Research Article

Anti-mutated citrullinatedvimentin antibody for predicting the activity of rheumatoid arthritis

Kamal Esalatmanesh[1], Zahra Soleimani[2]* and Meysam Orangi[3]

[1]Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
[2]Infectious Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
[3]Student Research Committee, Kashan University of Medical Sciences, Kashan, Iran

ABSTRACT

Background: Anti-citrullinated protein/peptide antibodies (ACPAs) are a heterogeneous family of autoantibodies, targeting citrullinated proteins. We investigated the relationship between serum levels of anti-mutated citrullinatedvimentin antibody (anti-MCV) and rheumatoid arthritis (RA) activity. **Method:** A total of 271 consecutive female patients with diagnosis of RA who met the ACR/EULAR criteria were enrolled. The disease activity was measured by the Disease Activity Score in 28 joints-erythrocyte sedimentation rate (DAS28-ESR). Anti-MCV was measured by enzyme-linked immunosorbent assay using a commercial kit with a cut-off value for positivity >20 U/mL. **Results:** Anti-MCV was positive in 153 (56.5%) patients. Disease in anti-MCV positive patients was significantly more active. A new composite index for predicting disease activity was constructed by replacing ESR with anti-MCV in the DAS28 model. There was a correlation between the absolute scores of DAS28-anti-MCV and DAS28-ESR scores. The new composite index best cut-off values corresponding to DAS28-ESR values of 2.6, 3.2, and 5.1 were 2.94, 3.17, and 4.87, respectively. The patients were re-categorized based on the new threshold values calculated by ROC curve analysis. There was agreement between the DAS28-anti-MCV categories and DAS28-ESR disease activity categories. **Conclusions:** Based on the correlation between anti-MCV levels with RA disease activity index, we conclude that anti-MCV may be a useful test to determine disease activity in RA.

Keywords: rheumatoid arthritis, disease activity scores in 28 joints (DAS28), anti-mutated citrullinatedvimentin (anti-MCV)

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by chronic erosive arthritis with involvement of small and large joints, pannus formation, as well as the presence of autoantibodies includingrheumatoid factor (RF) and anti-citrullinated protein/peptide antibodies (ACPAs). ACPAs are a heterogeneous family of autoantibodies, targeting citrullinated proteins like pro-filaggrin (anti-perinuclear factor), filaggrin (anti-keratin antibodies), citrullinated fibrinogen, and others [1].ACPAs have an important role in the pathogenesis of RA and have been introduced as a very specific marker for the diagnosis of RA [2]. Anti-citrullinated cyclic peptide antibodies (anti-CCP) are the most widely used ACPAs for the diagnosis of RA. Anti-CCP has been included in the most recent classification criteria for

the diagnosis of RA [3]. Anti-mutated citrullinatedvimentin (anti-MCV) antibodies are another type of ACPAs, which have been proposed recently as a tool for the diagnosis of RA. Anti-MCV recognizes citrullinatedvimentin, which is a widely expressed protein by macrophages in the synovium of patients with RA and in which arginine residues are replaced by glycine [4]. Anti-MCV has a diagnostic and prognostic value comparable with anti-CCP [5].

In addition to the role of ACPAs in the diagnosis of RA, they are frequently used by clinicians to assess RA. ACPAs are a marker of disease severity and radiographic joint damage [5-7]. Despite a good diagnostic and prognostic value of anti-CCP, reports about the correlation of anti-CCP and disease activity are controversial [8-15]. A few studies also evaluated the correlation between anti-MCV and RA activity and raised the probability that anti-MCV is a better predictor of RA disease activity [16-20]. However, the

results are contradictory.According to the existing conflicts contradiction, we investigated the relationship between serum levels of anti-MCV and RA disease activity.

MATERIALS AND METHODS

This study was conducted in the outpatient rheumatology clinic of Kashan University of Medical Sciences from Apr 2014 to Jan 2016. The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences. The study was performed in accordance with the Declaration of Helsinki and informed consents were obtained from the participants. A total of 271 consecutive female patients with diagnosis of RA who met the ACR/EULAR criteria[3] were enrolled. The disease activity was measured by the Disease Activity Score in 28 joints-erythrocyte sedimentation rate (DAS28-ESR) [21]. The 28-joint count of tender and swollen joint was performed by an expert rheumatologist. Patient global assessment of pain and general health was measured using visual analogue scale (VAS) method and was reported as millimeter (zero = no pain, and 100 =worst possible pain). The established cut points for the disease activity using DAS28-ESR are as follows: remission < 2.6; 2.6 ≤ low disease activity ≤ 3.2;3.2 < moderate disease activity ≤ 5.1; high disease activity > 5.1.

