.[10] Patients were divided into two groups: Group I: 30 patients with Early RA (< 1year duration), Group II: 30 patients with Late RA. The 25 normal controls were considered Group III. Patients with early and late rheumatoid arthritis aged >18 years, were included in the study. Children and patients with other autoimmune diseases, such as systemic lupus erythematosus (SLE) and multiple sclerosis were excluded from the study.

Complete history taking was recorded, including general and demographic data such as age, sex, weight, height, family history, extra-articular manifestations, and disease duration. Body mass index (BMI, kg/m2) was calculated. Disease activity for RA patients was assessed by disease activity score-28 (DAS-28) score with C-reactive protein [CRP].

Blood sampling and measurements

Venous blood samples (8 mls) were collected, form each study participant under complete aseptic conditions and divided into four aliquots. The first aliquote, two mls blood were placed into an EDTA tube for complete blood counts (CBC), performed using a cell counter (Celldyn Ruby, Abbott, USA), according to the manufacturer's instructions. For erythrocyte sedimentation rate (ESR) measurement, about 1.6 ml was added to a tube containing 0.4 ml sodium citrate, and performed by Westergen tube method.

Into a plain tube, 4 mls were placed and sera separated for routine clinical analysis. An automated chemistry analyzer (Dimension RXL max, Siemens, USA) was used to measure glucose, creatinine, urea, and liver function tests, according to the manufacturer's instructions.

The remaining serum sample was stored at-20c for subsequent analysis of Rheumatoid factor (RF), CRP, Anti-Cyclic Citrullinated Peptides (Anti-CCP), and Anti-Mutated Citrullinated Vimentin (AMCV).

Rheumatoid factor (RF)

RF was measured by a latex enhanced immunoturbidimetric test, for quantitative determination of the concentration of RF level in human serum using the ADVIA1800 chemistry systems (Siemens Healthcare Diagnostics, USA), according to the manufacturer's instruction. The normal range is <14 IU/mL.

C-Reactive Protein (CRP)

CRP was done by a latex enhanced immunoturbidimetric test, for the quantitative determination of the concentration of CRP level in the human serum using the ADVIA 1800 chemistry systems (Siemens Healthcare Diagnostics, USA), according to the manufacturer's instruction. The normal range in adults is 0-5 mg/L.

Anti-Cyclic Citrullinated Peptides (Anti-CCP)

Anti-CCP was performed using a chemiluminescent assay kit (ARCHITECT i1000, Abbott, USA), according to the manufacturer's instruction. The Anti-CCP assay is a chemiluminescent microparticle immune assay (CMIA) for the semi-quantitative measurement of the IgG class of autoantibodies specific to the cyclic citrullinated peptide (CCP) in human serum. The level range of <5 U/ml is considered negative.

Serum Anti-MCV Quantitative Determination

Anti-MCV was performed using a Human Anti-Mutated citrullinated vimentin Antibody ELISA kit (Catalog No: SG-12389, SinoGeneClon Biotech Co., China), according to the manufacturer's instruction.

Statistical analysis

Data were collected and analyzed using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data were expressed as mean ±standard deviation (SD) or median (range), whereas nominal data expressed as frequency (percentage). The Chi-square (X^2)-test was used to compare the nominal data of various groups, while the student t-test was utilized to compare the mean of two different groups and the ANOVA test for more than two groups. Pearson correlation was used to determine the correlation between anti-MCV and other continuous variables. The diagnostic performance of anti-MCV in the diagnosis of RA was determined by the ROC curve analysis. The level of confidence was determined at 95%, and hence the level of significance was set at *P* value < 0.05.

Results

In the current study, 60 RA patients and 25 controls were included. The mean age of patients with RA was 42.53 ± 12.26 years, with a range between 18 and 74 years. While the mean age of the control group was 39.04 ± 8.13 years, with a range between 24 and 51 years. The majority of both groups (78.3% of the RA group and 72% of the control group) were females. The age, sex, and BMI were not different in both study groups.

Table 1 depicts clinical data for early and late RA patients. RA disease activity was significantly different between the two groups. In current study, 56.7% of patients with early RA had mild disease activity, while 53.3% of patients with late RA had moderate and severe activity.

Table 1. Clinical data for early and late RA patients.

