



Review

Diagnostic value of anti-mutated citrullinated vimentin in comparison to anti-cyclic citrullinated peptide and anti-viral citrullinated peptide 2 antibodies in rheumatoid arthritis: An Italian multicentric study and review of the literature

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ABSTRACT

In the last years, the detection of antibodies (Abs) against citrullinated peptides (ACPA) has largely replaced rheumatoid factor (RF) as the most helpful biomarker in the diagnosis of rheumatoid arthritis (RA). Current assays detect ACPA reactivity with epitopes on various different citrullinated proteins. Among these, anti-cyclic citrullinated peptide (CCP) Abs have been widely demonstrated to be an important diagnostic and prognostic tool because of their high specificity. Recently, citrullinated vimentin, a protein highly released in synovial microenvironment, has been identified as potential autoantigen in the pathophysiology of RA and an enzyme-linked immunosorbent assay (ELISA) for the detection of Abs directed against a mutated citrullinated vimentin (anti-MCV) was developed. Several recent studies evaluating the characteristics of anti-MCV in comparison to anti-CCP Abs, have given conflicting results. Anti-MCV have been demonstrated to perform better than anti-CCP as predictor of radiographic damage. Conversely, its additional diagnostic and prognostic role in comparison to anti-CCP in both early and established RA is controversial. Aim of this study was to evaluate the diagnostic performance of anti-MCV in RA and to compare it to anti-CCP and the recently developed assay targeting viral citrullinated peptide 2 (VCP2) in a large cohort of RA patients (n = 285), healthy subjects and other disease controls (n = 227). Anti-MCV resulted to have a sensitivity of 59% and a specificity of 92%. In comparison, anti-CCP and anti-VCP2 displayed a sensitivity of 77% and 61% and a specificity of 96% and 95%, respectively. Of interest, at the manufacturer recommended cutoff value of 20 U/mL, a high percentage of healthy subjects as well as Epstein Barr (EBV) and hepatitis C (HCV) virus infected patients resulted anti-MCV positive. In our large cohort of RA patients, anti-MCV demonstrated lower sensitivity than anti-CCP and VCP2 test, thus not allowing to confirm previously published data. Moreover, the high rate of detection in infectious diseases limits its diagnostic value in undifferentiated arthritis.

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1. Introduction

In the last years, the diagnostic approach to rheumatoid arthritis (RA), the most common chronic inflammatory joint disease, underwent significant changes. The urgent need to recognize and treat the disease as soon as possible to prevent joint disability allowed the development of a new set of classification criteria for RA to replace the outdated 1987 American College of Rheumatology (ACR) ones [1,2]. In particular, new criteria have been derived in order to increase sensitivity and specificity for RA diagnosis in an earlier phase and they have been shown to perform a good discriminative ability with respect to the older ones [3]. In the same time, studies pointed to the importance of developing more sensitive and specific early diagnostic and prognostic tests in order to set up the most appropriate treatment according to different disease outcome and course. Rheumatoid factor (RF) is a well established diagnostic and prognostic biomarker for RA and can be detected in 60–80% of patients with established disease [4]. 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. Of importance, RF is not helpful in the diagnosis of RA in its initial phases, being detected in less than 50% of patients with early disease [4]. 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 autoantigens.

The antibodies specific for citrullinated proteins, that are almost exclusively detectable in RA sera, recognize a variety of citrullinated antigens, including α -enolase, fibrinogen, type II collagen and filaggrin and are collectively identified as ACPA or anti-citrullinated peptide/protein antibodies. Interestingly, high concentrations of ACPA have been detected in RA inflamed synovial tissue as well as synovial fluid [5], thus suggesting their possible role in synovial inflammation.

The most frequently used ELISA assay for ACPA detection is based on cyclic citrullinated peptides (CCP). Anti-CCP antibodies (Abs) have been demonstrated to be as sensitive as RF, but highly specific for RA and more specific than RF in early disease [6]. On the basis of several data confirming their predictive and prognostic role, anti-CCP have been included as new serologic criterion in the 2010 RA classification criteria [1,7].

