Predictive factors for outcome of rheumatoid arthritis

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CHAPTER 3

Value of anti-MCV and anti-CCP3 compared to anti-CCP2 and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis

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ABSTRACT

Objective

Autoantibodies such as rheumatoid factor (RF) and the second generation anti-cyclic-citrullinated-peptide autoantibodies (anti-CCP2) are frequently measured in clinical practice because of their association with disease outcome in undifferentiated arthritis (UA) and rheumatoid arthritis (RA). Recently two new tests were developed: anti-CCP3 and anti-modified-citrullinated-vimentin autoantibodies (anti-MCV). To facilitate the decision of which autoantibody to test in daily practice, this study evaluates aforementioned autoantibodies and combinations of them for predicting three outcome measures: progression from UA to RA, the rate of joint destruction and achieving sustained DMARD-free remission in RA.

Methods

625 UA patients were studied for the progression to RA after 1 year. 687 RA patients were studied for achieving sustained DMARD-free remission and the rate of joint destruction during a median follow-up of 5 years. Positive predictive values (PPVs) for RA development and the associations with the disease course in RA were compared for single tests (anti-CCP2, anti-CCP3, anti-MCV, RF) and for combinations.

Results

Using a single test in UA patients revealed that anti-CCP2 tended to have the highest PPV for RA development (67.1%), but the 95% confidence intervals of the other tests overlapped. Using a single test in RA, all tests showed comparable associations with the rate of joint destruction and achievement of remission. In ACPA-positive and ACPA-negative RA, RF-presence did not associate with more joint destruction. For all outcome measures, combining two autoantibody tests did not increase the predictive accuracy compared to performing one test.

Conclusion

For clinical practice, a single autoantibody test is sufficient for risk estimation in UA and RA.

INTRODUCTION

Rheumatoid arthritis (RA) is considered to have an autoimmune origin because of the presence of self-reactive autoantibodies. In addition to rheumatoid factor (RF), to date the only serologic measure included in the 1987 American College of Rheumatology criteria for RA, in recent years several other autoantibodies have been described.[1] The discovery of anti-citrullinated protein autoantibodies (ACPA) has led to the development of various new tests for autoantibodies in RA. The first generation anti-cyclic-citrullinated-peptide (anti-CCP) test, directed against a synthetic citrullinated peptide, revealed a higher specificity than RF (91-96% vs. 74-91%).[2-6] Subsequently, a commercially available second generation anti-CCP test (anti-CCP2) was developed, showing an even better specificity (90-97%).[3-8] RF and anti-CCP2 autoantibodies can also be present in the preclinical phase and are associated with future RA development.[9,10] Consequently, tests for anti-CCP2 and RF are nowadays widespread used as diagnostic tools in clinical practice.

Recently two other serological tests emerged, anti-CCP3 and anti-MCV. The anti-CCP3 test has been reported to have sensitivities and specificities comparable to anti-CCP2 (69-83% and 93-95% respectively).[8,11] The second novel autoantibody test targets modified citrullinated vimentin (MCV). This test has its origin in the older anti-Sa autoantibody test that has been shown to target citrullinated vimentin.[12] Compared to anti-CCP2, studies reported somewhat lower specificities and higher sensitivities for anti-MCV (79-92% and 70-84%).[3-5,13]

The aforementioned data were obtained by case-control studies comparing RA patients with non-RA patients or healthy individuals and the resulting test characteristics quantify the proportion of patients that are identified as positive by the test (sensitivity) or the proportion of healthy individuals that are identified as negative (specificity). As such, these measures, as well as the likelihood ratio of a test, provide information on the quality of the test. In clinical practice, the value of determining ACPA or RF relates to their ability to predict the disease course. The chance for an individual patient to have a certain disease course is expressed by the positive predictive value (PPV) and negative predictive value (NPV). A clinical state in which knowledge on the presence of RF and ACPA can be particularly helpful is undifferentiated arthritis (UA). In this subgroup of early arthritis patients no diagnosis can be established according to existing classification criteria and the presence of RF or anti-CCP2 indicates an increased risk for RA development.[14,15] Thus far the PPV and NPV for the risk to develop RA in UA have not been studied for anti-CCP3 and anti-MCV and the four autoantibodies have not been subjected to a head-to-head comparison. Furthermore, the additive value of testing several combinations of autoantibodies for the prediction of RA development in individual UA patients has not been addressed. Therefore, the first aim of this study is to compare anti-CCP2, anti-CCP3, anti-MCV and RF in the prediction of the RA development in patients with UA and to explore whether testing combinations of autoantibodies increases the predictive accuracy.

