

# Serum Soluble CD163 and its association with various disease parameters in patients with systemic sclerosis

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## Abstract

**Objective:** Cluster of differentiation 163 (CD163) is a receptor that binds haptoglobin-hemoglobin complexes and is mainly expressed on macrophages and monocytes. As a result of shedding, the extracellular portion of CD163 circulates in the blood as a soluble CD163 (sCD163). This study aimed to measure serum sCD163 levels in patients with systemic sclerosis (SSc) and to assess its association with the clinical, laboratory, and radiological features of the disease.

**Material and Methods:** We measured serum sCD163 levels in 24 patients with SSc and in 30 healthy controls. Complete history of the patients was recorded and thorough clinical, rheumatological, and dermatological examinations were performed. For SSc, the skin thickness score was scored according to the modified Rodnan skin score method and pulmonary involvement was assessed in all patients using high-resolution computed tomography and by performing pulmonary function tests.

**Results:** The mean serum sCD163 levels in patients with diffuse and limited SSc ( $61.64 \pm 19.57$  and  $60.8 \pm 21.43$  ng/mL, respectively) demonstrated a highly statistically significant increase compared with the mean serum levels in healthy controls ( $36.97 \pm 16.37$  ng/mL) ( $p < 0.001$ ). Patients with SSc having elevated serum sCD163 levels had significantly higher pulmonary artery systolic pressure (PASP) than those with normal serum sCD163 levels ( $p < 0.05$ ). Furthermore, the serum sCD163 levels were significantly correlated with PASP ( $r = 0.53$ ,  $p < 0.05$ ) in patients with SSc. The mean serum sCD163 level in patients with SSc having digital ulceration (DU) ( $70.82 \pm 18.3$  ng/mL) demonstrated a statistically significant increase ( $p < 0.05$ ) compared with that in SSc patients without DU ( $53.23 \pm 18.09$  ng/mL).

**Conclusion:** The elevated serum sCD163 levels in patients with SSc and its association with pulmonary hypertension suggest a possible role of macrophages in the pathogenesis and vascular involvement of SSc.

**Keywords:** Systemic sclerosis, soluble CD 163, macrophages, modified Rodnan skin score

## Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disorder in which extensive fibrosis of the skin and an internal organ is considered to be the hallmark of the disease. Immune alteration, vasculopathy, and an excessive production of extracellular matrix with increased collagen deposition are essential in the pathogenesis of this disease (1).

Macrophages play an important role in the pathogenesis of both autoimmune and fibrotic disorders, and they represent a major part of the inflammatory infiltrates found in the skin and affected internal organs during early SSc (2). Activation of macrophages toward various phenotypes differs according to different stimuli. Macrophages are activated to the M1 phenotype by microbial products or interferon. M1 macrophages represent a major source of proinflammatory cytokines, such as interleukin (IL)-1b, tumor necrosis factor alpha (TNF- $\alpha$ ), and IL-6 (3). In contrast, macrophages can be activated toward M2 phenotype by IL-4, IL-13, and IL-10, which are known to be increased in SSc (4). These M2 macrophages have many receptors, such as the hemoglobin scavenger receptor known as the cluster of differentiation 163 (CD163) (5). M2 macrophages are associated with vascularization and wound healing and may play a role in the pathogenesis of fibrotic diseases by the secretion of transforming growth factor beta (TGF- $\beta$ ) (6).

CD163 is the macrophage scavenger receptor that is exclusively expressed in monocytes/macrophages. CD163 has both a membrane-bound variant and soluble variant, which are present in the plasma and other tissue fluids (7). Soluble CD163 (sCD163) has been proposed to be a product of shedding by proteolytic enzymes (8).

Oxidative stress or inflammatory stimuli can release sCD163 from the cell surface through proteolytic cleavage. Therefore, oxidative conditions, such as diabetes mellitus, can cause elevated serum sCD163 levels (9).



