THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

# Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis

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# 1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation that ultimately leads to joint destruction. Although the exact etiology of RA is still unknown, genetic predisposition, environmental factors like infectious agents or smoking, and sex hormones may all be involved. Ultimately, synovial inflammation and hyperplasia leads to progressive destruction of the cartilage and bone, and can result in disability. With new and very effective therapeutic approaches becoming available, it is imperative to recognize and treat RA as early as possible in order to prevent joint disability. In very early stages, however, when clinical and radiological manifestations may not be evident, diagnosis may be difficult. Thus, a specific and sensitive serological test may be of great help in differential diagnosis.

Anti-citrullinated protein antibodies (ACPA) are sensitive and specific serological markers of rheumatoid arthritis, providing superior alternative of the rheumatoid factor (RF) test. They belong to the family of antibodies directing to epitopes containing the non-standard amino acid citrulline. The first members of this autoantibody family were anti-perinuclear factor (APF) and anti-keratin antibodies (AKA). Both APF and AKA recognize citrullinated epitopes of filaggrin. Citrullination is a post-translational modification of arginine by deimination, physiologically occurring during apoptosis and inflammation. The presence of several citrullinated proteins including filaggrin, fibrinogen, vimentin, α-enolase and viral peptides has been demonstrated in the RA synovium.

The identification of citrullinated epitopes as targets for anti-filaggrin antibodies led to the development of first and further generation anti-cyclic citrullinated peptide (anti-CCP) antibody assays. The anti-Sa antibody has been identified a decade ago; however, it has been confirmed that anti-Sa is directed against citrullinated vimentin.

ACPAs are highly specific for RA, only very few patients with other types of arthritis or connective tissue diseases are ACPA positive. The variability of different ACPA assays suggest that the combination of various citrullinated epitopes may drive autoimmunity in RA. ACPA production has been associated with genetic factors including HLA-DR antigens and PTPN polymorphisms, as well as environmental factors, primarily smoking. ACPAs have important relevance for prognosis, as ACPA positive patients exert significantly faster radiological progression and poorer outcome.

## 2. Aims

1. We were among the first ones to use the new anti-MCV test in the world. We wished to determine the diagnostic performance of these ELISA with standard second generation anti-CCP2 test.

2. Since several researches have been published in connection with anti-CCP IgG isotypes but we have not had information concering IgA and IgM isotypes, our goal was to find a method which would show IgA and IgM anti-CCP. We wished to analyze the diagnostical value of these antibodies and its changes during the course of a disease.

3. When applying the third generation anti-CCP3 and anti-CCP3.1 ELISA method, we wanted to find out whether the development of anti-CCP test means a diagnostical advantage as opposed to the second generation test.

## 3. Patients and methods

*3.1. Patients and controls*

Serum samples were obtained from 119 consecutive patients with RA. All patients met the ACR classification criteria for the disease. For comparisons, we tested 118 control subjects, including 74 patients with other well-defined rheumatic diseases, such as 37 patients with primary Sjögren's syndrome (pSS), 30 with polymyositis or dermatomyositis (PM/DM) and 7 with osteoarthritis (OA), as well as 44 healthy subjects. Serum samples were stored at -80 ℃ for less than 1 year until the present analysis. The RA group consisted of 100 women and 19 men. The mean (± SD) age of this group was 52.7±12.5 years (range: 19-77 years), which was not statistically different from that of the control subject. The mean duration of RA was 10.2±9.1 years at the time of the study.

*3.2. Methods*

*Anti-CCP2 IgG ELISA*

Anti-CCP2 IgG levels were measured using a second generation ELISA (QUANTA Lite™ CCP ELISA, INOVA Diagnostics Inc., San Diego, CA) utilizing synthetic citrullinated peptides bound to the surface of a microtiter plate as antigen. The test was performed according to the manufacturer's instructions. Instead of categorizing the results by the manufacturer recommended cutoff value (20 U/ml), receiver operating characteristic (ROC) curve analysis was performed, and the optimal cut-off level (12 U/ml) was established by choosing the combination of the highest possible sensitivity and specificity.

