Concise Report

Antibodies against cyclic citrullinated peptide are associated with the DRB1 shared epitope and predict joint erosion in rheumatoid arthritis

S. Kaltenhäuser, M. Pierer, S. Arnold, M. Kamprad¹, C. Baerwald, H. Häntzschel and U. Wagner

Objective. To evaluate antibodies against cyclic citrullinated peptide (anti-CCP antibodies) for their predictive value for severe joint destruction in rheumatoid arthritis (RA) and to examine their relationship to shared epitope (SE)-positive DRB1 alleles.

Methods. Concentrations of anti-CCP antibodies were determined in sera from 126 patients with recent onset RA who had been followed prospectively for 6 yr. Progression of joint destruction was evaluated according to Larsen by scoring radiographs from the hand and feet taken at baseline and after 1, 2, 4 and 6 yr of observation. In addition to clinical parameters, the presence of SE-positive DRB1 alleles and of rheumatoid factor IgM and IgA was determined.

Results. Anti-CCP antibodies were found more frequently and in higher concentrations in both DRB1*01-positive and in DRB1*04-positive SE-positive patients compared with SE-negative patients. Severe joint destruction as defined by a Larsen score in the upper third of the study population was predicted by positivity for anti-CCP antibodies, by the presence of SE-positive DRB1*04 alleles and by the presence of erosive disease at initial presentation. Multiple logistic regression analysis revealed that SE-positive DRB1*04 alleles and anti-CCP antibodies exerted a significant influence on the progression of joint destruction.

Conclusion. The association of anti-CCP antibodies with DRB1*01 and with SE-positive DRB1*04 alleles implies a functional role for the SE sequence motif. The determination of SE-positive DRB1*04 alleles and of anti-CCP antibody positivity facilitates the prediction of disease course and prognosis at the time of initial presentation.

KEY WORDS: Rheumatoid arthritis, Anti-CCP antibody, Disease progression, HLA-DR

In rheumatoid arthritis (RA), antibodies against cyclic citrullinated peptide have emerged as clinically relevant diagnostic markers due to their high specificity for RA. Their diagnostic value is particularly evident in early disease [1], and they are usually already detectable at the onset of disease or even prior to the clinical phase [2, 3].

The targets of anti-CCP antibodies are protein side chains, in which an arginine residue is deiminated to peptidyl citrullin [4–6]. The process is catalysed by the peptidylarginine deiminase (PAD) enzymes, which are expressed in RA predominantly in monocytes and macrophages in the synovial membrane [7]. A haplotype of PAD4, which influences enzymatic activity, has been found to be associated with an increased risk of developing rheumatoid arthritis [8, 9]. The substrates for this group of enzymes include cytoskeletal proteins such as fillagrin, keratin, fibrin, vimentin and others (for review see [10]). Accordingly, anti-CCP antibodies have been shown to be targeted primarily towards citrullinated fibrin, fillagrin and vimentin [7, 11].

Modification of protein residues from arginine to citrulline is not restricted to RA, but also occurs in other inflammatory situations [12]. Examples are rodent arthritis models, where citrullinated peptides have been shown to be present in the synovitic joints [13], while no anti-CCP antibodies can be detected in those mice [14]. Consequently, additional factors must be present in RA to explain the disease specificity, and genetic markers including individual RA-associated DRB1 alleles or the RA-specific shared epitope are possible candidates.

Reports on the HLA association of anti-CCP antibodies have been conflicting, however [15–19]. Two recently published studies found the presence of anti-CCP antibodies to be associated with RA-associated DRB1*04 alleles [17, 20], but no significant association with SE-positive DRB1*01 alleles was detected.

The aim of our study was, therefore, the analysis of anti-CCP antibody concentrations in relation to the immunogenetic background and disease course in a clinically well-defined patient cohort which had been followed prospectively from the onset of disease over a period of 6 yr.

Patients and methods

Patients

The patient cohort analysed was recruited in the out-patient clinic of the Department of Medicine IV, Leipzig University, as part of...
a long-term prospective observational study. The median disease duration before study enrolment was 6 months [interquartile range (IQR) 3.6–10.5 months]. Eighty per cent of the patients were female, and the median age at study entry was 54 yr (IQR 44.7–60.5 yr). Written consent according to the Declaration of Helsinki was obtained from all patients prior to enrolment in the study. The study was approved by the University of Leipzig local ethical committee. Therapeutic decisions were made according to clinical requirements. Therapeutic ‘biologics’ inhibiting tumour necrosis factor-alpha (TNF-α) or interleukin-1 (IL-1) had not yet become available during the study and follow-up period. Patients were excluded from the study if lost to follow-up (n = 8).

Study visits were performed at baseline and after 1, 2, 4 and 6 yr of observation. At each study visit, clinical and laboratory parameters were determined and documented as described previously [21].

Radiographic progression

Radiographs of hand and feet were taken at each study visit and scored according to Larsen’s method [22] by two independent rheumatologists as described previously [23]. For all 126 patients, radiographs of hand and feet taken after 1, 2 and 4 yr were available, while the follow-up radiograph taken after 6 yr was available from 93 patients.

