Antibodies Against Mutated Citrullinated Vimentin Are a Better Predictor of Disease Activity at 24 Months in Early Rheumatoid Arthritis Than Antibodies Against Cyclic Citrullinated Peptides

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ABSTRACT. ***Objective.*** To evaluate the predictive values for disease progression of various antibodies against citrullinated peptide proteins (ACPA) and their relation to PTPN22 1858C/T polymorphism and HLA-DRB1 alleles in early rheumatoid arthritis (RA).

Methods. The ACPA, e.g., antibodies against mutated citrullinated vimentin (MCV), cyclic citrullinated peptides (CCP) type 2 and 3 (both of IgG isotype) and 3.1 (of both IgG and IgA isotypes), were analyzed at baseline in patients with early RA (n = 210) and in population controls (n = 102) using an enzyme immunoassay. A receiver-operating characteristic curve was constructed for each antibody. Disease activity [swollen and tender joints, visual analog scale for global health, and erythrocyte sedimentation rate (ESR)] was evaluated at baseline and regularly for 24 months. Radiographs of hands and feet were graded using the Larsen score.

Results. Patients with anti-MCV antibodies had significantly less reduction in Disease Activity Score (DAS28) over time ($p < 0.01$), and significantly increased area under the curve (AUC) for DAS28 ($p < 0.05$), ESR ($p < 0.01$), C-reactive protein ($p < 0.01$), and swollen joint count ($p = 0.057$) compared to those without. Corresponding differences were not found in patients with anti-CCP2, CCP3, and CCP3.1 antibodies. Radiological progression ($p < 0.0001$–0.01) and radiological outcome ($p < 0.0001$–0.01) at 24 months were significantly predicted by all ACPA after baseline adjustments. PTPN22 T variant and HLA-DRB1 alleles were not related to radiological progression or inflammatory activity over time.

Conclusion. Anti-MCV antibodies are associated with a more severe RA disease, as measured by DAS28, ESR, and swollen joint count over time, compared with anti-CCP2, CCP3, and CCP3.1 antibodies. Radiological progression was predicted equally by all 4 autoantibodies. (First Release April 1 2008; J Rheumatol 2008;35:1002–8)

Key Indexing Terms:

ANTI-CYCLIC CITRULLINATED PEPTIDE 2 ANTIBODIES
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Detection of antibodies against citrullinated peptides/proteins (ACPA) has become a valuable tool in the diagnosis of rheumatoid arthritis (RA) and in predicting clinical outcome[1-3]. It is important to distinguish patients with early disease who will develop mild disease with a good prognosis from those with a severe disease with a poor prognosis to optimize therapy for each patient.

The test described recently for antibodies against anti-mutated citrullinated vimentin (anti-MCV) is a further development of the protocol for detecting antibodies to naturally citrullinated vimentin, e.g., Sa antigen[4]. These anti-Sa antibodies are highly specific for RA and are predominant-

ly of the IgG isotype[4,5]. The occurrence of vimentin in synovial cells in RA, and the recent observation that vimentin is secreted and modified by macrophages depending on the proinflammatory signals, makes this protein an interesting autoantigen in RA[6,7]. An ELISA for quantitative measurement of IgG isotype autoantibodies against MCV in serum/plasma, with the same sensitivity as anti-cyclic citrullinated peptide antibody (anti-CCP2) but a lower specificity, has been described[6]. However, it has been proposed as a better prognostic marker for RA compared with the anti-CCP2 ELISA[7]. As a further development of the anti-CCP2 test, an anti-CCP3 test for detecting IgG antibodies has been developed, as has another test detecting both IgG and IgA antibodies (anti-CCP3.1 ELISA)[8].

