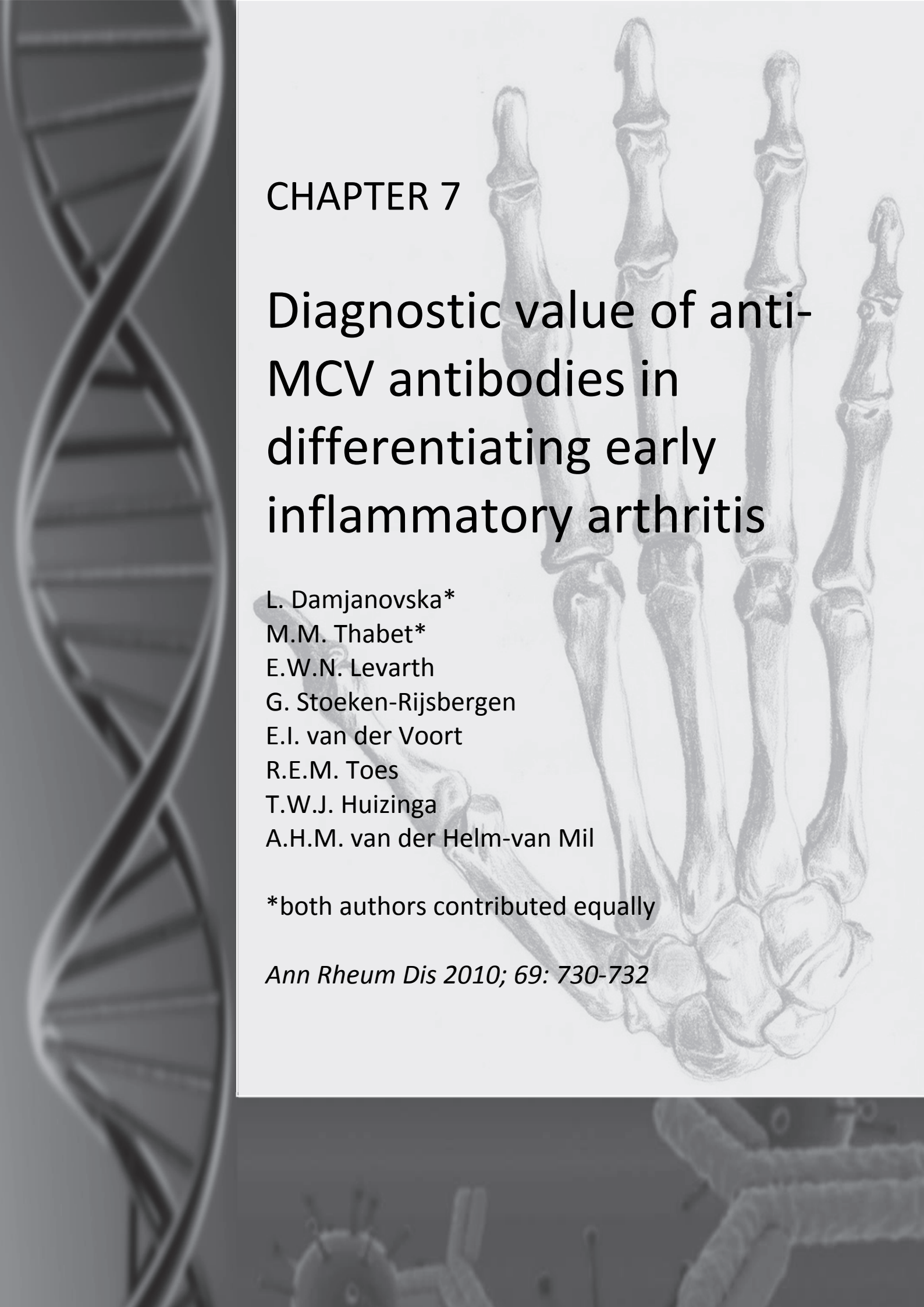




CHAPTER 7

Diagnostic value of anti-MCV antibodies in differentiating early inflammatory arthritis

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ABSTRACT

Objectives: To evaluate the diagnostic performance of the anti-CCP2, anti-CCP3 and anti-mutated citrullinated vimentin (anti-MCV) tests in differentiating rheumatoid arthritis (RA) from other forms of arthritis in a clinical setting of early arthritis.

