A Comparison Between IgG- and IgA-class Antibodies to Cyclic Citrullinated Peptides and to Modified Citrullinated Vimentin in Early Rheumatoid Arthritis and Very Early Arthritis

ANNA SVÄRD, ALF KASTBOM, MARIA K. SÖDERLIN, ÅSA RECKNER-OLSSON, and THOMAS SKOGH

ABSTRACT. ***Objective.*** Because of their slightly higher sensitivity, it has been argued that antibodies to modified citrullinated vimentin (anti-MCV) are superior to antibodies to cyclic citrullinated peptides (anti-CCP), while others claim that anti-CCP is preferable because of higher diagnostic specificity for rheumatoid arthritis (RA). We evaluated IgG- and IgA-class anti-MCV and anti-CCP as diagnostic and prognostic markers in early arthritis.

Methods. Two Swedish arthritis populations were examined: 215 patients with early RA (≤ 12 months' duration) from the Swedish TIRA-1 cohort, and 69 patients with very early arthritis (≤ 3 months' duration) from the Kronoberg Arthritis Incidence cohort, in which 22% were diagnosed with RA. IgG anti-CCP and anti-MCV antibodies were analyzed with commercial kits. These tests were modified for IgA-class antibody detection. Results were related to disease course, smoking habits, and shared epitope status.

Results. In the TIRA-1 cohort, occurrence of IgG anti-MCV and IgG anti-CCP showed a 93% overlap, although IgG anti-MCV had higher diagnostic sensitivity. Twenty-four percent tested positive for IgA anti-MCV compared to 29% for IgA anti-CCP. In the Kronoberg Arthritis Incidence cohort, 15% tested positive for IgG anti-MCV and 6% for IgA anti-MCV, compared to 10% positive for IgG anti-CCP and 3% positive for IgA anti-CCP, revealing that anti-CCP had higher diagnostic specificity for RA. As previously reported for IgA anti-CCP, IgA anti-MCV antibodies occurred in a small proportion of high-level IgG antibody-positive sera and were associated with a more aggressive disease course. Smokers were more often positive for antibodies to citrullinated proteins, most strikingly among the patients who were IgA anti-MCV-positive.

Conclusion. The occurrences of IgG-class anti-MCV and anti-CCP in early RA largely overlap. The sensitivity of anti-MCV is slightly higher, while the diagnostic specificity is higher for anti-CCP. In both instances a positive test predicts an unfavorable disease course, possibly slightly more so for anti-MCV. Although associated with a more active disease over time, IgA-class anti-CCP or anti-MCV do not add any diagnostic advantage. (J Rheumatol First Release April 1 2011; doi:10.3899/ jrheum.101086)

Key Indexing Terms:
ANTICYCLIC CITRULLINATED PEPTIDE ANTIBODIES VIMENTIN
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From the Rheumatology Clinic, Falun Hospital, Falun; and the AIR/Rheumatology Unit, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University Hospital, Linköping; and the Spenshult Rheumatology Centre, Oskarström, Sweden.

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A. Svärd, MD, Rheumatology Clinic, Falun Hospital; A. Kastbom, MD, PhD, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University Hospital; M.K. Söderlin, MD, PhD, Spenshult Rheumatology Centre; Å. Reckner-Olsson, MD, PhD; T. Skogh, MD, PhD, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University Hospital.

Address correspondence to Dr. A. Svärd, Rheumatology Clinic, Falun Hospital, SE-791 82 Falun, Sweden. E-mail: anna.svard@ltdalarna.se

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Testing for IgG-class antibodies to citrullinated proteins/peptides has become firmly established as an important diagnostic and prognostic tool in rheumatoid arthritis (RA)[1,2,3,4]. Compared to other anti-citrullinated protein antibody (ACPA) tests, the second-generation anti-cyclic citrullinated peptide antibody test (anti-CCP) is by far the best documented. A major advantage of the anti-CCP test compared to rheumatoid factor (RF) is its superior diagnostic specificity for RA. However, even before the anti-CCP era, anti-Sa antibody analysis had been shown to be a highly specific marker for RA[5]. Later it was demonstrated that the target antigen for anti-Sa antibodies is in fact citrullinated vimentin[6]. An enzyme-immunoassay for antibodies to modified citrullinated vimentin (anti-MCV) is now commercially available. Several studies report anti-MCV to be a slight-

