

# Association of Anti-Modified Citrullinated Vimentin with Subclinical Atherosclerosis in Early Rheumatoid Arthritis Compared with Anti-Cyclic Citrullinated Peptide

AMAL M. EI-BARBARY, ELHAM M. KASSEM, MERVAT A.S. EI-SERGANY, SALWA A-M. ESSA, and MOHAMED A. ELTOMEY

**ABSTRACT. Objective.** To investigate anti-modified citrullinated vimentin (anti-MCV) in early rheumatoid arthritis (RA), including correlation with disease activity and cardiovascular risk factors, compared with anti-cyclic citrullinated peptides (anti-CCP3).

**Methods.** Anti-MCV and anti-CCP3 concentrations were measured in 100 patients with early RA and 100 healthy controls at baseline to determine sensitivity and specificity. Patients received methotrexate (MTX) 0.2 mg/kg/week plus prednisone 10 mg/day. Anti-MCV, anti-CCP3, rheumatoid factor (RF), Disease Activity Score for 28 joints (DAS-28), lipid profile, erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein assay (hsCRP), homeostasis model assessment for insulin resistance (HOMA-IR) index, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 6 (IL-6), and carotid intima-media thickness (cIMT) were measured before and after 12 months of treatment.

**Results.** The sensitivity and specificity for anti-MCV antibody were 75% and 90%, respectively, and for anti-CCP3 antibody 71% and 96%. Serum anti-MCV and serum anti-CCP3 levels at baseline were positively correlated with hsCRP, IL-6, HOMA-IR index, serum RF levels ( $p < 0.001$ ), and cIMT ( $p < 0.05$ ). Serum anti-MCV was positively correlated with serum anti-CCP3 levels. There were significant positive correlations between the percentage of changes of anti-MCV levels versus changes in DAS-28, ESR, hsCRP, atherogenic ratios (TC/HDL-C and LDL-C/HDL-C), apolipoprotein A-I, IL-6, TNF- $\alpha$ , HOMA-IR index, and cIMT. These correlations were not found between changes in anti-CCP3 levels compared to clinical, laboratory, and radiological variables.

**Conclusion.** Anti-MCV was as sensitive as anti-CCP3 in diagnosing early RA. Anti-MCV testing appears to be useful for monitoring associated subclinical atherosclerosis in early RA. (J Rheumatol First Release March 1 2011; doi:10.3899/jrheum.101143)

## Key Indexing Terms:

RHEUMATOID ARTHRITIS

ANTI-CYCLIC CITRULLINATED PEPTIDE

ANTI-MODIFIED CITRULLINATED VIMENTIN

ATHEROSCLEROSIS

Rheumatoid arthritis (RA) is a chronic systemic disease affecting primarily the synovium, leading to joint damage and bone destruction<sup>1</sup>. Current therapeutic protocols in RA utilize more aggressive drugs as early as possible, which aim to control disease activity and give rapid and exact diagnosis. There is a need to improve the diagnostic specificity of commercial rheumatoid factor (RF) test kits and to discover new serological markers with high specificity for RA<sup>2</sup>.

*From the Department of Rheumatology and Rehabilitation, Department of Clinical Pathology, and Department of Radiology, Tanta University Faculty of Medicine, Tanta, Gharbeia, Egypt.*

*A.M. El-Barbary, MD, Lecturer; E.M. Kassem, MD, Assistant Professor; M.A.S. El-Sergany, MD, Assistant Professor, Department of Rheumatology and Rehabilitation; S.A-M. Essa, MD, Assistant Professor, Department of Clinical Pathology; M.A. Eltomey, MD, Lecturer, Department of Radiology, Faculty of Medicine, Tanta University.*

*Address correspondence to Dr. A.M. El-Barbary, Department of Rheumatology and Rehabilitation, Tanta University Faculty of Medicine, Elgesh Street, Tanta, Gharbeia, Egypt. E-mail: ml\_barbary@yahoo.com*  
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Anti-citrullinated protein/peptide antibodies (ACPA) have emerged as sensitive and specific serological markers of RA, providing a superior alternative to RF testing. ACPA production can precede the onset of clinical RA symptoms by years; ACPA-positive individuals with early, undifferentiated arthritis may have higher risk to develop RA. ACPA has an important prognostic role during the progression of RA and has also been associated with pronounced radiographic progression<sup>3</sup>.