Recent studies highlighted the good diagnostic performance of specific Abs targeting two other citrullinated antigens, the viral citrullinated peptide 2 (VCP2), a peptide corresponding to the sequence 338–358 of the Epstein–Barr virus encoded protein 2 (EBNA-2), and MCV, mutated/recombinant citrullinated vimentin. All three anti-VCP2 isotypes have been demonstrated to be a good and sensitive diagnostic tool, being detected almost exclusively in RA patients with respect to control subjects and patients with other autoimmune diseases [8]. Moreover, anti-VCP2 appears to display a high concordance with anti-CCP and results to be associated with a higher risk of erosive disease [8,9]. Citrullinated vimentin, the antigenic target of anti-Sa antibodies representing a highly specific marker for RA, has

been recently identified as a good candidate for RA diagnosis [10]. Vimentin is an intermediate filament widely expressed in the synovium. It is secreted and citrullinated by macrophages undergoing apoptosis, largely present in the RA synovial microenvironment due to impaired clearance [11]. Thus, citrullinated vimentin has been considered a potential autoantigen with possible diagnostic value and an ELISA detecting Abs directed against recombinant MCV (anti-MCV) has been recently developed. Anti-MCV Abs have been suggested to provide a significant adjunctive diagnostic value in early as well as long-standing RA [12]. Moreover, interesting results coming from recent studies suggest that anti-MCV, as already shown for anti-CCP Abs, may be associated with induction and progression of subclinical atherosclerotic damage in early RA patients as detected by ultrasound evaluation of carotid intima-media thickness [13,14]. Finally, their potential prognostic role in the prediction of disease radiographic progression has been hypothesized in a 10-year prospective study on a large cohort of RA patients [15].

A number of recent studies, investigating the diagnostic performance of anti-MCV Abs with respect to RF and anti-CCP Abs both in early and long-standing RA, reported conflicting results. This may be partly due to study heterogeneity, choice of study population (healthy controls or patients with infectious or other chronic inflammatory diseases) or to disease phase (very early, early or long-standing RA). Overall, both in early and in established RA patients, anti-MCV Abs appear to perform as a slightly more sensitive diagnostic marker with respect to anti-CCP, with 12–15% of anti-CCP negative patients resulting positive for anti-MCV [12,16,17]. On the other hand, anti-CCP provides better diagnostic performance in terms of specificity, especially in studies enrolling patients with other inflammatory disease as controls [12,18]. Moreover, in patients with early as well as established RA, anti-MCV Abs have been reported to be associated with a more active and severe disease and to perform better in classifying patients into broad and narrow responders and in predicting poor radiographic progression with respect to anti-CCP [15,19–21]. However, a firm conclusion on the additional clinical and diagnostic utility of anti-MCV in RA with respect to other ACPA cannot be drawn and the limited sample size of population enrolled in the majority of the published studies surely hampers results and data interpretation. Therefore, aim of the present multicenter study was to evaluate the diagnostic performance in RA of the newly developed anti-MCV compared to that of anti-VCP2 and anti-CCP assays in a large cohort of RA patients.

2. Materials and methods

A total of 512 frozen stored sera from 285 patients with established RA diagnosed according to the 1987 ACR classification criteria [2], 136 patients with other chronic autoimmune/inflammatory or infectious diseases and 91 healthy subjects were evaluated. Patients and controls were randomly selected and collected from 10 Italian Centers belonging to the FIRMA group, an Italian association of experts in the field of autoimmune and chronic inflammatory rheumatic

diseases. The disease control group included patients with systemic lupus erythematosus (n = 24), systemic sclerosis (n = 29), Sjögren's syndrome (n = 21), mixed connective tissue disease (n = 4), polymyositis/dermatomyositis (n = 2), psoriatic arthritis (PsA) (n = 19), EBV infection (n = 9) and HCV-related hepatitis (n = 28).

Anti-CCP Abs were measured by second generation routine method used in each participating Center (No. 6 Eurodiagnostica, No. 3 Phadia, No. 1 Inova, No. 1 Axis-Shield). As different methods were used, anti-CCP values were normalized and expressed as a ratio between the sample value and the cutoff value for each kit/method in order to obtain comparable data. Anti-VCP IgG was detected by the recently developed 2nd generation method (Astra Diagnostici, Milan, Italy) according to manufacturer's instructions [9]. Anti-MCV Abs were measured using a recently available ELISA kit (Orgentec Diagnostika GmbH, Mainz, Germany) according to manufacturer's instructions.

