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## Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis

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## ABSTRACT

**Background:** The preclinical phase of rheumatoid arthritis (RA) is characterized by specific autoantibodies and slightly elevated markers of inflammation, but the sequence of these events is unknown.

**Objective:** To investigate the temporal relationship between the onset of inflammation (as measured by secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) and C-reactive protein (CRP)) and the presence of autoantibodies (IgM rheumatoid factor (IgM-RF) and antibodies against citrullinated peptides (anti-CCP)) in the preclinical phase of RA.

**Methods:** Of 79 RA patients who had been blood donor before the onset of disease, a median of 13 serum samples per patient was available. sPLA<sub>2</sub> was measured in patient and matched control samples and related to previous measurements of CRP, IgM-RF and anti-CCP. The temporal relationship between the increased markers of inflammation and autoantibodies was analyzed with time-lag analysis.

**Results:** Both the IgM-RF concentration and the anti-CCP concentration were significantly associated ( $p < 0.001$ ) with the concentrations of sPLA<sub>2</sub>, CRP and the combination of sPLA<sub>2</sub> and CRP at the same time point. However, we found no stronger association between the two autoantibody tests and the three inflammation parameters 1, 2 and 3 years before or after in comparison with the measurements at the same time, in the group as a whole as well as in subgroups of IgM-RF and anti-CCP positive patients.

**Conclusion:** Both the acute phase response and autoantibody formation often develop years before the first symptoms of rheumatoid arthritis occur, and these phenomena are probably closely connected in time.

The preclinical phase of rheumatoid arthritis (RA) is characterized by the presence of specific autoantibodies, such as IgM rheumatoid factor (IgM-RF)<sup>1-3</sup> and antibodies against citrullinated peptides (anti-CCP).<sup>2, 3</sup> One half of blood donors, who developed RA later, were found to be positive for IgM-RF and/or anti-CCP at least once before the onset of RA.<sup>3</sup> C-reactive protein (CRP) levels, as a marker of the acute phase response, increase over time in preclinical RA patients, with the highest values at the start of the symptoms. However, it remains unclear whether increased inflammation, measured with CRP, occurs before, after or simultaneous with the development of antibodies (IgM-RF and/or anti-CCP) in the preclinical phase of RA.<sup>4</sup>

This issue is relevant to the understanding of the pathogenesis of RA: appearance of autoantibodies before a rise in inflammation markers could suggest an antibody-driven inflammatory response. Conversely, the detection of inflammation prior to the increase in autoantibodies would provide evidence that antibody formation only occurs after a detectable level of inflammation has been reached. In addition, evidence for a temporary concentration peak of a marker of inflammation prior to autoantibody formation would lend support to the possibility of an infectious process preceding the development of RA.

Therefore, in the present study secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) - another sensitive marker of the acute phase response - was measured to investigate the temporal relationship between the onset of inflammation and the presence of autoantibodies in the preclinical phase of RA. In addition, this relationship was also tested using CRP and the combination of sPLA<sub>2</sub> and CRP.

## PATIENTS AND METHODS

### *Study subjects*

Since 1984, the Sanquin Blood Bank North West Region in Amsterdam, The Netherlands, has stored serum from donated blood at -30°C. We identified 79 patients with RA registered at the Jan van Breemen Institute who donated blood, in general 2-4 times per year, before the onset of the symptoms, as described previously.<sup>3</sup> For each RA sample, 1 control sample was selected, matched for gender, age, and time of blood donation. sPLA<sub>2</sub> was measured with an in-house ELISA.<sup>5</sup> CRP, IgM-RF and anti-CCP had been determined previously.<sup>3, 4</sup> The study was approved by the local Institutional Review Board.

### *Statistical analysis*

In a first analysis, the progression of the sPLA<sub>2</sub> concentration over time in the patient and control groups, corrected for age, gender and CRP, was estimated with random coefficient analysis. This longitudinal regression technique was used, because each patient had a different number of measurements at different points in time.

In a second analysis, all patient samples were used to study the temporal relationship between the increased markers of inflammation and autoantibodies in preclinical RA with time-lag analyses.<sup>6</sup> Concentrations of IgM-RF and anti-CCP on the one hand were associated with concentrations of 1) sPLA<sub>2</sub>, 2) CRP and 3) the combination of sPLA<sub>2</sub> and CRP ( $Z\text{-score } \ln\_s\text{PLA}_2 + Z\text{-score } \ln\_CRP$ ) at the other hand, at the same time point as well as at the time points 1, 2, and 3 years before, and 1, 2 and 3 years after each other. By comparing the magnitude of the different regression coefficients, one can determine if a time lag is present. The regression coefficients were calculated with random coefficient analysis and corrected for age and gender.

Finally, the same time-lag analyses were repeated in two subgroups: 1) the relationship between the increase of IgM-RF and the inflammation markers over time was studied in all samples of the patients who were positive for IgM-RF at least once before the onset of the symptoms, and 2) the relation between anti-CCP and the inflammation markers was analyzed in all serum samples of the patients who were positive for anti-CCP at least once before the start of the symptoms.