ly more sensitive diagnostic marker of RA than anti-CCP[7,8,9,10], with 12%–15% of CCP-negative patients with RA being anti-MCV-positive[8,9]. Two studies report that anti-MCV and anti-CCP have similar specificities for RA (95% and 98%, respectively) using healthy blood donors as controls[8,11]. On the other hand, when patients with other inflammatory diseases have been used as referents, it has been reported that anti-CCP with specificities in the range 88%–99% is superior to anti-MCV with specificities of 78%–91%[7,9,10,12,13]. Recently, Raza, *et al* reported that, among patients with very early arthritis (duration of symptoms ≤ 3 months), anti-CCP has higher diagnostic specificity and higher positive predictive value for development of RA as compared to anti-MCV, although anti-MCV had marginally higher sensitivity[14]. In patients with established RA according to the 1987 American College of Rheumatology (ACR) classification criteria, IgG anti-MCV has been reported to be a better predictor of persistently active disease and radiographic progression than IgG anti-CCP[8,15].

A few studies have demonstrated that anti-CCP antibodies of the IgA class occur in a subpopulation of patients who are IgG anti-CCP-positive[16,17,18]. We reported that these patients had an even worse prognosis than patients with IgG anti-CCP alone, and that smokers more frequently produce IgA anti-CCP in addition to IgG anti-CCP[18]. Apart from IgG and IgA antibodies, anti-CCP antibodies of the IgM isotype occur[19] and, interestingly, IgE-class anti-CCP antibodies have been found to interact with circulating basophil granulocytes, indicating that ACPA of different isotypes can have distinct functional properties[20]. In a recent study in which anti-CCP of IgG_{1-4}, IgA, and IgM isotypes were evaluated, it was concluded that the presence of multiple ACPA isotypes was associated with more radiographic damage[21]. Our current study aims to compare the performance of IgG anti-MCV antibodies in 2 separate Swedish early arthritis cohorts, and to assess circulating IgA anti-MCV antibodies in relation to disease progression. In addition, we aimed to compare IgG and IgA anti-CCP and anti-MCV status in relation to smoking habits and genetic carriage of the shared epitope (SE).

MATERIALS AND METHODS

Patients. Two patient populations were investigated. First, the Swedish TIRA-1 (Swedish acronym for "early intervention in rheumatoid arthritis") cohort, as described[22]. It involves 320 patients with recent-onset RA (< 12 months since first joint swelling) who were followed up regularly over 3 years by recording the 28-joint count Disease Activity Score (DAS28)[23], serum C-reactive protein (CRP), the physician's global assessment of disease activity (PGA), and the Swedish version of the Health Assessment Questionnaire (HAQ)[24]. Of the TIRA patients, 97% fulfilled the 1987 ACR criteria, and the remaining 3% had symmetrical synovitis, small-joint arthritis (metatarsophalangeal and/or metacarpophalangeal, and/or proximal interphalangeal joints), and morning stiffness ≥ 60 min. In the TIRA-1 cohort, cigarette smoking and SE status were assessed as described, and defined as HLA-DRB1*01, *0401, *0404, *0405, *0408, *0409, *0410, *0413, *0416, *0419, *0421, or *10[25]. Serum samples for analysis of ACPA (IgG and IgA anti-MCV and anti-CCP) were available from 215 patients with RA. Data on smoking habits were available from all 215 patients; 140 never smoked and 75 had ever smoked (of whom 39 were current smokers). DNA was available from 145 of these patients, but no significant differences were found concerning age, sex, erythrocyte sedimentation rate (ESR), CRP, DAS28, or HAQ scores at inclusion, comparing the groups with and without serum samples and/or DNA.

The second study population was part of the Kronoberg Arthritis Incidence cohort[26]. It was designed as a prospective population-based incidence rate study in which 69 patients with very early arthritis (< 3 months' duration) were identified[26]. Serum samples for later analysis of ACPA were collected at inclusion. At the 2-year followup, the diagnoses as assessed by an experienced rheumatologist were RA (n = 16), reactive arthritis (n = 28), undifferentiated arthritis (n = 10), and other arthritides (n = 15), including 5 psoriatic arthropathy, 2 systemic lupus erythematosus, 2 sarcoid arthritis, 2 gluten enteropathy, 1 Lyme arthritis, 1 mixed connective tissue disease, 1 ankylosing spondylitis, and 1 polymyalgia rheumatica. All patients diagnosed with RA in the Kronoberg cohort fulfilled the ACR 1987 criteria. Criteria for the other diagnoses have been reported in detail[27]. Smoking status and DNA were not available in this cohort.