2.1. Statistical analysis

Data analysis was performed using SPSS 13.0 software. The diagnostic performance of anti-CCP, anti-VCP and anti-MCV assays was examined by receiver-operating characteristic (ROC) curve analysis

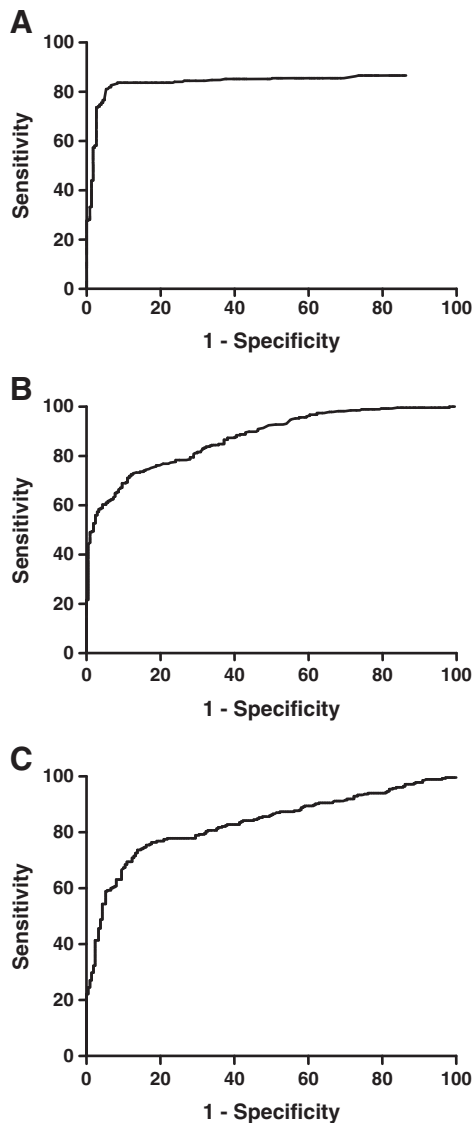


Fig. 1. A. ROC curve of anti-CCP assay. B. ROC curve of anti-VCP2 assay. C. ROC curve of anti-MCV assay.

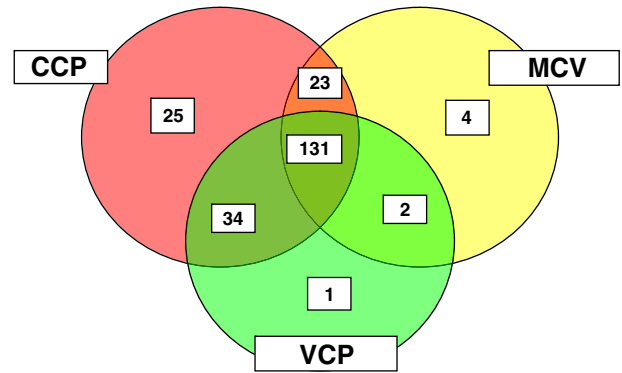


Fig. 2. Venn diagram showing the direct comparison of anti-VCP2, anti-CCP and anti-MCV positivity in RA patients.

by plotting sensitivity against 1-specificity at different cutoff values. Optimal cutoff values for the three antibody assays were determined based on ROC curve. Diagnostic sensitivities were compared at cutoff levels corresponding to 95% specificity.

3. Results

The ROC plot analysis identified a calculated AUC of 0.847 (95% CI, 0.808–0.887) for anti-CCP, 0.873 (95% CI, 0.844–0.903) for anti-VCP2 and 0.821 (95% CI, 0.785–0.858) for anti-MCV (Fig. 1). According to the obtained curves and considering a diagnostically acceptable 95% specificity, the optimal cutoff values resulted 5 (ratio) for anti-CCP, 21 U for anti-VCP2 and 55 U/mL for anti-MCV. Using the calculated cutoff values, sera from 167/285 RA patients (59%) resulted anti-MCV positive. In addition, 214 (77%) and 168 (61%) out of 277 RA patients, for which the two antibody determinations were available, resulted positive for anti-CCP and anti-VCP2, respectively.

