

Elevated Serum Concentrations of Triggering Receptor Expressed on Myeloid Cells-1 in Diffuse Cutaneous Systemic Sclerosis: Association with Severity of Pulmonary Fibrosis

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ABSTRACT. Objective. To determine serum concentrations and clinical association of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in patients with systemic sclerosis (SSc).

Methods. Serum sTREM-1 levels from 17 patients with limited cutaneous SSc (lSSc), 24 patients with diffuse cutaneous SSc (dSSc), and 29 healthy control individuals were examined by ELISA.

Results. Total SSc patients exhibited significantly elevated serum sTREM-1 levels relative to controls ($p < 0.01$). Serum sTREM-1 levels were significantly elevated in patients with dSSc compared to controls ($p < 0.005$) and lSSc patients ($p < 0.05$). By contrast, sTREM-1 levels in lSSc were similar to those in controls. Serum sTREM-1 levels were significantly elevated in SSc patients with decreased percentage vital capacity (%VC). Consistent with this, serum sTREM-1 levels in SSc patients correlated negatively with %VC ($r = -0.24$, $p < 0.005$). Among SSc patients with pulmonary fibrosis, sTREM-1 levels were significantly increased in patients with decreased %VC or decreased percentage of diffusion capacity for carbon monoxide relative to those with normal values ($p < 0.05$).

Conclusion. Serum sTREM-1 levels were elevated in dSSc patients and correlated with severity of pulmonary fibrosis, suggesting that serum sTREM-1 is a novel serological marker for the disease severity of SSc. (First Release Feb 15 2010; J Rheumatol 2010;37:787–91; doi:10.3899/jrheum.090664)

Key Indexing Terms:

SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS-1
SYSTEMIC SCLEROSIS PULMONARY FIBROSIS

Systemic sclerosis (SSc) is a connective tissue disease characterized by fibrotic change in the skin and other visceral organs. The leading cause of death in SSc is pulmonary involvement consisting of pulmonary fibrosis and pulmonary hypertension. Patients with SSc are commonly classified into 2 distinct subsets: limited cutaneous SSc (lSSc),

in which the skin lesions do not extend beyond the elbows and knees, or diffuse cutaneous SSc (dSSc), which also affects the thighs, arms, and torso. Patients with dSSc tend to have more rapid progression of their skin disease and are at higher risk of severe lung, heart, and kidney involvement than those with lSSc. Although the pathogenesis of SSc remains unknown, interactions among lymphoid cells, neutrophils, endothelial cells, and fibroblasts are likely to be central to the pathogenesis of the disease. Moreover, studies have shown that activated platelets and various cytokines released by leukocytes including polymorphonuclear leukocytes are related to the development of fibrosis in SSc^{1–3}.

The triggering receptor expressed on myeloid cells-1 (TREM-1) is identified as an activating receptor on neutrophils, monocytes, and macrophages⁴. A recent study indicates that a natural ligand for TREM-1 is present on platelets⁵. Expression of TREM-1 is upregulated by stimulation with lipopolysaccharide (LPS) and other microbial products. Activation of TREM-1 on monocytes by antibodies in the presence of Toll-like receptor ligands synergistically increases the production of proinflammatory chemokines and cytokines, and decreases the production of

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antiinflammatory cytokines⁴. In addition, the TREM-1/ligand interaction contributes to the amplification of LPS-induced activation of polymorphonuclear leukocytes⁵. A soluble form of TREM-1 (sTREM-1) is generated by proteolytic cleavage of membrane-bound TREM-1⁶, and can be measured in biological fluids including sera. The release of sTREM-1 represents a conserved mechanism for counter-regulation of the TREM-1 function⁷.

Several studies have shown that sTREM-1 levels are elevated in sera from patients with inflammatory diseases, such as sepsis⁸, chronic obstructive pulmonary disease⁹, and acute pancreatitis¹⁰. The finding that the activation of platelets and polymorphonuclear leukocytes depends in part on the TREM-1/ligand interaction suggests its contribution to the development of fibrosis in SSc; however, sTREM-1 levels were not previously investigated in SSc. Therefore, we examined serum sTREM-1 levels and their clinical correlation in patients with SSc in this study.