Autoantibody analyses. IgG-class anti-CCP antibodies of the second generation (RA immunoscan mark 2, EuroDiagnostica, Arnhem, The Netherlands) were analyzed as described[22], and a modification of this diagnostic kit was used to analyze anti-CCP antibodies of the IgA class[17]. Serum samples (stored in aliquots of 500 μl at –70°C until use) were diluted 1:100 with the diluent included in the kit. Polyclonal rabbit antihuman α-chain antibodies conjugated with horseradish peroxidase (HRP; DakoCytomation, Glostrup, Denmark) served as secondary antibodies at a dilution of 1:2000 in the kit diluent. A serially diluted high-level IgA anti-CCP patient serum was used as a calibrator, and the results were given in arbitrary units (AU/ml). Serum analyses were performed in duplicates and a cutoff limit of 25 AU/ml was determined based on the 99th percentile of 80 blood donors. The interassay coefficient of variation (CV) was 15% (9 separate analyses).

IgG-class anti-MCV antibodies (Orgentec Diagnostika, Mainz, Germany) were analyzed according to the manufacturer's directions, and a modification of this assay was developed to analyze anti-MCV antibodies of IgA class. Patient sera were diluted 1:100 using the diluent provided with the kit. As a secondary antibody, we used the HRP-conjugated rabbit antiserum, diluted 1:2000 with the anti-MCV kit diluent. (Anti-MCV kits were provided by Stefan Korpe, Electrabox Diagnostica AB, Tyresö, Sweden.) Similarly to the IgA anti-CCP assay, a 7-step serial dilution of a high-level IgA anti-MCV patient serum was used as a calibrator and the results expressed as AU/ml. Serum samples were analyzed in duplicate and the cutoff limit was set at 150 AU/ml, based on the 99th percentile of 105 blood donors. The interassay CV (12 separate analyses) was 15%. Anti-CCP antibodies had been analyzed earlier and anti-MCV antibodies were analyzed in connection with our current study.

Statistical analysis. Statistical analyses were performed using SPSS v.15.0 (SPSS, Chicago, IL, USA). Spearman's ρ correlation coefficient (r_s) was used to detect an association between levels of IgG anti-MCV and IgG anti-CCP. The difference in IgG anti-MCV levels between IgA anti-MCV-positive and IgA anti-MCV-negative sera was evaluated with the Mann-Whitney U test. The chi-squared test was used to analyze anti-MCV occurrence in relation to anti-CCP, as well as differences in antibody occurrence in relation to smoking and shared epitope status. Differences regarding disease activity measures at baseline and over time were tested by ANOVA for repeated measures.

The participating patients gave their written informed consent, and the study protocol was approved by the regional ethics committee in Linköping, Sweden.

RESULTS

The TIRA-1 cohort: IgG anti-MCV vs IgG anti-CCP. A total

of 215 baseline sera were available for analysis of both IgG anti-CCP and IgG anti-MCV. Out of these, 143 (67%) were IgG anti-MCV-positive and 137 (64%) IgG anti-CCP-positive. The distributions were largely overlapping, as shown in Table 1, but out of the 78 IgG anti-CCP-negative samples, 10 (13%) were IgG anti-MCV-positive and conversely, out of the 72 IgG anti-MCV-negative serum samples, 4 (6%) were IgG anti-CCP-positive ($p < 0.001$). The serum levels of IgG anti-CCP and IgG anti-MCV showed a strong correlation ($r_s = 0.87$).

The TIRA-1 patients testing positive for IgG anti-MCV had significantly higher disease activity as judged by ESR, serum CRP, DAS28, and PGA at 6, 12, 24, and 36 months compared to the IgG anti-MCV-negative patients (data not shown). No difference was seen in HAQ scores. Corresponding data regarding IgG and IgA anti-CCP, also from the TIRA-1 cohort, have been published[18]. The IgG anti-CCP-negative/IgG anti-MCV-positive patients (n = 10) had higher median ESR, DAS28, and HAQ scores than the IgG anti-CCP-negative/IgG anti-MCV-negative patients, with a consistent statistically significant difference over time regarding ESR (Figure 1). The few (n = 4) IgG anti-CCP-positive/IgG anti-MCV-negative patients had lower median levels of ESR (significant at 3 and 6 months) and DAS28 (significant at 3, 6, and 12 months) than the IgG anti-CCP-negative/IgG anti-MCV-negative patients.

Table 1. IgG anti-MCV and IgG anti-CCP status among 215 patients with early RA at the time of diagnosis (the TIRA-1 cohort).

