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ORIGINAL ARTICLE

Unique Correlation Between Mutated Citrullinated Vimentine IgG Autoantibodies and Markers of Systemic Inflammation in Rheumatoid Arthritis Patients

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Abstract Rheumatoid arthritis (RA) is the most common inflammatory systemic autoimmune disease, primarily affecting the peripheral joints. Anti-mutated citrullinated vimentin autoantibodies (anti-MCV) of IgG isotype were shown to be a useful diagnostic marker of RA especially in RA patients who were anti-cyclic citrullinated protein autoantibodies (anti-CCP) negative. Nevertheless, published data correlates rheumatoid factor (RF), anti-CCP or anti-MCV antibodies with either erythrocyte sedimentation rate (ESR) or serum C-reactive protein (CRP) as markers of disease activity, not investigated the possible correlations of RA autoantibodies towards ESR and CRP in comparison. Herein, we aim to evaluate the usefulness of anti-MCV as a dependable marker in established RA compared with anti-CCP and RF antibodies and to examine correlations between RF, anti-CCP and anti-MCV antibodies towards ESR and serum CRP. Serum RF-IgA, RF-IgM, anti-CCP and anti-MCV levels were measured in 30 patients with RA and 40 patients with other autoimmune diseases (non-RA) compared with 20 normal subjects. Specificity, sensitivity and AUC for RF antibodies, anti-CCP and anti-MCV were calculated towards RA diagnosis. Our results showed that ESR and CRP had significantly higher values in both RA and non-RA patients compared with our healthy controls with observed significant increment in RA patients compared with non-RA patients. An important finding from our study is that 33.3 % of RA patients were anti-CCP negative but being positive towards anti-MCV. Also, in-between 36.7 up to 40 % of RA patients were RF-IgA and RF-IgM negative while being anti-MCV positive. Anti-MCV antibodies showed the highest specificity and sensitivity (97.5 and 86.6 %, respectively) towards RA diagnosis with the highest AUC value (0.920) compared with anti-CCP and RF antibodies. Correlation analyses revealed that there was no significant correlation between ESR along with CRP towards RF-IgA, RF-IgM and anti-CCP while profound highly significant correlation exhibited between ESR and CRP towards anti-MCV data ($r = 0.879$ and 0.994, respectively). Thus, our data suggest that the assessment of serum anti-MCV autoantibodies along with ESR and CRP considered as a simple laboratory regime for monitoring RA patients to assess and follow-up disease activity. The addition of anti-MCV autoantibodies to serologic markers in the ACR/EULAR classification criteria for RA will add points for patients with negative anti-CCP and RF antibodies.

Keywords Rheumatoid arthritis · Anti-mutated citrullinated vimentine antibodies · Specificity · Sensitivity · C-reactive protein · Erythrocyte sedimentation rate

Introduction

Rheumatoid arthritis (RA) is the most common chronic, inflammatory autoimmune disease with a frequency of 0.5–1.0 % in the adult population of developed countries occurring more frequently in women than in men (2.5:1) [1]. It is marked by chronic synovial inflammation and associated damage to articular cartilage and underlying bone, leading to substantial disability [2]. RA can be

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diagnosed using the 1987 classification criteria of the American College of Rheumatology (ACR). A joint working group of the ACR and the European League Against Rheumatism (EULAR) was formed to develop new classification criteria of RA at 2010 to identify individuals at earlier stages of the disease. They classify a patient as having or not having definite RA, a history of symptom duration, joint involvement, and at least one serologic test (rheumatoid factor [RF] or anticyclic citrullinated peptide antibody [anti-CCP] and one acute-phase response measure (erythrocyte sedimentation rate [ESR] or C-reactive protein [CRP]) must be obtained [3, 4]. In patients with RA, the ESR correlates well with disease activity [5, 6]. Serum CRP measurement is comparable to ESR when used for screening the inflammatory response and its serum concentrations change more quickly than those in the ESR, and therefore CRP might be a better reflection of inflammation. The magnitude of inflammation directly relates to the concentration of CRP [6, 7].