Among controls, anti-MCV was detected in 3/91 (3%) of healthy subjects, in 6/80 (7%) of patients with other connective tissue diseases, in 3/28 (11%) of HCV related hepatitis, in 2/9 (22%) of EBV positive and in 3/19 (16%) PsA patients, corresponding to a specificity of 92%. Anti-CCP resulted positive in 10/227 (4%) and anti-VCP2 in 10/207 (3%) of disease and healthy controls for which both Abs determination was available. To note, the specificity of anti-CCP and anti-VCP2 was 96% and 95%, respectively.

A direct comparison of the diagnostic performance of the three different Abs was analyzed in the 277 RA patients for which all tests were available and is illustrated in Fig. 2. As reported, 57/277 (21%) of patients resulted negative and 131/277 (47%) positive for all three assays. Anti-MCV identified 4/277 (1%) of RA patients negative for anti-CCP and anti-VCP2, while the single anti-CCP positivity was depicted in 25/277 (9%) of RA subjects. Of interest, only one patient displayed the single anti-VCP2 positivity and two patients resulted positive for both anti-VCP2 and anti-MCV.

4. Discussion

There is growing evidence that early diagnosis and prompt therapeutic intervention in the course of RA represent an important tool to obtain more efficient disease control, less long-term joint damage and subsequent functional disability and better disease outcome. Therefore, recent research focused on the development of specific laboratory tests to be employed in the early identification of RA with respect to other inflammatory joint disease patients and in the prediction of disease course. In the present study, the diagnostic performance of 3 different ACPA (anti-MCV, anti-CCP and anti-VCP2 Abs) was compared for the first time in a large cohort of RA patients with respect to healthy controls and patients suffering from other autoimmune

Table 1
Specificity and sensitivity of anti-MCV antibodies in comparison to RF and anti-CCP in long-standing RA.

Author [ref]	Pts (n)	DC	HC	Sensitivity				Specificity			
				RF	CCP2	VCP2	MCV	RF	CCP2	VCP2	MCV
Roland [15]	156	158	50	45	51	na	57	90	95	na	91
Wagner [21]	193	332	na	na	69	na	71	na	98	na	81
Maraina [22]	100	153	na	85	71	na	80	74	95	na	59
Sghiri [23]	82	na	191	na	83	na	82	na	91	na	66
Besada [17]	75	69	na	73	69	na	76	59	96	na	96
Present study	285	136	91	na	77	61	59	na	96	95	92

Pts = patients; DC = disease controls; HC = healthy controls; RF = rheumatoid factor; Se = sensitivity; Sp = specificity; na = not assessed. All studies evaluated anti-MCV at cutoff of 20 U/mL except for present study.

and infectious diseases. In the last years, studies addressing the diagnostic value of anti-MCV in comparison to anti-CCP in RA documented a significant correlation with high level of agreement between the two tests. Indeed, their overall diagnostic performance resulted comparable suggesting that anti-MCV may be used as an alternative for anti-CCP [12]. Results driven from published studies suggest that at the cutoff value of 20 U/mL, anti-MCV Abs are slightly more or as sensitive as anti-CCP but less specific in RA diagnosis. In particular, as demonstrated in a recent meta-analysis, anti-MCV displayed a sensitivity ranging from 70% to 90% and a specificity from 79% to 96% according to the control population enrolled (healthy subjects or patients with other inflammatory or degenerative joint diseases) [12]. Subsequent studies performed on patients with long-standing RA confirmed these data, reporting a general better diagnostic sensitivity of anti-MCV with respect to anti-CCP, but a lower specificity (Table 1). Similar conclusions can be drawn analyzing data derived from studies including patients with a disease duration shorter than 3 years. As illustrated in Table 2, anti-MCV Abs displayed a higher sensitivity with respect to anti-CCP, ranging from 71% to 78% when healthy subjects were included as controls and from 57% to 62% when patients with other inflammatory diseases were considered. Specificity resulted lower in all studies, with a better diagnostic performance confirmed in studies enrolling only healthy subjects as control (from 90% to 96%) rather than patients with other rheumatic and rheumatic inflammatory diseases (78%–92%). In two studies evaluating a small cohort of patients with very early arthritis (<3 months of disease duration) anti-MCV did not provide any additional diagnostic value with respect to anti-CCP, resulting an equal sensitivity between the two tests but a higher specificity of anti-CCP (98% vs 91–92%)