Detection of anti-CCP antibodies

A commercially available, second-generation anti-CCP enzyme-linked immunosorbent assay (ELISA) (Immunoscan RA2, GA Generic Assays, Dahlewitz, Germany) was used for the quantification of anti-CCP antibodies in patient sera stored at baseline and after 2 yr of observation. As cut-off for the definition of anti-CCP positivity, 50 units/ml were used.

HLA DRB1 typing

HLA DRB1 alleles were determined by hybridization of sequence-specific oligonucleotide probes to amplified HLA DRB1 polymerase chain reaction (PCR) products as described previously [24].

Statistical analysis

Odds ratios (ORs) were calculated as described previously [24, 25]. The Mann–Whitney rank sum test or the two-sample t-test were used where appropriate for the comparison of the different groups. The Yates-corrected χ² and the corresponding P value are given in case of significance. In addition to the χ², the upper and lower 95% confidence intervals are indicated. Correlations were evaluated using the Spearman rank correlation coefficient method.

Multiple logistic regression analysis was performed to determine the prognostic value of the independent variables for prediction of the dependent dichotomized variable severe joint destruction (a Larsen score above 32 after 4 and above 38 after 6 yr of observation). For all calculations, the software packages SPSS or SigmaStat (SPSS Inc., Chicago, IL) were used.

Results

Anti-CCP antibody positivity, HLA DRB1 alleles and shared epitope carriership

Demographic data in the study population were comparable to previous reports [21, 23, 24]. Forty-three per cent of the patients were positive for rheumatoid factor (RF) IgM, 24% for RF IgA and 35 patients (28%) already had erosive disease at the initial presentation. HLA DRB1 high-resolution typing revealed a frequency of 59% for the shared epitope, of 35% for DRB1*04 alleles containing the shared epitope sequence and of 17.5% for the presence of two copies of the shared epitope.

Determination of anti-CCP antibody concentrations showed 87 patients (69%) to be seropositive at initial presentation (median concentration 194.8 U/ml, interquartile range 19.6–613.5 U/ml). Subsequent determination of anti-CCP antibodies after 2 yr of observation showed that five of the previously negative patients became positive, while 12 patients converted to anti-CCP seronegativity. The titres determined at the two time points correlated strongly (R = 0.758, P < 0.0001), and no trend of an overall increase or decrease was detectable in the study population (median 194.8 and 181.6 U/ml, P = 0.96).

Anti-CCP antibody positivity was found to be significantly more frequent in SE-positive compared with the SE-negative patients (82.2 vs 53.1%, P = 0.001). The frequency of anti-CCP antibodies was equally increased in SE+ DRB1*04-positive (81.4 vs 53.1%, P = 0.008) and in DRB1*01-positive patients (83.3%) when compared with the SE-negative group.

The concentrations of anti-CCP antibodies measured in patients positive for the shared epitope were also higher than in the SE-negative group (median 395 vs 54.5 IU/ml, IQR 97.5–979 vs 8–252 IU/ml, P < 0.001). This was equally true for patients positive for DRB1*01 and patients expressing an SE+ DRB1*04 allele (median 546, IQR 102–1600 in SE+DR1+ and median 297, IQR 71.2–856.6 in SE+DR4+ vs 56.8, IQR 8–254.8 IU/ml in SE-negative patients, P < 0.001 and P = 0.002, respectively). The observed difference was not due to the increased frequency of anti-CCP-negative patients among the shared epitope negatives. When anti-CCP-negative patients were excluded from the analysis, anti-CCP concentrations were still significantly higher in SE-positive compared with SE-negative patients (median 546 vs 210 IU/ml, IQR 247–1224 vs 105–429 IU/ml, P = 0.005). Again, these differences compared with SE-negative patients were significant for both SE+DR1+ and SE+DR4+ patients (P = 0.002 and P = 0.05, respectively).

Anti-CCP antibodies and radiographic progression of joint destruction

Analysis of the progression of erosive joint destruction was performed by determining Larsen scores prospectively at the time points indicated in Fig. 1. Patients positive for anti-CCP antibodies at initial presentation had significantly higher Larsen scores at all time points analysed (Table 1). Similarly, the presence of anti-CCP antibodies after 24 months of observation was also associated with higher Larsen scores after 24 months and at all later time points.

The simultaneous presence of both anti-CCP antibodies and a SE+ DRB1*04 allele in a patient was associated with even higher Larsen scores in comparison with patients positive for only one of the two markers (Fig. 1), indicative of a possible additive effect of the two markers. In contrast, patients negative for both markers were found to have significantly lower Larsen scores throughout the observation period (Fig. 1).

With regards to the production of RFs, a strong association of anti-CCP antibodies with severe radiographic outcome was observed in RF IgM-negative patients (mean ± S.D. 28.3 ± 23.054 vs 12.345 ± 14.958, P = 0.002).