*Anti-CCP2 IgA and IgM ELISA*

To measure anti-CCP2 IgA and IgM levels, citrullinated peptides coated plates, sample diluent, wash buffer, TMB substrate and stop solution were used from the QUANTA Lite™ CCP ELISA. Serum samples were diluted to 1:100, and were incubated in the wells of the ELISA plates for 60 minutes. Bound antibodies were detected by HRP-conjugated rabbit anti-human IgA and IgM (DAKO A/S, Glostrup, Denmark). Results were expressed as optical densities (ODs). Optimal cutoff values (0.198 for IgA anti-CCP2 and 0.513 for IgM anti-CCP2 antibodies) were determined by ROC curve analysis.

*Anti-CCP3 and anti-CCP3.1 ELISA*

Anti-CCP3 IgG and anti-CCP3.1 IgA/IgG levels were measured using a third generation ELISA (QUANTA Lite™ CCP3 and CCP3.1 ELISA, INOVA Diagnostics Inc.). These assays utilize a different citrullinated antigen compared to the second generation test. Moreover, the CCP3.1 IgA/IgG ELISA detects both IgA and IgG antibodies to CCP3.

*Anti-MCV IgG ELISA*

Anti-MCV IgG antibodies were assessed by ELISA (kindly provided by Orgentec Diagnostika GmbH, Mainz, Germany). This assay contains recombinant mutated citrullinated vimentin as antigen. This molecule is the recombinant form of a vimentin variant found in human monocytes, which differs from native vimentin in the presence of additional arginine residues and further sequence differences. The test was performed according to the manufacturer's instructions. The cutoff value for anti-MCV antibodies was 20 U/ml.

*IgM, IgA and IgG RF*

IgM, IgA and IgG RFs were asessed by ELISA (ImmuLisa™ RF IgM, IgA and IgG, Immco Diagnostics, Buffalo, NY) according to the manufacturer's instructions. Normal upper limits were 9 IU/ml for IgM RF, 25 EU/ml for IgA RF, and 25 EU/ml for IgG RF, respectively.

*3.3. HLA-DRB1 genotyping*

Genomic DNA was isolated from the peripheral blood of 85 RA patients using QIAamp Blood Mini Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions. HLADRB1 typing and subtyping was performed by polymerase chain reaction (PCR) with sequence specific primers (Olerup SSP™, GenoVision Inc., PA, USA). We investigated the presence of the following shared epitope alleles: HLADRB1*0101, HLA-DRB1*0102, HLADRB1*0401, HLA-DRB1*0404, HLADRB1*0405 and HLA-DRB1*0408.

*3.4. Statistical analysis*

Antibody levels between different groups were compared by the non-parametric Mann Whitney U test. The diagnostic performance of anti-CCP2 antibody ELISAs was examined by ROC curve analysis, and optimal cut-off levels were determined at the value resulting in the combination of the highest diagnostic sensitivity and specificity. Spearman's rank correlation was used to assess the relationship between IgA, IgM and IgG anti-CCP2 levels. Fisher's exact test was performed to investigate the association between the occurrence of anti-CCP2 antibodies and RFs of different isotypes, as well as between the frequency of IgA, IgM and IgG anti-CCP2 antibodies and disease duration or the presence of HLA-DRB1 SE alleles. *P* values $<0.05$ were considered significant. All statistical analyses were performed using the statistical package SPSS 11.0 (SPSS Institute Inc., Chicago, IL, USA).