Association between HLA-DRB1 and RA is well documented[9], and recently PTPN22 C1858T, a gene outside the HLA region, has also been associated with RA[10]. We have previously shown that the presence of anti-CCP antibodies together with carriage of either the shared epitope (SE) gene or the PTPN22 T-variant is associated with high relative risk for future development of RA[11,12]. The SE has been identified as a marker of more severe disease progression, although this is probably an effect of the association with anti-CCP antibodies[13,14]. Thus, the presence of anti-CCP antibodies in patients with RA seems to identify a more severe phenotype of the disease[15]. The effect of PTPN22 polymorphism for disease severity and progression is less well analyzed, although an increased radiological progression has been suggested in those carrying the T-variant[16-18].

Our aim was to evaluate, in patients with early RA followed for 24 months, the predictive values for disease activity and progression of the presence of various citrullinated antibodies, e.g., anti-MCV antibodies, anti-CCP2 and anti-CCP3 antibody tests of IgG isotype and a test of IgG and IgA isotypes together (anti-CCP3.1 antibodies), in relation to the SE and PTPN22 1858C/T polymorphism. The diagnostic power for RA of the various ACPA tests was compared using healthy controls.

MATERIALS AND METHODS

Patients and controls. Two hundred ten patients (145 female, 65 male) with early RA fulfilling the American Rheumatism Association classification criteria[19] attending the Department of Rheumatology, University Hospital, Umeå, were consecutively included into the study and followed regularly for 2 years. Age (mean ± SD) at the onset of disease was 52.5 ± 16.1 years for women and 57.7 ± 12.5 years for men. The mean duration of symptoms at inclusion was 6.4 months (range 0–12 mo). At baseline, 40.5% (n = 81) were prescribed prednisolone and 76% (n = 152) disease modifying antirheumatic drugs (DMARD), of whom 58 individuals were being treated with prednisolone and 43 with DMARD before inclusion in the study. During the 24 months, 95.7% (n = 201) of the patients were treated with DMARD, 36.5% (n = 77) with a combination therapy, and 52% (n = 109) with prednisolone. Ten patients were prescribed a tumor necrosis factor (TNF) blocker during the 24 months.

Control subjects (n = 102) were randomly selected, self-stated healthy individuals from the same geographical area and with the same ethnic background as the patients.

The Regional Ethics Committee approved this study at the University Hospital Umeå and all participants gave their written informed consent.

Enzyme immunoassays for ACPA. ACPA were measured in plasma from patients at baseline and in controls. Plasma was also sampled in the first 148 patients reaching 24 months. Anti-CCP2 antibodies were measured using the Diastat kit (Axis-Shield Diagnostics, Dundee, UK), with a cutoff value of 5 units/ml; and Quanta Lite™ anti-CCP3 using an ELISA for IgG (3rd generation antigen; Inova Diagnostics, San Diego, CA, USA) and for anti-CCP3.1 antibodies (Quanta Lite™, Inova) detecting both antibodies of IgG and IgA class (cutoff value 20 units/ml both tests). Tests for anti-CCP3 and anti-CCP3.1 antibodies were provided free to the Department of Clinical Immunology for testing evaluation. Anti-MCV antibodies were measured using the Orgentec 548 Anti-MCV ELISA (Orgentec Diagnostika GmbH, Mainz, Germany; cutoff value 20 units/ml) according to the manufacturer's instructions. There was no blood sample collection in 10 patients for various reasons (e.g., unwillingness of a patient to donate samples, empty tubes, forgetfulness by the patient or the nurse, etc.), leading to an incomplete number of samples for all antibodies being studied.

Waaler-Rose test for rheumatoid factor (RF). IgM class RF was analyzed by the Waaler-Rose hemagglutination test with sensitized sheep red blood cells.

Genotyping. PTPN22 1858C/T polymorphisms were determined using the 5'-nuclease assay[12]. Detection of the different genotypes was made using an ABI 7900HT Sequence Detector System (Applied Biosystems, Foster City, CA, USA) using primers and probes designed by Applied Biosystems. Data were processed using SDS 2.1 software.

