

Diagnostic value of antibodies against mutated citrullinated vimentin for Rheumatoid Arthritis.

Brief paper

Running head: Value of anti-MCV for RA

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DISCLOSURES: NONE

Abstract

Objective

To compare the diagnostic efficiency of anti-MCV, anti-CCP2 and RF detection for patients with RA.

Method

Cross-sectional study of patients with established rheumatic disease: Rheumatoid Arthritis (RA; n=75), Psoriatic Arthritis (PsA; n=25), 27 patients with Ankylosing Spondylitis (AS; n=27) and Connective Tissue Disease (CTD; n=17). Anti-CCP2, anti-MCV and RF were detected by enzyme-linked immunosorbent assays on stored serum according to the manufacturer's instruction.

Results

IgM-RF had the highest sensitivity, but the Positive Likelihood Ratio is just 1,43. The detection of anti-MCV has a higher sensitivity for RA (76%), a specificity similar to anti-CCP2 (96%) resulting in the lowest Negative Likelihood Ratio (0.25). Anti-MCV levels correlate well with anti-CCP2 levels ($R=0.74$; $p<0,01$). The mean level of anti-MCV is significantly higher in RA than in other subgroups (395 U/ml versus 14,4 U/ml, $\chi^2 =61,0$; $p<0,001$) and in each other subgroup (Mann-Whitney-Wilcoxon: $U=239$, $p<0,001$ for RA and PsA; $U=215$, $p<0,001$ for RA and AS; $U=192$, $p<0,001$ for RA and CTD). Among RA patients, anti-CCP2 levels have a dichotomous distribution whereas anti-MCV levels have a homogeneous distribution.

Conclusion

Anti-MCV could be a better test for diagnosing RA than anti-CCP2.

Introduction

Effective and early treatment can limit damage development in patients with Rheumatoid Arthritis (RA) and improve prognosis. Establishing an early diagnosis of RA is not always straightforward and biomarkers may aid clinicians in differentiating self-limiting synovitis from potentially persistent and destructive disease. The detection of antibodies against citrullinated peptides/proteins (ACPA) has largely replaced the presence of Rheumatoid Factor (RF) as the most helpful biomarker. ACPA are specific for RA, develop often before disease onset and are associated with genetic risk factors for RA such as Shared Epitope alleles and PTPN22 polymorphisms.

Citrullination of proteins is a post-translational conversion of arginine to citrulline residues by peptidylarginine deiminase enzymes (PAD) dependent of high calcium concentration. While citrullination of proteins in inflamed synovium is not specific for RA, the humoral response to citrullinated proteins is highly specific (1). ACPA are produced locally at the site of inflammation in the synovium and in the bone marrow by an antigen-driven maturation of specific B-cells in ACPA positive RA (2).

Current ACPA assays detect ACPA reactivity with epitopes on various different citrullinated proteins, such as fibrinogen, histones, vimentin, fibrinogen peptides and Sp α (a CD5 antigen-like protein). The identification of the Sa antigen as citrullinated vimentin (3) and the generation under oxidative stress of isoforms of vimentin with non-random citrullination and mutation (with transversion of a guanine base in carboxy-terminal domain) (4) make citrullinated mutated vimentin a potentially important autoantigen in RA (3). This study compared the diagnostic value of an enzyme-linked immunosorbent assay (ELISA) for antibodies against mutated citrullinated vimentin (anti-MCV) with standard anti-CCP2 and Rheumatoid factor (RF) for the detection of patients with RA in a cohort of Rheumatology outpatients.