MATERIALS AND METHODS

Serum samples. Blood samples were obtained from 41 Japanese patients with SSc (39 women, 2 men; age 52 ± 14 yrs). All patients fulfilled the criteria proposed by the American College of Rheumatology¹¹. These patients were grouped according to the classification system proposed by LeRoy, *et al*¹²: 17 patients (all women; age 50 ± 14 yrs) had ISSc and 24 (22 women, 2 men; age 53 ± 14 yrs) had dSSc. The disease duration in patients with ISSc and dSSc was 9.6 ± 9.6 and 3.1 ± 3.1 years, respectively. Disease duration was calculated from time of onset of the first clinical event (other than Raynaud's phenomenon) that was a clear manifestation of SSc. No patient had been treated with corticosteroid, D-penicillamine, or other immunosuppressive therapy at the evaluation. All patients with clinical signs of bacterial pneumonia were excluded from the study. Antinuclear antibody was determined by indirect immunofluorescence using HEP-2 cells as substrate, and autoantibody specificities were further assessed by enzyme-linked immunosorbent assay (ELISA) and immunoprecipitation. Anticentromere antibody (ACA) was positive for 16 patients (2 dSSc and 14 ISSc), anti-topoisomerase I antibody for 15 (12 dSSc and 3 ISSc), anti-U1RNP antibody for one (dSSc), anti-U3RNP antibody for one (dSSc), and anti-RNA polymerases I and III antibodies for 5 (all dSSc). Twenty-nine age- and sex-matched healthy Japanese individuals were used as controls. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were stored at -70°C prior to use.

Clinical assessment. Complete medical histories, physical examinations, and laboratory tests were conducted for all patients at their first visit. Skin score was measured by the modified Rodnan total skin thickness score (modified Rodnan TSS)¹³. The 17 anatomic areas were rated as 0 (normal skin thickness), 1+ (mild but definite skin thickening), 2+ (moderate skin thickening), and 3+ (severe skin thickening), and the modified Rodnan TSS was derived by summing the scores from all 17 areas (range 0–51). Organ system involvement was defined as described¹⁴: pulmonary fibrosis = bibasilar fibrosis on chest radiography and high resolution computed tomography (HRCT); esophagus = hypomotility shown by barium radiography; joint = inflammatory polyarthralgias or arthritis; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; kidney = malignant hypertension and rapidly progressive renal failure without any other explanation; and muscle = proximal muscle weakness and elevated serum creatine kinase. Chest radiographs and HRCT were performed in all SSc patients and revealed pulmonary fibrosis in 22 (7 ISSc, 15 dSSc). Pulmonary function tests, including vital capacity (VC) and diffusion capacity for carbon monoxide (DLCO), were conducted for all patients

within 3 to 5 weeks after serum collection. When the DLCO and VC were $< 75\%$ and $< 80\%$, respectively, of predicted normal values, they were considered to be abnormal.

The protocol for the study was approved by our institutional review board, and informed consent was obtained from all patients according to the Declaration of Helsinki.

ELISA. Specific ELISA kits were used for measuring serum sTREM-1 levels (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Each sample was tested in duplicate.

Statistical analysis. Statistical analysis was performed using Mann-Whitney U test for comparison of serum sTREM-1 levels, Fisher's exact probability test for comparison of frequencies, and Bonferroni's test for multiple comparisons. Spearman's rank correlation coefficient was used to examine the relationship between 2 continuous variables. A p value < 0.05 was considered statistically significant.

RESULTS

Serum sTREM-1 concentrations in SSc. The levels and presence of sTREM-1 in serum samples from SSc patients and controls were assessed by ELISA (Figure 1). Total SSc patients exhibited significantly elevated serum sTREM-1 levels relative to controls (mean 625.0 ng/ml vs 290.7 ng/ml, respectively; $p < 0.01$). Regarding the disease subsets, serum sTREM-1 levels were significantly higher in dSSc patients (744.2 ng/ml) than ISSc patients (417.0 ng/ml) ($p < 0.05$) as well as in the controls ($p < 0.0005$). By contrast, there was no significant difference in sTREM-1

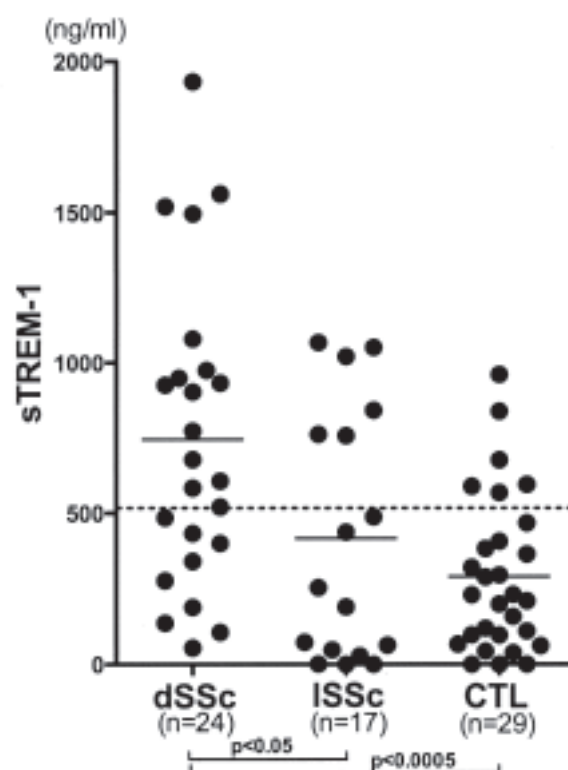


Figure 1. Serum sTREM-1 levels in dSSc patients, ISSc patients, and healthy controls were determined by a specific ELISA. Short bar indicates the mean value in each group. Broken line indicates the cutoff value (mean + 2 SD of control samples).