HLA-DRB1 genotyping was performed using polymerase chain reaction sequence-specific primers from a DR low-resolution kit and DRB1*04 subtyping kit (Dynal, Oslo, Norway) as described[11]. The SE alleles were defined as HLA-DRB1*0401 or DRB1*0404.

Measures of disease activity. Clinical examination was assessed with the 28-joint count of tender and swollen joints and with a global health visual analog scale (VAS), and erythrocyte sedimentation rate (ESR, mm/h), and blood levels of C-reactive protein (CRP, mg/l) were measured using routine methods. Disease Activity Score (DAS28) was calculated[20] at baseline and after 6, 12, 18, and 24 months. The response to therapy was determined according to the EULAR 28 response criteria based on DAS28[21].

Measures of disease outcome. Anterior-posterior radiographs of the hands, wrists, and feet obtained at baseline (n = 182) and after 2 years (n = 155) were graded in blinded fashion by 2 rheumatologists specially trained in evaluation of radiographs, according to the Larsen score[22], by comparison with standard reference radiographs[22]. Radiological progression was defined as an increase in Larsen score from baseline to 2 years that was greater than the median value.

Statistics. Differences in continuous data between 2 groups were analyzed using an independent t-test and those from 2 different timepoints for the same individual with a paired t-test. Nonparametric analyses were used when the variables were not normally distributed. Variations over time between groups were assessed by analysis of variance for repeated measurements (StatView v. 4.51; Abacus Concepts, Berkeley, CA, USA). The chi-square test was used for testing categorical data. Multiple regression analyses were performed using the ANOVA general linear model. Variables were chosen with respect to results of simple regression analyses and/or clinical assumptions. Backward logistic regression analyses were used to estimate the odds ratio for radiological progression at 2 years. The degree of explanation of variations in the dependent variable given by the independent variables was expressed as Nagelkerke R^2 or R-square. All p-values are 2-sided, and p-values ≤ 0.05 were considered statistically significant. Calculations were performed using SPSS for Windows (v. 11.5; SPSS, Chicago, IL, USA). Area under the curve (AUC) at the 24-month timepoint was calculated for all clinical and laboratory variables[23]. Receiver-operating characteristic (ROC) curves were constructed for each ACPA and kappa values calculated for concordance between tests.

RESULTS

ROC curves for the various ACPA (Figure 1) and the highest sensitivity for the best specificity in detecting RA are presented in Table 1. AUC of the ROC curves for anti-MCV ELISA were 0.91 (95% CI 0.88–0.95), for anti-CCP2 0.88 (95% CI 0.83–0.92), for anti-CCP3 0.90 (95% CI 0.86–0.93), and for anti-CCP3.1, 0.91 (95% CI 0.88–0.95). The highest sensitivity (80.5%) was for the presence of anti-CCP3.1 antibodies, with a specificity of 95.1%, followed by anti-CCP2 antibodies with a sensitivity of 80.4%. The specificity was 98% for both anti-CCP2 and anti-CCP3 antibodies, while that for anti-MCV antibodies was 96% (Table 1). The cutoff values for the antibodies, except that for anti-CCP2 antibodies, were in agreement with those given by the manufacturers (20 units/ml, respectively). The data for anti-CCP2 antibodies are presented using a cutoff value of 3 units; when using the cutoff value suggested by the manufacturer the sensitivity decreased to 74.6% with the same specificity of 98%. The likelihood ratio (LR) was highest (41.0) for anti-CCP2 antibodies, closely followed by anti-CCP3 antibodies (40.3; Table 1). The 95% CI for all antibodies were overlapping. At a fixed specificity chosen at 98% for all antibodies the sensitivity for anti-MCV antibodies decreased to 69.0% and for anti-CCP3.1 antibodies to 78.5%. The LR were still highest for anti-CCP2 and anti-CCP3 antibodies. The degree of concordance, according to the manufacturers' definitions, was high between the various ACPA, i.e., between 89.5% and 97.5% (data not shown), with kappa values of 0.689 to 0.923. The sensitivity for the presence of RF was 82.9% at a specificity of 95%. In patients who were RF-seropositive, ACPA were present in 84.1% to 88.8% (data not shown). There were significant associations between the SE and anti-MCV antibodies/anti-CCP2 antibodies/anti-CCP3 antibodies (chi-square = 16.4, 1