Patients and Methods

Cross-sectional study of patients with established rheumatic disease, who were followed at our Department: 75 patients with Rheumatoid Arthritis (RA) according to 1987 ACR criteria, 25 patients with Psoriasis Arthritis (PsA), 27 patients with Ankylosing Spondylitis (AS) according to New York criteria and 17 patients with Connective Tissue Disease (CTD) (SLE, Sjögrens` s syndrome and vasculitis, all fulfilling relevant classification criteria). Patients were selected from approved disease registries for which they had given written informed consent. Serological analyses were performed on stored samples using commercially available kits following the manufacturer`s instructions and inclusion of positive and negative controls. Anti-MCV levels were determined by enzyme-linked immunosorbent assay (ELISA) (Orgentec Diagnostika, Mainz Germany) and values greater than 20 arbitrary U/ml were considered positive. Quanta Lite TM (INOVA Diagnostics Norway) was used for the detection of IgG anti-CCP antibodies (second generation) with cut-off levels for positive samples ≥ 20 arbitrary U/ml. RF enzyme-linked immunosorbent assay (INOVA Diagnostics, Norway) was used for IgG, IgA, IgM isotypes detection with cut-off levels for positive samples > 6 arbitrary U/ml.

Data were analysed with SPSS version 16.0 (SPSS Ltd, Chicago IL, USA). Sensitivity (Se), Specificity (Sp), Youden index, both positive and negative Likelihood Ratio were calculated. Biomarker tests were analysed using ROC curves and calculation of the area under the curve (AUC). Spearman`s rank correlation coefficient, Anova test, Kruskal-Wallis test and Mann-Whitney-Wilcoxon test were used. P-values $< 0,05$ were considered significant.

Results

Patients' characteristics (Table 1):

The age and the sex distribution in the different disease groups are as expected: more women in the RA and CTD groups compared to the AS group; younger patients in the AS and CTD compared to the RA group.

62% of non-RA patients are IgM-RF positive. To a lesser extent, 41% and 52% of non-RA patients are positive respectively for IgG-RF and IgA-RF.

Two out of 17 CTD patients and one out of 25 PsA patients are positive for anti-MCV: titers of 40 and 155 U/ml for the two CTD patients and 267 U/ml for the PsA patient.

Five RA patients are anti-MCV positive and anti-CCP2 negative while there is no RA patient anti-CCP2 positive and anti-MCV negative.

Operating characteristics (Table 2 and figure 1):

Anti-MCV detection has a higher sensitivity with an equal specificity, a higher Youden index and a lower negative likelihood ratio than anti-CCP2. Both anti-MCV and anti-CCP2 have positive likelihood ratio over 10, indicating substantial contributions to the diagnostic process. ROC curve analysis shows that anti-MCV detection may be a more efficient biomarker for RA even though there is no statistical difference in the AUC between any of these tests.

While IgM-RF had the highest sensitivity, the positive likelihood ratio is just 1,43 because of its low specificity and the resulting low Youden index corroborates its poor efficiency.

Anti-MCV characteristics

Anti-MCV levels correlate well with anti-CCP2 levels (Spearman correlation of 0,74; $p < 0,01$, two-tailed). The mean level of anti-MCV is significantly higher in RA patients (395 U/ml) than in other disease subgroups (18,5 U/ml for PsA; 7,65 U/ml for AS; 19,1 U/ml for CTD) (Anova test; $F=14,5$; $p < 0,001$). The median level of anti-MCV is significantly higher in RA patients (164 U/ml) than in other disease subgroups (8,22 U/ml for PsA; 7,36 U/ml for AS;

9,38 U/ml for CTD) (Kruskal-Wallis test, $\chi^2 = 61,0$; $p < 0,001$). The difference of the median level of anti-MCV between RA patients and each of the other subgroups is significant (Mann-Whitney-Wilcoxon: $U = 239$, $p < 0,001$ with PsA; $U = 215$, $p < 0,001$ with AS; $U = 192$, $p < 0,001$ with CTD).

Distribution of anti-CCP2 and anti-MCV levels among RA patients (figure 2):

Anti-CCP2 level distribution is dichotomous, either definitely positive or definitely negative: the 23 RA patients negative for anti-CCP have a median level of 2,38 U/ml and 29 RA patients have anti-CCP level over 200 U/ml. Anti-MCV level distribution is more homogeneous with a top around cut-off: 33 RA patients have anti-MCV level under 100 U/ml with a median level of 18,8. The 18 RA patients negative for anti-MCV have a median of 7,97 U/ml and the 8 RA patients positive for anti-MCV with a level under 50 U/ml have a median of 35,2.