The novel autoantibody test for anti-modified citrullinated vimentin (anti-MCV), which targets modified citrullinated vimentin and has its origin in the older anti-Sa autoantibody test, was found to be highly specific for RA<sup>4</sup>.

RA is associated with increased morbidity and mortality due to cardiovascular disease (CVD), mostly accelerated atherosclerosis, and there is evidence that this occurs early in the inflammatory disease process. Both traditional and novel CVD risk factors as well as the effects of the RA dis-

ease process and its treatment interact and contribute to the development of CVD in RA<sup>5</sup>. A systemic inflammatory response may contribute to the development of accelerated atherosclerosis in patients with RA. Proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 1 $\beta$  (IL-1 $\beta$ ), and IL-6 generated in the synovial tissue can be released into the systemic circulation. These circulating cytokines are in a position to alter the function of distant organs, including adipose tissue, skeletal muscle, liver, and vascular endothelium, to generate a spectrum of proatherogenic changes that include endothelial dysfunction, insulin resistance, a characteristic dyslipidemia, prothrombotic effects, and pro-oxidative stress<sup>6</sup>.

Although CV events develop over years, the time course of epidemiologic and clinical studies has been reduced using B-mode ultrasound measurement of the carotid intima-media thickness (cIMT) and of carotid plaque to study early atherosclerotic changes noninvasively. cIMT values correlate closely with direct measurement of the local and systemic atherosclerosis and with clinical CV endpoints<sup>7</sup>.

Anti-citrullinated protein autoantibodies predict poor clinical outcome and may be implicated in the pathogenesis of RA<sup>8</sup>. However, the relationship between anti-MCV antibodies and CV comorbidity in patients with RA has not been well defined. We investigated anti-MCV in early RA, including correlations with disease activity and CV risk factors compared with anti-cyclic citrullinated peptides (anti-CCP3).

## MATERIALS AND METHODS

One hundred patients with RA were selected from the outpatient clinic of the rheumatology and rehabilitation department, Tanta University Hospitals. They fulfilled the 1987 American Rheumatism Association/American College of Rheumatology revised criteria for the diagnosis of RA<sup>9</sup>. All patients had disease duration < 1 year, without prior use of disease modifying antirheumatic drugs (DMARD) and/or systemic steroids. In addition, 100 healthy volunteers matched with patients for age and sex, who had no family history for autoimmune diseases such as RA and systemic lupus erythematosus or chronic musculoskeletal complaints, were included as healthy controls.

Patients with conditions that affect the lipid profile, such as diabetes mellitus, hypothyroidism, liver or kidney disease, Cushing syndrome, current smokers, obesity (body mass index > 30), and a history of familial dyslipidemia, were excluded. In addition, patients receiving medications affecting lipid metabolism (lipid-lowering drugs, beta-blockers, oral contraceptives, estrogen, progestin, thyroxin, and vitamin E) were excluded.

Approval for the study protocol was obtained from the local research ethics committee, and written informed consent was obtained from each participant.

**Study design.** RA patients were treated with methotrexate (MTX; 0.2 mg/kg/week) and prednisone (10 mg/day). The dose of MTX remained stable during the study, while the dose of prednisone was tapered according to the patient's clinical response. But this procedure was forbidden within 4 weeks of clinical and laboratory assessments.

Disease activity was assessed by the disease activity for 28 joint indices score (DAS-28). Components of DAS-28 are erythrocyte sedimentation rate (ESR), patient-assessed global score (0–100), and swollen and tender joint counts (0–28)<sup>10</sup>.