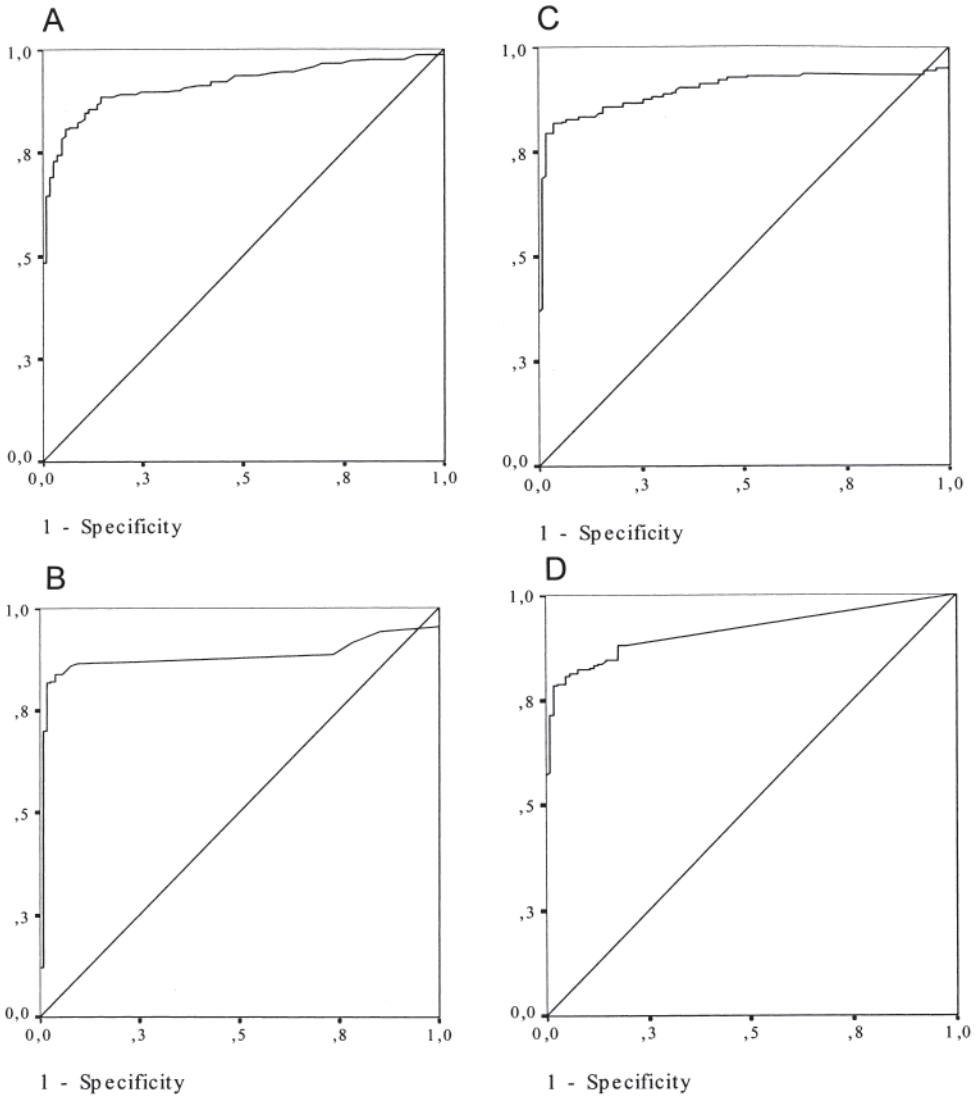

Figure 1. ROC curves in patients with early RA compared with healthy controls: A. anti-MCV; B. anti-CCP2; C. anti-CCP3; D. anti-CCP3.1.