The past decade has brought several revolutionary changes in the therapeutic approach to RA, based on the improved understanding of the joint inflammation pathogenic mechanism, the hallmark of RA. Historically, RF, an antibody directed to the Fc fragment of human immunoglobulin G, is a well established diagnostic and prognostic biomarker for RA and can be detected in 60–80 % of patients with established disease [2]. However, it can be detected in other autoimmune disorders, such as Sjögren's syndrome (SS), and in various non autoimmune conditions, including infectious diseases, as well as in healthy subjects. In this setting, the deeper knowledge of the pathogenic mechanisms underlying synovial rheumatoid damage allowed the identification of a variety of citrullinated proteins that may act as potential auto antigens [8]. A true breakthrough in the RA serologic profile was the identification of amino acid citrulline as the main compound of the antigenic determinant recognized by RA specific autoantibodies [2, 9]. Anti-citrullinated protein autoantibodies (ACPA) had been identified in the serum of RA patients which being sensitive and specific serological markers of RA, providing superior alternative of RF test in the laboratory diagnostics of RA [10]. Anti-perinuclear factor antibodies (APF), anti-keratin antibodies (AKA), anti-CCP and anti-mutated citrullinated vimentin antibodies (MCV) had an adequate specificity for supporting clinical and therapeutic decisions with RA [11]. Anti-CCP antibodies had been widely demonstrated to be an important diagnostic and prognostic tool with RA because of their high specificity [1].

Vimentin is an intermediate filament widely expressed in the synovia. It is secreted and citrullinated by macrophages undergoing apoptosis, largely present in the RA synovial microenvironment due to impaired clearance [12, 13]. Citrullinated vimentin had been identified as potential auto antigen in the pathophysiology of RA and an enzyme-linked immunosorbent assay (ELISA) was developed for the detection of autoantibodies directed against a mutated citrullinated vimentin [2]. Vossenaar et al. [14] and Nicaise Roland et al. [15] showed that anti-MCV of IgG isotype antibodies were shown to be a useful diagnostic marker of RA especially in RA patients who were anti-CCP negative. Nevertheless, published data correlates RF, anti-CCP or anti-MCV antibodies with either ESR or serum CRP as markers of disease activity, not investigated the possible correlations of RA autoantibodies towards ESR and CRP in comparison. Therefore, we aim to evaluate the usefulness of anti-MCV as a dependable marker in established RA compared with anti-CCP and RF antibodies and to examine correlations between RF, anti-CCP and anti-MCV antibodies towards ESR and serum CRP.

Patients and Methods

Patients

Patients were selected from external clinic of Rheumatology Department at Ain Shams University Hospital in Cairo (Egypt). RA patients were identified on the basis of questionnaire, laboratory investigations and clinical findings. A complete clinical history for all patients was obtained. Subjects who had visited the external clinic of Ain Shams University Hospital (Cairo) for routine health check-up programs without any specific problem and with no abnormality in the clinical and laboratory findings were considered 'normal controls'. Twenty healthy individuals (7 males and 13 females) of 33 up to 48 years old are served as controls. Patients with chronic renal failure, cardiovascular disorders, malignancy, diabetes mellitus, HIV, HBV, HCV and pregnant women were excluded from the study.

RA and non-RA autoimmune patients represented by 70 patients had given their written informed consent to be included in the study. Based on clinical diagnosis and investigations, patients were divided into two main groups:

1) RA group: This group included 30 patients (8 males and 22 females) of 25 up to 65 years old. RA patients diagnosed according to ACR classification criteria (1987) based on clinical and laboratory findings, i.e. morning stiffness, arthritis of three or more joints, symmetric arthritis, rheumatoid nodules, positive rheumatoid factor (RF-IgM), and radiographic changes.
2) Non-RA group: This group included 40 patients (10 males and 30 females) of 34 up to 74 years old.

According to diagnosis, 25 Systemic lupus erythematous, five Sjogren's syndrome, five mixed connective tissue disease, and five scleroderma patients were participated in the study.

Laboratory Investigations

All collected serum specimens were aliquot following blood sampling, stored at −80 °C and thawed only once before being assayed with all kits investigated. ESR was assessed by Westergren method in a period of 1 h. Normal ESR values were ≤20 mm/h. Serum CRP was measured by CRP-Latex Turbidimetric kit (LINEAR Chemicals, Barcelona, Spain). Expected values in normal individuals in serum were ≤6 mg/L. RF-IgM and RF-IgA in serum were assessed by ELISA (OrgenTec Diagnostika GmbH, Germany) according to manufacturer guidance. The normal cut-off value for RF-IgM and RF-IgA in serum was ≤20 U/mL. Determination of anti-CCP IgG antibodies in serum was done by AxSYM automated micro particle enzyme immunoassay system (MEIA) (Abbott Laboratories, USA). Expected values for normal individuals in serum were ≤5 U/mL. Serum anti-MCV IgG antibodies were assessed by ELISA according to manufacturer's instructions (OrgenTec Diagnostika GmbH, Germany). The normal cut-off value recommended by the manufacturer for anti-MCV antibodies in serum was ≤20 U/mL.

