Clinical Evaluation of Anti-Mutated Citrullinated Vimentin by ELISA in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Anti-cyclic citrullinated peptide (CCP) antibodies have emerged as sensitive and specific serological markers of rheumatoid arthritis (RA). However, antibodies to several other citrulline-containing proteins, including citrullinated fibrin and vimentin, have been detected in patients with RA, suggesting that citrulline is an essential constituent of autoantigens for RA-specific autoantibodies. We examined the diagnostic performance of the newly developed anti-mutated citrullinated vimentin (MCV) antibody assay.

Methods. Concentrations of anti-MCV, anti-CCP2, and rheumatoid factors (RF) were determined in the sera of 237 individuals: 119 patients with RA and 118 controls, including patients with other rheumatic diseases and healthy subjects. Diagnostic properties were compared by receiver-operating characteristic curve analysis.

Results. Using manufacturer's recommended cutoff values, sensitivity and specificity of anti-MCV antibodies were 75.6% and 91.5% in RA, compared to 66.4% and 98.3% for anti-CCP2. Introducing cutoff values to obtain the same 95% specificity resulted in decreased sensitivity of the anti-MCV test (69.7%) and increased sensitivity of the anti-CCP2 test (74.8%). At optimal cutoff levels, 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. Double-positivity for anti-MCV and anti-CCP2 provided 98.3% specificity with 97.5% positive predictive value in RA.

Conclusion. Overall, the performance of the novel anti-MCV ELISA for the diagnosis of RA is similar to that of the anti-CCP2 test [area under the curve 0.853 (95% CI 0.801–0.905) vs 0.910 (95% CI 0.873–0.946); p not significant]. As the diagnostic spectrum of the anti-MCV assay is somewhat different from that of anti-CCP2, the combined application of the 2 assays can improve the laboratory diagnostics of RA. (First Release July 1 2007; J Rheumatol 2007;34:1658–63)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
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Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation that ultimately leads to joint destruction[1]. 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[2]. Ultimately, synovial inflammation and hyperplasia lead to progressive destruction of the cartilage and bone, and can result in disability[1,2]. With new and very effective therapeutic approaches becoming available[3], 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.

Antibodies to citrullinated antigens, such as anti-cyclic citrullinated peptide (anti-CCP), have recently been identified as potential diagnostic and prognostic markers in RA[4-6]. They belong to the family of antibodies directing to epitopes containing the non-standard amino acid citrulline. Anti-citrullinated protein antibodies also include anti-perinuclear factor[7], anti-keratin[8], and anti-filaggrin antibodies[9-11]. Anti-CCP antibodies are not only highly specific for RA[6,12], but can be detected very early, sometimes even during the preclinical

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phase of the disease[13,14]. Anti-CCP positivity has been also associated with more destructive joint damage[15,16]. Recently, citrullinated vimentin was identified as the antigenic target for anti-Sa[17], thus making it a member of the family of anti-citrullinated protein antibodies.

These antibodies are present not only in the sera, but also in the synovial fluids of patients with RA[18-20]. They appear to be produced by local plasma cells in the inflamed joints, as they constitute a 7.5-fold greater proportion of IgG in the rheumatoid pannus than in paired sera[18]. Culture supernatants from synovial tissue fragments obtained from anti-fillagrin antibody-positive RA patients contain significant amounts of antibodies[18]. Moreover, B cells isolated from the synovial fluid of anti-CCP-positive RA patients were found to actively produce IgM anti-CCP antibodies[20]. These data suggest antigen-driven maturation of citrullinated protein-specific B cells at the site of inflammation. Indeed, synovial fluid CD38+ B cells from patients with RA show the imprints of an antigen-dependent process of somatic hypermutation and clonal selection[21]. Locally produced autoantibodies form immune complexes and may contribute to initiating and sustaining synovial inflammation by triggering monocyte and granulocyte activation and cytokine production.