# 4. Results

## 4.1. Anti-MCV autoantibodies

*4.1.1 Anti-MCV levels in the study population*

Patients with RA had significantly higher anti-MCV titers (median 60.8 U/ml, interquartile range 21.2–348.4 U/ml) than healthy subjects (median 8.9 U/ml, IQR 5.4–13.3 U/ml) and patients with other rheumatic diseases (median 9.8 U/ml, IQR 3.7–14.7 U/ml; $p < 0.0001$ for both).

*4.1.2 Diagnostic performance of anti-MCV, anti-CCP2, and RF assays using manufacturer recommended cutoff*

When ranking the results using the manufacturers' suggested cutoff levels, anti-MCV positivity was the most prevalent antibody in the RA group, resulting in 75.6% diagnostic sensitivity. This exceeded the sensitivity of IgM RF by 4%, and that of anti-CCP2 by 9%. IgA and IgG RF were characterized by equally low prevalence rate, yielding 36.9% and 37.8% sensitivity, respectively. Comparing RA patients with healthy individuals only, the specificity of all autoantibody assays was excellent (between 95.5% and 100%).

Anti-MCV positivity was observed in 4 patients with pSS, in 3 patients with PM/DM, and in one patient with OA, resulting in 91.5% overall specificity in our cohort. Anti-CCP2 was present in one patient with pSS and one with PM/DM, yielding 98.3% specificity.

The high prevalence of all 3 RF isotypes in pSS and PM/DM patients resulted in low overall specificity of these antibodies (82.2%, 88.9%, and 87.3% for IgM, IgA, and IgG RF, respectively). The occurrence of IgM RF was 40.5% in the pSS group, and 16.6% in the PM/DM group. IgM RF levels in the pSS group were similar to those measured in patients with RA (data not shown).

ROC analysis was performed to examine the overall diagnostic performance of anti-MCV and anti-CCP2 assays. The calculated area under the curve (AUC) was 0.853 (95% CI 0.801–0.905) for anti-MCV and 0.910 (95% CI 0.873–0.946) for anti-CCP2 (difference is not significant).

AUC values for both anti-MCV and anti-CCP2 exceeded the calculated AUC for IgM RF (0.788; 95% CI 0.728–0.847).

*4.1.3 Diagnostic performance of anti-MCV and anti-CCP2 assays using optimal cutoff*

We examined if performance characteristics of anti-MCV and anti-CCP2 could be optimized by introducing different cutoff values. The optimal cutoff levels were determined based on ROC analysis. For anti-MCV and IgM RF, the optimal cutoff (20.3 U/ml and 8.3 IU/ml, respectively) was approximately the same as the manufacturer recommended value. For the anti-CCP2, however, the optimal cutoff level was calculated as 12 U/ml, and resulted in 74.8% sensitivity and 95.8% specificity.

To directly compare the sensitivity of the assays, we introduced cutoff levels (26.3 U/ml for anti-MCV, 11.7 U/ml for anti-CCP2, and 50.3 IU/ml for RF) to obtain the diagnostically acceptable 95% specificity. This resulted in 69.7% diagnostic sensitivity for the anti-MCV test and 74.8% for anti- CCP2. The sensitivity of IgM RF decreased to 33.6%.

*4.1.4. Relationship among anti-MCV, anti-CCP2, and IgM RF positivity*

Using optimal cutoff values for anti-MCV, anti-CCP2, and IgM RF tests, all 3 antibodies were present in 70 RA patients (58.8%); however, none of them tested positive in 18 subjects (15.3%).

The agreement rate between anti-MCV and IgM RF in the RA group was 81.5%, while between anti- MCV and anti-CCP2 tests it was 88.2%. Using the combination of anti-MCV and anti-CCP2, positivity for both or either of these antibodies resulted in sensitivity/specificity values of 66.4%/98.3% and 78.2%/91.5%, respectively. The PPV of double positivity for RA was 97.5%.

Importantly, 29.4% of IgM RF-negative cases (10 patients), as well as 13.3% of anti-CCP2-negative cases (4 patients) in the RA group were anti-MCV-positive.