Statistical Analyses

Results are expressed as mean ± standard deviation (SD). Receiver operating characteristic curves (ROC) were constructed and areas under the curve (AUC) were compared for ACPA and RF antibodies. Correlation coefficients between ESR and CRP with RF antibodies and ACPA using Pearson's test at 95 % confidence were determined. The statistical package for social science (SPSS) for Windows version 11 (SPSS® Chicago, IL, USA) program was used.

Results

Seventy patients comprising 30 RA patients and 40 non-RA patients were recruited for this study along with 20 control subjects (Table 1). It was showed that ESR and CRP had significantly higher values in both RA and non-RA patients compared with our healthy controls with observed significant increment in RA patients compared with non-RA patients (Table 1). It was observed that 33.3 % of RA patients were anti-CCP negative but being positive towards anti-MCV. Also, in-between 36.7 up to 40 % of RA patients were RF-IgA and RF-IgM negative while being anti-MCV positive (Table 2).

Anti-MCV antibodies showed the highest specificity and sensitivity (97.5 and 86.6 %, respectively) towards RA diagnosis when compared with anti-CCP and RF antibodies (Table 2). The performance characteristics of antibodies tests for discriminating between RA and non-RA subjects were evaluated using ROC curves analysis to determine the best sensitivity and specificity for ACPA and RF antibodies in RA diagnosis (Table 2). The AUC for our parameters ranged between 0.542 and 0.920. Anti-MCV autoantibodies had the highest AUC value (0.920) compared with anti-CCP and RF antibodies for RA patients. Correlation analyses revealed that there was no significant correlation between ESR and CRP towards RF-IgA, RF-IgM and anti-CCP antibodies while profound highly significant correlation between ESR and CRP towards anti-MCV antibodies was observed ($r = 0.879$ and 0.994; $p < 0.01$) (Table 3).

Discussion

The diagnostic approach to RA, the most common chronic inflammatory joint disease, underwent significant changes. The urgent need to recognize and treat the disease allowed the development of a new set of classification criteria for RA to replace the outdated 1987 ACR ones [3]. Anti-MCV of IgG isotype were shown to be a useful diagnostic marker for RA especially in RA patients who were anti-CCP negative [14, 15]. Nevertheless, published data correlated RF, anti-CCP or anti-MCV antibodies with either ESR or serum CRP as markers of disease activity, not investigated the possible correlations of RA autoantibodies towards ESR and CRP in comparison. Therefore, the main objective of our study to evaluate the usefulness of anti-MCV as a dependable marker in established RA compared with RF and anti-CCP antibodies and to examine correlations between RF, anti-CCP and anti-MCV antibodies towards ESR and serum CRP.

An important finding from our study is that 33.3 % of RA patients were anti-CCP negative but being positive

Table 1 Characteristics of RA and non-RA patients compared with normal subjects

Parameter	Groups		
	Controls ($n = 20$) (7♂ + 13♀)	RA patients ($n = 30$) (8♂ + 22♀)	Non-RA patients ($n = 40$) (10♂ + 30♀)
Age (years)	30.6 ± 8.6[a]	38.8 ± 14.9[a]	36 ± 12.1[a]
ESR (mm/h)	9.2 ± 1.3[a]	38.6 ± 6.4[b]	24.7 ± 4.3[c]
CRP (mg/L)	3 ± 0.43[a]	36.7 ± 7.2[b]	15.1 ± 2.1[c]

Mean ± SD in the same row sharing the same superscript are not significantly different ($p > 0.05$) (One-way ANOVA-protected least significance difference (PLSD)-Fisher post hoc test)

Table 2 Specificity, sensitivity and AUC for RF antibodies and ACPA within RA and non-RA patients

Parameter	Groups						
	RA patients		Sensitivity (%)	Non-RA patients		Specificity (%)	AUC (ROC)
	($n = 30$)			($n = 40$)			
	Positive (n)	Negative (n)		Positive (n)	Negative (n)		
RF-IgA	14	16	46.6	7	33	82.5	0.542
RF-IgM	15	15	50	8	32	80	0.563
Anti-CCP	16	14	53.3	1	39	97.5	0.611
Anti-MCV	26	4	86.6	1	39	97.5	0.920