Discussion

Our study has several limitations because of its small size and the study design. Nevertheless some results merit to be discussed.

Two out of 17 CTD patients and one out of 25 PsA patients are positive for anti-MCV which is comparable to previous studies: Bang et al found 43 out of 189 SLE patients (17,5%) and 11 out of 68 pSS patients (11,7%) positive for anti-MCV (4) ,while Tesija-Kuna et al reported anti-MCV in 2 out of 56 PsA patients (3,4%) (5).

The ELISA determined IgM-Rheumatoid factor has a specificity of 0,38. This result is dependent of the characteristics of the control group and is in accordance with a previous report from our area (6). Because of its low efficiency, there is no advantage in combining RF and anti-MCV in the sole detection of RA patients as it contributes little to the diagnostic process and is not cost-effective.

There is also no additional benefit in combining anti-MCV with anti-CCP2 as their operating characteristics are quite similar. This is in agreement with the meta-analysis by Luime et al that concluded that anti-MCV may be used as alternative for anti-CCP (7). Nevertheless the distributions of anti-MCV and anti-CCP2 levels among RA patients are quite different and anti-MCV testing could still be valuable for negative anti-CCP patients (8) as the diagnostic spectrum of anti-MCV is somewhat different to anti-CCP (9).

Due to the homogenous distribution of anti-MCV levels with a top around cut-off, the recommended cut-off of 20 U/ml works well in the detection of RA patients in term of the operating characteristics. Because of the difference in distribution of levels in RA patients – unimodal and homogenous for anti-MCV, bimodal and dichotomous for anti-CCP2 - the sensitivity of anti-MCV decreases more rapidly than the sensitivity of anti-CCP2 when stratified with a higher specificity. This explains why the sensitivity for anti-CCP2 was higher

than the sensitivity for anti-MCV in the meta-analysis of Pruijn et al when stratified with a specificity of 98% (10).

Anti-MCV recognizes citrullinated and mutated vimentin bearing more epitopes – 43 arginine residues and at least 10 known citrullination sites (4) - than a commercial anti-CCP kit with its undisclosed and well-thought out composition of citrullinated peptides. The increased sensitivity of anti-MCV compared with anti-CCP has been explained with the findings that MCV antigens are more likely to bind a variety of autoantibodies with different specificities including anti-CCP (11). This diversity of epitope recognition and affinity of anti-MCV specific antibodies has thus far no clear clinical implication (11).

In summary, anti-MCV testing is more efficient in the detection of RA patients than anti-CCP2 and RF testing due to its greater negative predictive value. Its homogenous level distribution and the range of auto-antibodies binding a single citrullinated protein found in the inflamed synovium could be valuable clinically.

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Figure 1

Receiver operating curves for various serological assays in the detection of patients with Rheumatoid Arthritis (RA). Anti-MCV: antibodies against mutated citrullinated vimentin. Anti-CCP2: antibodies against cyclic citrullinated proteins, second generation. RF: rheumatoid factor.