On the other hand, 27.7% of anti-MCV-negative RA patients (10 subjects) had anti-CCP2 antibodies. Both anti-MCV and anti-CCP2 belong to the family of antibodies against citrullinated antigens. To further examine the relationship between them, we assessed the correlation between serum anti-MCV and anti-CCP2 concentrations. A significant correlation was found between anti-CCP2 and anti-MCV levels in RA ($R = 0.783$; $p < 0.0001$).

Moreover, the median anti-MCV level was higher in anti-CCP2-positive/anti-MCV-positive RA patients (n = 79, median antibody level 206.5 U/ml, IQR 59.3–826.3 U/ml) than in those found to be anti-CCP2-negative/anti-MCV-positive (n = 4, median antibody level 45.9 U/ml, IQR 38.3–112.7 U/ml; $p < 0.0001$). Median anti-CCP2 levels were also significantly higher in double-positive patients (296.5 U/ml, IQR 120.3–490.2 U/ml) than in those with single anti-CCP2 positivity (n = 10, 20.4 U/ml, IQR 16.4–68.7 U/ml; $p < 0.0001$).

These data together with the 88.2% agreement rate suggest that anti-MCV and anti-CCP2 may bind to similar epitopes. However, our data show that citrullinated antigens in both tests contain unique epitopes, which are recognized exclusively by one antibody or the other.

Although RF is not related to anti-citrullinated protein antibodies, weak, but statistically significant correlation was found between the serum titers of IgM RF and those of either anti-MCV ($r = 0.250$; $p = 0.01$) or anti-CCP2 ($r = 0.269$; $p = 0.007$).

## 4.2. Anti-CCP2 isotypes in rheumatoid arthritis

*4.2.1. IgA and IgM anti-CCP2 levels in the study population*

Patients with RA had significantly higher IgA anti-CCP2 antibody levels (median OD: 0.211, interquartile range: 0.090-0.630) than healthy subjects (median OD: 0.085, interquartile range: 0.077-0.096) and patients with other rheumatic diseases (median OD:0.096, interquartile range: 0.076-0.125) ($p<0.0001$ for both). They also had significantly higher IgM anti-CCP2 antibody levels (median OD: 0.404, interquartile range: 0.245-1.221) compared to healthy controls (median OD: 0.278, interquartile range: 0.246-0.309) and disease controls (median OD: 0.244, interquartile range: 0.185-0.411) ($p<0.0001$ for both). IgA and IgM anti-CCP2 levels showed significant correlation with IgG anti-CCP2 levels, and with each other ($p<0.0001$ for each).

*4.2.2. Frequency of IgA and IgM and IgG anti-CCP2 antibodies in RA*

To examine the overall diagnostic performance of anti-CCP2 assays of different isotypes, ROC analysis was performed. The calculated area under the curve (AUC) value was 0.910 (95% CI: 0.873-0.946) for anti-CCP2 IgG, 0.744 (95% CI: 0.678-0.809) and 0.704 (95% CI: 0.636-0.772) for IgA and IgM anti-CCP2, respectively.

The difference between IgA and IgM anti-CCP2 was not significant. Categorizing the results according to optimal cut off values, IgG, IgA and IgM anti-CCP2 antibodies were positive in 74.8%, 52.9% and 44.5% of RA patients, respectively.

Although most IgA and IgM anti-CCP2 antibodies were present in IgG anti-CCP2 positive RA subjects, two single IgM, and one single IgA positivity was detected in the RA group. No anti-CCP2 antibodies of any isotypes tested positive in healthy controls. IgG, IgA and IgM anti-CCP2 antibodies occurred in 5, 5 and 9 patients in the disease control group, respectively. In four control subjects two or three antibodies of different isotypes were present. The overall diagnostic specificity of the IgA and IgM anti-CCP2 tests was 95.8% and 91.6%, respectively.

