Chapter 3

The role of the shared epitope in arthralgia with anti-cyclic citrullinated peptide antibodies (anti-CCP), and its effect on anti-CCP levels

Wouter H Bos
Jennie Ursum
Niek de Vries
Geertje M Bartelds
Gertjan Wolbink
Micheal T Nurmohamed
Irene E van der Horst-Bruinsma
Rob J van de Stadt
Jacob BA Crusius
Paul P Tak
Ben AC Dijkmans
Dirkjan van Schaardenburg

Abstract

Objective. Patients presenting with both arthralgia and antibodies to cyclic citrullinated peptide (anti-CCP) have an increased risk of developing rheumatoid arthritis (RA). To further characterize this patient group and shed more light on its relation with clinically manifest early arthritis and established RA, an immunogenetic and serological analysis was performed.

Methods. In a group of 111 patients with anti-CCP positive arthralgia, anti-CCP levels and shared epitope (SE) status were determined. Data were compared to 125 and 128 anti-CCP-positive patients with early arthritis and established RA, respectively.

Results. In anti-CCP-positive arthralgia patients, the frequency of SE allele positivity is significantly lower when compared to anti-CCP positive early arthritis and established RA (58% vs. 80%, and 58% vs. 92%, respectively, both $P < 0.001$). Median anti-CCP levels were higher in the group of SE positive arthralgia patients compared to the group of SE-negative arthralgia patients ($P = 0.02$). Median anti-CCP levels were similar in the groups of SE positive arthralgia and arthritis patients.

Conclusions. The lower frequency of SE positivity in arthralgia patients compared to RA patients indicates that, compared to SE-positive patients, SE negative patients as a group go through a longer arthralgia phase, or alternatively have a lower risk for transition from anti-CCP positive arthralgia to RA. Furthermore, the present results suggest that in this early stage the effect of the SE on disease risk may be mediated through higher anti-CCP levels.

Introduction

Antibodies to cyclic citrullinated peptide (anti-CCP) often precede the development of rheumatoid arthritis (RA) and can therefore be used to detect those at risk for the development of RA.[1-3] Another major risk factor for RA is the shared epitope (SE) at the HLA-DRB1 locus.[4,5] The SE hypothesis postulates that highly conserved amino acid sequences bordering the peptide binding groove of the HLA-DRB1 molecule are involved in the pathogenesis of RA, e.g. by enabling the presentation of arthritogenic peptides to T cells.[6] In support of the latter concept it has been shown that the DRB1*0401 peptide binding groove allows for a high affinity interaction with citrullinated peptides, resulting in efficient antigen presentation.[7] In a study analyzing preclinical blood samples of RA patients the highest risk for RA was associated with the presence of both anti-CCP and SE.[8] Recently, it was suggested that the increased risk for RA in SE positive undifferentiated arthritis is in fact not due to the SE, but to anti-CCP positivity.[9] In the latter study, the presence of SE alleles was associated with significantly higher levels of anti-CCP antibodies, suggesting that the SE alleles act as classic immune response genes. Therefore, the effect of the SE on RA development may be mediated through anti-CCP levels.

Patients presenting with both arthralgia and anti-CCP probably confer a high risk for the development of arthritis, although this patient group has not been extensively studied. We assessed the frequency of SE and levels of anti-CCP in these patients, and compared this with early and established RA.

Patients and methods

Study Population

The frequency of SE positivity in anti-CCP antibody positive unrelated Dutch Caucasians aged 18 or older was measured in three cohorts; arthralgia (possible preclinical RA) patients, early arthritis patients and RA patients with established disease starting anti-TNF treatment.

Between September 2004 and 2007, 190 anti-CCP or IgM-RF positive arthralgia patients were recruited at rheumatology clinics in the Amsterdam area of the Netherlands. Patients referred by the general practitioner with arthralgias were seen by a rheumatologist at our outpatient rheumatology clinic. In the absence of arthritis, anti-CCP positive patients (n=147) were referred for inclusion in the present study, a mean 2 months after the first visit to the outpatient clinic. At the first study visit, a trained medical doctor (WB) and a senior rheumatologist (DS) independently scored for absence of arthritis in all joints at physical examination. The senior rheumatologist was blinded for the reported joint complaints and the anti-CCP status. If clear absence of arthritis was seen by both doctors (n=125), and anti-CCP positivity was confirmed in the baseline serum sample (at least one month after the

initial positive sample; n=115), patients were included. Patients previously or at presentation treated with a disease modifying anti-rheumatic drug (DMARD and patients in whom history or chart review revealed past arthritis were excluded (n=4). In total, 111 anti-CCP positive arthralgia patients were eligible for analysis.

