

CONCISE REPORT

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

M M J Nielen, D van Schaardenburg, H W Reesink, J W R Twisk, R J van de Stadt, I E van der Horst-Bruinsma, M H M T de Koning, M R Habibuw, B A C Dijkmans



Ann Rheum Dis 2006;**65**:535–537. doi: 10.1136/ard.2005.040659

Objective: To investigate the temporal relationship between onset of inflammation (as measured by secretory phospholipase A₂ (sPLA₂) 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 rheumatoid arthritis (RA).

Methods: For 79 patients with RA who had been blood donors before the onset of disease, a median of 13 serum samples per patient was available. sPLA₂ was measured in patient and matched control samples and related to previous CRP, IgM RF, and anti-CCP measurements. The temporal relationship between the increased markers of inflammation and autoantibodies was analysed with time lag analysis.

Results: IgM RF and anti-CCP concentrations were significantly associated ($p < 0.001$) with concentrations of sPLA₂, CRP, and the combination of sPLA₂ and CRP at the same time point. However, we found no stronger association between the two autoantibody tests and the three inflammation measures 1, 2, and 3 years before or after a time point than for measurements at the same time, in the whole group or 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 RA occur, and these phenomena are probably closely connected in time.

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

This issue is relevant to an understanding of the pathogenesis of RA: appearance of autoantibodies before a rise in inflammation markers might suggest an antibody driven inflammatory response. Conversely, detection of inflammation before 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 before autoantibody formation

would lend support to the possibility of an infectious process preceding the development of RA.

Therefore, in the present study secretory phospholipase A₂ (sPLA₂)—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 sPLA₂ 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 a year, before the onset of the symptoms, as described previously.³ For each RA sample, one control sample was selected, matched for sex, age, and time of blood donation. sPLA₂ was measured with an in-house enzyme linked immunosorbent assay (ELISA).⁵ CRP, IgM RF, and anti-CCP had been determined previously.^{3,4} The study was approved by the local Institutional Review Board (ethics committee: Slotervaart Hospital, Jan van Breemen Institute and BovenIJ Hospital, Amsterdam, The Netherlands).

Statistical analysis

In a first analysis, the progression of the sPLA₂ concentration over time in the patient and control groups, corrected for age, sex, 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.⁶ Concentrations of IgM RF and anti-CCP, on the one hand, were associated with concentrations of (a) sPLA₂; (b) CRP; and (c) the combination of sPLA₂ and CRP (Z score \ln sPLA₂ + Z score \ln CRP), on the other hand, at the same time point as well as at 1, 2, and 3 years before, and 1, 2, and 3 years after that time. By comparing the magnitude of the different regression coefficients, one can determine if a time lag is present. The regression coefficients were calculated by random coefficient analysis and corrected for age and sex.

Finally, the same time lag analyses were repeated in two subgroups: (a) the relationship between the increase of IgM RF and the inflammation markers over time was studied in

Abbreviations: anti-CCP, antibodies against citrullinated peptides; CRP, C reactive protein; RA, rheumatoid arthritis; RF, rheumatoid factor, sPLA₂, secretory phospholipase A₂

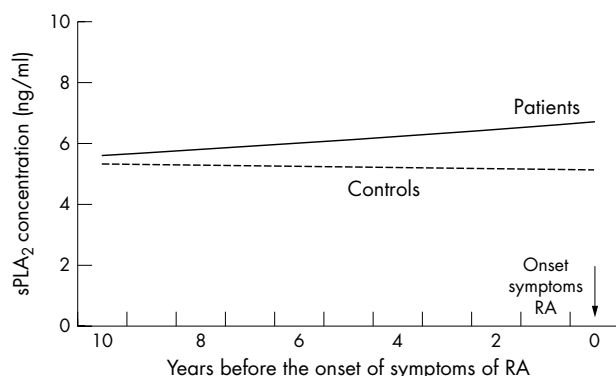


Figure 1 Secretory phospholipase A₂ (sPLA₂) levels before the onset of symptoms in the preclinical phase of patients with RA and in controls.

all samples of the patients who were positive for IgM RF at least once before the onset of the symptoms; and (b) the relation between anti-CCP and the inflammation markers was analysed 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 logs 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 (50 (62%) female; mean age at onset of symptoms 51 years) who had been blood donors 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). In total, 1078 patient sera and 1071 matched control serum samples were tested.

Figure 1 shows the sPLA₂ levels of the patients and controls before the onset of symptoms, corrected for age, sex, and CRP. 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 patients with RA. The concentrations of IgM RF and anti-CCP were 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 a point in time was no stronger than the association based on measurements at that 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 measures 1, 2, and 3 years before and after a point in time in comparison with the association based on measurements at that same time (data not shown).

DISCUSSION

Serum levels of sPLA₂ were increased in the preclinical phase of patients with RA in comparison with levels of healthy controls, in a manner similar to CRP, as published previously.⁴ Both measures 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 measures up to 3 years before or after a point in time in comparison with measurements at the same time.