Variables	Early RA (n= 30)	Late RA (n= 30)	*P* value
Family history of RA	7 (23.3%)	9 (30%)	NS
Extra-articular manifestations			
Weight loss	1 (3.3%)	1 (3.3%)	NS
Subcutaneous nodules	7 (23.3%)	6 (20%)	NS
Erythema	1 (3.3%)	0	NS
GIT upsets	1 (3.3%)	4 (13.3%)	NS
Dyspnea	1 (3.3%)	2 (6.7%)	NS
Morning stiffness	20 (66.7%)	22 (73.3%)	NS

Table 1. Continued.

Variables	Early RA (n= 30)	Late RA (n= 30)	*P* value
Duration of RA (year)	0.75 ± 0.12	4.50 ± 1.50	< 0.001
Disease activity			
Mild	17 (56.7%)	6 (20%)	
Moderate	8 (26.7%)	16 (53.3%)	0.01
Severe	5 (16.7%)	8 (26.7%)	

Data expressed as frequency (percentage), mean (±SD). *P* ≥ 0.05 is not significant (NS)
RA: rheumatoid arthritis. All data were compared with *Chi-square* test with exception of duration of RA that was compared with student t test.

Table 2 shows baseline laboratory data of patients with RA and the control group. There was no difference in baseline laboratory data among the two groups except for ESR, Hb, RF, Anti-CCP and anti-MCV. The ESR was significantly higher in patients with RA as compared to the control group (47.86 ± 6.25 vs. 6.48 ± 2.51 ml/h; *P*< 0.001), but ESR was not different between early and late RA groups. The hemoglobin level was significantly higher in the control group as compared to the RA group (13.43 ± 1.23 vs. 11.84 ± 1.77 mg/dl; *P*< 0.001), but Hb was not different between early and late RA groups.

RF, Anti-CCP and CRP were significantly higher in the patient group in comparison to control group *P*<0.001, for all, Table 2). Late RA patients showed a significantly higher mean Anti-CCP level (98.75 ± 83.43) compared to the early RA patients and the normal control group (0.72 ± 0.38 and 0.50 ± 0.01, respectively, *P*< 0.001). RF and CRP were not different between the early and late RA patients.

Table 2. Baseline laboratory data of patients with RA and control group.

Variables	Patients with RA (n= 60)		Control group (n= 25)		*P value*
	Range	Mean ± SD	Range	Mean ± SD	
ESR (ml)	12-130	47.86 ± 6.25	3-12	6.48 ± 2.51	< 0.001
WBCs (x10^9/l)	3.70-12	6.53 ± 1.91	3.80-11.40	7.02 ± 1.98	NS
Hb (g %)	5.8-15	11.84 ± 1.77	10.60-15.2	13.43 ± 1.23	< 0.001
Platelets (x10^9/l)	86-453	273.67 ± 80.89	168-437	267.6 ± 55.6	NS
RBS (mmol/l)	3.4-7.80	5.08 ± 1.23	3.80-7.80	5.63 ± 1.18	NS
Urea (mmol/l)	2.4-15	4.84 ± 2.25	2.40-6.90	4.29 ± 1.32	NS
Cr (mmol/l)	28-100	59.67 ± 17.14	63-121	83.88 ± 16.6	NS
Bili (μmol/l)	1-15	6.93 ± 3.08	3.40-14.10	5.86 ± 2.20	NS
D Bil (μmol/l)	0.20-5	1.81 ± 1.32	0.20-2	0.85 ± 0.56	NS
Protein (g/l)	58-84	72.38 ± 5.90	59-82.1	74.67 ± 5.45	NS
Albumin (g/l)	26-44.30	39.27 ± 3.87	32.46.30	40.98 ± 3.68	NS
ALT (U/L)	5-41	20.32 ± 3.87	7.50-36	19.81 ± 7.97	NS
AST (U/L)	6.7-54	21.10 ± 8.37	8.60-36.40	20.42 ± 8.86	NS
ALP (U/L)	38.4-141	88.89 ± 21.84	37.90-105	72.5 ± 18.73	NS
Anti-CCP (μmol/l)	0.50-280	76.58 ± 49.73	0.50-0.51	0.50 ± 0.01	< 0.001

Table 2. Continued.

Variables	Patients with RA (n= 60)		Control group (n= 25)		*P value*
	Range	Mean ± SD	Range	Mean ± SD	
Positive anti-CCP	30 (30%)		0		< 0.001
RF (μmol/l)	18-600	126.01 ± 84.15	2-6	3.19 ± 2.11	< 0.001
Positive RF	45 (75%)		0		< 0.001
CRP (mg/dl)	0-120	29.73 ± 25.85	3-4	3.11 ± 0.98	< 0.001
Positive CRP	44 (73.3%)		0		< 0.001

Data expressed as frequency (percentage), mean (±SD). *P* ≥ 0.05 is not significant (NS). RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; Anti-CCP: anti-citrullinated protein antibodies; WBCs: white blood cells; RBS: random blood sugar; Hb: hemoglobin; Cr: creatinine; Bili: bilirubin; D Bil: direct bilirubin; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; RF: rheumatoid factor; CRP: C-reactive protein. All continuous data were compared with student t test while nominal data were compared with *Chi square* test.

Table 3 shows the level of anti-MCV in the studied groups. The anti-MCV level was significantly higher in the RA patients than the control group (1.56 ± 0.56 vs. 1.20 ± 0.19 μmol/l; *P*< 0.001). The level of anti-MCV was significantly lower in patients with early RA (1.48 ± 0.73 μmol/l; *P*= 0.03) than patients with late RA (1.64 ± 0.28 μmol/l; *P*< 0.001), and than the control group (1.20 ± 0.19 μmol/l, *P*=0.03).

Table 3. Level of anti-MCV in enrolled groups.

Variables	Patients with RA (n= 60)	Early RA (N 30)	Late RA (n= 30)	Control group (n= 30)	*P*	*P*1	*P*2	*P*3
Anti-MCV (μmol/l)	1.56 ± 0.56	1.48 ± 0.73	1.64±0.28	1.20± 0.19	<0.001	NS	0.03	<0.001
Range	0.50-4.30	0.50-4.30	0.93-2.10	0.70-1.80				

Data expressed as mean (±SD). *P* ≥ 0.05 is not significant (NS). Anti-MCV: anti-modified citrullinated vimentin.
P value compared between all patients with RA and the control group with studied t test
*P*1 compares between early and late RA; *P*2 compares between early RA and control group;
*P*3 compare between late RA and control group and this was done by ANOVA test

As shown in Table 4, Anti-MCV did not differ according to disease activity grades.

Based on data in Table 5, the anti-MCV level did not differ in RA patients with positive or negative, rheumatoid factor, positive or negative CRP and patients with positive or negative anti-CCP.

Table 4. Level of anti-MCV in RA based on disease activity.

	Mild activity (n= 23)	Moderate activity (n= 24)	Sever activity (n=13)
Anti-MCV (μmol/l)	1.57 ± 0.76	1.56 ± 0.42	1.56 ± 0.35
Range	0.77-4.30	0.50-2.10	0.87-1.90
Significance	*P* 1	*P* 2	*P* 3
Anti-MCV	NS	NS	NS

Data expressed as mean (±SD). *P* ≥ 0.05 is not significant (NS). Anti-MCV: anti-modified citrullinated vimentin; Data compared with ANOVA test followed by post-hoc analysis. *P*1 compares between mild and moderate; *P*2 compares between mild and severe; *P*3 compare between moderate and severe.

Table 5. Level of anti-MCV in RA patients in relation to other markers.

	Anti-MCV (μmol/l) (range)
Rheumatoid factor	
Positive (n= 45)	1.55 ± 0.62 (0.50-4.30)
Negative (n= 15)	1.61 ± 0.30 (1.16-2.08)
P value	NS
C-reactive protein	
Positive (n= 44)	1.54 ± 0.56 (0.50-4.30)
Negative (n= 16)	1.61 ± 0.53 (0.93-3.01)
P value	NS
Anti-CCP	
Positive (n= 30)	1.64 ± 0.28 (0.93-2.10)
Negative (n= 30)	1.48 ± 0.73 (0.50-4.30)
P value	NS

Data expressed as mean (±SD). $P \geq 0.05$ is not significant (NS). Anti-MCV: anti-modified citrullinated vimentin; Anti-CCP: anti-citrullinated protein antibodies; RA: rheumatoid arthritis. Data was compared with student t test.

The ROC curve analysis indicated that anti-MCV at cut-off point > 1.2 μmol/l had 76% sensitivity and 100% specificity, with an overall diagnostic accuracy of 83.2% for diagnosing rheumatoid arthritis (Figure 1). For diagnosis of early RA, anti-MCV at cut off point > 1.2 μmol/l had 70% sensitivity and 100% specificity with an overall diagnostic accuracy of 79% (Figure 2, Table 6). Also, anti-MCV at cut-off point > 1.2 μmol/l, had 93.3% sensitivity and 100% specificity with an overall diagnostic accuracy of 96.3% for diagnosis of late RA (Figure 3, Table 6).

Table 6. Performance of anti-MCV in diagnosing rheumatoid arthritis.

Indices	RA	Early RA	Late RA
Sensitivity	76%	70%	93.3%
Specificity	100%	100%	100%
Positive predictive value	100%	100%	100%
Negative predictive value	64%	59%	93%
Accuracy	83.2%	79%	96.3%
Cutoff point	> 1.2	> 1.2	> 1.2
Area under curve	0.83	0.82	0.97
P value	< 0.001	< 0.001	< 0.001

$P \leq 0.05$ is significant. . Anti-MCV: anti-modified citrullinated vimentin.

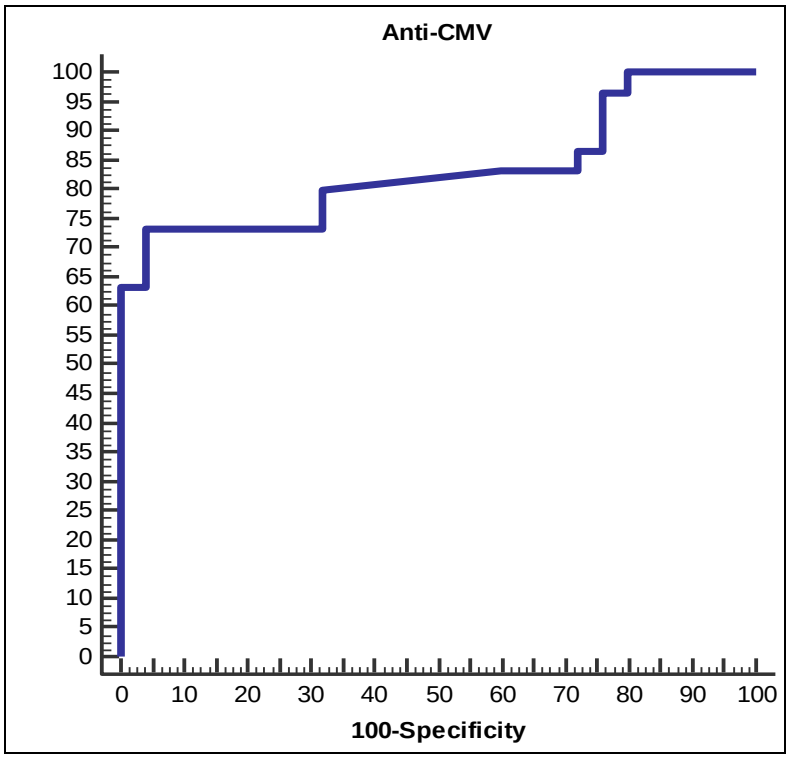

Figure 1. Performance of anti-MCV in diagnosing RA , anti-MCV at cut off point > 1.2 µmol/l had 76% sensitivity and 100% specificity.

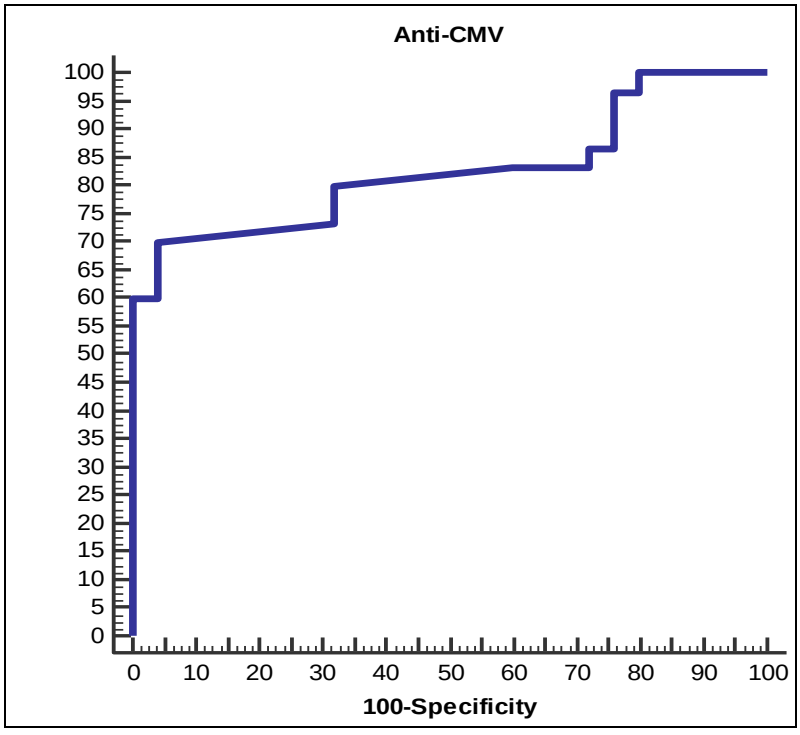

Figure 2. Performance of anti-MCV in diagnosing early RA, anti-MCV at cut off point > 1.2 µmol/l had 70% sensitivity and 100% specificity.

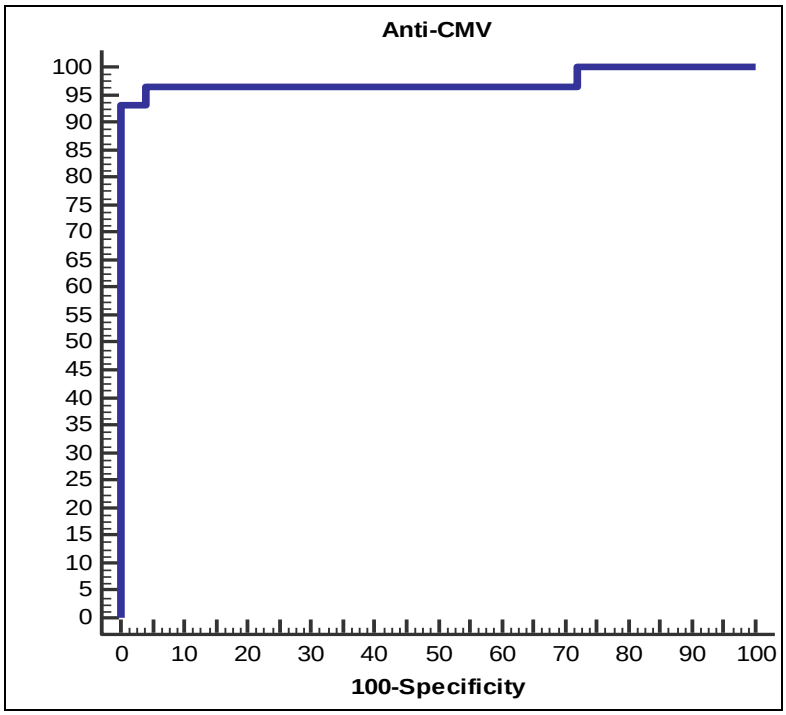

Figure 3. Performance of anti-MCV in diagnosing late RA, anti-MCV at cut off point > 1.2 μmol/l, had 93.3% sensitivity and 100% specificity.

Discussion

The present study focused on assessing the level of anti-MCV in patients with RA. In addition, the sensitivity and specificity of the anti-MCV test were determined in patients with RA.

Citrullinated vimentin is found in synovial membranes and is elevated in response to growth factors and pro-inflammatory cytokines, implying a role in the pathophysiology of RA.[11] In this study, the anti-MCV level in RA patients was significantly higher than in the control group ($P < 0.001$), which aligns with the results of studies by Abou el-Fetouh and Abo Zaid (2013)[8] and Osman et al., (2014)[12] A study by Nugraha et al., (2012)[13] demonstrated that the values of anti-MCV can be used for diagnosing rheumatoid arthritis in anti-CCP-negative patients. Consequently, anti-MCV outperforms anti-CCP.

In the present study, there was no difference between RA patients and controls from one side and between anti-MCV positive and negative patients with respect to age, disease duration, sex, and extra-articular manifestations. These finding are in agreement with the results found in the studies by Wagner et al., (2010),[14] Engelmann et al., (2009),[15] El Shazly et al., (2014),[16] and Pongratz et al., (2020).[17] Also, Sghiri et al., (2008)[18] reported no association between extra-articular involvement and anti-MCV.

In this research, anti-MCV did not differ according to grades of RA disease activity, which is compatible with findings of a study by Ursum et al., (2008,)[19] who found a low correlation between anti-MCV antibodies and disease activity in early RA patients. In contrast, a study by Mathsson et al., (2008)[11] suggested that anti-MCV antibodies are better predictors of RA disease activity.

