# Serum Amyloid A and C-Reactive Protein Concentrations Are Differently Associated with Markers of Autoimmunity in Patients with Primary Sjögren's Syndrome

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***ABSTRACT.*** ***Objective.*** Primary Sjögren's syndrome (pSS) is an autoimmune disease in which the concentration of the acute-phase protein serum C-reactive protein (CRP) is low. We investigated whether levels of another acute-phase protein, serum amyloid A (SAA), are increased in patients with pSS and whether the immunological markers in patients with pSS are associated with variation in SAA levels.

***Methods.*** Serum SAA concentrations were measured by ELISA in 74 patients with pSS and in 56 control subjects with sicca symptoms.

***Results.*** Median SAA levels did not differ significantly between patients with pSS and subjects with sicca symptoms. In patients with pSS SAA concentrations correlated significantly with age, leukocyte count, CRP, interleukin 6, and C4. Unlike CRP, there was a significant inverse correlation between SAA and serum IgG levels and anti-SSA antibody titers, as well as a trend towards an inverse correlation between SAA and antinuclear antibody and rheumatoid factor titers.

***Conclusion***. Our data imply that high SAA production could constitute a protective element in pSS: high SAA levels inhibit in particular various signs of B cell hyperreactivity, i.e., IgG and autoantibody production. (J Rheumatol First Release Oct 15 2009; doi:10.3899/jrheum.090300)

*Key Indexing Terms:*
AUTOANTIBODIES B CELL HYPERREACTIVITY C-REACTIVE PROTEIN
IgG PRIMARY SJÖGREN´S SYNDROME SERUM AMYLOID A PROTEIN

Primary Sjögren's syndrome (pSS) is a chronic rheumatic autoimmune disease, in which an elevated erythrocyte sedimentation rate (ESR), reflecting increased inflammatory activity, is a frequent finding. In contrast, Moutsopoulos and colleagues pointed out in the early 1980s that the concentration of the acute-phase protein serum C-reactive protein (CRP) is usually low in patients with pSS[1]. Apart from serum CRP, serum amyloid A (SAA) protein is another inflammatory marker involved in the pathogenesis of many diseases of inflammatory nature[2]. We sought to establish whether SAA concentrations in pSS behave like ESR and are markedly increased, or whether they behave like CRP, with only modest elevations. We measured concentrations of SAA in patients with pSS and, as controls, in patients with sicca symptoms but no pSS.

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*Supported by the competitive Funding of Pirkanmaa Hospital District, Tampere, Finland.*

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*Accepted for publication June 5, 2009.*

## MATERIALS AND METHODS

*Subjects and controls*. Serum samples were obtained with informed consent from 74 patients with pSS (72 women, 2 men), and 56 subjects presenting with sicca symptoms (46 women, 10 men) but not fulfilling the criteria for pSS served as controls. The mean age of the patients with pSS was 58 ± 12 years (range 29–82 yrs), and of the subjects with sicca symptoms 55 ± 13 years (range 28–80 yrs). The study protocol was approved by the Ethical Committee of Tampere University Hospital, Tampere, Finland. The data collections of the patients with pSS and controls have been described in detail[3,4].

*Standard laboratory tests*. Rheumatoid factor (RF) was determined by laser nephelometry and antinuclear antibodies (ANA) by indirect immunofluorescence using Hep-2 cells. Anti-SSA and anti-SSB antibodies were deter-

mined by enzyme immunoassay and serum concentration of beta-2 microglobulin by radioimmunoassay (Pharmacia beta-2-micro RIA kit, Pharmacia Diagnostics, Uppsala, Sweden).

*SAA determinations.* Serum SAA concentrations were determined with an ELISA kit with a detection limit of < 0.004 mg/l (Human SAA, Biosource International, Camarillo, CA, USA). The interassay coefficient of variation (CV) was according to the manufacturer 7.8% at a mean level of 0.0613 mg/l and 7.0% at a mean level of 0.5988 mg/l.

*Statistical methods.* Mann-Whitney U-test was used for comparisons of continuous variables and correlations were calculated with the Spearman correlation coefficient. Findings were considered statistically significant at $p < 0.05$. Statistical analyses were performed with SPSS 15.0 for Windows.

## RESULTS

The clinical characteristics of the patients with pSS are presented in Table 1. The median SAA concentration in these patients was 26.6 mg/l [interquartile range (IQR) 11.8, 55.9] and 21.4 mg/l (IQR 13.7, 40.0) in subjects with sicca symptoms ($p = 0.354$). The median SAA levels did not differ significantly between female and male subjects.

The correlations of serum SAA concentrations with various clinical and immunological manifestations of the patients with pSS are set out in Table 2. To compare, the analogous data are presented also regarding CRP (Table 2).