levels between ISSc patients and controls. However, sTREM-1 levels did not correlate significantly with modified Rodnan TSS ($r = 0.043$, $p = 0.19$). There were no significant correlations between sTREM-1 and titer of ACA and anti-topoisomerase I antibody (data not shown).

Values higher than the mean + 2 SD (517 ng/ml) of the control serum samples were considered to be elevated in this study. Elevated serum sTREM-1 levels were observed in 49% (20/41) of total patients with SSc, and especially in 63% (15/24) of dSSc patients, while they were detected in 35% (6/17) of ISSc patients and in 21% (6/29) of controls. Thus, serum sTREM-1 levels were elevated in patients with SSc, especially dSSc.

Clinical correlation of sTREM-1 levels in SSc. Next we assessed clinical correlation of serum sTREM-1 levels in SSc patients. Serum sTREM-1 levels were significantly increased in SSc patients with decreased %VC compared to those with normal %VC ($p < 0.005$; Figure 2a). Consistent with this, serum sTREM-1 levels correlated inversely with %VC ($r = -0.24$, $p < 0.005$; Figure 2b). However, there was no correlation of sTREM-1 with %DLCO in SSc patients ($r = -0.035$, $p = 0.24$). There were no significant correlations between the sTREM-1 levels and C-reactive protein ($r = 0.068$, $p = 0.119$). Further, elevation of sTREM-1 levels was not associated with any other organ involvement (data not shown).

To further determine the correlation of sTREM-1 with the severity of lung fibrosis, we investigated the association of sTREM-1 levels with pulmonary function tests among SSc patients with pulmonary fibrosis (Figure 3). Serum sTREM-1 levels were significantly higher in SSc patients with decreased %VC than in those with normal %VC ($p <$

0.05). Similarly, SSc patients with decreased %DLCO exhibited significantly elevated sTREM-1 levels relative to those with normal %DLCO ($p < 0.05$). In addition, dSSc and ISSc patients with pulmonary fibrosis exhibited significantly elevated serum sTREM-1 levels relative to those without pulmonary fibrosis ($p < 0.005$ and $p < 0.001$, respectively). Thus, elevated sTREM-1 levels were associated with the severity of pulmonary fibrosis.

DISCUSSION

In our study, sTREM-1 levels were increased in patients with dSSc, but not patients with ISSc, compared with healthy controls. In addition, serum sTREM-1 levels were elevated in SSc patients with decreased %VC. Consistently, serum sTREM-1 levels were found to be negatively correlated with %VC. Further, even when analyzed among SSc patients with pulmonary fibrosis, serum sTREM-1 levels were significantly increased in SSc patients with decreased %VC and %DLCO compared to those with normal %VC and %DLCO, respectively. Consistent with this, a previous study has shown that serum sTREM-1 levels correlated with lung function impairment in patients with chronic obstructive pulmonary disease⁹. The results of our study suggest that sTREM-1 levels correlate with the severity of skin and lung fibrosis in SSc.

The levels of sTREM-1 in serum or bronchoalveolar lavage fluid increase in patients with various inflammatory diseases, such as chronic obstructive pulmonary disease, sepsis, acute pancreatitis, inflammatory bowel disease, and pneumonia^{8-10,15,16}. Increased TREM-1 expression on monocytes is associated with both infectious and noninfectious inflammatory processes¹⁷. In addition, disease activity