In all analyses, the natural log of sPLA2, CRP, IgM-RF and anti-CCP were used, because of the non-normal distribution of these variables. Random coefficient analyses were performed with MLwiN (Multilevel Models Project, Institute of Education, University of London, London, UK), a statistical program for multilevel analyses.

## RESULTS

Seventy-nine patients (62% female; mean age at onset of symptoms 51 years) who had been blood donor before the onset of RA were identified. A median of 13 serum samples per patient (range 1–51) was available; the median time between the first donation and the onset of the symptoms was 7.5 years (range 0.1–14.5 years). In total, 1078 patient sera and 1071 matched control sera were tested.

The sPLA2 levels of the patients and controls before the onset of symptoms, corrected for age, gender and CRP, are plotted in figure 1. The mean sPLA2 level of the patient group increased significantly over time ( $p=0.005$ ) with the highest values at the onset of the symptoms, whereas the mean sPLA2 level of the controls remained stable ( $p=0.50$ ).

Table 1 shows the results of the time-lag analyses in the group of RA patients. The concentrations of IgM-RF and anti-CCP were statistically significantly associated ( $p<0.001$ ) with the concentrations of sPLA2, CRP and the combination of sPLA2 and CRP at the same point in time. In the group as a whole, the association between the two autoantibody tests and the three inflammation parameters as measured 1, 2 and 3 years before as well as 1, 2 and 3 years after each other was not stronger compared to the association based on measurements at the same point in time.

Also in the subgroups of IgM-RF positive and the anti-CCP positive patients there was no stronger association between the antibody tests and the inflammatory parameters 1, 2 and 3 years before and after in comparison with the association based on measurements at the same time (data not shown).

## DISCUSSION

Serum levels of sPLA2 were increased in the preclinical phase of RA patients in comparison with healthy controls, in a manner similar to CRP, as published previously.<sup>4</sup> Both parameters of inflammation were used to study the temporal relationship between the increased markers of inflammation and autoantibodies in preclinical RA. The sophisticated technique of time-lag analysis, did not detect a stronger association between the antibody tests and the inflammatory parameters up to 3 years before or after in comparison with measurements at the same time.

In a previous study we concluded that it remained unclear whether increased inflammation, measured with CRP, occurs before, after or simultaneous to the development of autoantibodies in preclinical RA.<sup>4</sup> sPLA2 is also increased in preclinical RA, but the patterns in time of sPLA2 and CRP differ between individuals. Therefore, both measures of inflammation were analyzed separately and combined in a new time lag analysis in an attempt to unravel the sequence of increased inflammation and elevated autoantibody concentrations in the preclinical phase of RA. Because again we did not find a time lag, it is unlikely that a time lag can be found by adding other inflammatory parameters to the analysis, and the possibility of a simultaneous occurrence of these phenomena becomes more likely.

A possible explanation for missing an actually existing time lag could be that the available blood samples, although numerous, were too far apart in time to be able to detect a short time lag. A possible temporary sPLA2 or CRP peak indicative of an infectious process involved in causing the

rheumatoid arthritis could also have been missed because the samples were too widely spaced in time. The opposite conclusion, that since there is no evidence for a time lag, the phenomena of inflammation and autoantibody formation must be intimately coupled, is likely to be true but cannot be established beyond doubt on the basis of the present data. Support for the possibility of simultaneous development comes from the observed link between local antibody production and level of inflammation in the rheumatoid synovium.<sup>7</sup> In the somewhat different situation of B-cell depletion in established RA, the relapse of active arthritis after recovery from this depletion coincides with increasing CRP levels, that are preceded by a rise in autoantibody levels.<sup>8</sup> In conclusion, both the acute phase response and autoantibody formation often develop years before the first symptoms of rheumatoid arthritis occur, and these phenomena are probably closely connected in time.

### **COMPETING INTEREST STATEMENT**

None of the authors have competing interest

### **SPONSORS**

This study was not sponsored

### **ETHICS APPROVAL**

The study was approved by the local Institutional Review Board. (Ethics committee: Slotervaart Hospital, Jan van Breemen Institute and BovenIJ Hospital, Amsterdam, The Netherlands)