Multivariate analysis of factors influencing radiographic outcome in RA

For multiple logistic regression analysis, patients were grouped according to the presence or absence of a Larsen scores above 32 after 48 months of observation. The cut-off was the value reached.
by only one-third of the study population, which is equivalent to severe destructive disease as described previously [23, 26–28].

For the multiple logistic regression analysis, the variables RF IgM positivity, RF IgA positivity, anti-CCP positivity and presence of SE+ DRB1*04 alleles were entered as independent variables. A significant influence in the final model ($-2 \times \log$ likelihood $= 116.535$) was detected for anti-CCP positivity (odds ratio $3.665$, $P = 0.042$) and presence of a SE+ DRB1*04 allele ($OR = 5.046$, $P = 0.001$, see supplementary material available at Rheumatology Online). The influence of anti-CCP antibody positivity fell below the required level of significance, however, when the presence of erosive disease at study entry was entered as an additional covariate, due to its highly significant influence on radiographic outcome in both bivariate and multivariate analysis (see supplementary material available at Rheumatology Online).

Calculation of sensitivity and specificity for the prediction of severe joint destruction showed that the presence of anti-CCP antibodies had the highest sensitivity for this prediction (88.6%, specificity 40.2%), while both the presence of a SE-positive DRB1*04 allele and of early erosions had higher specificities.

![FIG. 1. The presence of prognostic markers influences the radiographic progression of joint destruction. Larsen sores at the indicated time points are shown as medians and standard error of the mean. The study population was separated into patients positive for both a SE+ DRB1*04-positive allele and anti-CCP antibodies (circles), patients positive for only one of the two markers (squares) and patients negative for both markers (triangles). Asterisks indicate the level of significance of bivariate comparison with the middle group (patients positive for only one of the two markers): *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.](image)

![TABLE 1. Comparison of anti-CCP-positive and anti-CCP-negative RA patients](table)

Clinical and laboratory data are medians and interquartile range (IQR) for the comparison between anti-CCP-negative and anti-CCP-positive patients. Comparisons were performed for the values at the indicated times of determination of anti-CCP antibody positivity. Radiographic data are medians and IQRs of Larsen scores in the anti-CCP-negative and anti-CCP-positive patient groups according to the anti-CCP results at initial presentation and after 2 yr of observation. Levels of significance are given as determined by bivariate analysis.
(75.6 and 86.6%, sensitivity 60.0 and 54.3%, respectively). The most specific prediction of radiographic outcome was possible by using SE+ DRB1*04 alleles and anti-CCP antibodies as a compound marker. If patients were negative for both parameters, i.e. if the compound marker was absent, a less severe joint destruction could be predicted with a specificity of 91.4%, although sensitivity was low at 35.4% (see supplementary material available at Rheumatology Online).

Discussion

The association of anti-CCP antibody production with HLA DRB1 alleles in RA patients has been discussed controversially. Two recently published studies found the SE-positive alleles DRB1*0401 and *1001 to be associated with the production of anti-CCP antibodies [17, 20]. In contrast, the SE-positive allele DRB1*0101, which is also found in increased frequencies in RA patients, was not associated with the presence of anti-CCP antibodies [17].

The results presented here show, however, that the presence of SE+ DRB1*01 alleles is clearly associated with increased production of anti-CCP antibodies in a European patient population, which suggests that anti-CCP antibodies are indeed associated not only with individual DRB1 alleles but with SE carriership in general.

The pathogenetic mechanisms that cause the shared epitope association of anti-CCP antibodies are unclear. It has been suggested that the binding of antigenic peptides to MHC class II alleles might be facilitated by citrullination of arginine residues, which is supported by the higher affinity of antigenic peptides for DRB1*0401 after citrullination in side chain residues interacting with the MHC molecule [29].

From a clinical perspective, the results presented confirm previously published observations of a substantially faster progression of joint destruction in early rheumatoid arthritis in the presence of anti-CCP antibodies [17, 18, 20, 30]. The sequential determination of anti-CCP antibody levels at initial presentation and after 2 yr of observation showed only limited fluctuation in anti-CCP positivity and a high correlation between the quantitative antibody levels determined at the two time points. This consistency of anti-CCP levels in early RA, and the significantly higher Larsen scores seen in anti-CCP-positive patients suggests that anti-CCP positivity is indeed a useful prognostic marker.

The results presented here also support previous reports of the superiority of anti-CCP antibodies over the RF IgM as a prognostic parameter of radiographic progression [20, 31, 32].

The predictive values for the development of severe joint destruction also show, however, that no single marker predicts the severity of joint destruction with sufficient accuracy. The most clinically relevant information can be deduced from the simultaneous absence of at least two of the analysed markers, since milder courses of joint destruction are to be expected in those patients.

Acknowledgements

The work presented here was supported by grants from the German Ministry for Education and Science (Interdisziplinares Zentrum für Klinische Forschung Leipzig, Teilprojekt A 15 and A21 and Kompetenzzentrum Rheuma, Entzündlich-rheumatische Systemerkrankungen, Teilprojekt C2.7).

The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at Rheumatology Online.

References