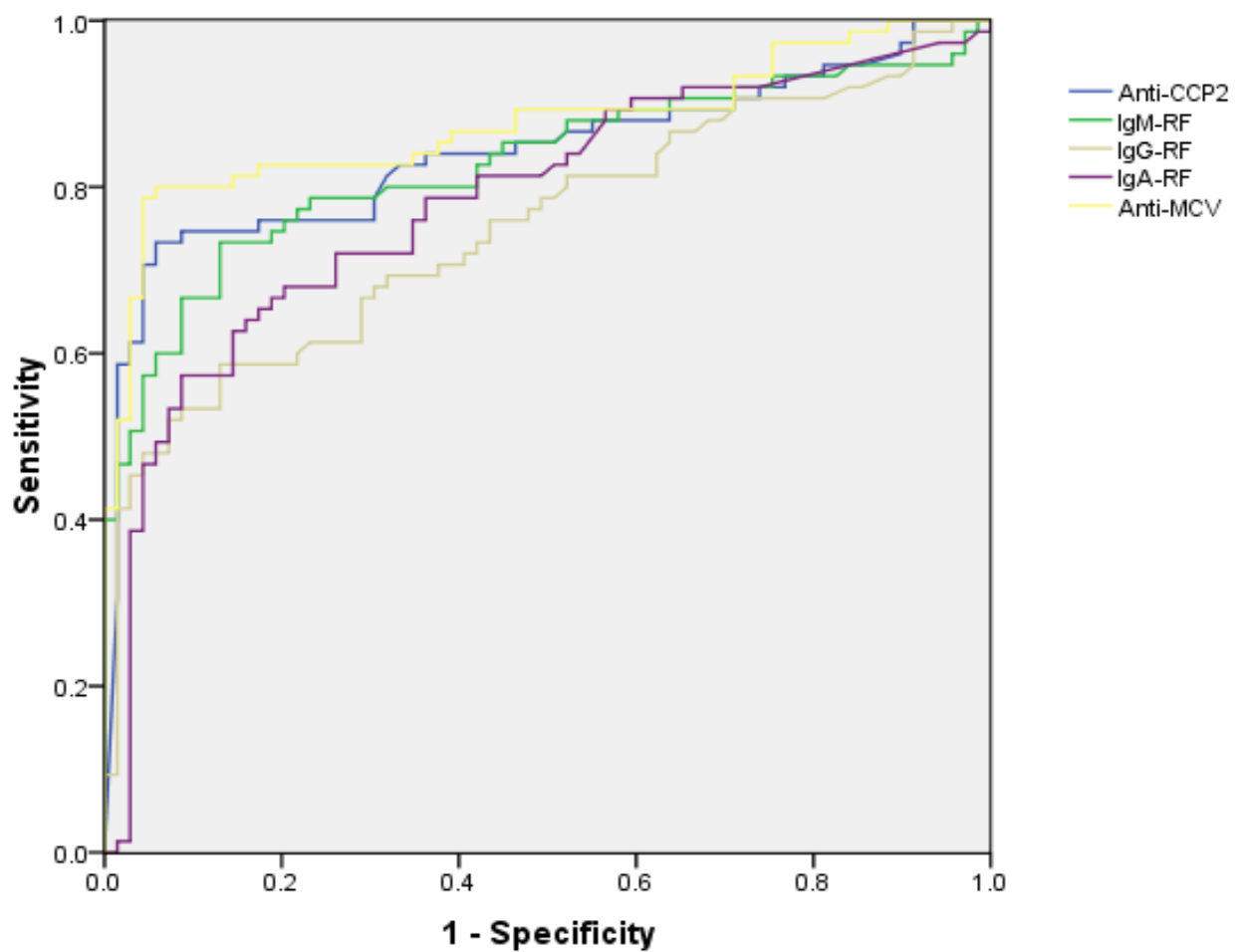


Figure 2

Bimodal distribution of anti-CCP levels whereas the distribution of anti-MCV levels is unimodal among patients with RA.