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Moreover, sCD163 is found to be increased during wound healing, giving a possible role of CD163 in the pathogenesis of fibrotic diseases and the remodeling of connective tissues (10).

CD163 is found to be increased in many chronic inflammatory conditions and is supposed to have a potential anti-inflammatory function (11). CD163-expressing macrophages can take up haptoglobin-hemoglobin complexes, leading to the increased secretion of IL-10 and the expression of hemoxygenase -1 (12).

This study aimed to estimate the serum sCD163 levels in SSc and to assess any possible association with the clinical, laboratory, and radiological features of the disease.

## Material and Methods

### Participants

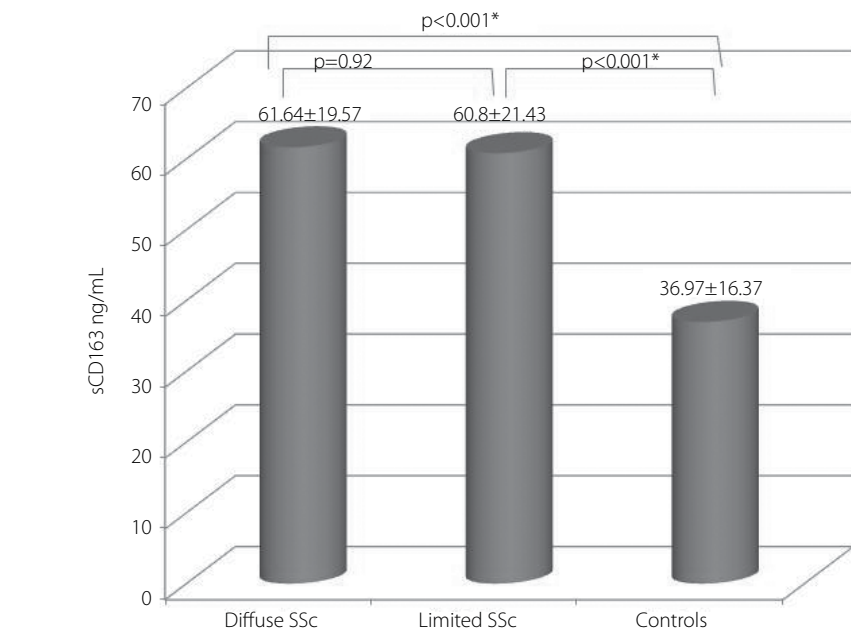
Twenty-four female patients, fulfilling the 2013 American college of rheumatology/European league against rheumatism classification criteria for SSc (13), were recruited from the in- and out-patients' clinic of the Rheumatology and Rehabilitation department of Benha University hospitals between January and December 2014. Furthermore, 30 age-matched apparently healthy females from the hospital personnel, undergraduates, medical, and nursing staff were included as controls.

We excluded patients with conditions that caused oxidative stress, such as patients with ischemic heart disease, heart failure, stroke, peripheral vascular disorders, and diabetes. Furthermore, patients with a recent history of infection were excluded.

Patients with SSc were sub-classified according to the extent of cutaneous affection into diffuse and limited SSc using the criteria of Le Roy et al. (14). The patients' evaluation included complete recording of history and a thorough physical examination emphasizing Raynaud, cutaneous manifestations, arthritis, gastrointestinal, lung manifestations, cardiovascular, and kidney diseases. Skin thickness was assessed in 17 areas using the modified Rodnan skin score (15), and internal organ involvement was defined as described by Steen et al. (16).

### Laboratory and radiological investigations

Routine laboratory investigations, including complete blood count, erythrocyte sedimentation rate, and liver and kidney function tests, were performed, and autoantibodies, including antinuclear antibodies testing by indirect immunofluorescence technique (IMMCO Diagnostics; New York, USA), anti-scleroderma 70,



**Figure 1.** Comparison between mean±SD of serum sCD163 levels in patients with diffuse (n=14) and limited (n=10) SSc and serum sCD163 levels in healthy controls (n=30)

\*significant  $p < 0.05$ .

sCD163: Soluble Cluster of differentiation 163; SSc: systemic sclerosis; SD: standard deviation

and anti-centromere antibodies, were determined using the enzyme-linked immunosorbent assay (ELISA) technique (Cusabio; Hubei, China). Lung involvement was assessed using the pulmonary functional test, and high-resolution computed tomography. In addition, color Doppler echocardiography was used to measure the pulmonary artery systolic pressure (PASP).