	IgG Anti-MCV+	IgG Anti-MCV–	Total
IgG anti-CCP+	133 (61%)	4 (2%)	137
IgG anti-CCP–	10 (5%)	68 (32%)	78
Total	143	72	215

MCV: modified citrullinated vimentin; CCP: cyclic citrullinated peptide antibodies; RA: rheumatoid arthritis.

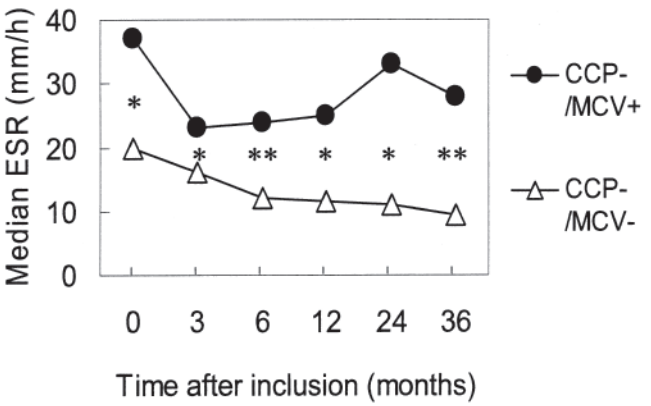

Figure 1. Median erythrocyte sedimentation rate (ESR) over 3 years after inclusion of IgG anticyclic citrullinated peptide (CCP)-negative patients with positive (n = 10) or negative (n = 68) IgG antimodified citrullinated vimentin (MCV) tests. *p < 0.05; **p < 0.01 (the TIRA-1 cohort).

The TIRA-1 cohort: IgA anti-MCV, smoking, and SE. Of the total number of 215 patients with RA, 52 (24%) were positive for IgA anti-MCV, all of whom tested positive for IgG anti-MCV and all but 1 testing positive for IgG anti-CCP. Among the IgG anti-MCV-positive patients, 36% were IgA anti-MCV-positive. The mean level of IgG anti-MCV was significantly higher ($p < 0.001$) in the IgA anti-MCV-positive patients (Figure 2).

The IgA anti-MCV-positive patients had significantly higher ESR and DAS28 throughout the 3-year followup period, compared to IgA anti-MCV-negative patients, according to ANOVA ($p < 0.001$ for ESR and $p = 0.002$ for DAS28; Figure 3A). Similar results were obtained using the Mann-Whitney U test at each timepoint. Among the IgG anti-MCV-positive patients, IgA anti-MCV-positive patients had significantly higher ESR at all times and a nonsignificant trend to higher DAS28 and HAQ (Figure 3B). Considering that IgA anti-MCV-positive individuals had higher levels of IgG anti-MCV, a condition known to be associated with higher disease activity, a comparison was done between 27 IgA anti-MCV-positive patients and 27 IgA anti-MCV-negative patients with similar levels of IgG anti-MCV. The median level of IgG anti-MCV in IgA anti-MCV-positive patients was 464 U/ml (25th percentile = 217, 75th percentile = 888) and 460 U/ml (25th percentile = 224, 75th percentile = 863) in IgA-negative patients. A comparison between these groups showed a remaining tendency to higher ESR over time in IgA anti-MCV-positive patients, but without reaching statistical significance (Figure 3C).

Smoking habits and prevalence of SE were assessed in the 3 groups with different ACPA status: IgG-negative/IgA-negative, IgG-positive/IgA-negative, and IgG-positive/IgA-positive. Chi-squared analyses of smoking habits

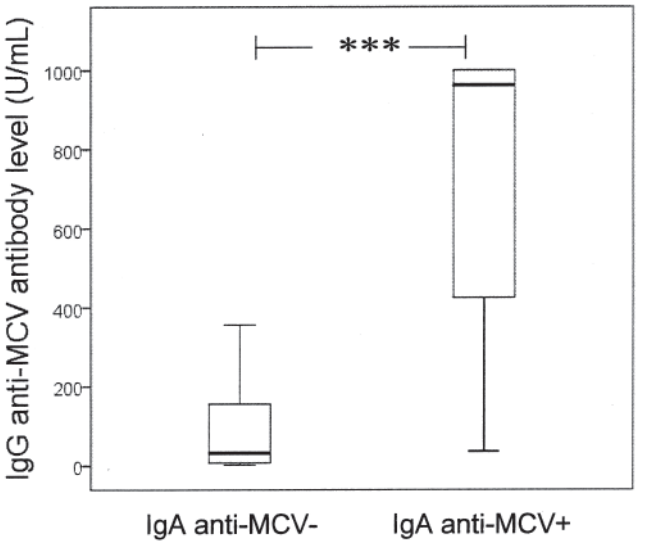

Figure 2. Levels of IgG antibodies to citrullinated vimentin (MCV; median/25th/75th percentile) in relation to IgA anti-MCV status. ***p < 0.001 (the TIRA-1 cohort).