RF and anti-CCP2 are not only important predictors for RA development, but are also some of the most potent predictors for the outcome of RA, as measured by the rate of radiological

joint destruction.[16-18] Thus far, only one study compared radiological progression for anti-MCV and anti-CCP2 in 273 RA patients and provided suggestive evidence that anti-MCV is a better predictor than anti-CCP2.[19] The effect of testing combinations of all four autoantibodies however was not studied. Thus, the second aim of the present study is to compare anti-CCP2, anti-CCP3, anti-MCV and RF in the prediction of the rate of joint destruction and to explore whether combinations of autoantibodies can increase the predictive ability, taking advantage of a longitudinal cohort of 687 RA patients with a median follow-up of 5 years.

A second disease outcome of RA is the achievement of remission that with the introduction of new aggressive treatment modalities has increasingly become an attainable goal. We chose a strict definition and defined sustained disease-modifying antirheumatic drugs (DMARD)-free remission as the persistent absence of synovitis for at least 1 year after cessation of DMARD-therapy.[20] Since the predictive value of the four autoantibodies in relation to remission is scarcely explored, the present study compares the four tests for their ability to predict sustained DMARD-free remission in RA patients treated with conventional DMARDs.

In summary, to support the choice on which autoantibody to test in daily practice, this study uses a large longitudinal cohort to evaluate the value of determining anti-CCP2, anti-CCP3, anti-MCV and RF for predicting three outcome measures: progression from UA to RA, the rate of joint destruction and the chance of achieving sustained DMARD-free remission in RA. In addition, the predictive value of combining several tests is investigated.

PATIENTS AND METHODS

Patients

All patients included in this study are selected from the Leiden Early Arthritis Clinic (EAC) cohort that was started in 1993.[21] Patients were referred by general practitioners when arthritis was suspected. Inclusion took place when arthritis was confirmed at physical examination and symptom duration was less than 2 years. Written informed consent was obtained from all participants. The study was approved by the local Medical Ethical Committee.

At inclusion, patients were inquired about their joint symptoms and subjected to a physical examination. Blood samples were taken for routine diagnostic laboratory screening (including IgM-RF) and stored to determine other autoantibodies at a later time. Follow-up visits were performed on a yearly basis and included radiographs of hands and feet.

Since the start of the EAC treatment strategies for RA have changed; four different strategies were applied depending on the inclusion period. Patients included between 1993 and 1995 were treated initially with analgesics and subsequently with chloroquine or salazopyrin if they had persistent active disease (delayed treatment).[22] From 1996 to 1998 RA patients were promptly treated with either chloroquine or salazopyrin (early treatment).[21,22] From 1998 to 2002 patients were promptly treated with either salazopyrin or methotrexate (early treatment) and patients included in 2002 or later were promptly treated with either salazopyrin or methotrexate com-

bined with treatment adjustments based on the disease activity (early and disease activity based treatment). Treatment of UA patients was not protocollized.

UA was defined as not fulfilling one of the existing classification criteria for rheumatologic diagnoses two weeks after the first presentation.[23] Thus other rheumatic diseases like Sjögren's syndrom, psoriatic arthritis, spondylarthropathies, etc. that were established at baseline were excluded (Figure 1). 625 patients with UA, consecutively included between 1993 and 2006, were studied. After 1 year of follow-up, 201 patients (32.2%) had progressed to RA, fulfilling the 1987 ACR criteria for RA. In addition, 687 patients with RA included between 1993 and 2006 were studied for their disease outcome; 486 patients fulfilled the ACR criteria for RA already at inclusion and 201 patients were initially diagnosed with UA and developed RA within the first year of follow-up (Figure 1).

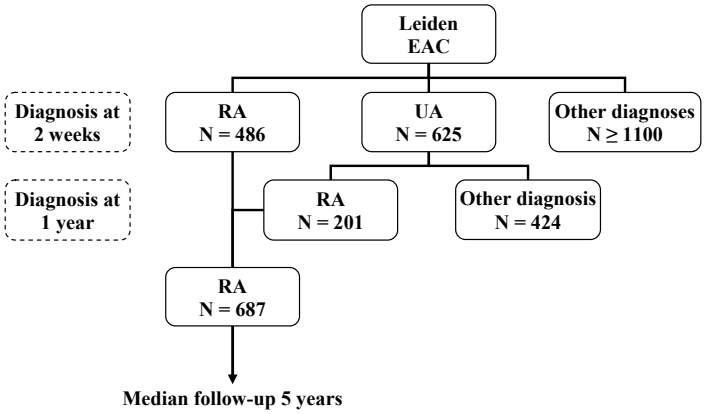

Figure 1. Flowchart of the study. From the 687 RA patients, 579 had radiographic data available and for 635 patients information on sustained DMARD-free remission was obtained. From all UA patients RF, anti-CCP2, anti-CCP3 and anti-MCV were determined in 623, 624, 597 and 597 patients respectively. From all RA patients with radiographs available RF, anti-CCP2, anti-CCP3 and anti-MCV were determined in 572, 565, 544 and 544 patients respectively. From all RA patients with data on sustained DMARD-free remission, RF, anti-CCP2, anti-CCP3 and anti-MCV were determined in 615, 603, 579 and 579 patients respectively

Autoantibodies

IgM–RF was determined by enzyme-linked immunosorbent assay (ELISA). Anti-CCP2 autoantibodies (total IgG) were measured by ELISA (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). The cutoff level for anti-CCP2 autoantibody-positivity was set at 25 arbitrary units, according to the manufacturer's instructions. Anti-CCP3 autoantibodies (IgA and IgG subforms) and anti-MCV autoantibodies were measured with ELISA as well (Quanta lite CCP 3.1 IgG/IgA, INOVA Diagnostics Inc., San Diego, CA, USA and Orgentec Diagnostika GmbH, Mainz, Germany respectively). According to the manuals, the cutoff level for both tests was 20 arbitrary units. The numbers of patients in which RF, anti-CCP2, anti-CCP3 and anti-MCV measurements were performed show some little variation (available data for RF, anti-CCP2,

anti-CCP3 and anti-MCV were respectively 665, 653, 629 and 629 out of 687 RA patients and 623, 624, 597 and 597 out of 625 UA patients). This is due to the difference in timing of performing the tests. RF was determined routinely at inclusion whereas the ACPA were determined using stored serum samples. The recently introduced anti-CCP3 and anti-MCV assays were performed on all available sera in 2008, whereas the anti-CCP2 assay was performed earlier.

Radiographs

Radiographs of hands and feet were taken on consecutive years starting at baseline and were scored according to the Sharp-van der Heijde method.[24] From all RA patients, 579 had data on both autoantibodies and radiographs. To encompass a reliable sample size during follow-up, radiographic data were restricted to a maximum of 7 years of follow-up. The number of available radiographs varied per time-point and declined from 552 at baseline to 478 after 1 year, 426, 358, 299, 270, 207 and 156 after 2 till 7 years of follow-up respectively. Due to the study design (an inception cohort) not all patients had a similar duration of follow-up (median 5 years). All radiographs were scored by one experienced scorer (ML) who was blinded with respect to the patient's autoantibody status, treatment and other clinical data. Scoring was performed with known time order, which is more sensitive to change compared to scoring with unknown time sequence.[25] From the total number of scored radiographs, 499 radiographs were rescored by the same reader, consisting of 149 baseline radiographs and 350 radiographs during follow-up belonging to 60 randomly selected RA patients. The intraobserver intraclass correlation coefficients were 0.91 for all scored radiographs, 0.84 for baseline radiographs and 0.97 for the radiographic progression rate.

Sustained DMARD-free remission in RA

Remission was defined in its most stringent form as the persistent absence of synovitis for at least one year after cessation of DMARD therapy and the identification of remission by the patient's rheumatologist.[26] The remission status could be reliably ascertained in 635 RA patients. Most patients who achieved remission were followed-up longer than the minimum requirement of 1 year; the median time of observation after discontinuation of DMARDs in the absence of swollen joints was 2.5 years. Patients, who had a recurrence of their arthritis after discharge, could easily return to the Leiden University Medical Center, the only referral center for Rheumatology in a health care region of approximately 400.000 inhabitants. The frequency of relapse was recorded and patients with relapse were included in the non-remission group (n=6).

Statistical analysis

The PPV (proportion of UA patients with a positive test that progressed to RA) and the NPV (proportion of UA patients that did not develop RA) were determined.