[26,28]. Moreover, anti-MCV Abs displayed a positive predictive value lower than anti-CCP in predicting progression of undifferentiated arthritis to RA, thereby suggesting no additional diagnostic value of anti-MCV in the differential diagnosis of undifferentiated arthritis [17,28]. Interestingly, a recent report evaluating the diagnostic value of the IgA anti-MCV class demonstrated higher specificity (95%) of this class of Abs compared to IgG anti-MCV and to IgG and IgA anti-CCP, but with very low sensitivity (51%) [24]. Moreover, IgA anti-MCV resulted and associated with a more active disease over time, but not with erosive risk or radiological progression [24,29]. Further studies on larger cohorts are needed to evaluate the diagnostic value of this class of Abs in RA. On the other hand, it has been suggested that anti-MCV determination may provide additional diagnostic performance in seronegative RA patients. Several studies reported higher sensitivity of anti-MCV in comparison to anti-CCP in seronegative RA, suggesting that anti-MCV testing could be employed to confirm diagnosis in RF and anti-CCP negative patients. In fact, RA patients identified by anti-MCV alone ranged from 4% to 18%, both in early and long-standing disease [13,16,18,21,22,29].

Results from our study, however, are not fully in agreement with such data and deserve some intriguing considerations. First of all, anti-MCV cutoff value of 20 U/mL could not be universally accepted. Indeed, when 20 U/mL was adopted as cutoff in our study, a high number of healthy subjects resulted anti-MCV positive (15%), while the correspondent specificity was only 65%. On the other hand, when the cutoff concentration of 55 U/mL was used to obtain an equal specificity for all three assays, anti-MCV demonstrated lower sensitivity than anti-CCP test, thus not allowing to confirm previously published data. Similar results were reported in a study aimed to compare diagnostic performance of anti-CCP and anti-MCV in RA patients as compared to patients with other inflammatory and non-inflammatory disorders [30]. In this study, at the recommended cutoff value of 20 U/mL, both tests had identical sensitivity but with a lower specificity for anti-MCV. In contrast, when 81.5 U/mL was employed to obtain an equal specificity for both tests, anti-MCV demonstrated lower sensitivity (54%). In addition, single anti-MCV positivity was detected in our RA cohort only in 1% of patients negative for both anti-CCP and anti-VC2 and in 2% of patients negative for anti-CCP alone, suggesting a limited additive diagnostic value of anti-MCV in seronegative patients.

According to previously published data, there is substantial agreement that anti-MCV Abs could provide an additional value over anti-CCP as markers of more persistent disease activity both in patients with early and established RA. In particular, although anti-MCV testing are not usually considered useful to monitor disease activity [25], it has been shown that anti-MCV positivity and levels significantly correlated with both parameters of disease activity (erythrocyte sedimentation rate, C reactive protein, swollen joint count) and

Table 2
Specificity and sensitivity of anti-MCV antibodies in comparison to RF and anti-CCP in early RA.

Author [ref]	Pts (n)	DD	DC	HC	Sensitivity			Specificity		
					RF	CCP2	MCV	RF	CCP2	MCV
Innala [18]	210	<1y	na	102	83	80	74	95	98	96
Mathsson [20]	273	<1y	na	100	na	58	71	na	96	95
van der Liden [16]	201	<2y	424	na	48	50	57	86	88	78
Ursum [24]	123	<3y	39	na	na	55	59	na	92	92
Liu [25]	170	<1y	76	60	72	62	78	80	96	93
Raza [26]	63	≤3 ms	112	na	na	48	54	na	98	91
Damjanovska [27]	566	<2y	351	99	na	57	62	na	93 vs DC 99 vs HC	83 vs DC 94 vs HC
El-Barbary [12]	100	<1y	na	100	62	71 (CCP3)	75	97	96 (CCP3)	90
Svärd [28]	16	<3 ms	53	na	na	40	40	na	98	92

Pts = patients; DD = disease duration; DC = disease controls; HC = healthy controls; RF = rheumatoid factor; Se = sensitivity; Sp = specificity; y = year; ms = months; na = not assessed. All studies evaluated anti-MCV at cutoff of 20 U/mL except for Liu (30 U/mL).

measures of functional status as detected by Health Assessment Questionnaire [16,19,21,22,25]. Moreover, there is growing evidence for a prognostic value of anti-MCV Abs in predicting erosive joint damage and radiological progression similar to that of anti-CCP [15,19,21,25,26].