Positive denotes for values above normal ranges, *Negative* denotes for values within normal ranges, *AUC* denotes for area under the ROC curve

Table 3 Correlation coefficients between ESR and CRP with RF antibodies and ACPA in RA patients

Parameter	RF-IgA	RF-IgM	Anti-CCP	Anti-MCV
ESR	−0.006	0.282	0.094	0.879*
CRP	−0.097	0.497	0.073	0.994*

* Pearson's correlation coefficient (r) at 95 % confidence was significant at 0.01 levels (two-tailed)

towards anti-MCV. Also, in-between 36.7 up to 40 % of RA patients were RF-IgA and RF-IgM negative while being anti-MCV positive. Anti-CCP antibodies have been demonstrated to be as sensitive as RF, but highly specific for RA and more specific than RF in early RA disease [3, 10, 16]. Luime et al. [13] showed that anti-MCV may be used as an alternative for anti-CCP from using diagnostic case–control studies about RA. In the study of Syversen et al. [17], it was found that anti-MCV and anti-CCP were strongly associated with regard to status and level of RA. A positive anti-MCV test increased the odds of radiographic progression by 7.3 compared to 5.7 for a positive anti-CCP. The odds of progression increased with increasing anti-MCV level. Vossenaar et al. [14] and Nicaise Roland et al. [15] showed that anti-MCV of IgG isotype antibodies were shown to be a useful diagnostic marker of RA especially in patients who were anti-CCP negative.

In our study, the specificity and sensitivity anti-MCV antibodies have been compared to that of anti-CCP and RF antibodies. Anti-MCV antibodies showed the highest specificity and sensitivity (97.5 and 86.6 %, respectively) towards RA diagnosis. It was observed that anti-MCV autoantibodies had the highest AUC value (0.920) compared with anti-CCP and RF antibodies for RA patients since the area under a ROC curve (AUC) quantifies the overall ability of the test to discriminate between those individuals with the disease and those without the disease. In our study, specificity and sensitivity values of anti-MCV for the established RA were most comparable to the findings of Ursum et al. [18]. Sahin et al. [19] demonstrated that the sensitivity and specificity of anti-MCV vary in the literature between 49 and 74 % up to 79 and 96 %, respectively. Mathsson et al. [20] showed that when patients with early RA are compared with healthy controls, analysis of anti-MCV yields greater sensitivity and unchanged specificity as compared with analysis of anti-CCP. Anti-MCV also appears to perform better than anti-CCP in identifying poor radiographic prognosis in patients with early RA. Keskin et al. [21] found that the measurement of serum anti-MCV levels is useful for diagnosis of RA and combined use of anti-MCV and RF antibodies may be more useful prognostic factor than RF and anti-CCP. The discrepancy between various studies can be explained by the difference in: (i) patient populations [22], (ii) early RA [23, 24] or established RA [12, 25], or (iii) number of cases studied [15].

ESR is a simple nonspecific laboratory measure of inflammation since it measures the effect of proteins alteration on RBC's sedimentation process, whereas serum CRP represents one of the proteins responsive to inflammation made in the liver under the control of cytokines such as interleukin-6 (IL-6), IL-1, and tumor necrosis factor alpha (TNF-α) [6]. Elevated levels of ESR and CRP in patients with RA suggest heightened disease activity while in contrast normal ESR and CRP results indicate low disease activity. However, elevations may also be due to other inflammatory conditions [26]. Lindqvist et al. [27] suggested that CRP levels during early RA may be predictive of long-term (10 years) disease progression. Interestingly, our correlation analyses revealed that there was no significant correlation between ESR and CRP towards RF-IgA, RF-IgM and anti-CCP antibodies while profound strong significant correlation exhibited towards anti-MCV antibodies. Our results were in agreements with Greiner et al. [28] who found that there was no significant correlation between anti-CCP antibodies towards CRP, ESR or

white blood cell count. Sizova [4] was observed no correlation between the anti-MCV titer and prevalent clinical indicators, radiographic signs, number of erosions in the joints, and quality of life scores, except for the moderate correlation of anti-MCV with duration of arthritis and ESR.

Conclusion

Our data suggest that the assessment of serum anti-MCV autoantibodies along with ESR and CRP considered as a simple laboratory regime for monitoring RA patients to assess and follow-up disease activity. The addition of anti-MCV autoantibodies to serologic markers in the ACR/EULAR classification criteria for RA will add points for patients with negative anti-CCP and RF antibodies.

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