When compared with the diagnostic performance of RFs, the specificity of IgA and IgM anti-CCP2 antibodies proved to be significantly higher than those of any RF isotypes, and their sensitivity exceeded the diagnostic sensitivity of IgA and IgG RFs Within the RA group, being positive for any of the two, or all the three anti-CCP2 isotypes corresponded to a specificity level of 96.6% and 99.2%, respectively.

*4.2.3 Association between anti-CCP2 antibodies and RFs of various isotypes*

We compared the proportions of RA patients in the IgM, IgA and IgG RF positive and negative groups who were positive for the same anti-CCP2 antibody isotypes. Significantly more RA subjects with IgM RF had IgM anti-CCP2 antibodies than those in the IgM RF negative population (56.8% versus 14.7%; $p<0.0001$). Also, the frequency of IgA anti-CCP2 antibodies was higher in the IgA RF positive group compared to the IgA RF negative patients (70.5% versus 42.6%; $p=0.004$). Although IgG anti-CCP2 positivity was also more prevalent among IgG RF positive patients (82.2% versus 70.2%), this association was not significant.

*4.2.4. Association between anti-CCP2 antibodies and HLA-DRB1 shared epitopes*

As the presence of the HLA-DRB1 SE is associated with IgG anti-CCP2 positivity in RA patients, we examined the possibility if the relationship can be extended to anti-CCP2 antibodies of IgA or IgM isotype. HLA-DRB1 typing and subtyping was performed in 85 RA patients, and one or two SE alleles were detected in 54.1% of them. When IgA and IgM anti-CCP2 antibodies were examined separately, only slightly higher IgA and IgM anti-CCP2 antibody frequencies were found in RA patients carrying SEs compared to SE negative ones (54.3% *vs.* 43.6% for IgA, and 45.7% *vs.* 33.3% for IgM; $p$=NS).

However, when anti-CCP2 antibodies of all isotypes were considered, we could demonstrate significant association between anti-CCP2 antibodies and the presence of SE ($p=0.03$). This association was stronger than the association between SE alleles and IgG anti-CCP2 antibodies alone ($p=0.04$). Antibody levels of anti-CCP2 antibodies of any isotype

(considering only those patients who tested positive) were not higher in the SE positive group compared to SE negative RA patients (data not shown).

*4.2.5 Association of anti-CCP2 antibodies of various isotypes with the duration of RA*

We examined the frequency and levels of anti-CCP2 antibodies in "early" (disease duration <3 years) and "longstanding" (disease duration >10 years) RA. The tendency of higher rate of positivity was observed for all three antibody isotypes in early RA. However, significant difference was detected only in the case of IgM anti-CCP2: the prevalence of this antibody was 71.4% in those with disease duration of less than 3 years, while it was 37.5% in those having RA for more than 10 years ($p$=0.03). Similar results were obtained when "early" RA was compared to the rest of the RA patients ($p$=0.04). The serum concentrations of anti-CCP2 antibodies were similar, and the frequency of RFs of various isotypes was not significantly different in "early" versus "longstanding" disease (data not shown).

## 4.3. The third generation anti-CCP3 and anti-CCP3.1 ELISA

*4.3.1. Frequency of anti-CCP3 and anti-CCP3.1 positivity in RA and controls*

The anti-CCP3 and anti-CCP3.1 tests were positive from 119 patients with RA in 92 and 102 patients (77.3% and 85.7%), from 74 disease controls in 3 and 6 (4.1% and 8.1%) and from 44 healthy controls in 1 and 6 (2.3% and 13.6%) cases. In RA population, the positivity was significantly higher, than in controls ($p<0.0001$)

*4.3.2. Diagnostic performance of anti-CCP3 and anti-CCP3.1 assays using manufacturer recommended cutoff*

Diagnostic sensitivity/specificity of the CCP2, CCP3 and CCP3.1 assays were 66.4/98.3%, 77.3/96.6% and 85.7/89.8%, respectively. The area under the curve values for these tests were 0.909 (95% CI: 0.872-0.946), 0.911 (95% CI: 0.868-0.953) and 0.945 (95% CI: 0.916-0.974).