Finally, data derived from the present study allows to draw other considerations. At the recommended cutoff value of 20 U/mL, anti-MCV can be more frequently detectable in other inflammatory, autoimmune and infectious diseases in comparison to anti-CCP Abs. In agreement with our results, anti-MCV positivity has been found in SLE, SS and PsA patients [22,27]. In fact, the peculiar structure of vimentin, a protein containing 43 arginine residues which can be citrullinated, allows its recognition by several Abs, thus enhancing anti-MCV assay sensitivity. However, the low number of patients and the lack of clinical data on disease presentation and evolution do not permit to draw definitive conclusions on the predictive diagnostic role of anti-MCV in patients with other autoimmune or chronic inflammatory joint diseases. Moreover, at the cutoff value of 20 U/mL, a high percentage of our patients with HCV-related hepatitis and EBV infection displayed anti-MCV positivity. In this setting, the observation that anti-MCV Abs were revealed in 12% of patients with hepatitis B virus infection is of interest [26]. Furthermore, a recent study demonstrated anti-MCV positivity in 30% of HCV patients with joint involvement and in 32% of patients without arthropathy, suggesting that anti-MCV is not helpful in differentiating HCV-related articular involvement from chronic inflammatory joint diseases [31,32]. Vimentin has been identified as an early marker of fibrosis progression in patients transplanted for hepatitis C and vimentin mRNA expression significantly increases in the process of HCV-related tumor genesis [33,34]. In this context, it may be postulated that vimentin may be a more ubiquitous protein than epitopes recognized by anti-CCP and different epitope recognition and affinity of anti-MCV Abs may be a plausible explanation. Noteworthy and in agreement with the present data, a high percentage of anti-MCV false positivity has been observed among patients with EBV infection in a study evaluating the diagnostic accuracy of commercially available tests measuring different citrullinated antigen substrates [35]. Moreover, it is to note that a role of EBV in triggering the autoimmune response in the pathogenesis of RA has been suggested and high titers of anti-EBV Abs have been demonstrated in the sera of RA patients [36].

Finally, the present investigation compared for the first time the diagnostic performance of anti-MCV with respect to anti-VCP2 assay in a large cohort of RA patients. It showed a similar sensitivity of the two tests, but higher specificity for anti-VCP2. This is in line with the results of our previously published study showing high sensitivity and specificity of anti-VCP2 [8].

In conclusion, our results confirm that anti-MCV assay does not appear to provide additional diagnostic performance over anti-CCP determination in RA. Nevertheless, preexisting results appear to show that anti-MCV determination may be helpful as diagnostic marker in seronegative patients, but at recommended cutoff value of 20 U/mL. According to our data, a small percentage of RA patients (2.5%) were positive for anti-VCP2 and/or anti-MCV (at a higher cutoff). We believe that further studies are needed to demonstrate the diagnostic value of these Abs in apparently seronegative RA subjects and to validate an universally and reliable accepted cutoff value for anti-MCV Abs in order to evaluate their diagnostic predictive value, their role in patients with other connective tissue diseases and to establish possible association of this Ab with peculiar clinical features of RA. Moreover, the availability of an international validated reference serum to standardize the results obtained by different methods for ACPA detection, might prove to be very useful to solve this issue and is urgently needed [37].

Take-home messages

- The present study on a large cohort of RA patients showed that, at the recommended cutoff value of 20 U/mL, anti-MCV can be detectable not only in 15% of healthy subjects, but also in a number of patients with chronic inflammatory and autoimmune disorders and infectious diseases, thereby reducing the Ab specificity to 65%.
- At cutoff value of 55 U/mL determined by ROC analysis, the anti-MCV assay showed a diagnostic sensitivity of 59% and a specificity of 92%.
- Results from the our study does not allow to confirm existing data reporting a better sensitivity with a lower diagnostic specificity of anti-MCV in comparison to anti-CCP Abs.
- Anti-MCV test displays a similar diagnostic sensitivity but a lower specificity with respect to anti-VCP2 test.
- In our population, anti-MCV Abs do not seem to provide substantial diagnostic value as adjunctive test in seronegative RA patients.

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Appendix

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