**Sampling.** After 12 hours of overnight fasting, venous blood samples (7 ml) were taken from the controls and RA patients before starting the recom-

mended line of treatment; 1.6 ml of blood was transferred into a vacutainer tube containing 0.4 ml sodium citrate for ESR measurement. The remainder of the blood was delivered into a plain glass tube, allowed to clot at room temperature, and centrifuged at 2000 rpm for 10 min, and serum was separated. Lipid profile, serum glucose concentration, and RF were determined immediately, and aliquots of the serum were stored at –70°C until analysis. Sampling was repeated for the patient group 12 months after the start of treatment.

Patient and control groups underwent the following laboratory investigations: ESR (mm/h) was determined by Westergren method as described<sup>11</sup>. hsCRP concentrations were measured using the Diamed Eurogen CRP ELISA kit<sup>12</sup>. Lipid profiles including serum triglyceride (TG) level<sup>13</sup>, serum total cholesterol (TC) level<sup>14</sup>, high-density lipoprotein cholesterol (HDL-C)<sup>15</sup>, and low-density lipoprotein cholesterol (LDL-C)<sup>16</sup> were analyzed using kits supplied by BioMerieux Vitek Inc. (Durham, NC, USA). Serum apolipoproteins B (apoB) and A-I (apoA-I) were measured by immunonephelometry from Behring Diagnostics GmbH (Liederbach, Germany). Fasting serum glucose level was determined according to the method of Trinder<sup>17</sup>, and fasting serum insulin was measured by ELISA (Millipore Corp., Natick, MA, USA). Insulin sensitivity was determined using the homeostasis model assessment for insulin resistance (HOMA-IR) index ( $[\text{fasting glucose (mmoles/liter)} \times \text{fasting insulin } (\mu\text{U/ml})]/22.5$ ). A HOMA-IR index value > 2.0 was considered the criterion of insulin resistance<sup>18</sup>. Serum TNF- $\alpha$  and IL-6 concentrations were determined by ELISA (Roche Diagnostics GmbH, Mannheim, Germany). RF was determined by nephelometry method (Behring, Marburg, Germany). Cutoff value was 15.0 U/ml according to the manufacturer's instructions. Serum anti-CCP3 was measured by ELISA (QuantaLite CCP version 3.1 for IgG/IgA; Inova Diagnostics, San Diego, CA, USA). Cutoff level for the anti-CCP3 test was 20 arbitrary units, according to the manufacturer's instructions. Serum anti-MCV concentrations were determined by an indirect solid-phase ELISA for quantitative measurement of IgG autoantibodies against mutated citrullinated vimentin in serum using commercial kits according to the supplier's instructions (Orgentec Diagnostika GmbH, Mainz, Germany). Values  $\geq$  20.0 U/ml were considered abnormal according to the manufacturer's recommendations. The sensitivity of the Orgentec anti-MCV ELISA for RA was determined at 1 U/ml<sup>19</sup>.

**Common carotid artery evaluation.** Common carotid arteries were assessed using B-mode ultrasound (Siemens G60S) with a linear transducer (midfrequency, 10 MHz). The radiologist for this study was blinded to other data for RA patients and controls. Patients and controls were examined in a supine position with the neck extended and the chin turned away from the side being examined. Measurement of cIMT was always performed at the same arterial wall 1 cm proximal to the carotid bifurcation. Images were obtained in longitudinal and axial projections. In longitudinal projection, the sound beam was placed perpendicular to the far wall of the common carotid artery, obtaining 2 parallel echogenic lines corresponding to the lumen/intima and media/adventitia interfaces. The distance between these 2 parallel lines corresponded to the cIMT. Values were expressed in millimeters<sup>20</sup>.

After screening, study visits were scheduled for 0 and 12 months.