### Measurement of serum sCD163 levels

The serum sCD163 levels were measured in all patients with SSc and healthy controls. All blood samples were allowed to clot, and the serum was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until analysis. sCD163 was analyzed using the ELISA technique with the kit supplied from My biosource; San Diego, California, USA. The assay procedures were followed according to the manufacturer's instructions. The detection range was 1.56–100 ng/mL.

The local ethics committee of our institution (Benha University School of Medicine) approved the study, and all the participants provided written informed consent before being enrolled in this study.

### Statistical analysis

The statistical analysis was conducted using the Statistical Package of Social Sciences 16.0 for Windows (SPSS Inc.; Chicago, IL, USA). The collected data were summarized in terms of mean±standard deviation (SD) for quantitative

data and frequency and percentage for qualitative data. Fisher's exact test was used to compare frequencies, as appropriate. The Student's t-test was used to detect the mean differences between two groups regarding quantitative data. The Pearson correlation coefficient was used to evaluate the correlation between sCD163 levels and some variables. Statistical significance was accepted at a p value of  $< 0.05$ .

### Results

Twenty-four patients with SSc (ages ranged from 23 to 53 years) with a mean age of  $35.5 \pm 8.4$  years and 30 age- and sex-matched apparently healthy controls (ages ranged from 19 to 58 years) with a mean age of  $35.32 \pm 8.46$  years were included in the study. Fourteen patients had diffuse SSc and 10 had limited SSc. The patients' clinical and laboratory features are shown in Table 1. The mean serum sCD163 levels in patients with diffuse and limited SSc ( $61.64 \pm 19.57$  and  $60.8 \pm 21.43$  ng/mL, respectively) demonstrated a highly statistically significant increase compared with the mean serum levels in healthy controls ( $36.97 \pm 16.37$  ng/mL) ( $p < 0.001$ ) (Figure 1). Although the serum sCD163 levels were higher in patients with diffuse SSc than those in patients with limited SSc, the difference revealed no statistical significance ( $p > 0.05$ ).

A high serum sCD163 level, defined as greater than the mean+2 SD of the value in healthy controls ( $69.7$  ng/mL), was found in nine patients with SSc (37.5%), while normal serum

**Table 1.** Clinical, laboratory and radiological characteristics of the systemic sclerosis patients (n=24)

	Patient with systemic sclerosis (n=24)	Range
<b>Continuous Variables (mean±SD)</b>		
Age (years)	35.5±8.4	23-53
Disease duration (years)	4.83±3.13	1-12
MRSS	15.67±6.57	7-32
FVC%	90.54±13.67	65-108
PASP (mmHg)	43.08±19.45	25-92
ESR (mm/ 1 <sup>st</sup> hr)	33.92±12.29	22-80
HB (g/dL)	10.8±1.63	8.4-14
WBC (thousands/mm <sup>3</sup> )	7.03±2.46	3.9-14
Platelets (thousands/mm <sup>3</sup> )	285.2±79.98	160-420
Creatinine (mg/dL)	1.08±0.63	0.5-3.1
<b>Categorical Variables [n (%)]</b>		
Type (diffuse:limited)	14:10	-
Raynaud's phenomenon	23 (95.83%)	-
Digital ulceration and pitting scars	9 (37.5%)	-
Calcinosis	5 (20.83%)	-
Diffuse pigmentation	15 (62.5%)	-
Pulmonary fibrosis on (HRCT)	10 (41.66%)	-
FVC <80%	9 (37.5%)	-
Pulmonary hypertension (>30 mmHg)	11 (45.83%)	-
Arthritis	1 (4.61%)	-
Renal	2 (8.33%)	-
Heart	3 (12.5%)	-
Anti-Scl 70 antibody	10 (41.66%)	-
Anti-centromere antibody	8 (33.33%)	-
<b>Treatment, no (%)</b>		
Corticosteroids	8 (33.33%)	-
Immunosuppressive medications	7 (29.16%)	-