Methods: In 917 patients with recent-onset arthritis (566 RA, 351 other diseases) and in 99 healthy-controls the anti-MCV, anti-CCP2 and anti-CCP3.1 tests were performed and the test characteristics compared.

Results: Comparison of the tests for differentiating RA from other causes of arthritis showed a lower specificity for anti-MCV (82.9%) than for anti-CCP2 (93.4%) and anti-CCP3.1 (90.0%). Similarly, the positive likelihood ratio for anti-MCV was also lower (3.6, compared with 8.7, 5.8 for anti-CCP2 and anti-CCP3.1). The anti-MCV test had a higher sensitivity (62% vs 56.9% and 58.1%, respectively). In psoriatic arthritis, spondyloarthropathy and other arthritis anti-MCV antibodies had a prevalence of 15.2%, 13.9% and 19.4%.

Conclusion: The diagnostic performance of the anti-MCV test in the differential diagnosis of early arthritis is lower than that of the anti-CCP tests.

Introduction

Autoantibody formation is characteristic for rheumatoid arthritis (RA). The best-known autoantibody is rheumatoid factor, which however lacks specificity. More recently, several tests have been developed that detect anti-citrullinated protein antibodies (ACPA). These tests include the commercially available anti-CCP2 and anti-CCP3 assays as well as the anti-mutated citrullinated vimentin (anti-MCV) test. The first two assays use citrullinated peptide(s) for detection of ACPA, whereas the later assay is based upon an entire protein, citrullinated vimentin [1, 2].

Several recent studies studied the test characteristics of the anti-MCV test and the anti-CCP2 [1, 3-9] and anti-CCP3 tests [3, 7], showing conflicting results (online supplementary table). This might be partly due to a limited sample size [3- 9]. In addition, in the majority of these studies RA patients were compared with healthy controls, which entails that the results cannot be easily extrapolated to clinical practice where these tests are used to differentiate early RA from other forms of early inflammatory arthritis. Therefore this study aims to evaluate the diagnostic performance of the anti-MCV test in the differentiation of RA from other forms of inflammatory arthritis in a large inception cohort of early arthritis patients and to compare these test characteristics with those of the anti-CCP2 and anti-CCP 3.1 tests.

PATIENTS AND METHODS

Patients

A total of 917 patients with early arthritis who were part of the Leiden Early Arthritis Clinic (EAC) were studied. The Leiden EAC is an inception cohort that started in 1993 at the Department of Rheumatology, Leiden University Medical Center, the Netherlands and includes patients with recent-onset arthritis (symptom duration <2 years) [10]. All patients with RA, psoriatic arthritis (PsA), spondylo-arthropathy (SpA) and baseline serum available, as well as a random selection of patients with other forms of arthritis were evaluated. The 917 patients had the following diagnoses: 566 RA according to the 1987 revised ACR classification criteria for RA [11]. 99 PsA, 72 SpA, 38 patients reactive arthritis, 32 inflammatory osteoarthritis, 30 remitting seronegative symmetrical synovitis with pitting edema (RS3PE), 21 connective tissue diseases, 26 sarcoidosis, 11 paramalignant arthritis, 6

gout and pseudo-gout, 6 Lyme disease, 2 juvenile idiopathic arthritis and 8 patients with other inflammatory arthritis. RA was diagnosed at baseline (75%) or within the first year of follow-up (25%). The other patients were diagnosed at baseline in 73% and within the first year in 27%. The diagnoses made by the treating physician were checked by an independent rheumatologist using chart review and existing criteria. 99 anonymous blood donors who served as healthy controls were also studied.