*Table 1.* Demographic, clinical, and immunological characteristics of 74 patients with primary Sjögren's syndrome.

| Characteristic | Value or Frequency |
|---|---|
| Age, mean ± SD yrs | 58 ± 12 |
| Disease duration, mean ± SD yrs | 9 ± 4 |
| Labial salivary gland histological grade 3–4* | 50 (68) |
| Arthralgia | 50 (68) |
| Raynaud's phenomenon | 42 (57) |
| Recurrent salivary gland swelling | 39 (53) |
| Myalgia | 36 (49) |
| Arthritis | 16 (22) |
| Purpura | 15 (20) |
| Peripheral nervous system symptoms | 14 (19) |
| Pleuritis | 10 (14) |
| Pulmonary fibrosis or alveolitis | 9 (12) |
| Lymphadenopathy | 8 (11) |
| Central nervous system symptoms | 5 (7) |
| Myositis | 0 |
| ANA-positive | 63 (85) |
| RF-positive | 51 (69) |
| Anti-SSA antibody-positive | 54 (73) |
| Anti-SSB antibody-positive | 39 (53) |

* Data are n (%) except where indicated. ANA: antinuclear antibodies; RF: rheumatoid factor. * Chisholm-Mason scale[5]. Alveolitis: findings in high-resolution computed tomography or bronchoscopy and histology; arthralgia and myalgia: patient-reported symptoms; arthritis: articular swelling observed by a clinician; lymphadenopathy: lymph node enlargement that indicated a nodal biopsy; myositis: histological findings; peripheral and central neurological symptoms; recorded from the history given by patients and from case histories; pulmonary fibrosis: findings in chest radiographs; purpura: a history of episodic palpable purpura lesions in the lower limbs or skin biopsy histology; Raynaud's phenomenon: history of cold-induced pallor and cyanosis of the fingers or toes.

SAA levels were significantly higher in pSS patients with myalgic symptoms compared with those without, and in patients with neurological symptoms compared with those without (Table 3). However, the patients with neurological symptoms were also older than those without (65 vs 56 yrs; $p = 0.011$). pSS patients with a history of purpura tended to have lower SAA levels than those without (Table 3). Age of pSS patients with myalgic symptoms or purpura did not differ from those without these manifestations (data not shown).

## DISCUSSION

SAA levels in patients with pSS did not differ from those in subjects of the same age with sicca symptoms but no pSS, but they were approximately twice as high as those we previously observed in healthy young adults[6]. SAA levels correlated significantly with leukocyte counts, CRP, interleukin 6 (IL-6), and C4. All these findings are biologically plausible: SAA is known to function in the priming of neutrophils[2], IL-6 stimulates the production of acute-phase proteins, and elevation in complement C4 concentration reflects an acute-phase reaction. However, there was no association between C4 and CRP.

Interestingly, there was a significant inverse correlation between SAA and serum IgG levels and anti-SSA antibody titers as well as a trend towards an inverse correlation between SAA and ANA and RF titers, but not between CRP and these autoantibodies. Apoptotic defects and impaired clearance of cellular debris are considered key events in the development of autoimmunity[7]. As levels of SAA correlated inversely with autoantibodies, it would appear that a capacity for high SAA production would protect from the autoimmune response. Support for these findings can be drawn from an experimental study where serum from amyloidotic mice was shown to suppress *in vitro* the antibody response to sheep red blood cells, and this suppression was removed by absorption of the sera with antiserum to SAA[8]. SAA has also been reported to have a protective role in vascular injury (as reviewed[9]). Inflammation is currently considered a link between atherosclerosis and autoimmune diseases[10], and CRP and SAA have been associated with cardiovascular risk in the general population[11], but no data exist on SAA and this risk in patients with pSS.

It is logical that SAA levels correlated positively with serum alkaline phosphatase, since liver is a major site of SAA synthesis[12]. The association of SAA levels with neurological symptoms could be due to older age of these patients, but age did not explain the association of SAA levels with myalgia. Low levels of SAA were found to be associated with purpura and low complement C4 levels, manifestations that have been found to be adverse predictors of development of non-Hodgkin lymphoma in pSS[13].

In conclusion, analogously to CRP and in contrast with ESR levels, SAA concentrations are only modestly elevated

*Table 2.* Correlation (r) of serum amyloid A (SAA) and serum C-reactive protein (CRP) with demographic, clinical, and immunological findings in 74 patients with primary Sjögren's syndrome (Spearman correlation coefficient).

| Variable | N | r for SAA | p | N | r for CRP | p |
|---|---|---|---|---|---|---|
| Age | 74 | 0.279 | 0.016 | 73 | 0.263 | 0.025 |
| Age at disease onset | 74 | 0.338 | 0.003 | 73 | 0.193 | 0.102 |
| Disease duration | 74 | –0.035 | 0.764 | 73 | 0.147 | 0.215 |
| Hemoglobin | 74 | 0.028 | 0.813 | 73 | –0.164 | 0.165 |
| Leukocytes | 74 | 0.465 | < 0.0001 | 73 | 0.137 | 0.247 |
| Thrombocytes | 74 | 0.194 | 0.098 | 73 | –0.093 | 0.436 |
| CRP at baseline | 68 | 0.281 | 0.020 | 67 | 0.267 | 0.029 |
| CRP | 74 | 0.299 | 0.010 | | | |
| Plasma IL-6 | 57 | 0.295 | 0.026 | 60 | 0.309 | 0.016 |
| ESR | 74 | –0.091 | 0.438 | 73 | 0.146 | 0.216 |
| Serum creatinine | 76 | 0.259 | 0.024 | 73 | 0.136 | 0.253 |
| ALAT | 74 | 0.022 | 0.851 | 73 | 0.054 | 0.649 |
| AFOS | 74 | 0.254 | 0.029 | 73 | 0.358 | 0.002 |
| Serum IgG at baseline | 74 | –0.272 | 0.019 | 73 | –0.094 | 0.431 |
| Gammaglobulin at baseline | 62 | –0.323 | 0.010 | 62 | –0.032 | 0.805 |
| Serum IgG | 74 | –0.307 | 0.008 | 73 | –0.212 | 0.072 |
| Serum IgA | 74 | –0.139 | 0.239 | 73 | –0.009 | 0.939 |
| Serum IgM | 74 | –0.048 | 0.687 | 73 | –0.090 | 0.448 |
| C3 | 74 | 0.190 | 0.104 | 73 | 0.037 | 0.754 |
| C4 | 74 | 0.275 | 0.018 | 73 | –0.021 | 0.860 |
| RF | 74 | –0.217 | 0.063 | 73 | –0.154 | 0.193 |
| ANA | 74 | -0.197 | 0.093 | 73 | –0.068 | 0.566 |
| Anti-SSA antibodies | 73 | –0.330 | 0.004 | 72 | –0.132 | 0.269 |
| Anti-SSB antibodies | 73 | –0.178 | 0.132 | 72 | 0.015 | 0.904 |
| Serum $ß_2$ microglobulin | 74 | 0.144 | 0.221 | 73 | 0.320 | 0.006 |

IL-6: interleukin-6; ALAT: alanine aminotransferase; AFOS: alkaline phosphatase; RF: rheumatoid factor; ANA: antinuclear antibodies.

*Table 3.* Serum amyloid A (SAA) concentrations in 74 patients with primary Sjögren's syndrome grouped by the presence of various extraglandular manifestations.

| Characteristic, n yes/no | SAA Concentration, mg/l (IQR) Yes | No | p* |
|---|---|---|---|
| Arthralgia, n = 50/24 | 22.8 (11.6, 60.4) | 28.8 (13.8, 55.3) | 0.560 |
| Raynaud's phenomenon, n = 42/32 | 26.6 (13.4, 55.2) | 27.9 (10.6, 84.9) | 0.815 |
| Recurrent salivary gland swelling, n = 39/35 | 21.0 (11.7, 55.2) | 36.6 (11.8, 95.7) | 0.555 |
| Myalgia, n = 36/38 | 39.0 (14.4, 95.1) | 20.4 (10.7, 47.8) | 0.049 |
| Arthritis, n = 16/58 | 47.2 (11.5, 146.0) | 23.8 (11.8, 51.8) | 0.215 |
| Purpura, n = 15/59 | 13.1 (10.7, 41.3) | 34.3 (13.5, 81.4) | 0.052 |
| Peripheral or central nervous system symptoms, n = 18/56 | 56.5 (36.2, 231.1) | 18.1 (10.7, 46.0) | < 0.0001 |
| Pleuritis, n = 10/64 | 32.2 (11.0, 189.2) | 23.8 (12.1, 55.2) | 0.477 |
| Pulmonary fibrosis or alveolitis, n = 9/65 | 86.0 (11.3, 182.2) | 23.8 (11.8, 54.7) | 0.234 |
| Lymphadenopathy, n = 8/66 | 15.2 (10.0, 48.3) | 31.1 (12.6, 58.5) | 0.300 |

* Mann-Whitney U-test. IQR: interquartile range.

in patients with pSS. Serum SAA and CRP concentrations are differently associated with various markers of autoimmunity in patients with pSS. Our data suggest that high SAA production could constitute a protective element in an autoimmune disease such as pSS: high SAA levels inhibit in particular various signs of B cell hyperreactivity, i.e., IgG and autoantibody production, and moreover protect from the development of hypocomplementemia C4 and purpura, signs linked in previous studies to the development of lymphoma in patients with pSS.


## ACKNOWLEDGMENT

We thank Sinikka Repo-Koskinen for skilful technical assistance.


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