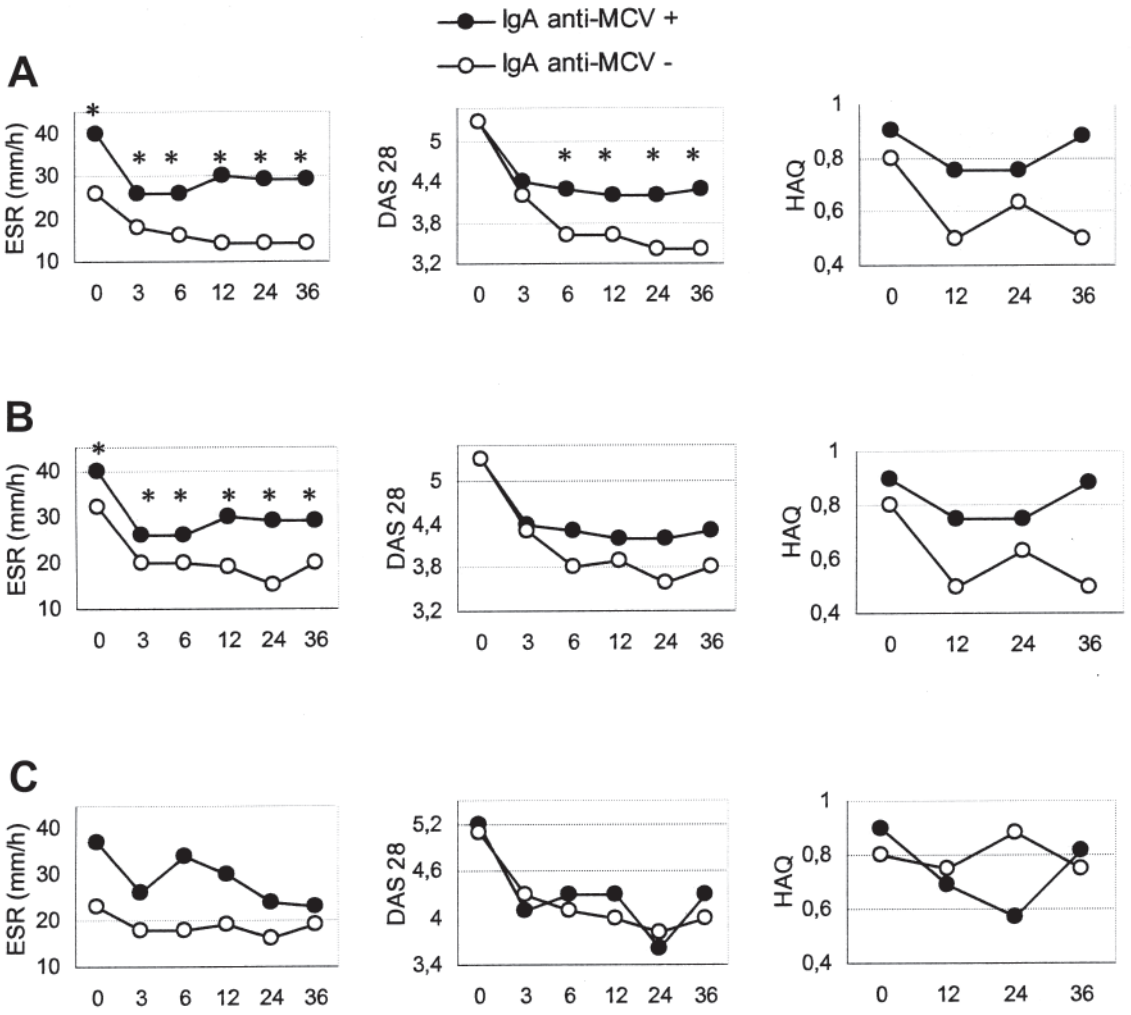

Figure 3. Median erythrocyte sedimentation rate (ESR), 28-joint count Disease Activity Score (DAS28), and Health Assessment Questionnaire (HAQ) in (A) all 215 patients, (B) all patients positive for IgG antimodified citrullinated vimentin (MCV; n = 143), and (C) 27 + 27 patients with pairwise comparable levels of IgG anti-MCV. *p < 0.05. Numbers along X axes indicate time, in months (the TIRA cohort).