**Statistical analysis.** All data were analyzed using SPSS software (version 11; SPSS Inc., Chicago, IL, USA). Baseline characteristics are presented as mean  $\pm$  standard deviation or as median (interquartile range) for continuous variables, and as frequency (percentage) for discrete variables. Receiver-operation characteristic curve analysis using cutoff values recommended by the manufacturer was used for determination of sensitivity and specificity of anti-MCV, anti-CCP3 and RF in RA patients. Comparisons between groups were conducted using the Student *t* test. Correlation between variables was examined using Pearson's correlation coefficient. A *p* value < 0.05 was considered statistically significant. Multiple linear regression analysis was performed. Change of anti-MCV between the end of the study and baseline were used as a dependent variable. Changes in clinical, biochemical, and radiological variables that could affect the anti-MCV were used as independent variables.

## RESULTS

A total of 100 patients with RA were recruited. Mean total dose of MTX/week was  $15.5 \pm 1.3$  mg. The dose of prednisone during the followup period ranged from 5 to 10 mg/day given to patients who were not under therapy with MTX. Then the dose was gradually tapered according to the clinical improvement. No patient received steroid within 4 weeks prior to clinical and laboratory assessments after gradually tapering the dose. No significant side effects were noted during the study, the drugs being well tolerated; 3 patients were lost to followup due to flares of disease and changes of the treatment protocol.

In the 100 RA patients, 65% were found to have both anti-MCV and anti-CCP3 antibody at baseline; 10% of patients were anti-MCV-positive and anti-CCP3 antibody-negative, whereas 6% of patients were anti-MCV-negative and anti-CCP3-positive. The percentage of patients who were concurrently anti-MCV-negative and anti-CCP3 antibody-negative was 19% (Table 1).

Analyses of anti-MCV, anti-CCP3, and RF at baseline revealed sensitivity for anti-MCV antibody of 75% (cutoff 20.0 U/ml) and specificity of 90%. Sensitivity of anti-CCP3 antibody was 71% (cutoff 20.0 U/ml) and specificity 96%. Sensitivity for RF was 62% (cutoff 15.0 U/ml) and specificity 97%. These results revealed no statistically significant differences (Table 2).

The demographic variables and biochemical and radiographic characteristics of RA patients before and after therapy and controls are described in Table 3. There were 75 women and 25 men with a median age 51 (37–65) years in the RA group. There were 80 women and 20 men, median age 48 (36–61) years, in the control group.

There was no significant difference of age, sex, and mean

body mass index values between RA patients and controls. Patients with early RA exhibited mild dyslipidemia characterized by significantly higher baseline TC, LDL-C, TG, and apoB compared to controls. In addition, HDL-C levels and apoA-I were significantly lower compared to controls. As a consequence, the atherogenic ratio of TC/HDL-C as well as LDL-C/HDL-C was significantly higher in RA patients compared to controls (Table 3).

All biochemical markers including serum hsCRP, IL-6, TNF- $\alpha$ , insulin resistance (HOMA-IR index), RF, anti-CCP3, and anti-MCV were significantly higher in RA patients than in controls ( $p < 0.001$ ). Moreover, cIMT was significantly greater in RA patients compared to controls ( $p < 0.001$ ; Table 3).

Altered anti-MCV status after followup was found in 9% of the patients (3 of the 25 patients who were initially anti-MCV-negative were found to be anti-MCV-positive; and 6 of the 75 patients who were initially anti-MCV-positive were found to be anti-MCV negative). However, 3% of the patients revealed altered anti-CCP3 and RF status after the followup period (3 of 29 initially anti-CCP3-negative and 38 RF-negative patients were found to be anti-CCP3-positive and RF-positive, respectively).

After 12 months of therapy, significant decreases were observed in the DAS-28 and ESR values as well as hsCRP levels (Table 3). There were significant increases in serum levels of TC, HDL-C, and apoA-I compared to baseline values before treatment, with no significant change in the serum levels of LDL-C, TG, and apoB in RA patients after treatment. Importantly, the atherogenic ratios TC/HDL-C and LDL-C/HDL-C were significantly reduced after treatment.