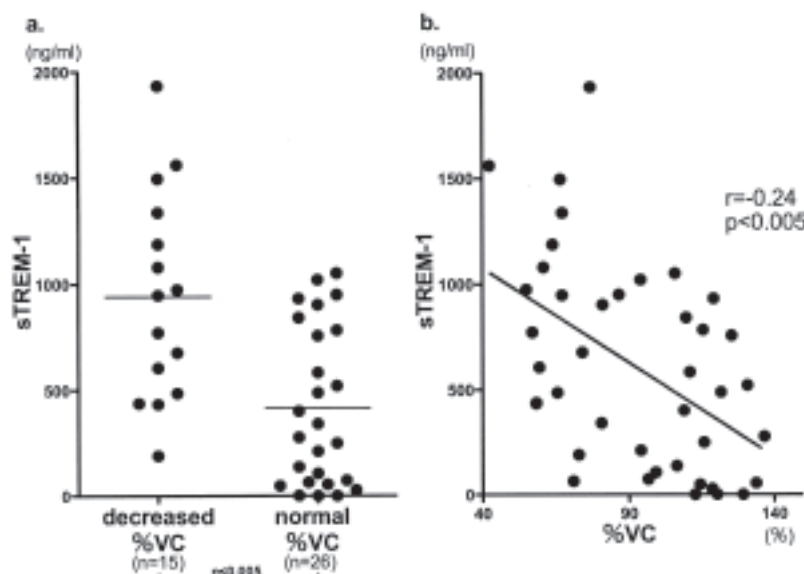


Figure 2. A. Serum sTREM-1 levels in SSc patients with decreased %VC compared to those with normal %VC. B. Correlation of serum sTREM-1 levels against %VC in SSc patients. Serum sTREM-1 levels were determined by ELISA.

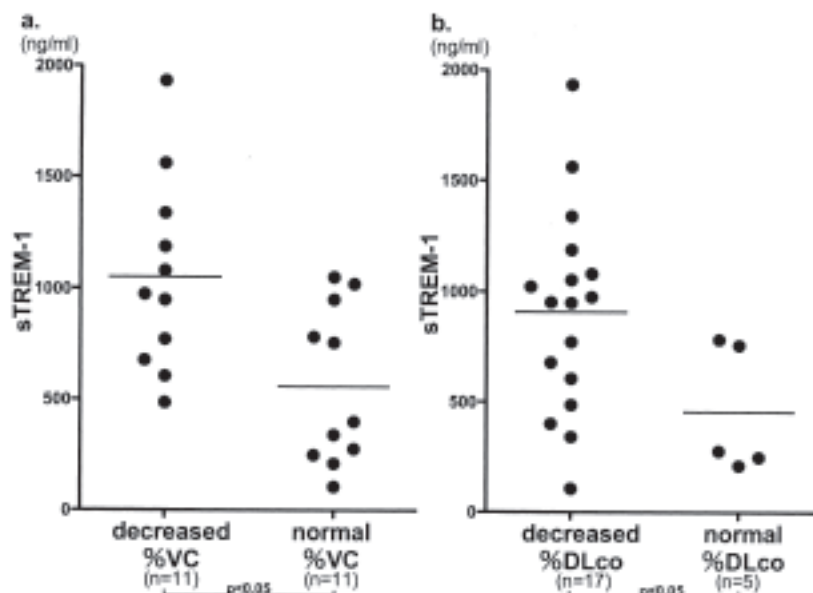


Figure 3. Correlations of serum sTREM-1 levels with %VC (A) and %DLCO (B) in SSc patients with pulmonary fibrosis. Serum sTREM-1 levels were determined by ELISA.

and inflammation in inflammatory bowel disease correlate with serum sTREM-1 levels¹⁶. Therefore, the significant elevation of serum sTREM-1 levels in dSSc patients in comparison with lSSc patients and healthy controls in our study may reflect the extent of inflammation, especially in lungs.

In SSc patients, platelet activation contributes to damage of blood vessels and production of inflammatory cytokines and fibrogenic growth factors². Further, the TREM-1 ligand is expressed on platelets⁵, suggesting that the increase in sTREM-1 levels may partially reflect platelet activation. The observation that platelet aggregation and activation are significantly increased in patients with dSSc compared to those with lSSc^{2,18} may be related to our finding that serum sTREM-1 levels were elevated in dSSc patients, but not lSSc patients. Further, since sTREM-1 has the same extracellular domain of TREM-1¹⁹, elevation of sTREM-1 may play a protective role as the result of competition with TREM-1 for its ligand in SSc.

The interaction of TREM-1/ligands plays an important role in LPS-induced activation of polymorphonuclear leukocytes⁵. Recently, we reported that elevated serum levels of polymorphonuclear leukocyte elastase are detected in SSc patients and are associated with decreased pulmonary function and increased serum levels of KL-6 and surfactant protein-D, both of which are serological markers for the severity of pulmonary fibrosis²⁰. There is recent evidence to suggest that high-mobility group box (HMGB)-1 and heat shock protein 70 could be ligands for TREM-1²¹. Interestingly, increased serum levels of HMGB-1 in SSc patients are associated with disease severity including lung severity²². Further, we also have shown that elevation of serum heat shock protein 70 levels is associated with pulmonary

fibrosis, skin sclerosis, renal vascular damage, oxidative stress, and inflammation in SSc²³. Thus, our results suggest that TREM-1 would be one of the markers of SSc lung fibrosis.

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