4.3.3. *Diagnostic performance of anti-CCP3 and anti-CCP3.1 assays using optimal cutoff*

Using ROC analysis, optimal cut-off values were established for each ELISA, and the data were recalculated. For the anti-CCP2, anti-CCP3 and anti-CCP3.1, the optimal cutoff levels were calculated as 12 U/ml, 15.6 U/ml and 23.7 U/ml. This resulted in 74.8/95.7%, 78.8/96.6% and 83.0/98.3% sensitivity and specificity for CCP2, CCP3 and CCP3.1 ELISA in RA. Quantitative anti-CCP3 and anti-CCP3.1 antibody levels correlated strongly.

*4.3.4. Association between anti-CCP3.1 and anti-CCP2 antibodies*

The serum levels of anti-CCP3.1 and anti-CCP2 antibodies were strongly correlated in RA population ($R=0.845$; $p<0.0001$). There were similarly strong correlation between positivity and negativity of anti-CCP3.1 and anti-CCP2 ($p=0.004$).

## 5. Discussion

### 5.1. Anti-MCV autoantibody

The newest member of this autoantibody family is anti-Sa, as citrullinated vimentin was identified as its target in 2004. An ELISA aiming at detection of antibodies against (modified) citrullinated vimentin was recently developed. The objective of this study was to assess the value of the anti-MCV assay and to compare it to the diagnostic performance of anti-CCP2 and RF tests.

Utilizing cutoff levels recommended by the manufacturers, anti-MCV showed 9% higher sensitivity than anti-CCP2, and 4% higher sensitivity than IgM RF; however, its diagnostic specificity was lower than that of anti-CCP2 (91.5% vs 98.8%). According to ROC analysis, the diagnostic performance of the anti-MCV ELISA for the diagnosis of RA was somewhat but not significantly lower than that of the anti-CCP2 test.

Categorizing the results by cutoffs resulting in the same 95% specificity for both tests, the sensitivity of the anti-MCV ELISA decreased (to 69.7%), while that of the anti-CCP2 assay increased (to 74.8%). Importantly, however, at optimal cutoff values, 29.4% of IgM RF-negative cases, as well as 13.3% of anti-CCP2-negative cases in the RA group, were anti-MCV-positive. Moreover, double positivity for anti- MCV and anti-CCP2 provided 98.3% specificity with 97.5% PPV.

### 5.2 Anti-CCP2 isotypes

Anti-CCP antibodies are considered as specific diagnostic and prognostic markers in RA. Second generation assays detect IgG antibodies against citrullinated epitopes in approximately 70-75% of RA patients. However, the prevalence and clinical significance of IgA and IgM anti-CCP antibodies have not been fully revealed. Although autoantibodies of IgG isotype are generally the most relevant ones for the assessment of various autoimmune diseases, IgA or IgM types may represent special significance in certain cases. In RA, RFs of all three isotypes can be present in the same patient. The prevalence of IgG and IgA RFs is usually lower than that of IgM RF, but the specificity of IgG and IgA isotypes may be higher.

Our results show that the levels of IgA and IgM anti-CCP2 antibodies are elevated in the sera of RA patients. Although these antibody isotypes are positive only in approximately half of the studied RA population, their diagnostic specificity exceeds that of any RF isotypes.

The presence of IgA or IgM anti-CCP2 antibody confirms the diagnosis of RA, and triple positivity increases the specificity of the anti-CCP2 test to 99.2%. Interestingly, significantly higher frequency of IgM anti-CCP2 antibodies was detected in early RA.