The early arthritis group consisted of 125 randomly selected anti-CCP positive patients included in the early arthritis clinic (EAC) of the Jan van Breemen Institute of whom SE status was available (n = 337/ ~1700). Random selection was performed by the Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, Illinois). Inclusion and exclusion criteria for this cohort have been described previously.[10] Ninety-seven patients fulfilled the ACR criteria for RA[11] at baseline and 9 additional patients during 2 years follow-up.

The established RA patients were 128 consecutive anti-CCP positive patients starting anti-TNF treatment at the Jan van Breemen Institute (n=113) and the Academic Medical Centre, Amsterdam, the Netherlands (n=15). All fulfilled the ACR criteria for RA;[12] mean disease duration was 12.6 years.

Laboratory investigations

Anti-CCP levels were determined using the serum samples that were obtained at inclusion by second-generation anti-CCP ELISA (Axis Shield, Dundee, United Kingdom). The cut-off level for anti-CCP antibody positivity was set at 5 Arbitrary Units/ml (AU) (according to the manufacturer's instructions). Sera reaching 1000 AU/ml were not further diluted.

HLA-DQ typing was performed as described previously. [13] The HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410 and *1001 alleles were taken to contain the SE. HLA-DRB1 SE carrier status (one or two SE copies) was inferred from HLA DQA1- DQB1 haplotypes using the strong linkage disequilibrium (LD) with HLA-DRB1 alleles in Caucasians.[14] This HLA-DRB1 typing procedure was validated in 87 established RA patients by sequence based high resolution typing (Sanquin, Amsterdam, the Netherlands). The technique correctly classified SE carriage in 86/87 patients. Independent confirmation in a second cohort of DRB1 and DQ-typed Dutch RA patients has shown that with DQA1 and DQB1 typing only two out of 167 patients would have been incorrectly classified.[15]

Analysis

Frequencies were analyzed by Fischer exact test, and odds ratios (ORs) were determined, whereas quantitative differences were analyzed using the Mann-Whitney test or *t*-test where appropriate. One-sided testing (P_1) was performed when appropriate.

Results

The 111 arthralgia patients (mean age 48 [range 22-81] years) were as expected significantly younger on average than both the 125 early arthritis and 128 established RA patients (mean ages 53 [range 23-76] and 55 [range 24-97] years, respectively). The sex distribution did not differ among the three groups (female sex 73, 66 and 73%, respectively). Table 1 shows the baseline characteristics of the arthralgia patients.

Table 1: Baseline characteristics of patients with anti-CCP positive arthralgia*

| Characteristic	
Age, mean (SD)	48 (12)
Female sex	81 (73)
Symptom duration at initial presentation in months, median (IQR) n=87†	12 (6-36)
Number of tender joints reported at baseline visit, median (IQR)	8 (2-14)
Distribution of tender joints	
Small Joints	24 (22%)
Large joints	24 (22%)
Small and Large joints	63 (57%)
Symmetric distribution tender joints	76 (69%)
Localisation of tender joints	
Upper extremities	49 (44%)
Lower extremities	9 (8%)
Upper and lower extremities	53 (48%)
Morning stiffness for more than one hour	15 (14%)
Pain on a 100 mm VAS, median (IQR)	28 (7-50)
Number of tender joints at physical examination, median (IQR)	0 (0-1)

* Except were indicated otherwise, values are the number of patients (%). SD=standard deviation, IQR=interquartile range, VAS=visual analogue scale. †data missing in remainder of patients

Low SE frequency in anti-CCP positive arthralgia compared to arthritis

Among anti-CCP positive individuals the fraction positive for SE was significantly higher in established RA than in early arthritis (Odds Ratio [OR] = 3.0; 95% Confidence Interval [95% C.I.] 1.4-6.4; P = 0.006), and significantly higher in patients with early arthritis than in those with arthralgia (OR = 2.9; 95% C.I. 1.6-5.2; P < 0.001). Compared to the arthralgia group, the odds ratio of SE positivity for anti-CCP positive established RA is even higher (OR = 8.7; 95% C.I. 4.1-18.3; P < 0.001) (figure 1).