There were significant decreases in biochemical markers including serum IL-6, TNF- $\alpha$ , HOMA-IR index, RF, anti-CCP3, and anti-MCV after treatment ( $p < 0.001$ ). Moreover, cIMT was significantly decreased after treatment, from  $0.84 \pm 0.27$  mm to  $0.64 \pm 0.18$  mm (Table 3).

In RA patients at baseline mean serum anti-MCV levels were positively correlated with hsCRP ( $r = 0.330$ ,  $p = 0.01$ ), IL-6 ( $r = 0.638$ ,  $p < 0.001$ ), HOMA-IR index ( $r = 0.77$ ,  $p < 0.001$ ), serum RF levels ( $r = 0.64$ ,  $p < 0.001$ ), serum anti-CCP3 levels ( $r = 0.70$ ,  $p < 0.001$ ), and cIMT ( $r = 0.45$ ,  $p < 0.05$ ). No significant correlation was found for anti-MCV levels compared to DAS-28, ESR, TNF- $\alpha$ , and the

Table 1. Distribution of anti-modified citrullinated vimentin (anti-MCV) and anti-cyclic citrullinated peptide (anti-CCP3) antibody in RA patients at baseline (n = 100).

Status	N
MCV+ CCP+	65
MCV+ CCP-	10
MCV- CCP+	6
MCV- CCP-	19
Total	100

Table 2. Sensitivity and specificity of anti-MCV, anti-CCP3, and rheumatoid factor (RF) in RA patients at baseline. One hundred RA cases and 100 healthy controls were studied. Receiver-operation characteristic curve analysis used cutoff value recommended by the manufacturer.

	PPV, %	NPV, %	Sensitivity, %	Specificity, %	Accuracy, %
Anti-MCV	88.24	78.26	75.00	90.00	82.50
Anti-CCP3	94.67	76.80	71.00	96.00	83.50
RF	95.38	71.85	62.00	97.00	79.50

PPV: positive predictive value; NPV: negative predictive value.

Table 3. Clinical, laboratory, and radiological findings in the study groups. Values represent mean  $\pm$  standard deviation or median (interquartile range).

Characteristic	Controls, n = 100	RA Patients	
		Baseline, n = 100	Posttreatment, n = 97
Age, yrs	48 (36–61)	51 (37–65)	—
Male/female	20/80	25/75	23/74
Disease duration, yrs	—	0.4 (0.3–0.6)	—
Body mass index, kg/m <sup>2</sup>	25.8 $\pm$ 3.7	25.6 $\pm$ 3.3	25.8 $\pm$ 3.0
DAS-28	—	6.09 $\pm$ 0.72	3.5 $\pm$ 0.71**
ESR, mm/h	9.10 $\pm$ 3.28	50.93 $\pm$ 20.81	25.6 $\pm$ 10.8**
hsCRP, mg/l	3.1 $\pm$ 1.84	6.49 $\pm$ 3.9	3.5 $\pm$ 2.23**
Total cholesterol, mg/dl	174.20 $\pm$ 14.09	225.66 $\pm$ 30.12*	235.66 $\pm$ 30.93***
Triglycerides, mg/dl	90.30 $\pm$ 18.80	135.33 $\pm$ 46.31*	135.13 $\pm$ 46.78
LDL-C, mg/dl	122 $\pm$ 16.46	141.33 $\pm$ 24.41*	140.03 $\pm$ 22.02
HDL-C, mg/dl	54.50 $\pm$ 11.81	43.06 $\pm$ 8.12*	58.06 $\pm$ 14.12**
TC/HDL-C	3.4 $\pm$ 0.27	5.61 $\pm$ 1.37*	4.3 $\pm$ 1.25**
LDL-C/HDL-C	2.5 $\pm$ 0.71	3.5 $\pm$ 1.02*	2.7 $\pm$ 1.04**
Apolipoprotein B, g/l	90 $\pm$ 29	120 $\pm$ 25*	122 $\pm$ 28
Apolipoprotein A-I, g/l	149 $\pm$ 43	125 $\pm$ 27*	152 $\pm$ 22**
IL-6, pg/ml	5.1 $\pm$ 3.0	52.3 $\pm$ 42.2*	22.5 $\pm$ 20.3**
TNF- $\alpha$ , pg/ml	3.51 $\pm$ 0.87	9.79 $\pm$ 3.42*	5.95 $\pm$ 2.64**
HOMA-IR index	0.5 $\pm$ 0.3	5.6 $\pm$ 3.06*	4.1 $\pm$ 2.12**
RF, IU/ml	14.6 $\pm$ 5.5	203 $\pm$ 200.3*	180 $\pm$ 160.2**
Anti-CCP3, U/ml	14.11 $\pm$ 7.45	350.5 $\pm$ 344.7*	305.4 $\pm$ 290.5**
Anti-MCV, U/ml	23.12 $\pm$ 12.04	665.77 $\pm$ 647.19*	476.55 $\pm$ 465.18**
cIMT, mm	0.54 $\pm$ 0.11	0.84 $\pm$ 0.27*	0.64 $\pm$ 0.18**