Table 1. Sensitivity, specificity, and positive likelihood ratios (LR) calculated using the ROC curves and at given specificity of 98% for all tests.

Antibodies	n	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity at 98%, Specificity (95% CI)	LR* (95% CI)
Anti-CCP2	192	80.4 (74.3–85.6)	98.0 (93.7–99.7)	80.4 (74.3–85.6)	41.0 (10.3–162.1)
Anti-MCV	200	74.0 (67.5–79.8)	96.1 (90.8–98.7)	69.0 (62.3–75.1)	35.2 (8.9–139.2)
Anti-CCP3	200	79.0 (72.9–84.2)	98.0 (93.6–99.7)	79.0 (72.9–84.2)	40.3 (10.2–159.2)
Anti-CCP3.1	200	80.5 (74.5–85.6)	95.1 (84.4–98.2)	78.5 (72.4–83.8)	40.0 (10.1–158.2)

* Calculated at a specificity for all of 98%.

df, $p < 0.001$; chi-square = 9.3, 1 df, $p < 0.01$; and chi-square = 5.62, 1 df, $p < 0.05$, respectively) and borderline significance between the SE and anti-CCP3.1 antibodies (chi-square = 3.30, $p = 0.068$). There was only a borderline significant association for carriage of PTPN22 T-variant with anti-CCP3 antibodies. Since the number of individuals included in all the ACPA groups was not the same, the data were recalculated based on the 192 RA patients tested for all antibodies, with essentially the same results, with differences only at the decimal level.

There was a significant reduction in DAS28 over time ($p < 0.0001$) from a mean ± SEM of 4.8 ± 0.9 to 3.3 ± 1.0. Patients with anti-MCV antibodies, defined by the ROC curve, had significantly less reduction compared with those without (ANOVA; F-value = 3.391, $p < 0.01$). No significant differences were found in DAS28 over time in patients positive for anti-CCP2, CCP3, or CPP3.1 antibodies analyzed separately, compared with patients negative for these antibodies. The AUC for DAS28 in anti-MCV antibody-positive patients was significantly ($p = 0.05$) higher compared with seronegative patients. The difference in AUC values between patients seropositive for any of the other ACPA, or for RF, compared with seronegative individuals did not reach statistical significance.

Patients with anti-MCV antibodies had significantly smaller reductions in the number of swollen joints during the 24 months compared with those without the antibodies (ANOVA $p < 0.01$ for both analyses; data not shown). There was also a significantly smaller reduction over time in anti-CCP2 antibody-positive patients compared with antibody-negative patients for using a cutoff value of either 3 or 5 units/ml. However, this significance was apparently associated with significantly different levels at baseline (data not shown). AUC for ESR and CRP were also significantly increased, and AUC was borderline for swollen joint count ($p = 0.057$) in anti-MCV antibody-positive patients compared with negatives; these differences were not detected for any other antibodies, with the exception of a borderline significance for the AUC for ESR in RF-positive versus RF-negative patients ($p = 0.053$).

The Larsen score increased significantly for the patients as a group during the 2 years of the study, from a median value (quartile range) of 7 (2–12) at baseline to 11 (6–17) at 24 months. When stratified for those positive or negative for ACPA, patients with any of the antibodies had significantly higher Larsen scores after 2 years (Table 2). The same pattern was seen for all of the ACPA and for anti-CCP2 using the manufacturer's cutoff of 5 units/ml (data not shown). No significant difference in Larsen score at baseline or at 2 years was detected between patients with and those without RF (Table 2). A higher Larsen score at 24 months was significantly predicted in multiple regression analyses by the baseline values for the Larsen score, swollen joint count, and for all 4 ACPA, for both cutoff levels for anti-CCP2 antibodies and for RF, with similar significance levels and degree of explanation (Table 3).