(never, former, current) compared to ACPA status revealed the lowest proportion of smokers in the IgG-negative/IgA-negative group, slightly higher in the IgG-positive/IgA-negative group, and the highest percentage of smokers in the IgG-positive/IgA-positive group (Figure 4). These differences were statistically significant for anti-MCV but not for anti-CCP.

Regarding SE, a large and highly significant difference in the number of SE copies was recorded between IgG-negative and IgG-positive patients, irrespective of IgA status, with similar results for anti-CCP and anti-MCV. There was no significant difference between the IgA-negative/IgG-positive and the IgA-positive/IgG-positive groups (Figure 5).

The Kronoberg Arthritis Incidence cohort. Analyses of IgG and IgA anti-CCP and anti-MCV were made on all 69 sera from the patients included in the Kronoberg Arthritis Incidence cohort. Sera from 2 patients (1 with RA and 1 with reactive arthritis) previously testing positive for IgG anti-CCP[26] were now negative in repeated tests (probably because of prolonged storage and repeated thawing), and these 2 patients were therefore excluded from our study. In the remaining 67 sera, all previous results were reproduced.

A total of 10/67 (15%) were positive for IgG anti-MCV and 4/67 (6%) positive for IgA anti-MCV, while 7/67 (10%) were positive for IgG anti-CCP and 2/67 (3%) positive for IgA anti-CCP. Among the patients with RA, 6/15 (40%) tested positive for IgG anti-CCP and IgG anti-MCV, but only 2 (13%) for IgA anti-CCP and IgA anti-MCV. Among the patients with reactive arthritis, 1/27 (4%) tested positive for IgG anti-CCP and 2 (7%) for IgG anti-MCV, but none for IgA anti-CCP or IgA anti-MCV. In the group with undifferentiated arthritis, 1 (10%) tested positive for IgG anti-MCV, and in the "Other" group there was 1 (7%) patient positive for IgG anti-MCV and 1 (7%) for IgA anti-MCV.

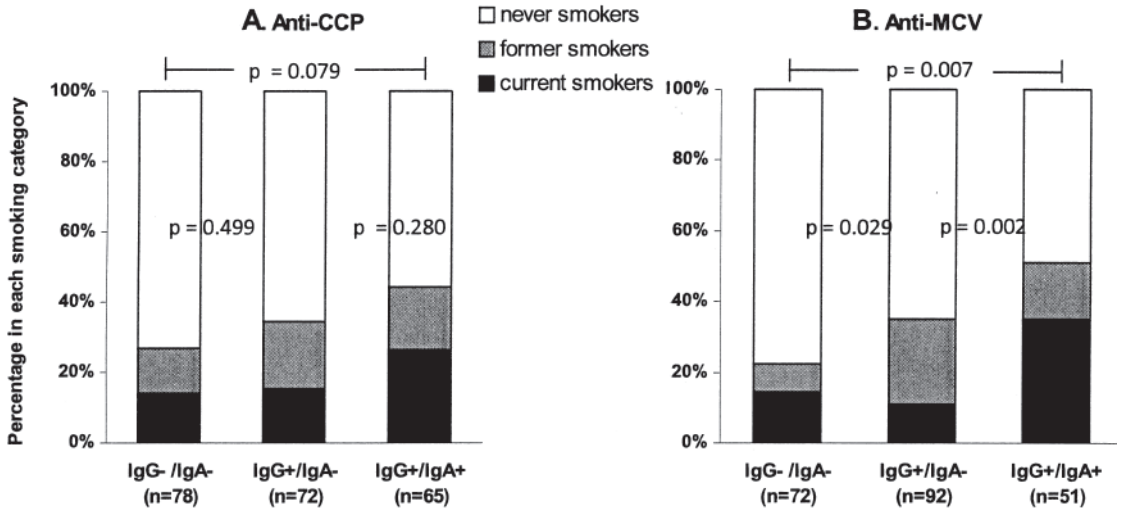

Figure 4. Smoking habits in groups with different antibody profiles (IgG- and IgA-negative, IgG-positive but IgA-negative, and IgG- and IgA-positive). A. Antibodies to cyclic citrullinated peptides (anti-CCP). B. Antibodies to modified citrullinated vimentin (MCV). P values refer to chi-squared tests, taking all 3 types of smoking status into account (the TIRA-1 cohort).