In the current study, there was no correlation between anti-MCV with RF or CRP in the RA patients group. Patients with positive or negative rheumatoid factor did not differ in the level of anti-MCV. This finding aligns with the results of studies by Abou-Elfattah et al., (2019)[20] and by Osman et al., (2014).[12] RF status also did not show a significant contribution to fluctuations of anti-MCV as reported in a study by Pongratz et al., (2020),[17] however, this disagreed with a study by Al-Shukaili et al.,

(2012),[21] who reported a significant positive correlation between anti-MCV and RF. Such differences in study findings could be attributed to the difference in the methods used in both studies.

In the present study, the level of anti-MCV did not vary according to the CRP being negative or positive. This is in consistent with data of studies by Abou-Elfattah et al., (2019)[20] and by Liu et al., (2009) [22] who showed no correlation between Anti-MCV and CRP.

In this study, patients with RA had a significantly higher ESR and CRP levels compared to the control group. These findings agreed with data of previous studies by Osman et al., (2014)[12], by Abou El-Fetou & Abozaid (2013),[8] as well as the results of the study by Shen et al., (2015),[23] they reported that ESR and CRP demonstrated a significant correlation in RA patients than the control group.

RA is characterized by the presence of several autoantibodies. The most well-known is the RF, an autoantibody listed among the diagnostic criteria.[24] In our study, 75% of RA patients had positive RF test results. This finding corresponds to data of Doğan et al., (2014)[25] and Porto et al., (2017)[26], who found that 59.3% and 55.4%, respectively of RA patients had positive RF test outcomes. Furtheremore, a study by Shen et al., (2015)[23] reported that 91.7% of RA patients had positive RF test results. RF is the most widely used and highly approved serologic test for RA. However, it is not specific in detecting early RA. In the normal population, 15% may be positive at low titrations, and this percentage increases with age.[27] RF may also be positive in other autoimmune rheumatologic disorders such as Sjogren's syndrome, SLE, cryoglobulinemia, pulmonary diseases, including interstitial fibrosis and silicosis, and many viral infections.[27] It is unknown how a group of chronic infections and inflammatory disorders cause enhanced RF generation.[28]

Anti-CCP can adequately diagnose RA in individuals with inflammatory arthritis early in the disease's course. It was found to be significantly related to various indices of RA disease activity and severity.[29] In the present study, anti-CCP was found to be positive in 50% of the RA patients, which is consistent with findings of a study by Porto et al., (2017)[26] who found that 66% of RA patients had positive Anti-CCP results. Furthermore, anti-CCP showed a significantly higher level in RA patients in comparison to the control group ($P< 0.001$). In agreement with our results, Shen et al., (2015)[23] reported that anti-CCP was significantly higher in RA patients than in the control group ($P<0.01$). Although anti-CCP assays are successful and frequently used for RA diagnosis, their sensitivity is reduced in patients with early RA.[19]

In the present study, anti-MCV at a cut off point of > 1.2 μmol/l had 76% sensitivity and 100% specificity with overall diagnostic accuracy of 83.2% for diagnosing rheumatoid arthritis. These agreed with data of a study by Abou El-Fetou & Abozaid (2013) who showed that the sensitivity and specificity of anti-MCV were 75.6% and 93.3% respectively.[8] In addition, the study by Abou-Elfattah et al., (2019)[20] found that the sensitivity of anti -MCV in the diagnosis of RA was 92.0%, and specificity 92.0%. In addition, a study by Liu, et al., (2009)[22] reported that the sensitivity and the specificity of anti-MCV were 78.2% and 93.4%, respectively. Furthermore, a study by Bang et al., (2007)[30] found that the sensitivity of anti MCV was 82% with a specificity of 88%, while Poulsom & Charles, (2008)[31] reported a sensitivity of 84% and a specificity of 87%. In contrast, El Shazly et al., (2014)[16] reported that the sensitivity of anti-MCV was 84%, and the specificity 80%. The discrepancy between these study results may be explained by the difference in patient populations.[32]

For early RA diagnosis, anti-MCV at cut off point of > 1.2 μmol/l had 70% sensitivity and 100% specificity with overall diagnostic accuracy of 79%, which agreed with data of a study by Liu et al., (2009) [22], where the anti-MCV antibody had a sensitivity of 65%–82%, specificity of 80%–97% for early RA diagnosis. Additionally, a study by Mathsson et al., (2008) [11] illustrated that when patients with early RA are compared with normal controls, analysis of anti-MCV yields greater sensitivity and unchanged specificity than analysis of anti-CCP.