ACPA measurement

From all patients and controls, stored baseline serum samples were used to perform the anti-MCV, anti-CCP2 and anti-CCP3.1 tests. Anti-MCV antibodies were measured by a commercially available anti-MCV ELISA (Orgentec Diagnostika, Mainz, Germany). The assay was performed according to the manufacturer's instructions and values >20 U/ml were considered positive. Anti-CCP2 antibodies were measured by Immunoscan CCPlus (Euro-Diagnostica, Arnhem, the Netherlands) and anti-CCP3.1 antibodies using Quanta Lite CCP 3.1 IgG/IgA (Inova, California, USA). The cut-off points were used as determined by manufacturers, 25 U/ml for CCP2 and 20 U/ml for CCP3.1.

Statistical analysis

Sensitivity and specificity with 95% confidence interval (CI) as well as the positive and negative likelihood ratios (LR+, LR-) were calculated for each of the tests. Receiver operating characteristic curves as (ROC) were constructed by plotting sensitivity (true positive rate) against 1-specificity (false positive rate) and the areas under the curve (AUC) were compared. For analysis SPSS version 16 (SPSS 16 Chicago, IL, USA) was used. In all tests, p values <0.05 were considered statistically significant.

RESULTS

Of the 917 early arthritis patients 566 patients had RA, 99 PsA, 72 AS and 180 other forms of inflammatory arthritis. To evaluate the diagnostic performance of the tests in the differentiation of RA from other forms of arthritis, the patients with PsA, AS and other diagnosis were grouped. Amongst the RA-patients 379 (67%) were female; the mean age was

56.9 +/- 15 years. In the group with all other early arthritis patients the mean age was 45.9 ± 16.8 years and 184 (52%) were female (both p-values <0.001 versus RA).

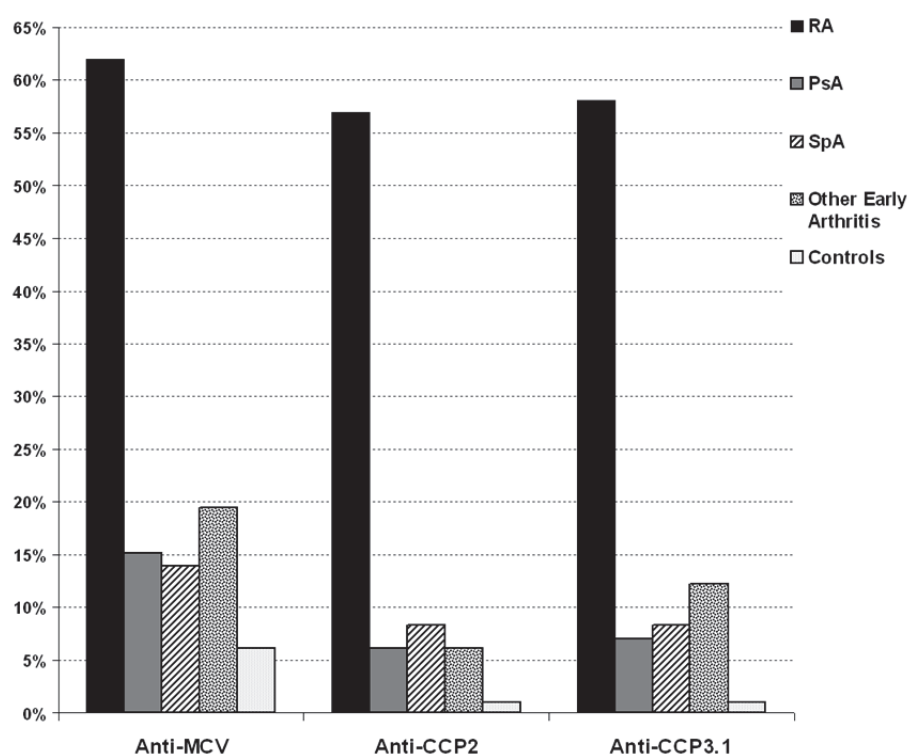


Figure 1: Prevalence of autoantibody positivity in early arthritis and in healthy controls. CCP, cyclic citrullinated peptide; MCV, anti-mutated citrullinated vimentin; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondyloarthropathy.