Five milliliters venous blood samples were taken from all the participants after an overnight fast. The sera of the participants were separated by centrifugation with the speed of 1500 rounds per minute for 10 min at room temperature. Then it was stored at -86°C until the biochemical analysis was performed. ESR was measured based on the amount of erythrocytes sediment in 1 hour and was reported as millimeter per hour (mm/h) according to Westergren. Anti-MCV was measured by the enzyme-linked immunosorbent assay (ELISA) using a commercial kit (ARP American, Affymetrix) with a cut-off value for positivity >20 U/mL.

Statistical analysis

The data was analyzed by SPSS version 16. Quantitative variables were described as mean and standard deviation (SD). Qualitative variables were described as number and percentage. The correlation between disease activity and serum anti-MCV was analyzed by linear regression, and correlation coefficient (Pearson) was calculated. Comparisons of mean serum anti-MCV in different disease activity categories were performed by analysis of variances (ANOVA) using Tukey post hoc tests. In order to consider the correlation between the disease activity based on DAS28-ESR and serum anti-MCV level, in this study, we decided to construct a new composite index for measuring disease activity by replacing the ESR with the serum anti-MCV level in the DAS28-ESR composite index. In order to assess the correlation validity of the new composite index, the correlation of the absolute scores of the new composite index was compared with the DAS28-ESR scores by linear regression analysis, and correlation coefficient was calculated.

ROC curve analysis was performed to set cut-off values for new composite index (based on anti-MCV) to diagnose RA patients in different disease activity categories, considering the DAS28-ESR cut-off values as the gold standard. The best cut-off value was calculated through the contact point of the ROC curve and the line with slope equal to one in which the sum of sensitivity and specificity was the highest. The patients were re-categorized based on the calculated threshold values by ROC curve analysis for the new composite index. The number of patients in each disease activity categories in new composite index was compared with the number of patients in DAS28-ESR disease activity categories by using cross tabs and calculating kappa statistics.

Table 1. Disease activity in anti-MCV positive and negative patients

	Anti-MCV positive 153	Anti-MCV negative 118	P-value
Age	34.1 ± 8.7	35.4 ± 11	0.004
DAS-28	4.4 ±1.5	3.6 ±1.2	0.001
Disease activity Remission Mild activity Moderate activity Severe activity	24 (15.7) 6 (3.9) 62 (40.5) 61 (39.9)	28 (23.7) 15 (12.7) 69 (58.5) 6 (5.1)	0.001

Anti-MCV: anti-mutated citrullinatedvimentin

Table 2. Relationship between disease activity and anti-MCV titer in the study patients

Disease activity	Frequency (%)	Anti-MCV titer (U/ml)	P-value
DAS-28 ≤ 3.2	73 (26.9)	20.9 ± 17.8	0.001
3.2 < DAS-28 ≤ 5.1	131 (48.3)	26.3 ± 18.7	
DAS-28 > 5.1	67 (24.7)	50.4 ± 19.2	

Anti-MCV: anti-mutated citrullinatedvimentin

RESULTS

Anti-MCV was measured in 271 female patients diagnosed with RA. Anti-MCV was positive in 153 (56.5%) patients. Disease in anti-MCV positive patients was significantly more active (Table 1). The

Constructing new composite index

The new composite index for predicting disease activity was constructed by replacing ESR with anti-MCV in the DAS28 model, as follows:

DAS anti-MCV= $0.56 \times \sqrt{}$ (number of tender joints)+ $0.28 \times \sqrt{}$ (number of swollen joints)+ $1.63 \times$ ln (anti-MCV) + $0.014 \times$ VAS − 3.06