Patients with radiological progression at 24 months already had at baseline significantly higher concentrations of anti-MCV (median levels 205 and 66 units/ml; $p < 0.05$), anti-CCP3 (305 and 222 units/ml; $p < 0.05$), and anti-CCP3.1 antibodies (326 and 219 units/ml; $p < 0.05$), compared to patients without progression. Patients positive for any of the 4 ACPA had significantly more frequent radiological progression compared with antibody-negative patients (chi-square = 11.15, $p < 0.001$ for anti-MCV antibodies; chi-square = 10.51, $p < 0.001$ for anti-CCP2 antibodies; chi-square = 17.23, $p < 0.001$ for anti-CCP3 antibodies; chi-square = 16.11, $p < 0.001$ for anti-CCP3.1 antibodies).

Backward stepwise logistic regression analyses were performed to identify predictors for radiological progression at 2 years. Baseline values of any of the ACPA or RF (yes/no), swollen joint count, ESR, Larsen score, SE, or carriage of the T-variant of PTPN22 and therapeutic response at 6/12/24 months, respectively, were included. Positive test for ACPA or RF and ESR at baseline significantly predicted radiological progression at 24 months. Therapeutic response at 6, 12, or 24 months included in the same analysis models significantly predicted less radiographic progression (Table 4). In the models with anti-CCP2 antibodies or with anti-MCV antibodies, 23% and 18% of the variation, respectively, was explained, and 65% and 67% of the cases were correctly classified (Table 4). As well, anti-CCP3 and anti-CCP3.1 antibodies and RF remained significant predictors for radiological progression calculated with therapeutic response at 6 months (OR 6.42, $p < 0.0001$; OR 7.45, $p < 0.0001$; OR 3.22, $p < 0.05$, respectively) and 12 and 24 months (data not

Table 2. Larsen score, given as median (interquartile range), at baseline and at 2 years, stratified for presence of citrullinated antibodies and IgM-RF.

	Anti-MCV		Anti-CCP2		IgM-RF	
	Positive	Negative	Positive	Negative	Positive	Negative
Baseline	7 (2–12) (n = 130)	5.5 (0–11.3) (n = 46)	6 (2–11) (n = 128)	5 (0–10) (n = 46)	6 (1–11) (n = 151)	6 (0–10.5) (n = 31)
2 years	12** (7–18) (n = 112)	9 (3–15.8) (n = 40)	12* (7–18) (n = 110)	7 (2–12) (n = 39)	12 (6–18) (n = 130)	9 (4–14.3) (n = 26)

n: Number of individuals who had radiographs. * $p < 0.05$, ** $p < 0.01$ for comparison of progression of values in positive versus negative groups. All comparisons between baseline versus 24 months were $p < 0.001$, except for anti-CCP2-negative values, $p < 0.01$ (analyzed for matched pairs).

Table 3. Multiple regression analyses for radiological outome at 24 months.

Baseline Variables	ß	p	ß	p	ß	p
Larsen score	0.98	< 0.0001	1.0	< 0.0001	1.0	< 0.0001
Swollen joints	0.30	< 0.01	0.24	< 0.009	0.21	< 0.05
IgM-RF	3.5	< 0.05				
Anti-CCP2 antibody (cutoff 3 u/ml)			5.1	< 0.0001		
Anti-MCV antibody					3.4	< 0.01
Adjusted R-squared*		47.2%		60.2%		58.4%

ß: regression coefficient. * Adjusted for number of predictors.

shown), with the exception for therapeutic response at 24 months for the anti-CCP3.1 test (data not shown). The percentage correctly classified in models with therapeutic response at 6 months was 66% for both anti-CCP3 and CCP3.1 antibodies and 62% for RF, and the variation was explained by 22%, 23%, and 15%, respectively (data not shown). Calculating the data with the cutoff at 5 units for anti-CCP2 antibodies gave very similar results (data not shown). The results were equivalent after adjustments for SE or PTPN22 T-variant (data not shown).