\*p < 0.001 compared to control group; \*\* p < 0.001 and \*\*\* p < 0.05 compared to baseline values. DAS-28: Disease Activity Score for 28 joints; ESR: erythrocyte sedimentation rate; hsCRP: high sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance; cIMT: carotid intima-media thickness.

lipid profile. Mean serum anti-CCP3 levels were positively correlated with the same variables as anti-MCV, except for hsCRP (p < 0.001).

We examined the correlation of changes in anti-MCV and anti-CCP3 levels after treatment with changes in clinical, laboratory, and radiological variables. There were significant positive correlations between changes in anti-MCV levels among anti-MCV-positive patients at baseline and changes in DAS-28, ESR, hsCRP, atherogenic ratio (TC/HDL-C and LDL-C/HDL-C), apoA-I, IL-6, TNF- $\alpha$ , HOMA-IR index, and cIMT. However, these correlations were not found between changes in anti-CCP3 levels and clinical, laboratory, and radiological variables (Table 4).

Multiple linear regression showed that the change in anti-MCV was statistically significantly associated with the change in DAS-28 ( $\beta$  = 0.021, p < 0.001), ESR ( $\beta$  = 0.011, p = 0.025), hsCRP ( $\beta$  = 0.061, p < 0.001), IL-6 ( $\beta$  = 0.001, p = 0.045), TNF- $\alpha$  ( $\beta$  = 0.013, p = 0.008), HOMA-IR index ( $\beta$  = 0.069, p < 0.001), and cIMT ( $\beta$  = 0.09, p < 0.001).

## DISCUSSION

It is clear that the description of antibodies reactive against

citrullinated proteins/peptides (ACPA) has been an important development in our ability to predict outcome in patients with early synovitis. Current widely used approaches for detection of these antibodies use synthetic cyclic peptides containing citrulline residues. Such assays have a very high specificity but a relatively low sensitivity. There has been great interest in identification of substrates that allow detection of ACPA in a larger proportion of RA patients than is possible using CCP as the target antigen. One such substrate is vimentin<sup>21</sup>.

Our results in patients with early RA revealed no significant difference of sensitivities and specificities between anti-MCV and anti-CCP3 antibodies.

Mathsson, *et al*<sup>22</sup> and Innala, *et al*<sup>23</sup> suggested that the anti-MCV test had better sensitivity compared with the anti-CCP2 test. They stated that both tests had nearly equal specificities (95% and 96%, respectively). Bizzaro, *et al*<sup>24</sup> reported a comparison of 11 different ACPA tests, including tests of anti-CCP2, anti-CCP3, and anti-MCV; the anti-CCP3 and anti-MCV tests had sensitivity and specificity higher than those of the anti-CCP2 test.