In a previous study we concluded that it remained unclear whether increased inflammation, measured by CRP, occurs before, after or simultaneously with the development of autoantibodies in preclinical RA.⁴ sPLA₂ is also increased in preclinical RA, but the patterns over time of sPLA₂ and CRP differ between individual patients. Therefore, both measures of inflammation were analysed separately and combined in a new time lag analysis in an attempt to unravel the sequence of increased inflammation and raised 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 measures 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 might be that the available blood samples, although numerous, were taken too far apart in time to be able to detect a short time lag. A possibly temporary sPLA₂ or CRP

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

	B (95% CI)		
	CRP	sPLA ₂	CRP + sPLA ₂
<i>IgM RF</i>			
Inflammation 3 years earlier	0.06 (−0.01 to 0.12)	0.07 (0.04 to 0.10)	0.18 (0.07 to 0.29)
Inflammation 2 years earlier	0.07 (0.01 to 0.13)	0.05 (0.02 to 0.08)	0.16 (0.06 to 0.25)
Inflammation 1 year earlier	0.12 (0.06 to 0.18)	0.05 (0.01 to 0.08)	0.19 (0.10 to 0.29)
Inflammation at same point in time	0.09 (0.04 to 0.13)	0.07 (0.04 to 0.09)	0.20 (0.13 to 0.27)
Inflammation 1 year later	0.06 (0.00 to 0.12)	0.04 (0.02 to 0.07)	0.15 (0.06 to 0.24)
Inflammation 2 years later	0.11 (0.05 to 0.16)	0.02 (−0.01 to 0.04)	0.14 (0.05 to 0.23)
Inflammation 3 years later	0.05 (−0.01 to 0.12)	0.02 (−0.01 to 0.05)	0.09 (−0.01 to 0.20)
<i>Anti-CCP</i>			
Inflammation 3 years earlier	0.05 (0.00 to 0.10)	−0.02 (−0.05 to 0.01)	0.00 (−0.09 to 0.09)
Inflammation 2 years earlier	0.03 (−0.03 to 0.08)	−0.01 (−0.03 to 0.02)	0.01 (−0.07 to 0.09)
Inflammation 1 year earlier	0.08 (0.03 to 0.13)	0.00 (−0.03 to 0.03)	0.08 (−0.01 to 0.16)
Inflammation at same point in time	0.05 (0.02 to 0.09)	0.05 (0.02 to 0.07)	0.13 (0.07 to 0.19)
Inflammation 1 year later	0.00 (−0.05 to 0.05)	0.01 (−0.02 to 0.03)	0.01 (−0.07 to 0.04)
Inflammation 2 years later	0.08 (0.03 to 0.14)	0.02 (0.00 to 0.05)	0.13 (0.04 to 0.21)
Inflammation 3 years later	0.06 (−0.01 to 0.13)	0.01 (−0.02 to 0.05)	0.09 (−0.01 to 0.19)

peak indicative of an infectious process involved in causing the RA might also have been missed because the samples were obtained too far apart. The opposite conclusion, that because 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.⁷ 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, which are preceded by a rise in autoantibody levels.⁸

In conclusion, both the acute phase response and autoantibody formation often develop years before the first symptoms of RA occur, and these phenomena are probably closely connected in time.

Authors' affiliations

M M J Nielen, D van Schaardenburg, R J van de Stadt, M H M T de Koning, B A C Dijkmans, Jan van Breemen Institute, Amsterdam, The Netherlands

D van Schaardenburg, I E van der Horst-Bruinsma, B A C Dijkmans, Department of Rheumatology, VU University Medical Centre, Amsterdam, The Netherlands

H W Reesink, M R Habibuw, Sanquin Bloodbank North West Region, Amsterdam, The Netherlands

J W R Twisk, Department of Clinical Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam, The Netherlands

Competing interest: None.

Correspondence to: Professor B A C Dijkmans, Vrije Universiteit Medical Centre, Department of Rheumatology, PO Box 7057, 1007 MB, Amsterdam; Secr.reumatologie@vumc.nl

Accepted 31 July 2005

Published Online First 3 August 2005

REFERENCES

- 1 **Aho K**, Heliövaara M, Maatela J, Tuomi T, Palosuo T. Rheumatoid factors antedating clinical rheumatoid arthritis. *J Rheumatol* 1991;**18**:1282–4.
- 2 **Rantapää-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.
- 3 **Nielen MMJ**, 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.
- 4 **Nielen MMJ**, van Schaardenburg D, Reesink HW, Twisk JW, van de Stadt RJ, van der Horst-Bruinsma IE, *et al*. Increased levels of C-reactive protein in serum from blood donors before the onset of rheumatoid arthritis. *Arthritis Rheum* 2004;**50**:2423–7.
- 5 **Wolbink GJ**, Schalkwijk C, Baars JW, Wagstaff J, van den Bosch H, Hack CE. Therapy with interleukin-2 induces the systemic release of phospholipase-A2. *Cancer Immunol Immunother* 1995;**41**:287–92.
- 6 **Twisk JWR**. *Applied longitudinal data-analysis for epidemiology: a practical guide*. Cambridge, UK: Cambridge University Press, 2003.
- 7 **Vossenaar ER**, van Venrooij WJ. Anti-CCP antibodies, a high specific marker for (early) rheumatoid arthritis. *Clin Applied Immunol Rev* 2004;**4**:239–62.
- 8 **Cambridge G**, Leandro MJ, Edwards JCW, Ehrenstein MR, Salden M, Bodman-Smith M, *et al*. Serological changes following B lymphocyte depletion therapy for rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:2146–54.