Anti-MCV antibodies were present in 351 out of 566 (62%) RA patients and in 60 out of 351 (17.1 %) of all other forms of inflammatory arthritis. Dividing this group into disease categories revealed that anti-MCV antibodies were present in 13.9% of SpA, 15.2% of PsA and 19.4% of patients with other forms of arthritis. As depicted in **Figure 1** the prevalence of anti-CCP2 and anti-CCP3.1 antibody positivity in these conditions were significantly lower. **Figure 2** shows the prevalence of autoantibodies in RA, AS, PsA, and other forms of early inflammatory arthritis given they were positive in the anti-CCP2/3, respectively anti-MCV assay. This figure indicates that most anti-MCV positive RA patients (82%) are also positive in the anti-CCP assays. More importantly, the results depicted in **figure 2** illustrate that only 40% of anti-MCV positive patients with other inflammatory arthritides are anti-CCP positive.

These results indicate that the anti-MCV assay is less specific for RA compared to the two anti-CCP assays.

Figure 2: Prevalence of anti-MCV and anti-CCP2/3 antibodies in different diagnoses. AB+, antibody positive; AB-, antibody negative; CCP+, patients tested positive for either anti-CCP2 and/or anti-CCP3; MCV+, patients tested positive for anti-MCV; CCP+ MCV+, patients tested positive for (either anti-CCP2 and/or anti-CCP3) and positive for anti-MCV. CCP, cyclic citrullinated peptide; MCV, anti-mutated citrullinated vimentin; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondyloarthropathy.

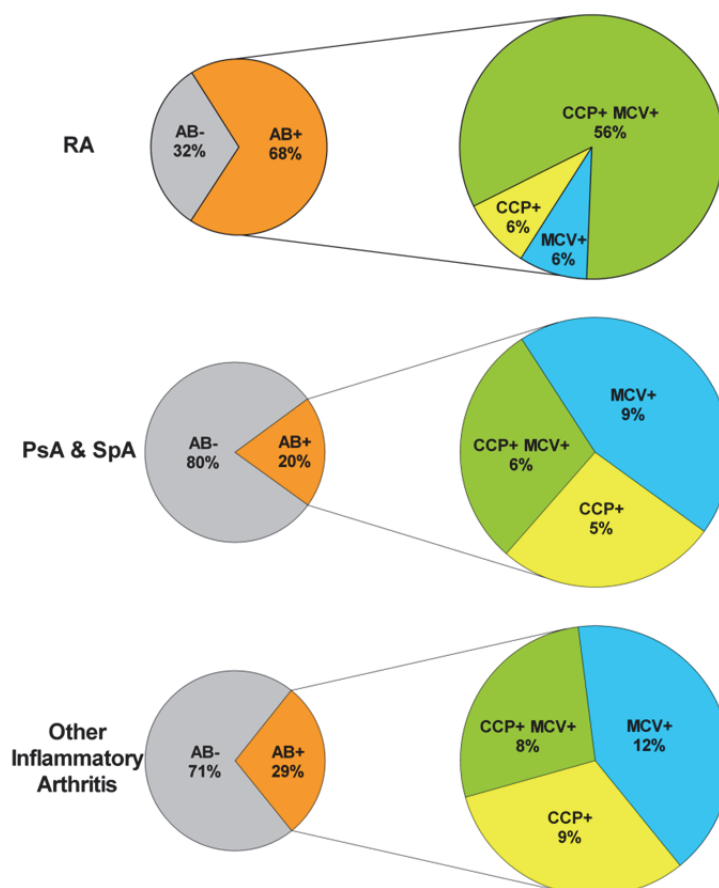


Table 1: Diagnostic performance of anti-MCV, anti-CCP2 and anti-CCP3.1 in differentiating RA from other early arthritis and from healthy controls