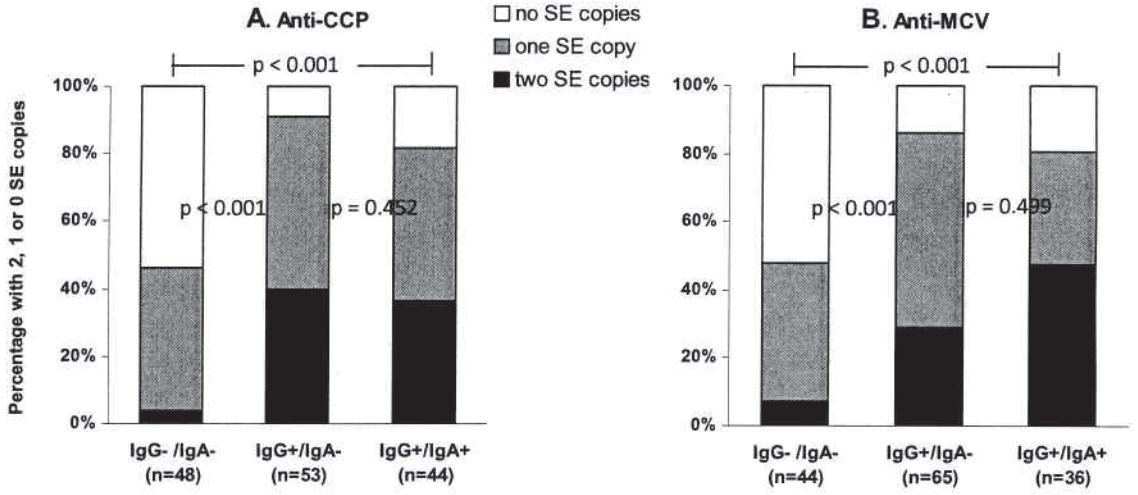

Figure 5. Prevalence of shared epitope (SE) in groups with different antibody profiles (IgG- and IgA-negative, IgG-positive but IgA-negative, and IgG- and IgA-positive). A. Antibodies to cyclic citrullinated peptides (anti-CCP). B. Antibodies to modified citrullinated vimentin (MCV). P values refer to chi-squared tests, taking all 3 types of SE status into account (the TIRA-1 cohort).

In this cohort of patients with very early arthritis progressing into clinically distinct diagnoses, IgG anti-CCP and IgG anti-MCV had equal sensitivity for very early RA (40%), but the specificity and positive predictive value of IgG anti-CCP was higher (Table 2).

DISCUSSION

We found that circulating IgA-class antibodies to MCV occur in a subpopulation of patients with early RA who have high serum levels of IgG anti-MCV. This is in keeping with our previous report on IgA-class anti-CCP[18]. The presence of IgA anti-MCV (similarly to IgA anti-CCP) was associated with a less favorable disease course compared to IgA anti-MCV antibody-negative cases[18]. In our current study we also compared the diagnostic utility of anti-MCV with anti-CCP antibodies, by analyzing data from 2 separate early arthritis cohorts. The Swedish TIRA-1 cohort was exploited to assess the prognostic value of the different IgG and IgA ACPA tests and their relation to disease activity, smoking, and SE status. The Kronoberg Arthritis Incidence cohort, in which patients with very early arthritis were progressing into a variety of rheumatologic diagnoses, was used

Table 2. Performance profiles of IgG- and IgA-ACPA in relation to rheumatoid arthritis diagnosis in the very early arthritis cohort (n = 67; the Kronoberg Arthritis Incidence cohort). Data are percentages.

ACPA Type	Sensitivity	Specificity	PPV
IgG anti-CCP	40	98	86
IgA anti-CCP	13	100	100
IgG anti-MCV	40	92	60
IgA anti-MCV	13	98	50

ACPA: anti-citrullinated protein antibodies; PPV: positive predictive value; MCV: modified citrullinated vimentin.

to evaluate the specificity of the ACPA tests regarding the diagnosis of very early RA. We found higher specificity of IgG anti-CCP but higher sensitivity of IgG anti-MCV, in keeping with other studies[4,7,9,12].