We have shown a significant positive correlation of

anti-MCV levels at baseline compared to RF and anti-CCP3. However, no significant correlation was found between anti-MCV levels at baseline and DAS-28. This result was in accord with the results of Liu, *et al*<sup>25</sup>, who concluded that anti-MCV had significant correlation with anti-CCP2 and RF and no significant correlation with other factors such as ESR, CRP, global visual analog scale score for joint pain, and DAS-28. In contrast with our results, Keskin, *et al*<sup>26</sup> recorded a correlation between disease activity score for RA and anti-MCV levels.

After 12 months of MTX therapy, a significant decrease in serum anti-MCV and anti-CCP3 was observed. The magnitude of the change in anti-MCV level showed a high degree of correlation with changes in clinical and laboratory variables related to disease activity, especially DAS-28 and ESR. However, the change in anti-CCP3 levels did not reveal the same correlation with changes in clinical and laboratory findings. Our results were in accord with those of Mathsson, *et al*<sup>22</sup>, who demonstrated the association between marked decrease in anti-MCV levels and clinical improvement, which implies that anti-MCV might be a more sensitive indicator of disease activity than anti-CCP. However, Ursum, *et al*<sup>27</sup> recorded that anti-MCV-positive patients exhibited higher levels of inflammation than those of anti-MCV-negative patients at baseline; but during 2 years of followup, the correlation between anti-MCV levels and measures of disease activity was very low.

Our study demonstrated increased levels of TC, LDL-C, TG, and apoB, with decreased HDL-C and apoA-I levels in patients with early active RA. As a consequence, the atherogenic ratios TC/HDL-C and LDL-C/HDL-C were significantly higher compared to controls. These changes are associated with an increased incidence of CVD in the general

population. There have been studies reporting increased, decreased, or similar levels for TC, LDL-C, and HDL-C in comparison to control subjects<sup>28,29,30</sup>. The observed discrepancies in lipid values might be due to differences in study populations as well as in disease activity. After 12 months of MTX therapy, a significant increase in serum TC, HDL-C, and apoA-I levels was observed that resulted in reduction of the atherogenic ratios TC/HDL-C and LDL-C/HDL-C. This improvement was associated with a reduction in disease activity and in the acute-phase reactants. These observations indicate that active disease with a maintained inflammatory response, as expressed by a persistently elevated CRP, may be responsible for the development of early atherosclerosis in RA. Georgiadis, *et al*<sup>31</sup> documented that effective suppression of the disease activity at an early stage with MTX was associated with a reduction in dyslipidemia and a decrease in mortality.

Atherosclerotic disease, the major cause of cardiovascular events, is considered to be a multifactorial condition in which inflammation plays a crucial role. One of the inflammatory mediators is IL-6, which is produced by smooth-muscle cells in the tunica media and corresponds to the proliferation of these same cells. Persistent inflammation stimulates artery wall remodeling and foam-cell formation that is the hallmark of the early atherosclerotic lesion<sup>32,33,34</sup>. In our study, IL-6 and TNF- $\alpha$  showed a significant increase in patients with early active RA, with levels that were significantly decreased after MTX therapy. Hadda, *et al*<sup>35</sup> stated that DMARD control the inflammation, which leads to normalization of functions of circulating cytokines like TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 that alter the function of distant tissues, including adipose tissue, skeletal muscle, liver, etc., which leads to dyslipidemia.

Table 4. Correlation between percentage change in anti-MCV and anti-CCP3 levels and changes in clinical, laboratory, and radiological variables. Only patients who were anti-MCV-positive and patients who were anti-CCP3-positive at baseline were included.