All the analyses for anti-MCV and anti-CCP3.1 were also undertaken at 98% specificity. The AUC calculations revealed that the significant difference found in the anti-MCV antibody test remained for swollen joint count, ESR, and CRP ($p < 0.05$) and was borderline for DAS28 ($p = 0.074$). The AUC differences between anti-CCP3.1 antibody-positive or negative patients were unchanged, i.e., not significantly different (data not shown). The analyses for Larsen score at baseline and at 24 months, and prediction of radiological outcome and of radiological progression by these 2 antibodies were practically the same as calculated on ROC curve data. The predictability for radiological outcome ($ß = 1.9$, $p < 0.086$ and $ß = 4.1$, $p < 0.001$, respectively) and the OR for radiological progression (OR 2.5, $p < 0.01$ and OR 5.02, $p < 0.0001$) was slightly decreased for both.

The concentrations at baseline of any of the ACPA did not differ in patients already receiving DMARD (n = 43) or prednisolone (n = 58) before baseline, compared with DMARD-naive patients. ACPA were retested at the followup in 148 patients. There was a slight reduction in the concentrations of all antibodies; however, the differences were not significant. When the data were stratified for response to therapy as nonresponders versus good–moderate

Table 4. Predictors of radiological progression at 2 years in patients with early RA. Variables included in the backward stepwise logistic regression analyses were baseline values of the antibodies, swollen joint count, ESR, Larsen score, SE, and therapeutic response at 6 months.

Variables	Model 1 OR (95%CI)	p	Model 2 OR (95% CI)	p
ESR (baseline)	1.02 (1.001–1.037)	< 0.05	1.02 (1.000–1.035)	0.051
Therapeutic response at 6 months*	0.407 (0.19–0.859)	< 0.05	0.430 (0.209–0.883)	< 0.05
Anti-CCP2 antibodies (baseline)**	7.76 (2.51–23.97)	< 0.001		
Anti-MCV antibodies (baseline)			3.45 (1.47–8.08)	< 0.01

* Therapeutic response at 12 (OR 0.429 and 0.523; p = 0.036 and 0.089, respectively) or 24 months (OR 0.44 and 0.483; p = 0.046 and 0.069, respectively). ** Cutoff at 3 units according to ROC curve.

responders, anti-MCV-positive patients with a therapeutic response at 6 months ($p < 0.05$) had, at 24 months, a significant reduction in antibody concentration and almost significant reduction in antibody concentration with therapeutic response at 24 months ($p < 0.08$). There was also a reduction of antibody concentration in patients having a therapeutic response at 12 months; however, this was not statistically significant. In none of the other ACPA was there a significant difference in concentrations, irrespective of therapeutic response.

DISCUSSION

Our objective was to analyze the predictive values of the presence of ACPA at the onset of RA for disease progression and outcome, rather than to evaluate the different antibody tests per se. Presence of anti-MCV antibodies, defined as positive by the ROC curve or calculated at a preset specificity of 98%, indicated a more persistent disease activity as measured by DAS28, number of swollen joints, or ESR/CRP. In the study of Bang, *et al* the anti-MCV antibodies correlated better with disease activity, compared with the anti-CCP antibodies[7]. Our observation that the presence of anti-MCV antibodies at disease onset is associated with a more severe disease course, measured as higher level of inflammatory activity (e.g., smaller reduction of DAS28 and higher numbers of swollen joints over time) compared with anti-CCP2 or CCP3 antibodies, is consistent with these results.

Patients with any of the ACPA studied had increased radiological progression and worse radiological outcome 2 years after diagnosis compared to those without these antibodies. However, the predictability of all antibodies for radiological progression or radiological outcome after 2 years was equal. Boire, *et al* showed that the presence of anti-Sa antibodies was associated with more persistent arthritis and severity as measured by modified Health Assessment Questionnaire and/or radiography compared with anti-CCP antibodies or RF[24]. Thus, our findings of increased DAS28 and swollen joint count in anti-MCV-positive patients are concordant with those of persistent arthritis, but not the radiological progression that was equal for both anti-MCV and anti-CCP antibodies.