In the current study, anti-MCV at a cut-off point of > 1.2 μmol/l, had 93.3% sensitivity and 100% specificity with an overall diagnostic accuracy of 96.3% for diagnosing late RA. In contrast to this finding, study by Morbach et al., (2010)[33] found no significant difference in anti-MCV between RA patients and normal controls. It was suggested that vimentin contains 43 arginine residues with ten citrullination sites experimentally confirmed, and anti-MCV antibodies are considered a heterogeneous group of antibodies directed against different epitopes on the citrulline molecule.[18]

We concluded that serum levels of Anti-MCV can be used as a diagnostic test in RA. The increased serum levels of anti-MCV regardless of the serum RF and anti-CCP status highlight the importance of anti-MCV as an independent serum marker in predicting the diagnosis of RA. Serum Anti-MCV may be suggested as a specific tool to facilitate the early diagnosis of patients with rheumatoid arthritis.

Author Contributions

HHA and DAN contributed to the study conception and design. DK and JH contributed to material preparation, data collection and analysis. DAN and JH performed the clinical pathology and laboratory work. DK provided clinical support. DAN wrote the manuscript draft. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Medical Ethics Committee of the Faculty of Medicine, Assiut University , Assiut, Egypt (approval date: 12/9/2017).

Informed consent

Written informed consent was obtained from all patients and control subjects who participated in this study after being informed about the nature of the study.

References

1. Scott D. L. (2004). Radiological progression in established rheumatoid arthritis. *J Rheumatol. Supplement*, 69, 55–65.

2. Sakkas LI, Bogdanos DP, Katsiari C. (2014). Anti-citrullinated peptides as autoantigens in rheumatoid arthritis-relevance to treatment. *Autoimmun Rev* , 13(11):1114–1120.

3. Syversen SW, Gaarder PI, Goll GL, et al. (2008). High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Ann Rheum Dis* , 67(2):212–217.

4. de Brito Rocha, S., Baldo, D. C., & Andrade, L. (2019). Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. *Advances in rheumatology (London, England)*, *59*(1), 2.

5. Rönnelid, J., Turesson, C., & Kastbom, A. (2021). Autoantibodies in Rheumatoid Arthritis - Laboratory and Clinical Perspectives. *Frontiers in Immunol. 12*, 685312.

6. Muller, S., & Radic, M. (2015). Citrullinated Autoantigens: From Diagnostic Markers to Pathogenetic Mechanisms. *Clinical reviews in allergy & immunology*, *49*(2), 232–239.

7. Demoruelle, M. K., & Deane, K. (2011). Antibodies to citrullinated protein antigens (ACPAs): clinical and pathophysiologic significance. *Current rheumatology reports*, *13*(5), 421–430.

8. Abou El-Fetouh S & Abozaid HS (2013). Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. *Open J. Rheumatol. & Autoimmune Dis.*, 3(4): 185-191.5.

9 . Khalifa IA, Lotfy AM, Elsayed MA, et al. (2013). Anti-mutated citrullinated vimentin antibodies in rheumatoid arthritis compared with anti-cyclic citrullinated peptides. *J Am Sci*, 9: 160-166.

10. Aletaha, D., Neogi, T., Silman, A. J., et al. (2010). 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Annals of the Rheumatic Diseases*, *69*(9), 1580–1588.

11. Mathsson, L., Mullazehi, M., Wick, M. C., et al. (2008). Antibodies against citrullinated vimentin in rheumatoid arthritis: higher sensitivity and extended prognostic value concerning future radiographic

progression as compared with antibodies against cyclic citrullinated peptides. *Arthritis and rheumatism*, 58(1), 36–45.

12. Osman KS, Aly LH, Saedii AA, et al. (2014). Anti-Mutated Citrullinated Vimentin (Anti-MCV) Antibodies as a Diagnostic Aid for Rheumatoid Arthritis. *J Clin Cell Immunol*, 5:263.

13. Nugraha J, Rahmawati F and Soeroso J (2012). Diagnostic Value of AntiMutated Citrullinated Vimentin and Rheumatoid Factor with Immuno chromatographic Method in Early Rheumatoid Arthritis Patients. *J Rheumatoid Arthritis*, 62: 189-193.

14. Wegner, N., Lundberg, K., Kinloch, A., et al. (2010). Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. *Immunological reviews*, *233*(1), 34–54.

15. Engelmann, R., Brandt, J., Eggert, M., et al. (2009). The anti-mutated citrullinated vimentin response classifies patients with rheumatoid arthritis into broad and narrow responders. *The Journal of Rheumatology*, *36*(12), 2670–2674.