As radiographic data are not normally distributed, non-parametric Mann-Whitney tests were used to compare the Sharp-van der Heijde scores at individual time points for patients with and

without autoantibodies or for autoantibody combinations. In addition, to take advantage of the prospective character of the data consisting of repeated measurements, and to avoid multiple testing by performing statistical tests for each time point, a linear mixed model with an autoregressive correlation structure with heterogeneous variances was used. This model estimates the linear progression rate in radiological joint destruction using normalized, log-transformed Sharp-van der Heijde scores, taking missing observations into account. This means that it compares the progression rates for the different patient groups. In the mixed model analyses, corrections were applied for age, gender and inclusion period/treatment strategy. Correction for treatment strategy was performed by including the inclusion period in the linear mixed model. This was done because treatment modalities improved over time and an influence of the treatment strategy (reflected by the inclusion period) on the progression of radiographic joint damage was observed previously as well as in the present study (data not shown). In addition, the available follow-up duration differed between patients and therefore the number of radiographs per time point declined during follow-up. As the patients with the longest follow-up were included in the earliest inclusion period and thus have been treated with the least aggressive treatment strategy, correction for inclusion period was performed. In order to prevent overfitting of the data no corrections were applied for other variables. Analysis of sustained DMARD-free remission was performed by Cox regression analysis, to take into account the differences in follow-up times among patients. For patients that achieved remission the dependent variable is the "time-to-event", indicating the time until reaching remission. For non-remission patients the time to last follow-up was used, with a maximum of 10 years. SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used. P-values <0.05 were considered significant. All reported p-values are two-sided.

RESULTS

Progression from UA to RA

From the total EAC, consisting of >2,000 patients with early arthritis, UA patients (n=625) were selected and studied for progression to RA after 1 year of follow-up. At inclusion, the mean age was 50.9 (±17.0) years, 371 (59.1%) patients were female and the self-reported symptom duration was 5.5 (±8.5) months. Anti-CCP2, anti-CCP3, anti-MCV and RF were present in 149 (23.9%), 172 (28.7%), 199 (31.7%) and 155 (24.8%) patients respectively. The presence of autoantibodies overlapped; UA patients who tested positive for anti-CCP2 were also frequently positive for other autoantibodies (Figure 2).

The PPV was compared for the four autoantibodies (Table 1). Anti-CCP2 had the highest PPV, 67.1%, compared to 64.0%, 56.3% and 61.7% for anti-CCP3, anti-MCV and RF respectively. The NPVs of all four tests were comparable (~80%). Thus, in case one autoantibody test is performed, a positive anti-CCP2 test tends to correlate with the highest risk of RA development, but overlapping 95%CI's hamper a definite differentiation.

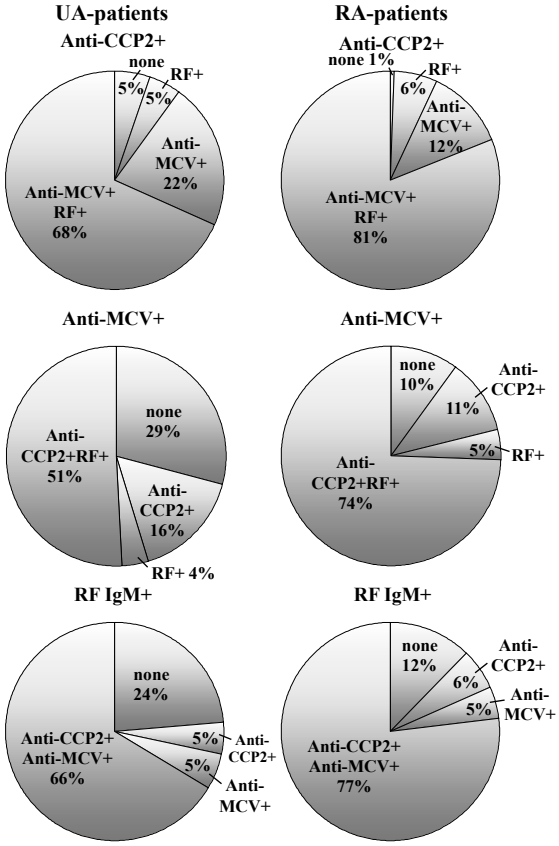

Figure 2. Prevalence of other autoantibodies in case of a positive test result for anti-CCP2, anti-MCV and RF for both UA and RA. Figure 2 illustrates the prevalence of other autoantibodies given one positive autoantibody test. This figure is derived using 596 UA and 540 RA patients for whom data on all autoantibodies was present. none: no other autoantibodies present

Next it was determined whether performing two autoantibody tests results in a better estimation of the risk for RA than performing one test. Since anti-CCP2 and anti-CCP3 are related tests and anti-CCP2 positive patients were in 90% also positive for anti-CCP3, the possible combinations of anti-MCV, anti-CCP2 and RF were first assessed. The proportions of patients that developed RA in case of two positive test results (PPV) were: anti-CCP2+/anti-MCV+ 69.9% (95%CI 62.1-77.7), anti-CCP2+/RF+ 74.1% (95%CI 65.8-82.3) and anti-MCV+/RF+ 70.6% (95%CI 62.1-79.2). Additional analyses using anti-CCP3 instead of anti-CCP2 yielded comparable results (data not shown). Altogether, these data show that when two tests are performed none of these combinations is clearly superior to the other. Furthermore, no additive value of performing two instead of one autoantibody test could be observed.