MRSS: modified Rodnan severity score; FVC: forced vital capacity; PASP: pulmonary artery systolic pressure; ESR: erythrocyte sedimentation rate; HB: haemoglobin; WBC: white blood cells; HRCT: high resolution computed tomography; Anti-Scl 70: anti-scleroderma 70; n: number

sCD163 levels were found in 15 patients with SSc (62.5%). Patients with SSc having elevated serum sCD163 levels had significantly higher PASP (53.22±23.98 mmHg) than those with normal serum sCD163 levels (37±13.67 mmHg) ( $p<0.05$ ). There was no statistically significant difference in the incidence of other clinical or laboratory features (Table 2). However, the mean serum sCD163 level in patients with SSc having digital ulceration (DU) (72.33±17.62 ng/

mL) demonstrated a statistically significant increase compared with those with SSc without DU (52.25±18.74 ng/mL) ( $p<0.05$ ) (Figure 2).

Regarding the presence of pulmonary fibrosis (PF), the mean serum sCD163 level in patients with SSc having PF (68.6±19.11 ng/mL) demonstrated no statistically significant difference ( $p>0.05$ ) compared with patients with SSc but without PF (56.07±19.44 ng/mL).

Serum sCD163 levels revealed a statistically significant correlation with PASP ( $r=0.53$ ,  $p<0.05$ ) (Table 3). There was no other statistically significant correlation between sCD163 and the other clinical or laboratory features of SSc.

## Discussion

At early stages of SSc, monocytes/macrophages represent major components of the inflammatory infiltrates found in the skin and affected organs (17). CD163 is considered to be a marker of differentiation of monocytes into activated macrophages (M2 macrophages) that release anti-inflammatory mediators and may contribute to connective tissue remodeling (18). Moreover, M2 macrophages are suggested to play a major role in the development of many fibrotic disorders by secreting pro-fibrotic cytokines, such as TGF- $\beta$  (19).

In our study, serum sCD163 levels were statistically significantly increased in both patients with diffuse and limited SSc than its serum level in healthy controls. Our results confirmed the results of others who found an increased serum sCD163 level in patients with SSc compared with that in healthy controls (20-22).

Shimizu et al. (22) suggested that increased sCD163 was attributed to the presence and degree of oxidative stress, and SSc is considered to be one of the oxidative stresses, and they found a positive correlation between sCD163 and serum 8-isoprostane, which is considered to be a marker of lipid peroxidation, in patients with SSc. Furthermore, Guiducci et al. (23) suggested that the main function of sCD163 to bind haptoglobin-hemoglobin complexes and to prevent vessel wall injury is beneficial in SSc, as microangiopathy is also present as a prominent feature of SSc and might lead to increased hemolysis and the release of free hemoglobin.

Vascular involvement and microcirculation disturbance are essential components of SSc-related pathophysiology. The involvement of pulmonary vessels can lead to the development of pulmonary arterial hypertension, which has a significant impact on disease prognosis (24). Biomarkers may be used to recognize patients with a high-risk of pulmonary hypertension, who may require more invasive investigations, such as right heart catheterization. Furthermore, these biomarkers can predict prognosis and provide guidance for vasodilator therapy (25).