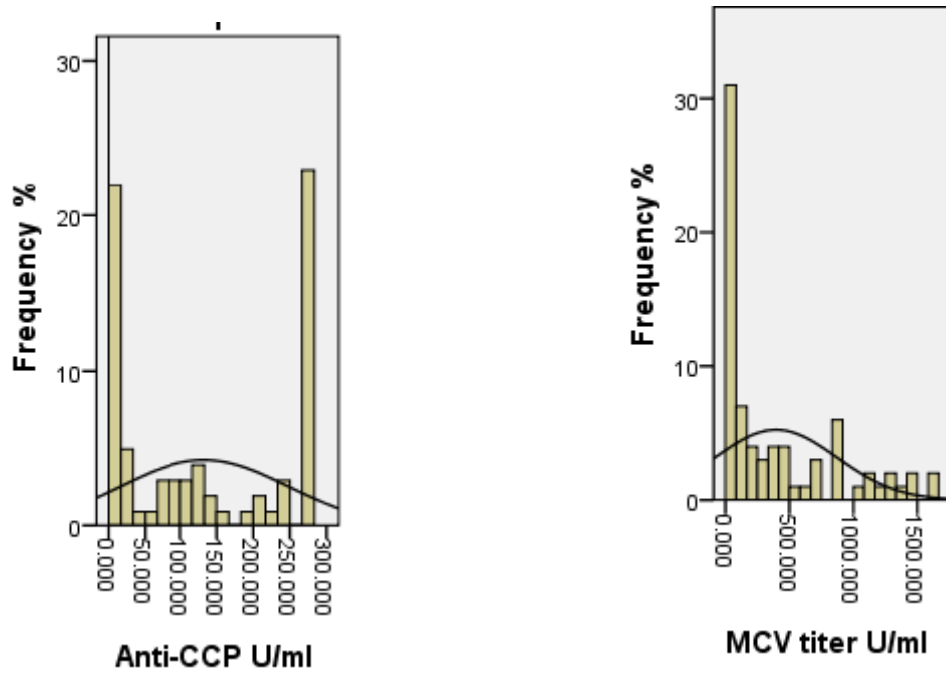


Table 1 Disease group characteristics and results of serological assays

| | Sex | Age | Anti- | Anti- | IgM-RF | IgG-RF | IgA-RF |
|--------|-------|---------|----------|----------|----------|----------|----------|
| | F/M | (y) +/- | MCV | CCP2 | Positive | Positive | Positive |
| | ratio | SD | positive | Positive | | | |
| RA | 59/16 | 64 +/- | 57 | 52 | 67 | 55 | 62 |
| (n=75) | | 15 | | | | | |
| PsA | 10/15 | 56 +/- | 1 | 1 | 15 | 10 | 9 |
| (n=25) | | 12 | | | | | |
| AS | 6/21 | 44 +/- | 0 | 1 | 17 | 11 | 15 |
| (n=27) | | 14 | | | | | |
| CTD | 12/5 | 53 +/- | 2 | 1 | 11 | 7 | 12 |
| (n=17) | | 17 | | | | | |

n: number of patients; RA: Rheumatoid Arthritis, AS: Ankylosing Spondylitis, PsA: Psoriatic Arthritis, CTD: Connective Tissue Disease. Anti-MCV: antibodies against mutated citrullinated vimentin. Anti-CCP2: antibodies against cyclic citrullinated proteins, second generation. RF: rheumatoid factor.

Table 2 Operating characteristics of the various assays for the detection of patients with Rheumatoid arthritis (RA).

| | Sensitivity | Specificity | Likelihood Ratio(+) | Likelihood Ratio(-) | AUC (Confidence interval) | Youden index |
|------------------|--------------------|--------------------|--------------------------------|--------------------------------|--|-------------------------|
| Anti-MCV | 0,76 | 0,96 | 17,27 | 0,25 | 0,875 (0,815-0,936) | 0,72 |
| Anti-CCP2 | 0,69 | 0,96 | 15,68 | 0,32 | 0,841 (0,773-0,909) | 0,65 |
| IgM-RF | 0,89 | 0,38 | 1,43 | 0,29 | 0,828 (0,759-0,898) | 0,27 |
| IgG-RF | 0,73 | 0,59 | 1,80 | 0,45 | 0,753 (0,673-0,832) | 0,32 |
| IgA-RF | 0,83 | 0,48 | 1,58 | 0,36 | 0,784 (0,708-0,860) | 0,31 |

Anti-MCV: antibodies against mutated citrullinated vimentin. Anti-CCP2 : antibodies against Cyclic citrullinated proteins, second generation. RF: rheumatoid factor. AUC: area under the curve.