Several types of citrullinated proteins were identified in the joints of patients with RA that can serve as potential autoantigens. Citrullinated proteins were observed in the lining layer, the sublining layer, and in extravascular fibrin deposits in inflamed synovium[22]. Extracellular fibronectin aggregates were also found to be citrullinated in RA synovial tissue[23]. Moreover, citrullinated fibrinogen was detected as a soluble autoantigen in RA synovial fluids[24]. The major synovial targets of the RA-specific antifilaggrin autoantibodies are thought to be the deiminated forms of the α and ß chains of fibrin[25]. This is supported by the demonstration of cross-reactivity between autoantibodies to filaggrin and citrullinated fibrin[26]. However, although a few studies suggest that the presence of citrullinated proteins in the synovial tissue is specific for RA[23,27], there is general agreement now indicating that citrullination commonly occurs in various types of synovitides[22,28]. An abnormal humoral response to citrullinated proteins, rather than their synovial expression, may be the specific feature in RA[22,28].

Anti-Sa is a specific diagnostic and prognostic marker in RA[29]. It has recently been shown that anti-Sa specifically recognizes citrullinated vimentin[17]. Vimentin is secreted and citrullinated by macrophages in response to apoptosis, or proinflammatory signals like tumor necrosis factor-α (TNF-α)[30,31]. Inflammatory leukocytes express 2 isoforms of the peptidylarginine-deiminase (PAD) enzyme, PAD2 and PAD4[32], responsible for the posttranslational generation of citrulline from arginine residues[33]. These results suggest that citrullinated vimentin may be a promising autoantigen in RA. To detect antibodies to citrullinated vimentin, an ELISA system was recently developed. The assay utilizes genetically modified citrullinated vimentin (MCV) to improve the performance of the test.

We assessed the diagnostic characteristics of the newly developed anti-MCV test in RA, and compared them to those of anti-CCP2, as well as IgG, IgA, and IgM rheumatoid factor (RF) assays. Our results indicate that the diagnostic performance of the anti-MCV assay was similar to that of anti-CCP2, but its diagnostic spectrum is somewhat different, which makes it a useful addition to the diagnostic repertoire of RA.

MATERIALS AND METHODS

Patients and controls. Serum samples were obtained from 119 patients with RA. All patients met the American College of Rheumatology classification criteria for the disease[34]. For comparisons, we tested 118 controls including 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 volunteers. The clinical records of the patients were reviewed for classification. All patients undergo regular followups at outpatient clinics of our institution. Serum samples were stored at –80°C for less than 1 year until the present analysis. The RA group consisted of 100 women and 19 men. The mean (± standard deviation) age of this group was 52.7 ± 12.5 years (range 19–77 yrs), which was not statistically different from that of the control subjects. The mean duration of RA was 10.2 ± 9.1 years (Table 1).

Autoantibodies. IgM, IgA, and IgG RF were determined by ELISA (ImmuLisa RF IgM, IgA and IgG; Immco Diagnostics, Buffalo, NY, USA) 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.

Anti-CCP2 IgG levels were measured using a second generation ELISA (Quanta Lite™ CCP IgG ELISA; Inova Diagnostics Inc., San Diego, CA, USA) utilizing synthetic citrullinated peptides bound to the surface of a microtiter plate as antigen. The test was performed according to the manufacturer's instructions, and values above 20 IU/ml were considered positive.

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.

Statistical analysis. Diagnostic sensitivity and specificity and positive (PPV) and negative predictive values were calculated for RF, anti-CCP2, and anti-MCV. The diagnostic performance of antibody assays was also examined by receiver-operating characteristic (ROC) curve analysis, by plotting sensitivity against 1–specificity at different cutoff values. Optimal cutoff values for anti-MCV and anti-CCP2 tests were determined based on ROC curve. Diagnostic sensitivities were compared at cutoff levels resulting in 95% specificity. Antibody levels between different groups were compared by the nonparametric Mann-Whitney U-test. Spearman's rank correlation was used to assess the

Table 1. Demographic and clinical characteristics of the study population.