In this study, serum sCD163 levels were positively correlated with PASP ( $r=0.53$ ,  $p<0.05$ ), PASP was also significantly elevated ( $p<0.05$ ) in patients with raised serum sCD163 levels

**Table 2.** Clinical and laboratory features of systemic sclerosis patients with elevated or normal sCD163 serum levels

	Patient with elevated sCD163 levels (n=9)	Patient with normal sCD163 levels (n=15)
<b>Continuous Variables (mean±SD)</b>		
Age (years)	37.11±8.64	34.53±8.42
Disease duration (years)	4.44±2.96	5.07±3.31
MRSS	17.44±7.75	14.6±5.77
PASP	53.22±23.98*	37±13.67
FVC	88.33±15.65	91.87±12.73
ESR (mm/ 1 <sup>st</sup> hr)	35.22±18.27	33.13±7.46
<b>Categorical variables [n (%)]</b>		
Type (diffuse:limited)	5:4	9:6
Raynaud's phenomenon	9 (100%)	14 (93.33%)
Digital ulceration and pitting scars	5 (55.55%)	4 (26.67%)
Calcinosis	2 (22.22%)	3 (20%)
Diffuse pigmentation	6 (66.66%)	9 (60%)
Pulmonary fibrosis on (HRCT)	5 (55.55%)	5 (33.33%)
FVC <80%	4 (44.44%)	5 (33.33%)
Pulmonary hypertension (>30 mmHg)	6 (66.67%)	5 (33.33%)
Arthritis	0(0%)	1 (6.67%)
Renal	1 (11.11%)	1 (6.67%)
Heart	1 (11.11%)	2 (13.33%)
Anti-Scl 70 antibody	3 (33.33%)	7 (46.67%)
Anti-centromere antibody	4 (44.44%)	4 (26.67%)
<b>Treatment, no (%)</b>		
Corticosteroids	4 (44.44%)	4 (26.67%)
Immunosuppressive medications	4 (44.44%)	3 (20%)

sCD163: soluble cluster of differentiation 163; MRSS: modified Rodnan severity score; FVC: forced vital capacity; PASP: pulmonary artery systolic pressure; ESR: erythrocyte sedimentation rate; HRCT: high resolution computed tomography; Anti-Scl 70: anti-scleroderma 70; n: number  
\*significant  $p < 0.05$ .

compared with patients with normal serum sCD163 levels. This is in agreement with many studies showing that patients with SSc having elevated serum sCD163 levels had significantly higher PASP compared with patients with normal serum sCD163 levels (20, 21).

This may suggest that M2 macrophages may play a potential role in the pathogenesis of pulmonary hypertension associated with SSc. The histological analysis of hypertensive pulmonary arteries show the proliferation of endothelial cells, smooth muscle cells contraction, and macrophages infiltrates that produce proinflammatory cytokines that may contribute to tissue injury (26, 27).

In contrast to our results, the study of Bielecki et al. (28) did not reveal any association between the greater release of sCD163 in peripheral blood mononuclear cells and pulmonary hypertension and attributed this discrepancy to differences in study methodology and the differences in patient populations involved in other studies. This made analysis of associations between the ex vivo production of sCD163 by PBMC and severity of the disease less accurate.

In our study, the mean serum sCD163 level in patients with SSc and DU (72.33±17.62 ng/mL) showed a statistically significant increase ( $p < 0.05$ ) compared with patients with SSc but

without DU (52.25±18.74 ng/mL). Furthermore, patients with SSc and increased serum sCD163 levels were more likely to have DU than those with normal serum sCD163 levels, but this difference was not statically significant ( $p > 0.05$ ). This result is inconsistent with other studies that found digital ulcers to be more frequent in patients with SSc having elevated serum sCD163 levels (20, 21).