Figure 1: Carriership of the shared epitope (SE) in anti cyclic citrullinated peptide antibody (anti-CCP) positive arthralgia, early arthritis and established rheumatoid arthritis (RA) patients
The frequency of SE carriage was 58%, 80% and 92% for anti-CCP positive arthralgia, early arthritis and established RA, respectively. The associated odds ratios are reported in the text.
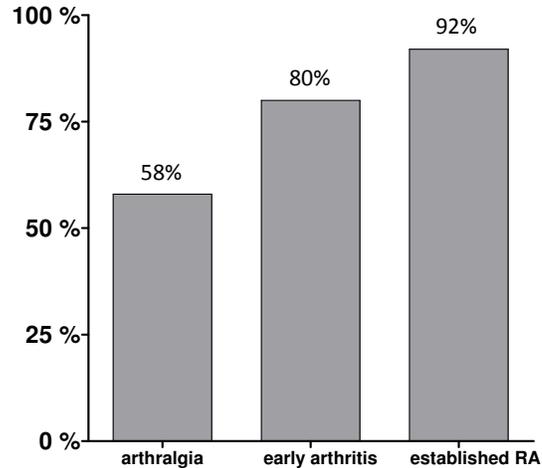
SE carriage is associated with higher anti-CCP levels in arthralgia patients only

SE carriage is associated with higher anti-CCP levels in anti-CCP positive arthralgia patients (median level 69 versus 18 AU, P_1 = 0.02, see figure 2). This association is not present in the early arthritis (median level 75 versus 73 AU, P_1 = 0.43) and established RA group (median level 91 versus 236 AU, P_1 = 0.1).

SE negative arthralgia patients have low anti-CCP levels when compared to arthritis patients

Anti-CCP levels were higher in established RA when compared to arthralgia patients (median levels 101 [IQR 31-397] versus 48 [IQR 14-135] AU, P_1 < 0.001). Early arthritis patients had intermediate anti-CCP levels (75 [IQR 23-162] AU), these levels were higher than in arthralgia patients (P_1 = 0.02) and lower than in established RA (P_1 = 0.01).

Anti-CCP levels were similar among the SE positive individuals in the three groups (median levels 69, 75 and 91 AU for arthralgia, early arthritis and established RA, respectively, see figure 2). However, in SE negative individuals, the median anti-CCP levels were lower in the arthralgia group (18 AU) when compared to the early arthritis group (73 AU; P_1 = 0.02), the established RA group (236 AU; P_1 = 0.002) or the pooled arthritis group (early arthritis and established RA, 82 AU; P_1 = 0.003). The median anti-CCP levels in the SE positive patients were similar in the arthralgia and the pooled arthritis groups (median levels 69 versus 76 AU, P_1=0.06).
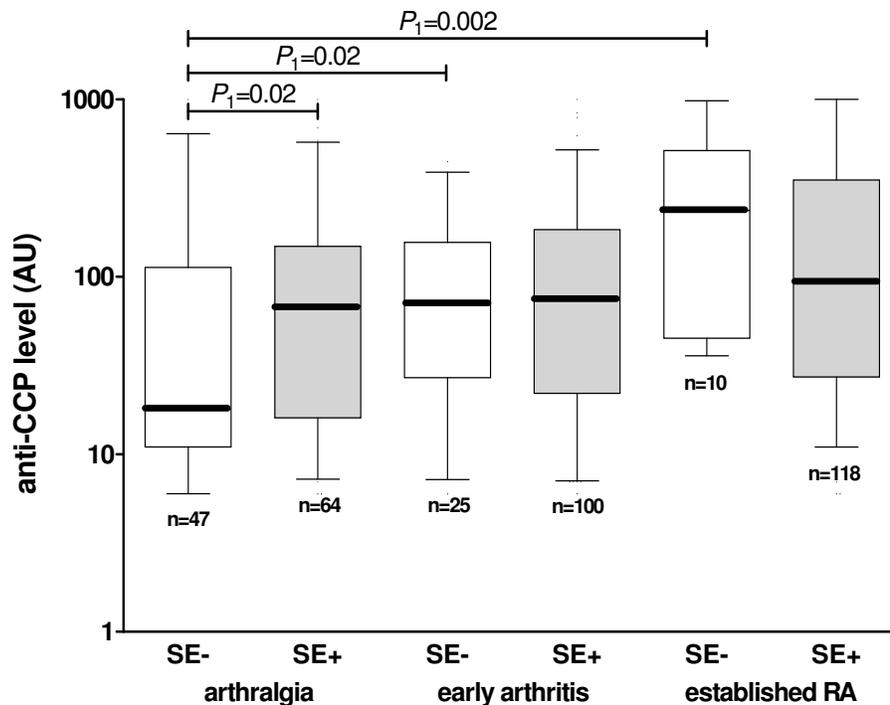
Figure 2: Baseline anti-cyclic citrullinated peptide antibody (anti-CCP) levels in arthralgia, early arthritis and established rheumatoid arthritis (RA) patients, stratified for the presence of the shared epitope (SE) allele.
The median anti-CCP antibody levels were 18 (interquartile range [IQR] 11-113), 73 (IQR 27-156) and 236 (IQR 45-515) Arbitrary Units (AU) in the SE negative arthralgia, early arthritis and established RA, respectively. In the SE positive patients, the median anti-CCP levels were 69 (IQR 16-149), 75 (IQR 22-184) and 91 (IQR 27-352) AU in arthralgia, early arthritis and established RA, respectively. Boxes depict median and interquartile range; whiskers show 5th en 95th percentile. One-sided *P* values are reported.