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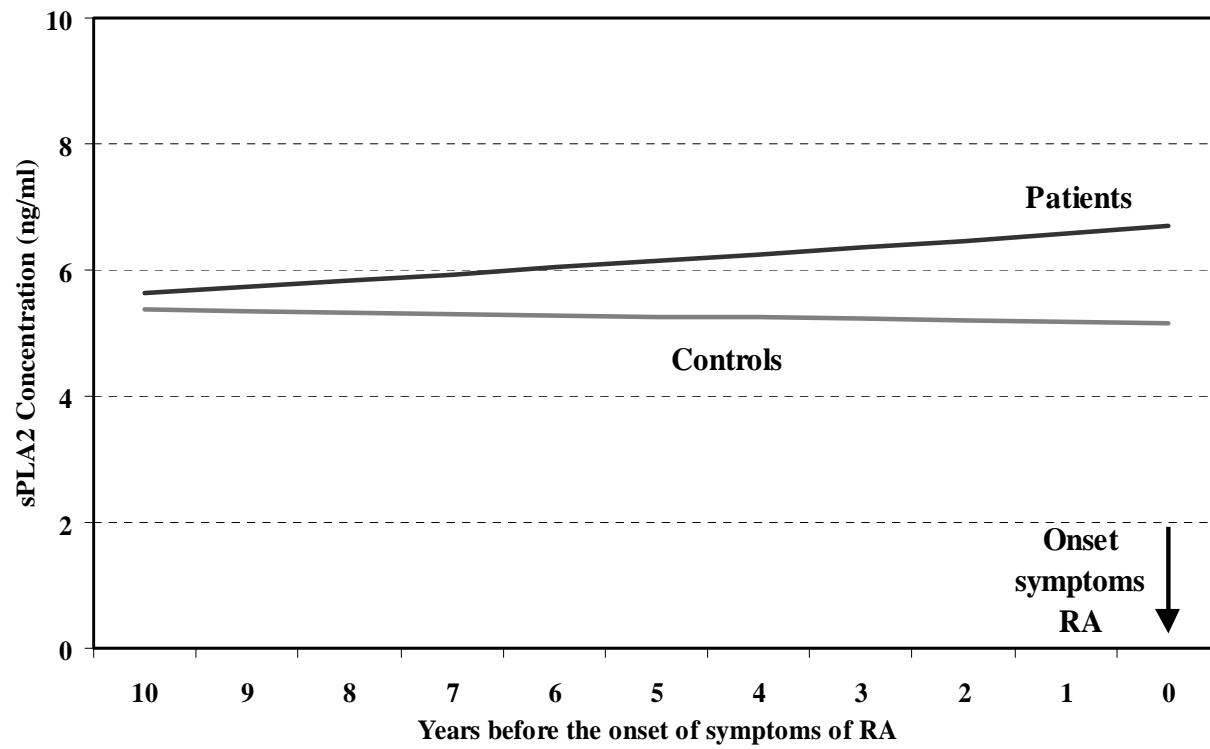
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**Table 1** Association between autoantibodies and parameters of inflammation (CRP, sPLA2 and the combination of CRP and sPLA2) at different points in time

IgM-RF	B (95% CI)		
	CRP	sPLA2	CRP + sPLA2
Inflammation 3 years earlier	0.06 (-0.01 – 0.12)	0.07 (0.04 – 0.10)	0.18 (0.07 – 0.29)
Inflammation 2 years earlier	0.07 (0.01 – 0.13)	0.05 (0.02 – 0.08)	0.16 (0.06 – 0.25)
Inflammation 1 year earlier	0.12 (0.06 – 0.18)	0.05 (0.01 – 0.08)	0.19 (0.10 – 0.29)
<b>Inflammation at same point in time</b>	<b>0.09 (0.04 – 0.13)</b>	<b>0.07 (0.04 – 0.09)</b>	<b>0.20 (0.13 – 0.27)</b>
Inflammation 1 year later	0.06 (0.00 – 0.12)	0.04 (0.02 – 0.07)	0.15 (0.06 – 0.24)
Inflammation 2 years later	0.11 (0.05 – 0.16)	0.02 (-0.01 – 0.04)	0.14 (0.05 – 0.23)
Inflammation 3 years later	0.05 (-0.01 – 0.12)	0.02 (-0.01 – 0.05)	0.09 (-0.01 – 0.20)

Anti-CCP	B (95% CI)		
	CRP	sPLA2	CRP + sPLA2
Inflammation 3 years earlier	0.05 (0.00 – 0.10)	-0.02 (-0.05 – 0.01)	0.00 (-0.09 – 0.09)
Inflammation 2 years earlier	0.03 (-0.03 – 0.08)	-0.01 (-0.03 – 0.02)	0.01 (-0.07 – 0.09)
Inflammation 1 year earlier	0.08 (0.03 – 0.13)	0.00 (-0.03 – 0.03)	0.08 (-0.01 – 0.16)
<b>Inflammation at same point in time</b>	<b>0.05 (0.02 – 0.09)</b>	<b>0.05 (0.02 – 0.07)</b>	<b>0.13 (0.07 – 0.19)</b>
Inflammation 1 year later	0.00 (-0.05 – 0.05)	0.01 (-0.02 – 0.03)	0.01 (-0.07 – 0.04)
Inflammation 2 years later	0.08 (0.03 – 0.14)	0.02 (0.00 – 0.05)	0.13 (0.04 – 0.21)
Inflammation 3 years later	0.06 (-0.01 – 0.13)	0.01 (-0.02 – 0.05)	0.09 (-0.01 – 0.19)



**Figure 1.** Secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) levels before the onset of symptoms in preclinical rheumatoid arthritis patients and controls.