# 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

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 predisease stage are absent. The phase preceding clinically apparent inflammatory arthritis (IA) can be distinguished into an asymptomatic and symptomatic (ie, clinically suspect arthralgia, CSA) subphase. 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 patients with CSA that progress compared with patients with CSA 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 patients with CSA that did and did not progress.

In serum from 147 patients with CSA, 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 nine autoantibody measurements per patient per timepoint. Autoantibody-response maturation was defined as increase in number of autoantibody reactivities or isotypes, and/or increase in autoantibody levels. Patients with CSA 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 patients with IA that fulfilled the 2010 or 1987 criteria at the time of IA development). Detailed description of methods and baseline characteristics is shown in the online supplemental file.

In patients negative for all autoantibodies at baseline, 17% of patients that progressed to IA became positive, compared with 6% of 'non-progressors' (figure 1A, p=0.12). In patients with ≥1 autoantibody reactivity at baseline progressing to IA,
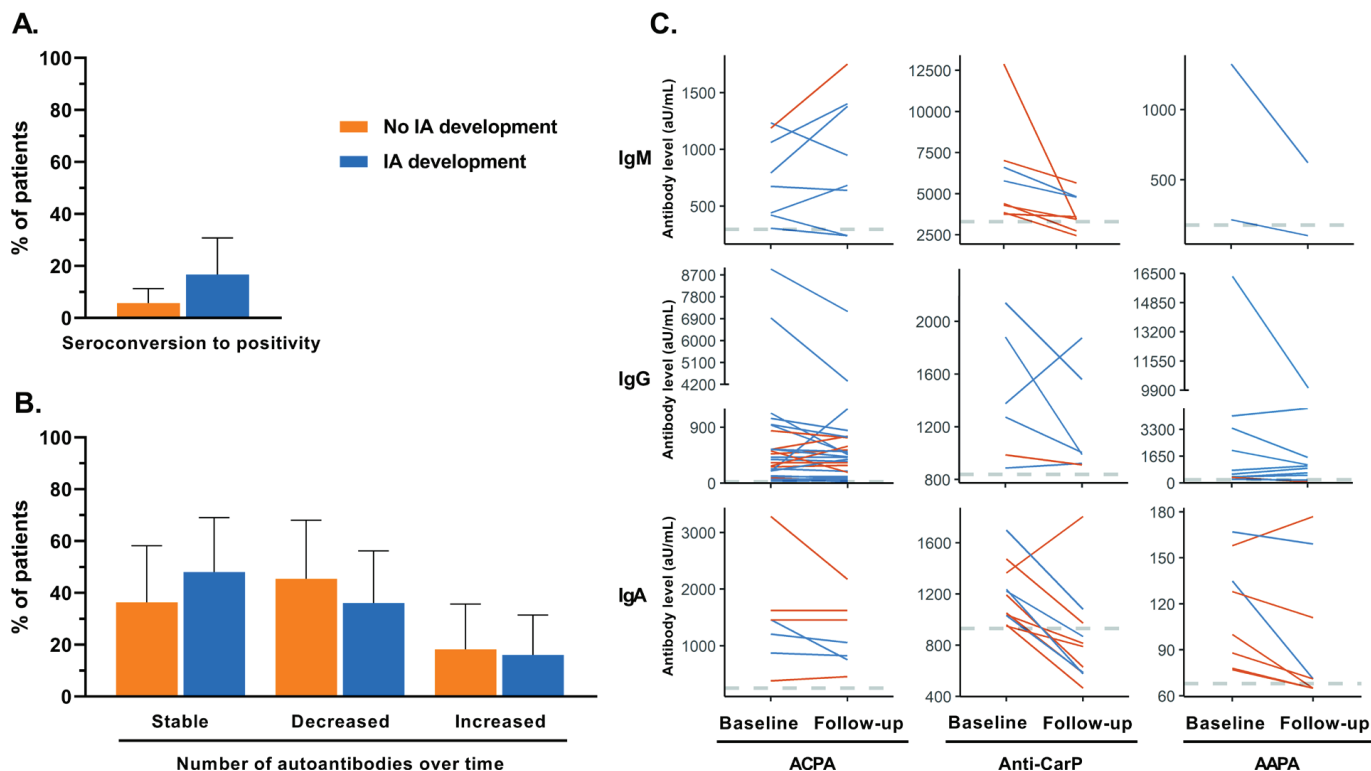
Figure 1 Changes in autoantibody response over time: (A) percentage of patients with seroconversion to positive in patients negative for all autoantibodies at baseline, (B) percentage of patients that has an increasing, decreasing or stable number of positive measurements over time in patients positive 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 patients with clinically suspect arthralgia that did and did not progress to clinically apparent inflammatory arthritis (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. (A) Autoantibody negativity at baseline was defined as negative for the nine studied measurements (n=100), (B) autoantibody positive was defined as at least one (out of nine) positive measurement at baseline (n=47). Error bars in (A) and (B) represent 95% CI. Dashed grey horizontal lines in (C) indicate the cut-off values for each autoantibody. ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; AAPA, anti-acetylated protein antibodies.

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 patients with non-progressing CSA 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 (online supplemental 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 (online supplemental 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 patients with CSA 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 (online supplemental figure S3, online supplemental table S4). Although we cannot exclude that the results of this study would be different with a larger sample size (especially in patients with CSA 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 patients with CSA 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 patients with CSA before presenting with symptoms and broadening of the autoantibody response is not specific for progression from arthralgia to clinical arthritis.

Fenne Wouters ![ORCID] ,[1] **Ellis Niemantsverdriet** ![ORCID] ,[1] **Nazike Salioska,**[1] **Annemarie L Dorjée,**[1] **René E M Toes** ![ORCID] ,[1] **Annette H M van der Helm-van Mil** ![ORCID] [1,2]

[1]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Rheumatology, Erasmus Medical Center, Rotterdam, The Netherlands

Correspondence to Fenne Wouters, Department of Rheumatology, Leiden University Medical Center, Leiden 2300 RC, The Netherlands; f.wouters@lumc.nl

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ORCID iDs
Fenne Wouters http://orcid.org/0000-0002-4375-4043
Ellis Niemantsverdriet http://orcid.org/0000-0002-5781-3817
René E M Toes http://orcid.org/0000-0002-9618-6414
Annette H M van der Helm-van Mil http://orcid.org/0000-0001-8572-1437

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