	Age, yrs, mean ± SD	Male/Female Ratio
RA, n = 119	52.7 ± 12.5	19/100
pSS, n = 37	55.9 ± 15.2	2/35
PM/DM, n = 30	47.8 ± 14.5	6/24
OA, n = 7	56.6 ± 16.6	2/5
Healthy subjects, n = 44	45.8 ± 10.6	13/29

RA: rheumatoid arthritis; pSS: primary Sjögren's syndrome; PM/DM: polymyositis/dermatomyositis; OA: osteoarthritis.

relationship between anti-MCV, anti-CCP2, and RF levels. P values < 0.05 were considered significant. All statistical analyses were performed using the SPSS for Windows 11.0 statistical package.

RESULTS

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).

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 (Table 2), resulting in 75.6% diagnostic sensitivity (Table 3). 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 (Table 2).

Comparing RA patients with healthy individuals only, the specificity of all autoantibody assays was excellent (between 95.5% and 100%; Table 3). 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 (Table 2). 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) (Figure 1).

Table 3. Diagnostic sensitivity and specificity of anti-MCV and anti-CCP2 assays using optimal cutoff values.

Test, %	Anti-MCV	Anti-CCP2	Anti-MCV and Anti-CCP2	Anti-MCV and/or Anti-CCP2
Sensitivity	75.6	74.8	66.4	78.2
Specificity	91.5	95.8	98.3	91.5

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 (Table 3).

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%.

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 (Table 3). 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 (Table 4). On the other hand, 27.7% of anti-MCV-negative RA patients (10 subjects) had anti-CCP2 antibodies (Table 4).

Table 2. Diagnostic sensitivity and specificity, and positive and negative predictive values of IgM RF, anti-CCP2, and anti-MCV tests in RA at manufacturer recommended cutoff levels.

Test, %	RF IgM	RF IgA	RF IgG	Anti-CCP2	Anti-MCV
Sensitivity	71.4	36.9	37.8	66.4	75.6
Specificity* (RA/healthy subjects)	97.7	97.7	100	100	95.5
Specificity* (RA/all controls)	82.2	88.9	87.3	98.3	91.5
Positive predictive value	80.2	77.2	75.0	97.6	90.0
Negative predictive value	74.0	58.3	58.2	74.4	78.8

* Specificity was calculated for RA versus healthy controls only (RA/healthy subjects), and for RA versus healthy + disease controls (RA/all controls). RF: rheunatoid factor; CCP: cyclic citrullinated peptide; MCV: mutated citrullinated vimemtin; RA: rheumatoid arthritis.

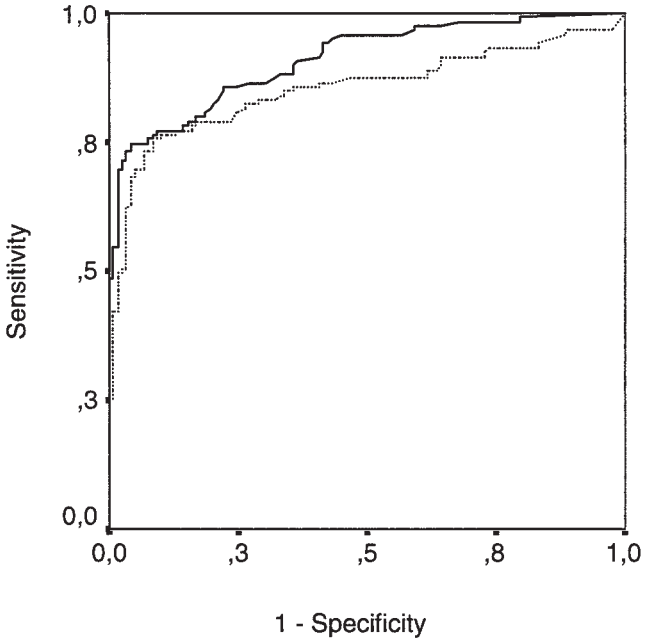

Figure 1. Receiver-operating characteristic curves of anti-MCV and anti-CCP2 ELISA. Area under the curve (AUC) value is a measure of the diagnostic efficacy of the test. Broken line: anti-MCV ELISA (AUC 0.853, 95% CI 0.801–0.905); solid line: anti-CCP2 ELISA (0.910, 95% CI 0.873–0.946). NS: not significant.

Table 4. Relationship between anti-MCV, anti-CCP2, and IgM RF positivity in the RA group (n = 119) at optimal cutoff levels. Data are shown as number of cases.

A. Agreement between anti-MCV and IgM RF results.

	Anti-MCV positive	Anti-MCV negative
RF IgM positive	73	12
RF IgM negative	10	24

B. Agreement between anti-MCV and anti-CCP2 results.

	Anti-MCV positive	Anti-MCV negative
Anti-CCP2 positive	79	10
Anti-CCP2 negative	4	26

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; Figure 2). 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.

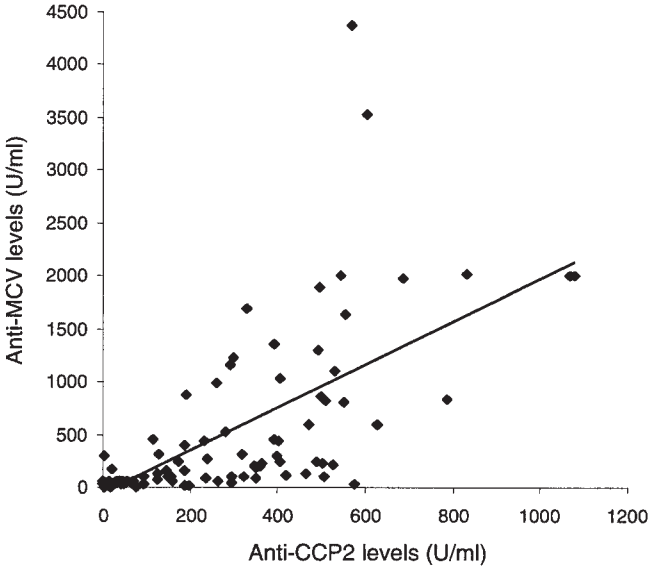

Figure 2. Correlation between anti-MCV and anti-CCP2 concentrations in patients with RA. Strongly significant correlation was found between anti-CCP2 and anti-MCV levels (Spearman's r = 0.783; p < 0.0001).

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).

DISCUSSION

Antibodies against citrullinated antigens have recently emerged as specific diagnostic and prognostic markers in RA[35]. The newest member of this autoantibody family is anti-Sa, as citrullinated vimentin was identified as its target in 2004[17]. An ELISA aiming at detection of antibodies against (modified) citrullinated vimentin was recently developed. The objective of our 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 [AUC 0.853 (95% CI 0.801–0.905) vs 0.910 (95% CI 0.873–0.946)].

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.

Our data confirm the results of Dejaco, *et al*, who found that using a cutoff value for the anti-MCV ELISA to obtain identical specificity compared to that of the anti-CCP2 test resulted in decreased sensitivity of the assay compared to anti-CCP2[36]. The authors of the latter study used a different anti-CCP2 assay, and although they found almost identical sensitivity for anti-MCV and anti-CCP2, they do not mention whether positivities were parallel, or if single-positive anti-MCV cases were present[36]. We found strong correlation between anti-MCV and anti-CCP2 titers in RA sera, and high levels of agreement between their occurrences. However, the presence of anti-MCV-positive patients in the anti-CCP2-negative group suggests that these antibodies may be directed to citrullinated epitopes present only in the anti-MCV assay, and the anti-MCV test is able to identify a number of patients with RA who tested seronegative for IgM RF or anti-CCP2 antibodies.

The composition of the antigen that initiates the autoimmune process in RA remains to be identified. Vimentin, however, is a promising candidate molecule, as it is present and citrullinated in macrophages invading the RA synovium[30,31]. Vimentin contains 43 arginine residues, which can be potentially citrullinated by PAD enzymes, resulting in numerous different citrullinated epitopes[17]. These data raise the possibility that antibodies to citrullinated vimentin are not only sensitive and specific serological markers of the disease, but are directly involved in the pathogenesis of RA[37].