16. El Shazly RI, Hussein SA, Raslan HZ, et al. (2014). Anti-mutated citrullinated vimentin antibodies in rheumatoid arthritis patients: Relation to disease activity and manifestations. *The Egypt Rheumatologist* , 36(2):65–70.

17. Pongratz G, Frieser R, Brinks R, et al. (2020). Association between autoantibody level and disease activity in rheumatoid arthritis is dependent on baseline inflammation. *Clinical and experimental rheumatology*, *38*(4), 691–698.

18. Sghiri, R., Bouajina, E., Bargaoui, D., et al. (2008). Value of anti-mutated citrullinated vimentin antibodies in diagnosing rheumatoid arthritis. *Rheumatology international*, *29*(1), 59–62.

19. Ursum, J., Nielen, M. M., van Schaardenburg, D., et al. (2008). Antibodies to mutated citrullinated vimentin and disease activity score in early arthritis: a cohort study. *Arthritis Research & Therapy*, 10(1), R12.

20. Abou-Elfattah, Nalgaa , Sarah Younes Abozaid , Azza Ali Althoqapy , et al. (2019). Anti-Mutated Citrullinated Vimentin antibodies (Anti-MCV): A Relation with Other Diagnostic Markers in Rheumatoid Arthritis Patients. *Clinical Medicine & Diagnostics*, 9(4): 61-67.

21. Al-Shukaili, A., Al-Ghafri, S., Al-Marhoobi, S., et al. (2012). Evaluation of anti-mutated citrullinated vimentin antibodies, anti-cyclic citrullinated Peptide antibodies and rheumatoid factor in omani patients with rheumatoid arthritis. *Inter. J.f Rheumatol.*, 285854.

22. Liu, X., Jia, R., Zhao, J., et al. (2009). The role of anti-mutated citrullinated vimentin antibodies in the diagnosis of early rheumatoid arthritis. *J. Rheumatol. 36*(6), 1136–1142.

23. Shen, R., Ren, X., Jing, R., et al. (2015). Rheumatoid Factor, Anti-Cyclic Citrullinated Peptide Antibody, C-Reactive Protein, and Erythrocyte Sedimentation Rate for the Clinical Diagnosis of Rheumatoid Arthritis. *Lab. Med.*, 46(3), 226–229.

24. van Delft, M., & Huizinga, T. (2020). An overview of autoantibodies in rheumatoid arthritis. *J. autoimmunity*, *110*, 102392.

25. Doğan M, Küçüksaraç S, Tüfekçi O, et al. (2014). Comparison of the diagnostic values in rheumatoid arthritis: Anti-CCP antibodies and other serological tests. *Biomed Res,* 25(3):381–386.

26. Porto, L. S., Tavares, W. C., Júnior, Costa, D. A., et al. (2017). Anti-CCP antibodies are not a marker of severity in established rheumatoid arthritis: amagnetic resonance imaging study. *Revista brasileira de reumatologia*, *57*(1), 15–22.

27. Birtane, M., Yavuz, S., & Taştekin, N. (2017). Laboratory evaluation in rheumatic diseases. *World J. Methodol. 7*(1), 1–8.

28. Tiwari, V., Jandu, J. S., & Bergman, M. J. (2021). Rheumatoid Factor. In *StatPearls*. StatPearls Publishing. .

29. Shang, L. N., Di, G. X., Wei, F. J., et al. (2020). Study on the evaluation of rheumatoid arthritis via doppler ultrasonography and anti-cyclic citrullinated peptide antibody analysis. *Pakistan J. Med. Sci*, *36*(3), 569–573.

30. Bang, H., Egerer, K., Gauliard, A., et al. (2007). Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis & Rheumatism*, *56*(8), 2503–2511.

31. Poulsom, H., & Charles, P. J. (2008). Antibodies to citrullinated vimentin are a specific and sensitive marker for the diagnosis of rheumatoid arthritis. *Clinical reviews in allergy & immunology*, *34*(1), 4–10.

32. Van Steendam, K., Tilleman, K., & Deforce, D. (2011). The relevance of citrullinated vimentin in the production of antibodies against citrullinated proteins and the pathogenesis of rheumatoid arthritis. *Rheumatol. (Oxford, England)*, *50*(5), 830–837.

33. Morbach, H., Dannecker, H., Kerkau, T., et al. (2010). Prevalence of antibodies against mutated citrullinated vimentin and cyclic citrullinated peptide in children with juvenile idiopathic arthritis. *Clin. & Exp. Rheumatol.* 28(5), 800.