There was a correlation between the absolute scores of DAS28-anti-MCV and DAS28-ESR scores ($R^2 = 0.91$, P value < 0.014). ROC curves for determining new composite index values corresponded to the DAS28-ESR values of 2.6, 3.2, and 5.1. The new

mean serum anti-MCV level in patients with more disease activity was significantly higher (Table 2). Also Pearson correlation test results showed that there is a significant relationship between the anti-MCV and DAS-28 in patients with rheumatoid arthritis (p=0.0001) and (r=0.379).

composite index best cut-off values corresponding to DAS28-ESR values of 2.6, 3.2, and 5.1 were 2.94, 3.17, and 4.87, respectively (Table 3). The patients were re-categorized based on the new threshold values calculated by ROC curve analysis. The distribution of the patients in each disease activity category in new composite index based on the new threshold values and DAS28-ESR disease activity categories are shown in Table 4. There is agreement between the DAS28-anti-MCV categories and DAS28-ESR disease activity categories (Kappa = 0.69, P value < 0.01).

Table 3. New composite index values corresponding to DAS28-ESR cut-off values

DAS28-ESR	DAS28-anti-MCV	Sensitivity (%)	Specifity (%)
2.6	2.94	92	89
3.1	3.17	95.4	91
5.2	4.87	91	93

Anti-MCV: anti-mutated citrullinatedvimentin
DAS28-ESR: Disease Activity Score in 28 joints-erythrocyte sedimentation Rate

Table 4. Distribution of the patients in different disease activitycategories in DAS28-anti-MCV and DAS28-ESR

	DAS28-anti-MCV categories	DAS28-ESR categories
Remission (%)	64 (23.6)	52 (19.2)
Mild activity (%)	14 (5.2)	21 (7.7)
Moderate activity (%)	118 (43.5)	131 (48.3)
Severe activity (%)	75 (27.7)	67 (24.7)

Anti-MCV: anti-mutated citrullinatedvimentin
DAS28: Disease Activity Score in 28 joints

DISCUSSION

This study showed that RA activity in patients with positive anti-MCV is higher than patients with negative anti-MCV and a positive correlation exist between anti-MCV level and disease activity. Regarding the high specificity of anti-MCV in predicting disability in RA, a new composite index for

measuring disease activity was developed by replacing ESR with anti-MCV in the DAS28 model. The positive correlation between the new composite index scores and the DAS28-ESR scores showed the correlation validity of the new model. Considering the DAS28-ESR as gold standard, the high area under curve (AUC) in the ROC curve analysis indicates the great diagnostic accuracy of the calculated cut-off

values for DAS28-anti-MCV in determination of patients in different disease activity categories. The best cut-off values for new composite index were calculated to diagnose RA patients in different disease activity categories. Considering the distribution of the patients in different disease activity categories in DAS28-anti-MCV with kappa statistic showed a substantial agreement with DAS28-ESR. The main limitation of our study was lack of follow-up analysis.

In agreement with this study, some studies showed a correlation between anti-MCV level and RA activity; however, other studies did not find this correlation. Bang et al. in a study on 1151 RA patients and the follow-up analysis of anti-MCV response in 42 patients found a significant correlation between anti-MCV and disease activity [22]. In Mansour et al. study on 64 patients with RA anti-MCV positive patients there was a significantly higher DAS-28 and more erosion on peripheral joints than anti-MCV negative patients [23]. Zahran et al. in a study on 30 patients with RA considered the value of anti-MCV, RF, and anti-CCP as a marker of disease activity [24]. They found that only anti-MCV can be used as a marker of disease activity. In a study by Ursum and colleagues, the association between anti-MCV levels and DAS28 was very low [25]. They conclude that monitoring disease activity with anti-MCV levels is not useful. In Mathsson et al. study on 273 patients with RA, no relationship was found between disease activity in anti-MCV positive and negative patients [26]. Yousefghahari et al. also did not report any relationship between disease activity and anti-MCV [27].

CONCLUSION

Based on the correlation found between anti-MCV levels and disease activity index, we conclude that anti-MCV may be a useful test to determine disease activity in RA. A disease activity index that applies serum anti-MCV has a substantial agreement with DAS28-ESR.

Conflict of Interest

The authors have no conflict of interest to disclose.

Authors' Contribution

Kamal Esalatmanesh developed the study concept and design and the acquisition of data, interpretations of data, and drafting of the manuscript. Davood Kheirkhah, Zahra Soleimani and Meysam Orangi developed the protocol, analysis of data and drafting of the manuscript.

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