The duration of RA is calculated from the time of the diagnosis, which, according to several studies, sometimes is established with substantial delay after the first symptoms. Anti-

CCP antibodies of IgG isotype can be present in as much as half of RA patients before the development of symptoms. The predictive value of IgA and IgM anti-CCP antibodies has not been studied so far. The high prevalence of IgM anti-CCP2 antibodies in early RA strongly suggests, though, that these antibodies can be present along with, or even earlier than IgG anti-CCP2.

Differentiating RA from other rheumatic diseases, especially Sjögren's syndrome accompanied with RA-like polyarthritis, is a difficult diagnostic problem. The possibility of developing RA cannot be ruled out, and the anti-CCP2 positive pSS and PM patients in our study group (especially those with two or three anti-CCP2 antibody isotypes) require close clinical, laboratory and radiographic follow-up.

In summary, IgA and IgM anti-CCP2 antibodies are present in patients with RA, and they are similarly specific for the disease as IgG anti-CCP2. The production of IgA and IgM anti-CCP2 antibodies has been closely associated with that of IgG anti-CCP2 and with the presence of HLA-DRB1 SE alleles. IgM and IgA anti-CCP2 positivity strongly confirms the diagnosis of RA, as triple positivity represents 99.2% diagnostic specificity. IgM anti-CCP2 antibodies are more prevalent in early than in longstanding RA, which suggests that they are mostly produced during the first phase of immune response against citrullinated antigens. Our data suggest that the antibody response declines along the course of the disease in some RA patients, while it remains sustained in others.

### 5.3. Anti-CCP3 and – CCP3.1 assays

In our hands (using either manufacturer-suggested or optimal cut-off levels), CCP3 ELISA performed better than CCP2, and CCP3.1 performed better than CCP3. Anti-CCP3.1 ELISA, in addition to detecting IgA, seems to be more sensitive in detecting IgG antibodies, too, and represents significant improvement in the laboratory diagnosis of RA.

## 6. Summary- New results

1. We were among the first ones to use the new anti-MCV test in the world. We have established that the diagnostical value of anti-MCV and the earlier used second generation anti-CCP assay are similar, some of the patients show anti-CCP negativity while showing anti-CCP positivity. For this reason using a combination of the two tests we can identify a greater majority of patients.

2. In our experiments the third generation anti-CCP3 and even more the further developed anti-CCP3.1 assay shows a better diagnostical value than second generation anti-CCP2 test.

3. We were the first to examine the possible differential diagnostical role of anti-CCP isotypes. Besides the widely measured IgG isotype, the examination of IgA and IgM isotypes can have a role in RA. The appearance of IgM isotype in the early stage of the disease means that the examination of this isotype can make the recognition of RA easier.

In conclusion, the performance of the novel anti-MCV ELISA for the diagnosis of RA is similar to that of the anti-CCP2 test, however, as the diagnostic spectrum of the anti-MCV assay is somewhat different from that of anti-CCP2, the combined application of the two assays can improve the laboratory diagnostics of RA. In our hands, the third generation CCP3 and CCP3.1 ELISAs performed better than the CCP2 assays. The CCP3.1 assay may represent a significant improvement over the other two assays. Furthermore, IgA and IgM anti-CCP2 antibodies are present in RA patients, and they are similarly specific for RA as IgG anti-CCP2. The higher frequency of IgM anti-CCP2 antibodies in early RA suggests that they are mostly generated during the first phase of immune response; nonetheless, their production seems to be sustained in some patients. The introduction of anti-MCV, anti-CCP3.1 assays and the determination of anti-CCP isotypes may enable a more refined characterization of RA patients.

## Publications

**List of publications establishing the dissertation:**

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2. **Soós L**., Szekanecz Z., Szabó Z., Fekete A., Zeher M., Horváth IF., Dankó K., Kapitány A., Végvári A., Sipka S., Szegedi G., Lakos G.: Clinical evaluation of anti-mutated citrullinated vimentin by ELISA in rheumatoid arthritis. J Rheumatol, 34: 1658-1663, 2007.

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