We found using ROC curves for each antibody that sensitivity was highest for anti-CCP3.1 antibodies, followed by anti-CCP2 antibodies, for patients with early RA compared with healthy controls. The specificity was highest, 98%, for anti-CCP2 and anti-CCP3 antibodies. Further, the cutoff values were equivalent to those of the manufacturers' values, except for the anti-CCP2 antibody test, which gave the highest sensitivity using a cutoff value of 3 units/ml based on the calculated ROC curve. We found essentially the same accuracy for all 4 ACPA as a diagnostic marker, the AUC for each being within the same confidence limits (0.88–0.92).

The highest likelihood ratio for diagnosing RA was for anti-CCP2 and anti-CCP-3 antibodies, albeit with overlapping confidence intervals between all of the antibodies. The anti-CCP3.1 antibody ELISA test detects both IgG and IgA antibodies, giving it higher sensitivity than anti-CCP2 and anti-CCP3 antibody tests. The observations are consistent with our previous finding that IgA-RF has higher sensitivity for predicting RA compared with IgG-RF in samples from individuals analyzed before disease onset[25]. With a comparable specificity set at 98% for anti-MCV and anti-CCP3.1 antibodies, the sensitivity of these 2 antibodies decreased, giving the lowest LR for the anti-MCV antibody test of all ACPA. Applying the cutoff value for anti-CCP2 antibodies cited by the manufacturer (5 units/ml) decreased the sensitivity, but the predictive values for inflammatory activity and radiological progression were unchanged.

In another study, comparison between anti-MCV antibody and anti-CCP2 antibody tests showed that at the same level of sensitivity (65.3%), the specificity of the anti-MCV antibody ELISA was clearly lower (91.5% compared with 98.7%)[6]. However, that study included patients with established RA and the controls were non-RA patients, whereas we compared patients with early RA and healthy controls. The magnitude of the LR was almost identical in both studies. In another recent study of patients with established RA, both the sensitivity and the specificity were higher for anti-MCV antibodies[7]. The cutoff level for anti-MCV antibodies in that study was set at mean + 3 SD antibody reactivity in sera from blood donors[7]. A comparable performance of 6 different ACPA assays, including 3 used in our study, using the manufacturer's cutoff values, was described for early and established RA[8]. Among the 3 comparable tests there was also a tendency for lower sensitivity for the anti-MCV antibody test. However, in general the specificity is higher when healthy controls are used rather than non-RA populations to define cutoffs. The quality of the various ACPA tests will be challenged when they are used to distinguish RA from other arthritides early in the disease course.

Carriage of the PTPN22 T-variant and/or the SE did not, separately or in combination with any of the ACPA, increase the risk for radiological progression or higher inflammatory activity. However, the number of patients in our study was relatively small, thus it could be difficult to determine differences. Our findings are consistent with other studies of larger patient cohorts showing a lack of influence of the SE or PTPN22 on disease severity[15,26,27]. The relationship between anti-CCP3.1 antibodies and SE alleles did not reach statistical significance, in contrast to findings in the other ACPA. This could suggest that ACPA of the IgA isotype are not associated with the SE; this represents a contrast to the findings of a relationship between ACPA of the IgG isotype and the SE[15].

In our study of patients with early RA, persistent inflammatory activity, measured by the DAS28, ESR, CRP, and swollen joint count, was identified best by the anti-MCV antibody test at baseline compared with the other ACPA and

RF. A significant decrease in the concentration of anti-MCV antibodies corresponded to a therapeutic response. Radiological progression seemed to be predicted equally by the various assays for ACPA or RF. The accuracy as a diagnostic marker was similar for all 4 ACPA.

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