Unravelling the anti-carbamylated protein antibody response in rheumatoid arthritis

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Chapter 4

Anti-Carbamylated Protein antibodies in Rheumatoid Arthritis patients of Asian descent

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Anti-Carbamylated protein (anti-CarP) antibodies were recently described to be present in Rheumatoid Arthritis (RA) patients[1]. The presence of anti-CarP antibodies has been found prior to disease onset, associates with the conversion towards arthralgia and with a more severe disease course in patients negative for anti-citrullinated protein antibodies (ACPA) [2,3]. The presence of anti-CarP antibodies has been described in other cohorts of RA patients as well [4-6]. So far, all of the investigated patient cohorts were Caucasian. However, the development of autoantibodies in RA patients can depend on genetic background. The presence of ACPA, for example, is strongly associated with certain HLA-DRB1 genes[7]. For anti-CarP antibodies, genetic associations have not yet been investigated thoroughly, but no specific association has been found with HLA-DRB1 in previous studies[4]. Since Caucasians and Asians have a different genetic background and positivity for autoantibodies, also for ACPA, can differ between ethnicities[4,8,9], we set out to investigate the levels of anti-CarP antibodies in serum samples from Asian RA patients. We now describe, for the first time, the presence of anti-CarP antibodies in a Japanese cohort.

Samples were obtained from Japanese RA patients on first visit at the Konan Kakogawa Hospital between April 2003 and March 2006 as described previously[9]. Written informed consent was obtained from the patients, according to the declaration of Helsinki and the study was approved by the Kobe Univ Hospital Review Board and Kohnan Kakogawa Hospital Review Board for Ethics. The average disease duration of the RA patients was 3,6 years. The presence of anti-CarP antibodies was determined in 268 RA patients and 324 healthy local controls by an enzyme-linked immunosorbent assay (ELISA) as described before[1]]. For this study, we measured the presence of IgG antibodies directed against carbamylated fetal calf serum (Ca-FCS). The levels were determined in arbitrary units per mL (aU/ml) using a standard curve.

RA patients display increased levels of anti-CarP antibodies when compared to healthy controls (mean ± standard deviation: 449 ± 544 vs 71 ± 133, Mann-Whitney U test: $P < 0{,}001$)(figure 1A). In order to determine positivity for anti-CarP antibodies, we fixed the cut-off at a specificity of 97%, as was the case in the Leiden early arthritis clinic (EAC) study (Figure 1B)[1]. Using this cut-off detection of anti-CarP antibodies had a sensitivity of 45,2% in RA. Further subdivision of the cohort on the basis of both anti-CarP antibodies and anti-CCP2 antibodies revealed four patient populations (Figure 1C). We observed 42,2% anti-CarP$^+$CCP2$^+$, 31,7% anti-CarP$^-$CCP2$^+$, 23,1% anti-CarP$^-$CCP2$^-$ as well as 3.0 % anti-CarP$^+$CCP2$^-$ patients (11,4% of all CCP2$^-$ patients). Similar percentages were observed when measuring CCP3 instead of CCP2. These proportions differ slightly from the Leiden EAC cohort, but are more similar to the epidemiological investigation of rheumatoid arthritis (EIRA) cohort, likely because of the higher frequency of CCP+ patients (EAC 53%, EIRA 63%, Japan 74%)[1,4].

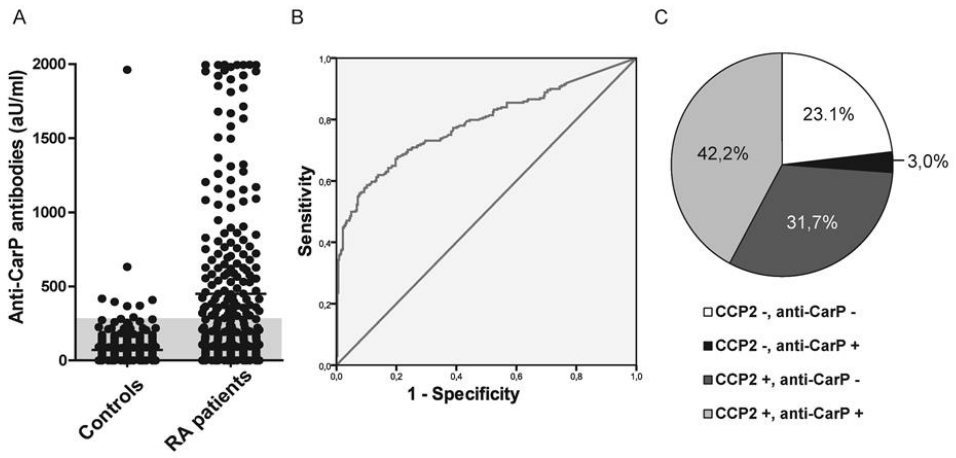

Figure 1 – Anti-CarP antibodies are present in the sera of Japanese RA patients. (A) The levels of anti-CarP antibodies as measured in the serum of RA patients and healthy controls by ELISA. (B) ROC curve using the presence of RA as the positive disease state and the anti-CarP antibody levels as the test variable. The area under the curve is 0.786. (C) The percentage of RA patients that are positive for CCP2 antibodies and / or anti-CarP antibodies. The cut-off for anti-CarP antibodies was set at the 97th percentile, while the cut-off for CCP2 antibodies was set according to the manufacturer's instructions.

Here we show for the first time that anti-CarP antibodies are also present in Asian RA patients. Importantly, also in the Japanese cohort, patients are present that are positive for anti-CarP antibodies, but not for ACPA. Also, some patients are positive for ACPA but not for anti-CarP antibodies. Previously, it has been suggested that anti-CarP antibodies and ACPA may be cross-reactive[6]. This data shows that although cross-reactivity might be present in the patients that show positivity for both autoantibodies, ACPA and anti-CarP antibodies are not always cross-reactive. In the future, it would be interesting to further investigate whether anti-CarP antibodies associate with disease progression in Asian RA patients.

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