When performing a single autoantibody test, no information about the presence or absence of the other autoantibodies is obtained. However, the eventual coexisting presence of other

Table 1. PPV, NPV, sensitivity, specificity and likelihood ratios of anti-CCP2, anti-CCP3, anti-MCV and RF for predicting progression from UA to RA

Autoantibody Test	PPV		NPV		Sensitivity		Specificity		Likelihood Ratio	
	% (95%CI)	True pos./ all pos. tests	% (95%CI)	True neg./ all neg. tests	% (95%CI)	True pos./ all RA	% (95%CI)	True neg./ all non-RA	LR+	LR-
Anti-CCP2	67.1 (59.6-74.7)	100/149	79.0 (75.3-82.6)	375/475	50.0 (43.1-56.9)	100/200	88.4 (85.4-91.5)	375/424	4.33	0.57
Anti-CCP3	64.0 (56.8-73.1)	110/172	80.0 (76.2-83.8)	340/425	56.4 (49.5-63.4)	110/195	84.6 (81.1-88.1)	340/402	3.66	0.52
Anti-MCV	56.3 (49.4-63.2)	112/199	79.2 (75.2-83.1)	315/398	57.4 (50.5-64.4)	112/195	78.4 (74.3-82.4)	315/402	2.66	0.54
RF	61.7 (54.0-69.4)	95/154	77.8 (74.1-81.6)	365/469	47.7 (40.8-54.7)	95/199	86.1 (82.8-89.4)	365/424	3.43	0.61

UA: undifferentiated arthritis; PPV: positive predictive value; NPV: negative predictive value; 95%CI: 95% Confidence Interval; pos.: positive; neg.: negative; LR: likelihood ratio

unmeasured autoantibodies can affect the risk for RA. To determine the risk for RA as conferred to by the individual autoantibodies or by the number of autoantibodies, the PPVs for progression to RA were determined in the group of 596 UA patients for whom information on all three autoantibodies was available. The difference with above mentioned data is that the presence of two autoantibodies now indicates that the third is absent, whereas in above mentioned data only two tests were performed and the third autoantibody test could be positive as well as negative. The PPV for RA development in patients without autoantibodies was 18.8% (95%CI 14.7-22.9). In the presence of one autoantibody the PPV was 26.5% (95%CI 17.9-35.0) and increased significantly to 59.6% (95%CI 45.5-73.6) in the presence of two autoantibodies (Table I, supplementary data). The PPV was the highest in the presence of 3 autoantibodies (73.3%, 95%CI 64.6-81.9).

In conclusion, when performing one autoantibody test in clinical practice, none of the four tests is clearly superior. Although the presence of 2 autoantibodies significantly increased the risk for RA compared to the presence of 1 autoantibody, for clinical use performing two tests does not significantly increase the predictive performance compared to performing one test. This finding is likely explained by the presence of other, non-measured autoantibodies that affect the risk for RA.

Joint destruction in RA

Baseline characteristics of the 579 studied RA patients were: mean age 56.2 (±15.5) years, 405 patients (69.9%) were female, anti-CCP2, anti-CCP3, anti-MCV and RF were present in 313 (55.4%), 322 (59.2%), 331 (60.8%) and 334 (58.4%) patients respectively. Anti-CCP2 positive patients also tested positive for anti-MCV and RF in 93% and 87% of cases respectively (Figure 2).

First, the association between autoantibody-positivity and rate of joint destruction was assessed when performing only one autoantibody test. For all autoantibody tests (anti-CCP2, anti-CCP3, anti-MCV and RF) a positive test result was associated with a higher Sharp-van der Heijde score at all time points except baseline (M-W, $p<0.001$) and a higher rate of joint destruction over a period of 7 years (mixed model, $p<0.001$) compared to a negative test result. Figure 3A shows that there is no difference among the four tests with regard to predictive ability for joint destruction.

Next, it was investigated whether the addition of a second autoantibody test increased the predictive value for the rate of joint destruction. As depicted in Figure 3B no differences were seen between testing positive for anti-CCP2, anti-MCV or RF alone and combinations of these autoantibodies. These results indicate that for clinical use, a positive test for one of these autoantibodies predicts a severe disease course, and a second or third autoantibody test does not increase the predictive accuracy. Testing for anti-CCP3 instead of anti-CCP2 gave comparable results (data not shown).

