

# New Potential Biomarkers for Disease Activity and Fibrosis in Systemic Sclerosis

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**S**ystemic sclerosis (SSc) is a slowly progressing chronic inflammatory disease that leads to inflammation and fibrosis of the skin and internal organs [1]. Clinically, systemic sclerosis is a heterogeneous disease of different subsets characterized by the extent of skin fibrosis, presence of distinct circulating autoantibodies, and the involvement of internal organs. It is a rare disease with a prevalence ranging from 50 to 300 cases per million with female gender preference.

## THE INFLAMMATORY RESPONSE IN SSC

One of the major pathogenetic mechanisms of SSc is the inflammatory process. It is composed mainly of activated T cells, which release pro-inflammatory and pro-fibrogenic cytokines. Defective T-regulatory (Treg) cells have also been described in these patients, thus the immunoregulation in this disease is flawed. It is believed that transforming growth factor-beta (TGF $\beta$ ), platelet-derived growth factor (PDGF), and several other interleukins are released from early inflammatory infiltrates and are crucial for the activation of fibroblasts and the transition of fibroblasts to myofibroblasts [2], thus contributing to the process of fibrosis. Semaphorin 3A (sema3A), a secreted member of the semaphorin family, is now recognized as a potent immunoregulator during all immune response stages – from early initiation to the late phase of inflammatory processes [3].

Sema3A expression on Treg cells has been recognized as a suppressive marker, contributing to the regulatory properties of these cells [4]. In 2010 Catalano [5] was the first to report on the defective expression of sema3A in CD4<sup>+</sup> T cells derived from patients with rheumatoid arthritis (RA). The altered expression on T cells was shown to correlate with the progression of RA [5]. Recently we reported the presence of low serum semaphorin3A levels in systemic lupus erythematosus (SLE) patients, in correlation with SLEDAI score and reflecting dis-

ease activity [6]. As a front player in the regulation of immune responses and the maintenance of self-tolerance, sema3A should be expected to be involved in the pathogenesis of many autoimmune diseases. Thus we designed a study in which we evaluated the level of sema3A in the serum and its expression on Treg cells in SSc patients, healthy controls and SLE patients as the disease control, and correlated the results with clinical and serological parameters. Serum levels of sema3A were lower in SSc patients compared to healthy controls ( $14.38 \pm 5.7$  vs.  $27.14 \pm 8.4$  ng/ml,  $P < 0.0001$ ) and similar to SLE patients ( $15.7 \pm 4.3$  ng/ml). The expression of sema3A on Treg cells was also lower in SSc patients compared to healthy controls ( $61.7 \pm 15.7\%$  vs.  $88.7 \pm 3.7\%$ ,  $P < 0.0001$ ). Semaphorin 3A serum level correlated inversely with the duration of disease ( $r = -0.4$ ,  $P = 0.036$ ) and with low C4 level ( $r = 0.66$ ,  $P = 0.026$ ). SCL-70 antibody positivity was associated with a lower sema3A level in serum (difference in mean of 3.44,  $P = 0.06$ ). Sema3A expression was found in this study to be lower in SSc serum and also on Treg cells, in inverse correlation with disease duration. The finding of reduced expression of sema3A on Treg cells in SSc is in line with former studies that suggested either increased or decreased numbers of these cells but denoted inefficient T regulatory activity in autoimmune diseases such as SSc [7-9]. Thus, the finding of reduced sema3A expression on Treg cells in SSc patients may reflect their impaired regulatory function, thereby contributing to our understanding of the immune pathogenesis of the disease and this sema3A level may serve as a future target for follow-up and treatment.

## DEVELOPMENT OF FIBROSIS IN SSC

Fibrosis is the main complication of SSc and its pathophysiology is complex. Despite our growing understanding of this process and the many available targets, our therapeutic success in ameliorating fibrosis in SSc is minimal [10]. Moreover, even today, assessment of skin fibrosis is usually determined by the modified Rodnan skin score (mRSS), a score based on clinical inspection of 17 parts of the body, which has significant inter-observer variability and is rather subjective, hence the need for other objective and specific markers for assessing fibrosis.

Collagen I is the most abundant structural protein of connective tissues such as the skin. The formation of collagen is an active

process that reflects a balance between degradation and synthesis. Under physiological conditions, the chemical cross-linking of collagen molecules incorporated in collagen fibrils is critical for the mechanical stability of these fibrils. Moreover, the presence of chemical cross-links makes fibril-incorporated collagen molecules more resistant to proteolysis. Formation of cross-links is an enzymatic process catalyzed by lysyl-oxidase (LOX).

LOX is a copper-dependent amine oxidase that initiates the covalent cross-linking of collagen and elastin by catalyzing oxidative deamination of lysine and hydroxylysine residues to aminoaldehydes. These highly reactive semi-aldehydes can spontaneously condense to assure extracellular matrix (ECM) stability. LOX activity is essential to maintain the tensile and elastic features of connective tissues of skeletal, pulmonary, and cardiovascular systems, among others. LOX is synthesized as a pre-pro-LOX and is secreted into the extracellular environment where it is proteolytically processed to release the mature and active 32 kDa form and its pro-peptide.

LOX has been evaluated in patients with diffuse cutaneous systemic sclerosis by immune staining of the skin and was found to be increased in interstitial fibroblastic cells compared with normal skin, but was not increased in SSc patients with skin atrophy [14]. LOX has been evaluated in other states of fibrosis, including primary myelofibrosis (PMF), hepatic and myocardial fibrosis [10], and was found to be overexpressed in the relevant tissues. We therefore conducted a study to evaluate LOX serum levels in SSc patients compared to normal controls and patients with PMF, as a disease control, and correlated these levels with clinical parameters. The study population comprised 26 SSc patients who were compared with healthy and primary myelofibrosis patients as the disease control. Ten SSc patients had diffuse disease with lung fibrosis and 16 had limited cutaneous disease. LOX serum concentration in SSc patients was higher than in healthy controls and similar to the disease control ( $58.4 \pm 4.8$  vs.  $28.4 \pm 2.5$  ng/ml vs.  $44.6 \pm 9.4$  ng/ml respectively,  $P < 0.001$ ). LOX serum level was significantly higher in patients with diffuse vs. limited disease ( $73 \pm 6.6$  vs.  $49.3 \pm 5.5$  ng/ml,  $P$

$< 0.01$ ). LOX serum concentration correlated with mRSS ( $P < 0.01$ ) and with severity score ( $P < 0.001$ ) in patients with SSc. This was the first study to demonstrate high serum levels of LOX in SSc patients, which specifically correlate with skin fibrosis and disease severity.

These correlations suggest that LOX levels can serve as a novel biomarker for fibrosis and severity in SSc. Future studies are warranted to determine LOX as a potential therapy target in SSc.

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