Soon after the discovery of IgG-class antibodies to citrullinated proteins/peptides, anti-CCP analysis was introduced into clinical routine as an important diagnostic tool in early arthritis[1]. Apart from its high diagnostic specificity, the IgG anti-CCP antibody assay identifies a distinct subpopulation of patients with unfavorable prognosis[2,3,4,22]. The strong association between IgG anti-CCP and SE has been firmly established[28] and has also clearly been confirmed in our early arthritis cohort TIRA-1[18]. Among exposure and lifestyle factors, cigarette smoking is so far the strongest identified risk factor for development of anti-CCP-positive RA[28]. This risk factor has also been found in the TIRA-1 cohort[29], and it has been shown to be a risk factor for extraarticular manifestations[30,31]. Further, a strong gene-environment interaction between SE and smoking has been demonstrated in 3 European studies[32,33,34]. In one study based on 3 European RA cohorts, it was demonstrated that the association between ACPA positivity, smoking, and genetic susceptibility factors to a large extent can be attributed to a subset of patients positive for IgG antibodies to citrullinated α-enolase[35]. In a recent Dutch study it was reported that the interaction between genotype, smoking, and ACPA positivity was stronger in a subset of anti-CCP-positive patients with RA who had antibodies against citrullinated vimentin as compared to anti-CCP-positive patients with antibodies against citrullinated fibrinogen[36]. In contrast to the European studies, one North American study found only a weak gene-environment interaction, while in 2 other North American studies, no interaction was found between SE and smoking with regard to susceptibility to anti-CCP-positive RA[37]. Thus, although several investigations have confirmed a genetic dose-dependent interaction with cigarette smoking and development of IgG anti-CCP-positive RA, conflicting reports exist[37,38]. In a study by Verpoort, *et al*, it was noted that the occurrence of anti-CCP of multiple isotypes among patients with RA, most notably IgA and IgM, was more prevalent in former or current smokers and without association to SE[39]. Our study partly supports this notion. Strong correlations were seen both to smoking and to SE among IgG anti-MCV-positive patients, but the even stronger correlation seen between IgA anti-MCV and smoking was not accompanied by a further enhanced occurrence of SE. This implies that IgG- and IgA-ACPA production in patients with RA may be influenced in part by different factors. Further studies on larger patient groups are required to shed more light on this matter.

Although the functions of mucosal IgA are well established, the roles of circulating IgA are not. It is conceivable that monomeric IgA-ACPA can mediate proinflammatory as well as antiinflammatory Fcα-receptor-dependent responses in phagocytes[40,41], but the importance of Fcα-mediated effects compared to Fcγ-mediated effects is completely unknown. A proinflammatory action of IgA-ACPA may seem logical, since their presence correlates to aggressive disease. Nevertheless, a factor correlating to disease activity does not necessarily mean that such a factor is a driving proinflammatory force. On the contrary, it could be speculated that IgA-ACPA may act as a downregulator of inflammation by tuning down proinflammatory responses mediated by IgG-class antibodies.

In our very early arthritis cohort (≤ 3 months' disease duration), IgG anti-MCV did not provide any important diagnostic information beyond that of IgG anti-CCP for the diagnosis of very early RA, as the sensitivity was equal, while the specificity of IgG anti-CCP was slightly higher, as also shown by others[14]. A limitation of these results is the low number of patients in this cohort (n = 67). In the TIRA-1 cohort, on the other hand, a substantial number of IgG anti-CCP-negative/IgG anti-MCV-positive patients were identified. These patients had a similarly unfavorable disease course to those testing positive for IgG anti-CCP. Because the IgG anti-CCP-negative/IgG anti-MCV-positive patients (in the absence of anti-MCV results and relying on the anti-CCP test alone) may erroneously be considered to have a favorable prognosis, there could be a risk of overlooking their need for close surveillance and early aggressive treatment. However, 7 out of these 10 patients tested positive regarding IgM-class RF, and as long as we continue analyzing RF, the "serological risk" of misjudging the prognosis of anti-CCP-negative/anti-MCV-positive patients is lowered, because a positive RF test is associated with an aggressive disease course in RA, even in the absence of anti-CCP[42].

Our study demonstrates a large overlap between anti-CCP and anti-MCV antibodies of IgG as well as IgA class in serum. Although the ACPA test of choice in clinical routine may largely be a matter of personal preference, our first choice remains IgG anti-CCP2 because of its superior diagnostic specificity. IgA-class ACPA is not needed for routine diagnostic purposes. However, further studies are required to investigate functional (proinflammatory or antiinflammatory) properties of IgG-class and IgA-class antibodies. The

indication that IgA-ACPA-positive patients may constitute a distinct subpopulation of patients with RA with poor prognosis, and where smoking may be a trigger, needs further scrutiny.

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