Discussion

In this first report on HLA typing in patients with anti-CCP positive arthralgia we observed that the anti-CCP positive arthralgia group includes a relatively large SE-negative subgroup of patients compared to anti-CCP positive early arthritis group and established RA group. However, anti-CCP positive arthralgia patients are clearly more often SE positive than Caucasian population healthy controls (58% versus 26-46 %).[16-18] The present study also shows that the SE-negative arthralgia group has relatively low anti-CCP levels compared to an anti-CCP positive early arthritis group and an established RA group. In contrast, SE positive arthralgia seems to reflect pre-rheumatoid arthritis since anti-CCP levels in patients with SE positive arthralgia are comparable to those found in SE positive arthritis patients.

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The current data shed more light on recent seemingly contrasting results on the role of SE and anti-CCP in RA. The presence of both SE and anti-CCP antibodies was associated with the highest risk for the development of RA in a retrospective analysis of preclinical RA patients[8], suggesting that the SE independently contributes to the risk for RA. However, a redundant role of the SE in the progression from anti-CCP positive undifferentiated arthritis to RA was recently reported.[19]

Our study reconciles both results since we show that only in anti-CCP positive arthralgia the SE is associated with higher anti-CCP levels, suggesting that the SE only operates on anti-CCP levels in the early phase up to early arthritis, but not in the later transition from early arthritis to established RA. This supports the conclusion that the SE does not itself predispose to development of RA but rather operates through higher anti-CCP levels, not only in the clinical phase[19] but also in the preclinical stages of RA. The presence in the arthralgia group of a relatively large group of SE negative patients with low anti-CCP levels compared to the arthritis groups suggests that among SE-negative arthralgia patients development of arthritis is restricted to patients with high anti-CCP levels. Alternatively, this could mean that a large part of the SE positive RA patients do not go through a stage of anti-CCP-positive arthralgia but rather simultaneously develop frank arthritis with the onset of arthralgia. The differences in observed anti-CCP levels were probably not due to age effects. In line with previous literature[20], we did not observe an effect of age on anti-CCP levels in the three patient groups, neither when analyzed individually nor when combined. Furthermore, age was similar among the SE negative and positive arthralgia patients (data not shown).

In summary, anti-CCP positive arthralgia patients have a relatively low frequency of the SE compared to anti-CCP positive early and established RA. Furthermore, these patients have relatively low anti-CCP levels. In later stages, the SE is not associated with differences in anti-CCP levels. This correlates with observations reported earlier that the presence of the SE, together with anti-CCP positivity increases the risk for RA, but that the SE does not influence the transition of anti-CCP positive early arthritis to rheumatoid arthritis. Together these findings suggest that the SE may influence RA risk through effects on anti-CCP levels. Longitudinal follow-up of arthritis development in arthralgia patients may further clarify the effect of the SE and anti-CCP (levels) on RA development.

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