	Specificity (95% CI)	True negative/all other early arthritis or controls	Sensitivity (95% CI)		True positives/all RALR+ (95% CI)	LR– (95% CI)	AUC (SEM)
RA vs other early arthritis							
Anti-MCV	82.9 (78.5 to 86.6)	291/351	62.0 (57.9 to 66.0)	351/566	3.6 (2.8 to 4.6)	0.46 (0.41 to 0.51)	0.725 (0.017)
Anti-CCP2	93.4 (90.2 to 95.7)	328/351	56.9 (52.7 to 61)	322/566	8.7 (5.8 to 13)	0.46 (0.42 to 0.51)	0.752 (0.016)
Anti-CCP3.1	90.0 (86.3 to 92.9)	316/351	58.1 (53.9 to 62.2)	329/566	5.8 (4.2 to 8)	0.47 (0.42 to 0.51)	0.741 (0.016)
RA vs healthy controls							
Anti-MCV	93.9 (86.6 to 97.5)	93/99	62.0 (57.9 to 66.0)	351/566	10.2 (4.7 to 22.3)	0.40 (0.36 to 0.45)	0.789 (0.020)
Anti-CCP2	99.0 (93.7 to 99.9)	98/99	56.9 (52.7 to 61)	322/566	56.3 (8 to 396)	0.43 (0.39 to 0.48)	0.78 (0.019)
Anti-CCP3.1	99.0 (93.7 to 99.9)	98/99	58.1 (53.9 to 62.2)	329/566	57.5 (8.2 to 405)	0.42 (0.38 to 0.47)	0.785 (0.019)

LR+, positive likelihood ratio; LR-, negative likelihood ratio. The LR are measures of the degree to which a positive test result increases the probability of a disease (LR+) and a negative test result decreases the probability of a disease (LR-).

AUC, area under the receiver operator characteristic curve; CCP, cyclic citrullinated peptide; MCV, anti-mutated citrullinated vimentin; RA, rheumatoid arthritis; SEM, standard error of the mean.

In the 99 healthy controls 6 individuals had anti-MCV antibodies (6.1%), compared to 1 individual positive for anti-CCP2 and 1 individual positive for anti-CCP3.1. The test characteristics in differentiating RA from healthy controls showed that the anti-MCV test had the highest sensitivity but the lowest specificity (93.9% compared to 99% for anti-CCP2 and anti-CCP3.1) (**Table 1**). In addition, the LR+ was markedly higher for anti-CCP2 and anti-CCP3.1 compared to anti-MCV (56.3, 57.5 and 10.2 respectively).

DISCUSSION

The present study evaluated the diagnostic performance of a novel commercially available anti-MCV ELISA test for the detection of anti-MCV antibodies in differentiating RA patients from other forms of early inflammatory arthritis, taking advantage of a large inception cohort. Using the cut-off values as indicated by the manufacturer, the anti-MCV assay displayed a higher sensitivity but a lower specificity compared to a second and third generation anti-CCP test. In addition, the LR+ was higher for anti-CCP2 and anti-CCP3.1 than for anti-MCV, suggesting that a positive anti-CCP2 or anti-CCP3.1 test indicates a larger increase in the probability on RA compared to a positive anti-MCV test. This finding is further supported by the observation of a high prevalence of anti-MCV antibodies in other forms of arthritis, reducing its discriminative ability in an early arthritis setting.

An increased prevalence (6-10%) of anti-CCP2 antibodies has been reported previously in PsA [12- 14]. We observed a prevalence of anti-CCP2 of 6.1% in PsA and approximately three times higher percentage of anti-MCV positivity. The antibodies detected by the anti-MCV test were not only relatively frequently present in PsA but also in SpA and other forms of inflammatory arthritis, suggesting that these antibodies are not highly specific for RA.

The current observation of a higher sensitivity but a lower specificity in differentiating RA from other inflammatory arthritis is in line with a previous study comparing the test characteristics in 92 RA-patients and 150 patients with other forms of arthritis [8]. The strengths of the present study are the sample size (n=917) and the early arthritis setting representing the situation in clinical practice where these auto-antibody tests are used.

Generally, the autoantibody tests are performed in patients with recent-onset arthritis in order to differentiate between different diagnoses.

In conclusion, the diagnostic performance of the anti-MCV test in the differential diagnosis of early arthritis is lower compared to the anti-CCP tests. Anti-MCV antibodies are present in 13.9-19.4% of non-RA arthritis. It will be interesting to know whether the presence of the antibodies detected by the anti-MCV assay is related to arthritis only or whether they will be found in other inflammatory disorders as well.

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