To identify the contribution of the individual autoantibodies to the association with the rate of joint destruction, the effect of the number of positive autoantibodies (with the known absence of the other autoantibodies) was investigated using data on RF, anti-CCP2 and anti-MCV. The

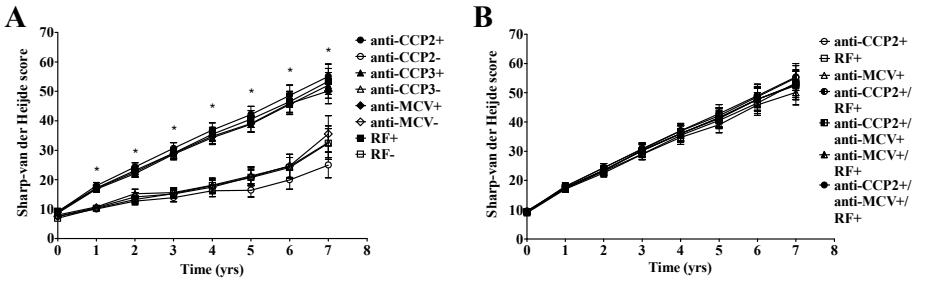

Figure 3. Mean Sharp-van der Heijde scores (±SEM) for the different autoantibody tests (A) and for combinations of positive tests (B). The number of patients in Figure 3A for anti-CCP2-positive/negative, anti-CCP3-positive/negative, anti-MCV-positive/negative and RF-positive/negative were respectively 313/252, 322/222, 331/213 and 334/238. *Mann-Whitney, p<0.001, for comparison of positive with negative test. For all four tests the p-value was <0.001 using mixed models. In Figure 3B, for anti-CCP2+, RF+, anti-MCV+, anti-CCP2+/RF+, anti-CCP2+/anti-MCV+, anti-MCV+/RF+ and anti-CCP2+/anti-MCV+/RF+ the numbers of patients were 313, 334, 331, 271, 283, 259 and 244 respectively

presence of either 2 or 3 autoantibodies was associated with a higher rate of joint destruction compared to 0 or 1 autoantibody (Figure 4A) (mixed model, p <0.001 for both 2 and 3 compared to 0 as well as 1 autoantibody). No significant difference was observed between the presence of 2 or 3 autoantibodies or between 0 and 1 autoantibody. It should be noted that the group with 1 autoantibody consisted almost exclusively of patients that were anti-MCV+ (n=33) or RF+ (n=39); only two patients were positive for anti-CCP2 and negative for anti-MCV and RF.

To more specifically investigate the role of RF in relation to ACPA (anti-CCP2, anti-CCP3 or anti-MCV) and the rate of joint destruction, the additional effect of RF in the presence or

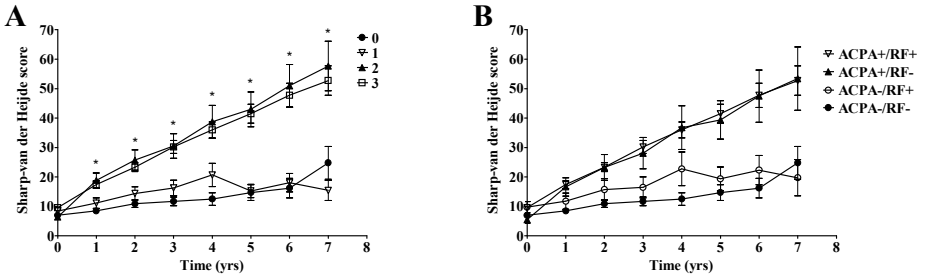

Figure 4. Mean Sharp-van der Heijde scores (±SEM) for the number of positive autoantibody tests (A) and for the effect of RF in the presence or absence of ACPA (B). In Figure 4A the mean Sharp-van der Heijde scores are depicted for the number of positive autoantibody tests (with the other tests negative) studying data on RF, anti-CCP2 and anti-MCV (total n=540). The numbers of patients positive for 0, 1, 2 or 3 autoantibodies were 152, 74, 70 and 244 respectively. Comparing 3 with 0, 3 with 1, 2 with 1 and 2 with 0 autoantibodies revealed a p<0.05 at all time points except baseline (M-W) and a p<0.001 for the 7 year period (mixed model). In Figure 4B the mean Sharp-van der Heijde scores are depicted for the following groups ACPA-RF-, ACPA-RF+, ACPA+RF- and ACPA+RF+, the patients numbers per group were 152, 39, 36 and 244. P=0.864 for comparison of ACPA-RF- with ACPA-RF+ and p=0.702 for comparison ACPA+RF- and ACPA+RF+ (both mixed model)

absence of ACPA was determined (Figure 4B). This revealed that in the presence of ACPA, but also in the absence of ACPA, RF did not significantly contribute to the rate of joint destruction.