Variable	Change in Anti-MCV, % (n = 75)		Change in Anti-CCP, % (n = 71)	
	r	p	r	p
DAS-28	0.520	< 0.001*	0.121	NS
ESR	0.263	0.03*	0.054	NS
CRP	0.210	0.02*	0.140	NS
TC/HDL-C	0.542	< 0.001*	0.051	NS
LDL-C/HDL-C	0.592	< 0.001*	0.121	NS
ApoA-I	0.551	< 0.001*	0.054	NS
IL-6	0.542	< 0.001*	0.131	NS
TNF- $\alpha$	0.439	< 0.001*	0.111	NS
HOMA-IR	0.432	< 0.001*	0.063	NS
cIMT	0.441	< 0.001*	0.055	NS

\*Spearman rank correlation. Anti-MCV: anti-modified citrullinated vimentin; anti-CCP: anti-cyclic citrullinated peptide; DAS-28: Disease Activity Score for 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; apo: apolipoprotein; IL-6: interleukin 6; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; HOMA-IR: homeostasis model assessment for insulin resistance; cIMT: carotid intima-media thickness; NS: nonsignificant.

We observed that the HOMA-IR index was significantly elevated in RA patients at baseline compared to controls, with values that were significantly decreased after MTX therapy. Rho, *et al*<sup>36</sup> concluded that leptin is associated with increased insulin resistance in RA that is associated with coronary calcification.

Inflammation has a strong association with increased atherosclerosis in RA. Indirect evidence of accelerated atherosclerosis in RA comes from studies measuring cIMT<sup>37</sup>. We found that patients with early RA exhibited higher common carotid artery IMT values, and both the lipid profile and cIMT values were significantly improved after treatment. Our findings were in accord with results from Georgiadis, *et al*<sup>31</sup>.

We showed a significant positive correlation between anti-MCV and anti-CCP3 levels at baseline versus hsCRP, IL-6, TNF- $\alpha$ , HOMA-IR index, and cIMT. These results reflect the association of anti-MCV and anti-CCP3 with CVD risk, which may reflect the effect of a more aggressive disease. This finding is fully consistent with the recent report that RA patients with circulating anti-CCP antibodies exhibit signs of accelerated subclinical atherosclerosis as detected by ultrasound evaluation of cIMT<sup>37</sup>. Moreover, our study demonstrated that changes in anti-MCV levels after treatment were strongly associated with changes in CV risk factors (hsCRP, IL-6, TNF- $\alpha$ , HOMA-IR index, and cIMT). However, we did not find any correlation between changes in anti-CCP3 levels and changes in CV risk factors. These results indicate that changes in anti-MCV levels following treatment may indicate improvement in subclinical atherosclerosis.

Bang, *et al*<sup>38</sup> concluded that antigenic properties of vimentin were determined by mutation and citrullination. Anti-MCV antibodies are a novel diagnostic marker for RA. They identified mutated glycine residues within the vimentin DNA caused by at least 1 single-nucleotide polymorphism. Moreover, it was shown that mutated vimentin is also citrullinated in synovial fluid of patients with RA. These data indicate that citrullination by peptidylarginine deiminase is influenced by amino acid residues that flank arginine, resulting in a nonrandom modified protein. Thus, citrullination and mutation of vimentin represents an independent trigger of the antigenic properties of the antigen in RA. Use of the mutated and citrullinated recombinant human antigen vimentin for the diagnosis of RA in a standardized ELISA clearly documented a preserved high diagnostic specificity of the antigen<sup>38</sup>. Therefore, the diagnostic significance of anti-MCV antibodies must be confirmed in a multicenter study. Importantly, the assay showed better sensitivity than the anti-CCP ELISA.

A weakness of our study is the control population. Even though we found comparable specificity for anti-MCV and anti-CCP3 using healthy blood donors as a control group, the specificity analysis should be repeated using clinically

relevant control groups, including patients with other rheumatic diseases.

Anti-MCV was as sensitive as anti-CCP3 in diagnosing RA. Anti-MCV testing may represent a useful tool for monitoring associated subclinical atherosclerosis in early RA.

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