Anti-Sa was originally identified by immunoblot in the sera of patients with RA using human spleen and placenta extracts as antigens[38]. Although it was present in only 50% of RF-positive and 27% of RF-negative subjects, it was essentially found only in RA (specificity 98.9%), and provided a 96.7% PPV[38]. Another study also found relatively low sensitivity (43.6%), but specificity and PPV as high as that of anti-CCP (specificity 93.6% vs 94.4%, PPV 86.3% vs 87.5%, respectively)[39]. Although the antigen of anti-Sa is citrullinated vimentin, we observed that anti-MCV antibodies are much more sensitive, but are similarly specific to RA than anti-Sa. This can be partly explained by technical differences in the detection (immunoblot vs ELISA), but presumably by the improved reactivity of the (modified) antigen, too. Anti-Sa and anti-CCP results were discordant in about half of the patients with RA in the above mentioned report, and the authors have suggested anti-Sa as a useful complementary assay[39]. In our study, we found high agreement between the results of anti-MCV and anti-CCP2; nonetheless, we also detected 14 single anti-CCP or anti-MCV-positive patients, justifying the usefulness of combined application. Anti-Sa has also been associated with disease severity[29,40], and was superior to anti-CCP in predicting more rapid and more severe joint damage in previous cohorts[40]. Direct comparison of the performance of anti-Sa and anti-MCV antibodies should be an important direction of future research, to explore if the anti-MCV assay in fact combines the excellent diagnostic and prognostic features of anti-Sa with the ease and objectivity of the ELISA method. The capability of anti-MCV for monitoring disease activity also remains to be investigated.

The anti-MCV ELISA is a new, sensitive, and specific serological test in RA. It is characterized by diagnostic performance similar to the anti-CCP2 assay; however, our results show that its diagnostic spectrum is somewhat different. The combined application of anti-CCP2 and anti-MCV tests can improve the laboratory diagnosis of RA, thus promoting early diagnosis and treatment.

REFERENCES

1. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev 2005;4:130-6.
2. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001;358:903-11.
3. Goldblatt F, Isenberg DA. New therapies for rheumatoid arthritis. Clin Exp Immunol 2005;140:195-204.
4. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998;101:273-81.
5. Girbal-Neuhauser E, Durieux JJ, Arnaud M, et al. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. J Immunol 1999;162:585-94.
6. Schellekens GA, Visser H, de Jong BA, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63.
7. Nienhuis RLF, Mandema EA. A new serum factor in patients with rheumatoid arthritis. The antiperinuclear factor. Ann Rheum Dis 1964;23:302-5.
8. Young BJ, Mallya RK, Leslie RD, Clark CJ, Hamblin TJ. Anti-keratin antibodies in rheumatoid arthritis. BMJ 1979;2:97-9.
9. Simon M, Girbal E, Sebbag M, et al. The cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies," autoantibodies specific for rheumatoid arthritis. J Clin Invest 1993;92:1387-93.
10. Vincent C, Simon M, Sebbag M, et al. Immunoblotting detection of autoantibodies to human epidermis filaggrin: a new diagnostic test for rheumatoid arthritis. J Rheumatol 1998;25:838-46.
11. Vincent C, Nogueira L, Sebbag M, et al. Detection of antibodies to deiminated recombinant rat filaggrin by enzyme-linked immunosorbent assay: a highly effective test for the diagnosis of rheumatoid arthritis. Arthritis Rheum 2002;46:2051-8.
12. Zendman AJ, Vossenaar ER, van Venrooij WJ. Autoantibodies to citrullinated (poly)peptides: a key diagnostic and prognostic marker for rheumatoid arthritis. Autoimmunity 2004;37:295-9.
13. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies

against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741-9.
14. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004;50:380-6.
15. Meyer O, Labarre C, Dougados M, et al. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis 2003;62:120-6.
16. Kroot EJ, de Jong BA, van Leeuwen MA, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831-5.
17. Vossenaar ER, Despres N, Lapointe E, et al. Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. Arthritis Res Ther 2004;6:R142-50.
18. Masson-Bessiere C, Sebbag M, Durieux JJ, et al. In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. Clin Exp Immunol 2000;119:544-52.
19. Iwaki-Egawa S, Matsuno H, Ogawa Y, Watanabe Y. Production of anti-CCP antibodies and matrix metalloproteinase-3 by human rheumatoid arthritis synovial tissues using SCID mice. Ann Rheum Dis 2005;64:1094-5.
20. Reparon-Schuijt CC, van Esch WJ, van Kooten C, et al. Secretion of anti-citrulline-containing peptide antibody by B lymphocytes in rheumatoid arthritis. Arthritis Rheum 2001;44:41-7.
21. Van Esch WJ, Reparon-Schuijt CC, Hamstra HJ, et al. Human IgG Fc-binding phage antibodies constructed from synovial fluid CD38+ B cells of patients with rheumatoid arthritis show the imprints of an antigen-dependent process of somatic hypermutation and clonal selection. Clin Exp Immunol 2003;131:364-76.
22. Vossenaar ER, Smeets TJ, Kraan MC, Raats JM, van Venrooij WJ, Tak PP. The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. Arthritis Rheum 2004;50:3485-94.
23. Chang X, Yamada R, Suzuki A, Kochi Y, Sawada T, Yamamoto K. Citrullination of fibronectin in rheumatoid arthritis synovial tissue. Rheumatology Oxford 2005;44:1374-82.
24. Takizawa Y, Suzuki A, Sawada T, et al. Citrullinated fibrinogen detected as a soluble citrullinated autoantigen in rheumatoid arthritis synovial fluids. Ann Rheum Dis 2006;65:1013-20.
25. Masson-Bessiere C, Sebbag M, Girbal-Neuhauser E, et al. The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. J Immunol 2001;166:4177-84.
26. Sebbag M, Moinard N, Auger I, et al. Epitopes of human fibrin recognized by the rheumatoid arthritis-specific autoantibodies to citrullinated proteins. Eur J Immunol 2006;36:2250-63.
27. Baeten D, Peene I, Union A, et al. Specific presence of intracellular citrullinated proteins in rheumatoid arthritis synovium: relevance to antifilaggrin autoantibodies. Arthritis Rheum 2001;44:2255-62.
28. Chapuy-Regaud S, Sebbag M, Baeten D, et al. Fibrin deimination in synovial tissue is not specific for rheumatoid arthritis but commonly occurs during synovitides. J Immunol 2005;174:5057-64.
29. Hayem G, Chazerain P, Combe B, et al. Anti-Sa antibody is an accurate diagnostic and prognostic marker in adult rheumatoid arthritis. J Rheumatol 1999;26:7-13.
30. Asaga H, Yamada M, Senshu T. Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. Biochem Biophys Res Commun 1998;243:641-6.
31. Mor-Vaknin N, Punturieri A, Sitwala K, Markovitz DM. Vimentin is secreted by activated macrophages. Nat Cell Biol 2003;5:59-63.
32. Vossenaar ER, Radstake TR, van der Heijden A, et al. Expression and activity of citrullinating peptidylarginine deiminase enzymes in monocytes and macrophages. Ann Rheum Dis 2004;63:373-81.
33. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays 2003;25:1106-18.
34. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
35. Vincent C, Nogueira L, Clavel C, Sebbag M, Serre G. Autoantibodies to citrullinated proteins: ACPA. Autoimmunity 2005;38:17-24.
36. Dejaco C, Klotz W, Larcher H, Duftner C, Schirmer M, Herold M. Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. Arthritis Res Ther 2006;8:R119.
37. Lundberg K, Nijenhuis S, Vossenaar ER, et al. Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. Arthritis Res Ther 2005;7:R458-67.
38. Despres N, Boire G, Lopez-Longo FJ, Menard HA. The Sa system: a novel antigen-antibody system specific for rheumatoid arthritis. J Rheumatol 1994;21:1027-33.
39. Lopez-Longo FJ, Rodriguez-Mahou M, Sanchez-Ramon S, et al. Anti-cyclic citrullinated peptide versus anti-Sa antibodies in diagnosis of rheumatoid arthritis in an outpatient clinic for connective tissue disease and spondyloarthritis. J Rheumatol 2006;33:1476-81.
40. Boire G, Cossette P, de Brum-Fernandes AJ, et al. Anti-Sa antibodies and antibodies against cyclic citrullinated peptide are not equivalent as predictors of severe outcomes in patients with recent-onset polyarthritis. Arthritis Res Ther 2005;7:R592-R603.