Sustained DMARD-free remission in RA

From a total of 635 RA patients, 78 patients achieved sustained DMARD-free remission after a median follow-up of 39.5 months. These 78 patients had a mean age of 59.4 (±15.7) years, 57 (73.1%) were female. Anti-CCP2 autoantibodies were present in 11.8% and anti-CCP3, anti-MCV autoantibodies and RF in 21.9%, 28.8% and 25.0%.

The four autoantibody tests were compared for their association with the achievement of remission (Figure 5A). The Hazard Ratio (HR) of each of the four tests for not achieving sustained DMARD-free remission was 11.6 (95%CI 5.8-23.4) for anti-CCP2, 6.0 (95%CI 3.4-10.4) for anti-CCP3, 4.9 (95%CI 3.0-8.2) for anti-MCV and 4.7 (95%CI 2.8-8.0) for RF.

Subsequently, the additive value of performing two autoantibody tests compared to one test was investigated. The HRs were 15.6 (95%CI 6.7-36.4), 14.0 (95%CI 6.4-31.0) and 11.5 (95%CI 5.4-24.5) for combinations of anti-CCP2 and RF, anti-CCP2 and anti-MCV and anti-MCV and RF respectively. These data indicate that to predict the chance on remission, performing two tests has no additional value compared to anti-CCP2 alone.

To investigate whether the number of present autoantibodies affected the chance of achieving sustained DMARD-free remission, the HRs were determined for the presence of 1, 2 or 3 positive autoantibodies (with the other autoantibodies known to be absent) using data on anti-CCP2, anti-MCV and RF (Figure 5B). The HRs for not achieving sustained DMARD-free remission were as follows, 3.7 (95%CI 1.1-12.3), 15.5 (95%CI 5.9-41.2) and 17.1 (95%CI 6.8-43.3) for the presence of, respectively, 1, 2, and 3 autoantibodies compared to no autoantibodies. Thus, however the 95%CIs were overlapping, the results suggestively indicate that the more autoantibodies are present, the lower the chance is to achieve sustained DMARD-free mission.

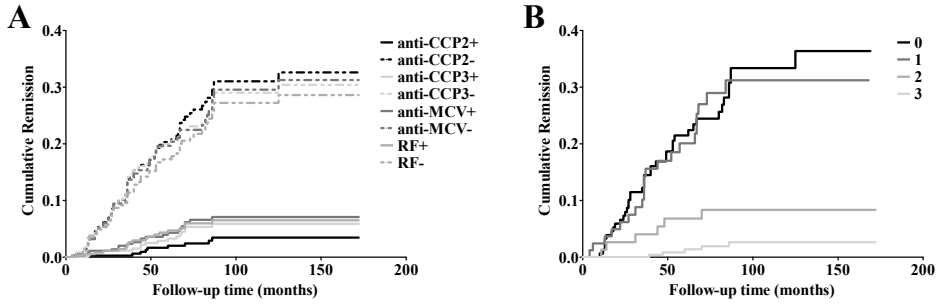

Figure 5. Effect of the presence of a positive autoantibody test (A) and the number of autoantibodies on sustained DMARD-free remission (B)

DISCUSSION

Among the autoantibodies tested in RA, only RF and ACPA are considered clinically useful. In clinical practice a physician or patient is interested in the chance for the individual to progress to RA or not, given a positive or negative test result respectively. These risks are reflected by the PPV and NPV. In addition, in early arthritis the predictive value of these autoantibody tests is valued the most in patients in whom at presentation no definite diagnosis can be established (UA), as only one-third of these patients progresses to RA after one year.[14] Also in RA, the autoantibody tests form one of the most potent predictors to obtain an indication on the severity of the future disease course. As such, information on the results of the autoantibody tests can influence treatment decision in individual patients with UA and RA.[23] Nevertheless, it is thus far unknown which test, or which combination of tests, is most powerful in predicting the progression from UA to RA and the disease progression in RA and therefore the present study was undertaken.

