Do autoantibody-responses mature between presentation with arthralgia suspicious for progression to rheumatoid arthritis and development of clinically apparent inflammatory arthritis? – a longitudinal serological study

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Several nested case-control studies have shown that autoantibody-response maturation in rheumatoid arthritis (RA) precedes clinical arthritis-development.[1–3] This suggests a role in disease triggering. However, nested case-control studies have, similar to case-control studies, the disadvantage that controls are selected and that prospective data from non-progressing patients in a similar pre-disease stage are absent. The phase preceding clinically apparent inflammatory arthritis (IA) can be distinguished into an asymptomatic and symptomatic (i.e. clinically suspect arthralgia, CSA) sub-phase. It is unknown whether autoantibody-response maturation occurs in the symptomatic phase. Likewise, its role in progression to clinical arthritis is undetermined; if autoantibody-response maturation relates to disease-development, maturation is expected to be more pronounced in CSA-patients that progress compared to CSA-patients that do not. To better understand the relation between autoantibody-response maturation in time and development of clinical arthritis (RA/IA), we performed a longitudinal study on autoantibody-response maturation in CSA-patients that did and did not progress.

In serum from 147 CSA-patients, we determined with in-house ELISAs the presence and levels of IgM, IgG, IgA anti-citrullinated, anti-carbamylated and anti-acetylated protein
antibodies (ACPA, anti-CarP, AAPA), resulting in 9 autoantibody measurements per patient per time-point. Autoantibody-response maturation was defined as increase in number of autoantibody-reactivities or isotypes, and/or increase in autoantibody levels. CSA-patients with paired samples at first presentation at the outpatient clinic and at IA-development (n=55) or else after 2-years (n=92) were selected. Analyses were repeated with the outcome RA (the subgroup of IA-patients that fulfilled the 2010-or 1987-criteria at the time of IA-development). Detailed description of methods and baseline characteristics are shown supplementary.

In patients negative for all autoantibodies at baseline, 17% of patients that progressed to IA became positive, compared to 6% of “non-progressors” (figure 1A, p=0.12). In patients with ≥1 autoantibody-reactivity at baseline progressing to IA, the median number of autoantibody-reactivities was 1.0 (IQR 1.0-3.5, max. 6) at baseline and 1.0 (IQR 1.0-4.0, max. 6) at IA-development (p=0.29). In non-progressing CSA-patients with ≥1 autoantibody-reactivity at baseline, this was 1.0 (IQR 1.0-2.0, max. 4) at baseline and 1.0 (IQR 0.0-2.3, max. 5) after 2-years (p=0.07). As shown in figure 1B; an increase in the number of autoantibody-reactivities was infrequent (16% in progressors, 18% in non-progressors (p=1.00)). Most changes in autoantibody-positivity were explained by fluctuations around the cut-off (data not shown). Levels of autoantibodies did not significantly change over time (p-values ranging 0.21-1.00) both in progressors and non-progressors (figure 1C). Similar results were found with the outcome RA (figure S1), though remarkably, the number of autoantibody-reactivities in patients not-progressing to RA significantly decreased over time (1.0 (IQR 1.0-2.0) at baseline and 1.0 (IQR 0.0-2.0) after 2-years, p=0.015). Finally, when evaluating number of autoantibody-reactivities and autoantibody-level changes within the entire study population (instead of within patients with ≥1 autoantibody-reactivity at baseline) no significant increases were found (figure S2).

To the best of our knowledge, this is the first study evaluating multiple isotypes and three anti-modified protein autoantibodies over time in CSA. Our data indicate that the presence and levels of IgM, IgG and IgA ACPA, anti-CarP and AAPA did not significantly increase over time, and that this was similar for CSA-patients that did or did not develop IA.

Autoantibody maturation in terms of cross-reactivity, affinity maturation and involvement of individual B-cell clones was not studied here, which is a limitation. We did not observe changes in isotype-usage over time, indicating that isotype switching was infrequent in both groups (figure S3, table S4). Although we cannot exclude that the results of this study would be different with a larger sample size (especially in CSA-patients autoantibody-negative at first presentation), the current data suggests that autoantibody-response maturation already occurs before presenting with CSA and that it does not increase substantially during progression to IA. Our results on characteristics of the ACPA, anti-CarP and AAPA-response expand on previous longitudinal studies showing similar ACPA- and RF-levels[4, 5], and absence of change in the ACPA antigen-recognition repertoire in ACPA-positive arthralgia.[6] The data together imply that maturation occurs predominantly in the asymptomatic phase, a finding to be confirmed in population-based studies. Moreover, in relation to a multiple-hit model for RA-development, our data suggest that autoantibody-response maturation in the CSA-phase is not related to the “final hit” as maturation was
similar in CSA-patients not developing RA. These results increase the comprehension of the pathogenesis of RA.

In conclusion, autoantibody-response maturation as measured in this study occurs in the vast majority of CSA-patients before presenting with symptoms and broadening of the autoantibody-response is not specific for progression from arthralgia to clinical arthritis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

[1]. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004; 50:380–6. [PubMed: 14872479]

[2]. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 2003; 48:2741–9. [PubMed: 14558078]

[3]. Gan RW, Trouw LA, Shi J, Toes RE, Huizinga TW, Demoruelle MK, et al. Anti-carbamylated protein antibodies are present prior to rheumatoid arthritis and are associated with its future diagnosis. J Rheumatol. 2015; 42:572–9. [PubMed: 25593232]

[4]. Ten Brinck RM, van Steenbergen HW, van Delft MAM, Verheul MK, Toes REM, Trouw LA, et al. The risk of individual autoantibodies, autoantibody combinations and levels for arthritis development in clinically suspect arthralgia. Rheumatology (Oxford). 2017; 56:2145–53. [PubMed: 28968865]

[5]. van Beers-Tas MH, Stuiver MM, de Koning M, van de Stadt LA, Geskus RB, van Schaardenburg D. Can an increase in autoantibody levels predict arthritis in arthralgia patients? Rheumatology (Oxford). 2018; 57:932–4. [PubMed: 29401313]

[6]. Janssen KM, Westra J, Chalan P, Boots AM, de Smit MJ, van Winkelhoff AJ, et al. Regulatory CD4+ T-Cell Subsets and Anti-Citrullinated Protein Antibody Repertoire: Potential Biomarkers for Arthritis Development in Seropositive Arthralgia Patients? PLoS One. 2016; 11:e0162101. [PubMed: 27585422]
Figure 1.
Changes in autoantibody-response over time: A) percentage of patients with seroconversion to positive in patients negative for all autoantibodies at baseline, B) percentage of patients that has an increasing, decreasing or stable number of positive measurements over time in patients positive for ≥1 autoantibody-reactivity at baseline, C) autoantibody levels over time in patients positive for the respective autoantibody at baseline.
All results are shown separately for CSA-patients that did and did not progress to IA.
The mean time between first presentation and IA development was 5.6 months (SD 9.2).
In patients that did not progress the second serum sample was obtained after 2-years.
Figure 1A autoantibody negativity at baseline was defined as negative for the nine studied measurements (n=100), figure 1B autoantibody positive was defined as at least one (out of nine) positive measurement at baseline (n=47). Error bars in figure 1A and 1B represent 95% CI. Dashed grey horizontal lines in figure 1C indicate the cut-off values for each autoantibody.
IA: clinically apparent inflammatory arthritis, ACPA: anti-citrullinated protein antibodies, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies.