

# High acute phase protein levels correlate with pulmonary and skin involvement in patients with diffuse systemic sclerosis

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## Abstract

**Objective:** This study was performed to evaluate the serum amyloid A (SAA) and C-reactive protein (CRP) levels in patients with diffuse systemic sclerosis (dSSc) in relation to a control group, disease duration, and skin and pulmonary involvement.

**Methods:** This case-control study included 18 patients with early dSSc, 15 patients with late dSSc, and 15 healthy controls. The SAA and CRP levels, modified Rodnan skin score (mRSS), and diffusing capacity of the lungs for carbon monoxide (DLCO) were determined in all patients.

**Results:** The SAA and CRP levels were significantly higher in patients with early and late dSSc than in healthy controls. The frequency of detection of elevated SAA and CRP levels was approximately 66% and 85%, respectively. A significant correlation was found between the SAA and CRP levels in patients with dSSc. The SAA and CRP levels were inversely correlated with DLCO. The CRP level was positively correlated with the mRSS.

**Conclusions:** High SAA and CRP levels could serve as biomarkers for pulmonary involvement. The serum CRP level accurately reflects the extension of skin thickening in patients with dSSc.

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## Keywords

Acute phase response, C-reactive protein, scleroderma, serum amyloid A, diffuse systemic sclerosis, modified Rodnan skin score

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## Introduction

Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by progressive fibrosis of the skin and internal organs. The disease course and severity have various clinical characteristics ranging from a relatively benign condition to a rapidly progressive disease with high mortality.<sup>1</sup> Clinically, SSc can be classified into two major subtypes: limited SSc (lSSc), defined as fibrosis distal to the elbow and knee joint (including the face), and diffuse SSc (dSSc), which is characterized by proximal fibrosis. dSSc is associated with a poorer prognosis than lSSc because of earlier and more severe organ involvement. Interstitial lung disease (ILD) is the most serious complication and major cause of death in patients with dSSc.<sup>1</sup>

Inflammation is a prominent feature in the early stages of dSSc, preceding the development of fibrosis. Inflammatory infiltrates are observed in a variety of affected organs and are accompanied by elevated circulating levels of inflammatory cytokines and chemokines.<sup>2</sup> Although activation of the immune system and inflammation modulate the systemic acute phase reaction and the hepatic acute phase protein response, the role of acute phase reactants for identifying inflammation in SSc have received little attention to date.<sup>3,4</sup> Some authors have indicated that the acute phase response is impaired in patients with SSc.<sup>5</sup> C-reactive protein (CRP) and serum amyloid A (SAA) are the two major acute phase proteins in humans. In recent years, CRP is being increasingly recognized as a marker

of prognosis and disease activity in SSc.<sup>6</sup> In three studies, the CRP levels were elevated in patients with SSc and correlated with the diffuse cutaneous disease type and lung involvement.<sup>3,4,7</sup> Similarly, some authors have shown that patients with more active SSc have moderately or significantly elevated SAA levels.<sup>7,8</sup> One of these studies showed that CRP was correlated with SAA and that the latter could be a biomarker for pulmonary involvement in patients with SSc.<sup>7</sup> The CRP level is a general marker of inflammation. SAA is an acute phase protein that contributes to deposition of amyloid in tissues; however, only a few studies have reported amyloidosis A in patients with SSc.<sup>9–12</sup> SAA may also play a role in the pathogenesis of autoimmune diseases, atherosclerosis, and tumors.<sup>13</sup>

## Objective

Because of the scarcity of clinical data regarding serum CRP and SAA levels in patients with SSc, we evaluated a cohort of patients with dSSc and focused on those with the highest levels of both markers in previous studies. This study was designed to evaluate the SAA level in patients with dSSc in relation to healthy controls, disease duration, skin and pulmonary involvement, and the serum CRP level.

## Methods

Patients with dSSc and age- and sex-matched healthy controls were investigated.

All patients fulfilled the SSc diagnostic criteria<sup>14</sup> and had skin thickening above the elbows and knees and on the chest. Patients with lSSc and those who had been treated with immunosuppressive agents and/or steroids within the previous 3 months were excluded from the study. The exclusion criteria were overlap syndromes, acute or chronic infections, malignancies, and other systemic diseases.

The patients were divided into two subgroups: those with an overt disease duration of  $\leq 3$  years (early dSSc) and those with a disease duration of  $> 3$  years (late dSSc). The disease onset was defined as the time point at which the first skin thickening symptoms were observed. Skin thickening was evaluated using the modified Rodnan skin score (mRSS). Diagnostic procedures for specific organ and systemic involvement were typically performed.<sup>15</sup> A diagnosis of ILD was based on the radiologic abnormalities and low diffusing capacity of the lung for carbon monoxide (DLCO).<sup>15</sup> All patients underwent high-resolution computed tomography of the chest on the same day that pulmonary function tests were performed. DLCO was measured using the single-breath technique. The DLCO was corrected for the lung volume and hemoglobin level. The observed values were compared with those predicted for the age, sex, and height of each individual. The results are expressed as a percentage of the predicted value (% predicted) and were considered abnormal when the DLCO was  $< 80\%$ . The SAA and serum CRP levels were determined in all patients. The SAA level was evaluated using an enzyme-linked immunoassay with a detection limit of 0.005 mg/L (Human SAA; BioSource Europe S.A., Nivelles, Belgium). The serum CRP level was measured by immunoturbidimetric assay. The protocol was approved by the local ethics committee of the Medical University of

Silesia, and informed consent was obtained from all patients.

### **Statistical analysis**

The SAA and serum CRP levels were analyzed in relation to the clinical data: early dSSc versus late dSSc, mRSS, and DLCO. Calculations were performed with Statistica Version 10 software (StatSoft, Tulsa, OK, USA). The Shapiro–Wilk test was performed to check the normality of the distribution. Statistical comparisons were performed using the Mann–Whitney U-test. The cut-off was calculated as the 95th percentile of the healthy controls. Spearman’s rank order correlation coefficient ( $r$ ) was used to test for an association between clinical/laboratory parameters. A  $p$  value of  $< 0.05$  was considered significant.

### **Results**

Thirty-three patients with dSSc (24 women and 9 men) and 15 age- and sex-matched healthy controls were investigated. The patients’ ages ranged from 25 to 75 years with a median of 52 years (interquartile range, 40–57 years). The disease duration ranged from 3 to 191 months with a median of 25 months (interquartile range, 9–66 months). Twenty-one patients had ILD and 12 had no pulmonary involvement. Eighteen patients had early dSSc and 15 had late dSSc.

Table 1 shows the SAA and CRP levels in the dSSc and control groups. The SAA level was significantly higher in both patients with early dSSc and late dSSc than in controls ( $p = 0.0004$ ,  $U = 20$  and  $p = 0.0012$ ,  $U = 20$ , respectively). The serum CRP level was also higher in both patients with early dSSc and late dSSc patients than in controls ( $p = 0.0003$ ,  $U = 18.5$  and  $p = 0.0002$ ,  $U = 10.5$ , respectively). There was no difference in the SAA

**Table 1.** Serum amyloid A and C-reactive protein levels in patients with diffuse systemic sclerosis and healthy controls.

	Serum amyloid A (mg/mL)					Serum C-reactive protein (mg/mL)				
	Median	LQ	UQ	dSSc subgroup versus controls		Median	LQ	UQ	dSSc subgroup versus controls	
				p-value	Z-score				p-value	Z-score
dSSc (n = 33)	67.4	44.8	74.7	0.00013	3.8	47.7	12.8	197.6	0.00004	4.1
Early dSSc (n = 18)	69.9	44.9	76.5	0.0004	3.5283	74.3	19.6	224.5	0.0003	3.5957
Late dSSc (n = 15)	65.2	42.2	70.1	0.0013	3.2178	19.6	12.2	92.6	0.0002	3.7108
dSSc with ILD (n = 12)	74.6	64.2	77.9	0.0005	3.4773	159.8	67.3	339.3	0.0005	3.4773
dSSc without ILD (n = 21)	56.7	36.8	70.3	0.0009	3.3328	18.8	11.0	84.7	0.0002	3.7692
Controls (n = 11)	21.4	6.8	28.7			2.5	1.7	28.7		

dSSc, diffuse systemic sclerosis; ILD, interstitial lung disease; LQ, lower quartile; UQ, upper quartile.

**Table 2.** Correlation of serum amyloid A and C-reactive protein levels with skin and pulmonary involvement in patients with diffuse systemic sclerosis.

	Serum amyloid A		Serum C-reactive protein	
	Correlation with mRRS	Correlation with DLCO	Correlation with mRRS	Correlation with DLCO
Spearman correlation coefficient (r)	0.101	-0.681	0.45	-0.55
Significance (p)	0.58	0.02	0.008	0.07

DLCO, diffusing capacity of the lung for carbon monoxide; mRRS, modified Rodnan skin score.

level or serum CRP level between patients with dSSc with a short disease duration and those with overt disease lasting >3 years. Using a cut-off value of 48 mg/mL for SAA, approximately 66% of patients with early and late dSSc had elevated SAA levels (12/18 and 10/15 patients, respectively). The frequency of detection of elevated CRP levels in patients with early and late dSSc using a cut-off level of 6 mg/mL was approximately 85% (15/18 and 13/15 patients, respectively). A significant correlation was found between SAA and CRP in patients with dSSc ( $r = 0.62$ ,  $p = 0.04$ ). There was no correlation in the control group ( $r = -0.14$ ). Table 2 shows the correlation of circulating SAA and CRP levels

with skin and pulmonary involvement. The serum CRP and SAA levels were inversely correlated with DLCO, and both markers were significantly higher in patients with ILD. Although the serum CRP level was positively correlated with mRSS, no correlation was found between the SAA level and mRSS.

## Discussion

CRP and SAA have long been known to be elevated in some patients with SSc, but such studies are scarce. Our study showed significantly elevated SAA and CRP levels in patients with dSSc than in control subjects during both early and late disease.

Moreover, the SAA and serum CRP levels were similar between patients with a long and short disease duration. SAA was positively correlated with CRP in patients with dSSc. Because the CRP and SAA were very low in most of the healthy controls, this probably resulted in no correlation. The elevation of both parameters in early dSSc might explain their role as acute phase reactants and indicate a stronger acute phase response in the early inflammatory stage of SSc. In patients with a longer disease duration, fibrosis is a much more dominant phenomenon than inflammation, and the increase in the SAA and CRP levels might have a different role. Notably, in dermal and lung fibroblasts, SAA could act as a direct stimulus for the synthesis of interleukin (IL)-6 and IL-8, mediators implicated in the pathogenesis of SSc and its pulmonary complications.<sup>7,16</sup> CRP possesses both proinflammatory and anti-inflammatory properties.<sup>17</sup> Whether CRP inhibits or promotes systemic inflammatory response syndrome in patients with SSc has not yet been studied. A positive correlation was found between the mRSS and CRP level but not with the SAA level in our study. In contrast, the CRP and SAA levels correlated with DLCO ( $r = -0.55$   $p = 0.07$  and  $r = -0.681$   $p = 0.02$ , respectively). Some authors have evaluated the effects of CRP and SAA on human lung fibroblasts and suggested that these acute phase proteins might be endogenous modulators of lung fibrosis.<sup>7,18</sup> Moreover, Lakota et al.<sup>7</sup> observed that patients with elevated CRP and SAA levels had significantly impaired pulmonary function but that the correlation with DLCO was poor, with a lower Spearman coefficient than in the current study ( $r = -0.358$ ,  $p = 0.03$  and  $r = -0.294$ ,  $p = 0.08$ , respectively). The discrepancy in these results could be explained by the disease subtype (ISSc and dSSc versus dSSc alone in our study), stage, severity of tissue fibrosis, and various circumstances

of the acute phase response. We found elevated SAA and CRP levels in most of our patients with dSSc. Previous studies included patients with ISSc and dSSc, and only a minority of patients with SSc had elevated CRP and SAA levels (25.7%–29% and 25%–38%, respectively).<sup>3,4,7</sup> Both CRP and SAA are highly elevated after microbial infection and mainly synthesized by hepatocytes in the liver.<sup>13</sup> This effect is executed by IL-6 and enhanced by IL-1 $\beta$ .<sup>17</sup> Therefore, the sensitivity and specificity of acute phase reactants seem low. It is important to note that inflammation or infection anywhere in the body can result in increased CRP and SAA values.

In conclusion, our findings suggest spatial and temporal differences in the role of CRP and SAA in patients with SSc. Our patients with dSSc had elevated CRP and SAA levels, presumably as a result of their active ILD. Increased serum CRP levels additionally indicate the extent of skin thickening. Further studies are needed to evaluate the role of CRP and SAA in patients with dSSc and their impact on clinical and other biomarker correlations.

### Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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