To evaluate the prediction from UA to RA using a single test, the PPVs of the four tests were compared. This revealed that a positive anti-CCP2 test tended to have the highest predictive value (PPV 67% compared to 62% and 56% for RF and anti-MCV), but due to overlapping 95%CIs a definite differentiation could not be made. In addition, performing a second test did show a tendency for a higher chance on RA development in case both tests were positive (highest risk for RA in anti-CCP2+, RF+ patients, PPV 74%), but also here the 95%CIs overlapped. To formally conclude whether the PPVs of 67% and 74% are statistically significantly different or not, >1800 UA patients would be required (using a p-value of 5% and a power of 90%). Based on the present data it is concluded that addition of a second test does not result in an increased predictive accuracy. Notably, UA patients who tested positive for anti-CCP2 were also frequently positive for other autoantibodies (Figure 2). Thus the finding of comparable prognostic performances of one or two tests is likely due to co-existing presence of autoantibodies that are unmeasured with a single test but that do affect the risk-estimation for RA.

The development of RA was assessed after 1 year of follow-up. This time-point was chosen in order to have a similar duration of follow-up for all studied patients. However, this may have introduced misclassification as patients with UA may have progressed to RA after more than 1 year of follow-up. With all available follow-ups, 25 patients (4.4%) progressed to RA later than 1 year, indicating that the current PPVs may be marginally underestimated. Since this misclassification is present in the total group of UA patients this does not hamper a comparison of tests.

Two measures were studied for the severity of the disease course in RA: achieving sustained DMARD-free remission and the level of radiological joint destruction during a median follow-up of 5 years. Although Figure 3A and 5A may lead to the impression that anti-CCP2 positive patients have a higher rate of joint destruction and achieve sustained DMARD-free remission less frequently than patients positive for the other autoantibodies, these differences were not statistically significant. Similar to the data on RA development, performing a second test appeared not to result in a more accurate prediction for the disease outcome in RA. This observation,

which is in contrast to an earlier report,[27] can be explained by the presence of non-measured autoantibodies that are associated with a progressive course of RA.To obtain a more detailed comprehension of the current results, the contribution of the individual autoantibodies to disease progression (RA development, joint destruction and achievement of remission) was investigated. This showed that the presence of two autoantibodies indicated a significantly increased risk compared to the presence of one autoantibody. However, the group with one autoantibody present consisted mostly of anti-MCV or RF-positive patients. Patients that were positive only for anti-CCP2 were very rare. Therefore it cannot be excluded that the increased risk in the presence of two compared to one autoantibody is due to the effect of anti-CCP2 rather than to the effect of an additional autoantibody. Nevertheless, in general it was observed that a higher number of autoantibodies present resulted in a higher risk for RA or for progressive disease. This is in line with recent published data showing that a broader autoantibody response associated with disease progression.[28]

As the association of RF with the presence of RA is primarily explained by its interaction with ACPA,[29] we investigated whether we could observe a similar effect for the progression of joint destruction in RA. Intriguingly, the rate of joint destruction both in the ACPA-positive and ACPA-negative groups was not affected by the presence or absence of RF. This finding further supports the notion that RF does not, by itself, contribute to disease progression.

In order to get an impression of the effect of anti-MCV itself on the rate of joint destruction, RA patients that were positive only for anti-MCV (n=33) were studied. This revealed that the rate of joint destruction was comparable to that of the patients with no autoantibodies (data not shown), indicating that anti-MCV alone does not strongly affect the level of joint damage in RA.

In conclusion, our results indicate that for risk estimation of the disease course in clinical practice, performing a single autoantibody test is sufficient, both in UA and RA.

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Supplementary data, Table I. Positive predictive values of the number of positive antibodies for progression from UA to RA

Number of Positive Antibodies	PPV % (95% CI)	True pos./ all pos. tests	Antibody Combinations	PPV % (95% CI)	True pos./ all pos. tests
0	17.9 (13.9-22.0)	61/340	-	-	-
1	27.5 (18.8-36.1)	28/102	Anti-CCP2+/anti-MCV-/RF-	12.5 (-10.4-35.4)	1/8
			Anti-CCP2-/anti-MCV+/RF-	29.3 (17.6-41.0)	17/58
			Anti-CCP2-/anti-MCV-/RF+	27.8 (13.2-42.4)	10/36
2	61.2 (47.6-74.9)	30/49	Anti-CCP2+/anti-MCV+/RF-	58.1 (40.7-75.4)	18/31
			Anti-CCP2+/anti-MCV-/RF+	83.3 (53.5-113.2)	5/6
			Anti-CCP2-/anti-MCV+/RF+	58.3 (30.4-86.2)	7/12
3	71.9 (62.9-80.9)	69/96	-	-	-

These data are based on the patients with data available of all three antibodies (anti-CCP2, anti-MCV and RF, n=587). UA: undifferentiated arthritis; PPV: positive predictive value; 95%CI: 95 % Confidence Interval; pos.: positive