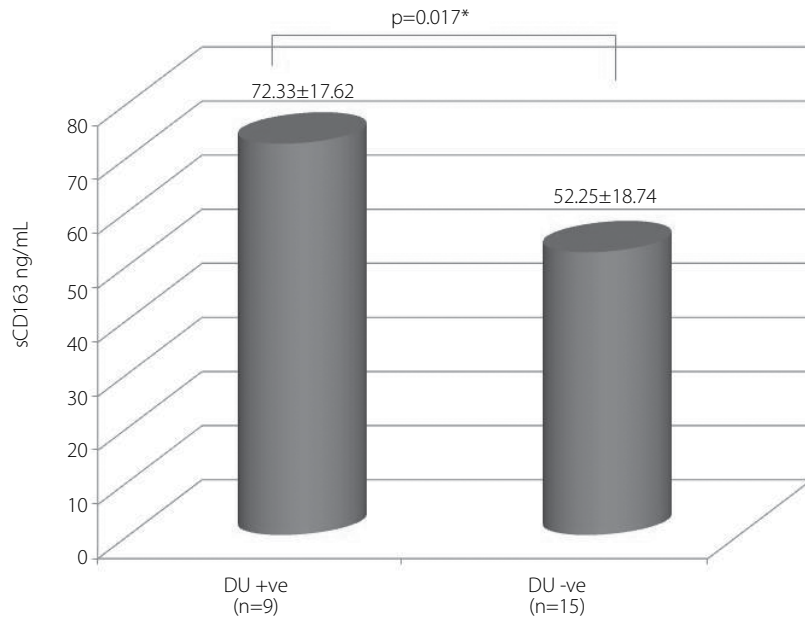
This is explained by stating that DUs are related mainly to vascular injury of the digital vessels, characterized by increased intimal thickness and occlusion of the vessel lumen. Ischemic tissue has a reduced nutritional supply and decreased healing capacity, and DUs tend to occur more likely in ischemic tissue (29). The vascular pathology found in digital vessels in SSc is very similar to that observed in pulmonary arteries in patients with pulmonary hypertension and in renal vessels in patients with renal crisis (30).

CD163 has been found to inhibit the function of a TNF-like weak inducer of apoptosis (TWEAK), a cytokine that is involved in tissue remodeling and angiogenesis. TWEAK can also be incorporated within CD163-positive macrophages, leading to a decreased TWEAK level, which has been established to be associated with more severe microvascular injury and the active pattern on capillaroscopic examination (31).

On the other hand, Kowal-Bielecka et al. (21) found a lower risk of digital ulceration with elevated serum sCD163 levels and suggested that CD163-positive cells can induce new blood vessel formation through stimulation of endothelial cells proliferation, which may directly contribute to the healing of DU.

In our study, the mean serum sCD163 level in patients with SSc and PF (68.6±19.11 ng/mL) showed no statistically significant difference compared to patients with SSc but without PF (56.07±19.44 ng/mL) ( $p > 0.05$ ). On the other hand, Shimizu et al. (22) found patients with SSc and PF to have significantly increased sCD163 levels compared with those without PF. However, there was no significant correlation between sCD163 levels and the pulmonary function tests. They suggested that sCD163 was related to the level of oxidative stress, which results in lung damage in patients with SSc, and recommended further research to explore the exact mechanism.

This discrepancy can be explained by stating the role of sCD163 in the pathogenesis of fibrosis is controversial, as CD163 positive macrophages can have fibrotic function (32). On the



**Figure 2.** Comparison between mean±SD of serum sCD163 levels in patients with SSc (n=9) and without (n=15) DU and pitting scars

\*significant p<0.05.

SSc: systemic sclerosis; sCD163: Soluble Cluster of differentiation 163; DU: digital ulceration; SD: standard deviation

**Table 3.** Correlations between sCD163 serum levels and different variables in systemic sclerosis patients

sCD163	r	p
Age (years)	0.13	>0.05
Disease duration(years)	0.17	>0.05
MRSS	0.22	>0.05
FVC (%)	-0.21	>0.05
PASP (mmHg)	0.53	<0.05*
ESR (mm/1 <sup>st</sup> hr)	0.21	>0.05
CRP	0.24	>0.05

sCD163: soluble cluster of differentiation 163; MRSS: modified Rodnan severity score; FVC: forced vital capacity; PASP: pulmonary artery systolic pressure; ESR: erythrocyte sedimentation rate; CRP: C reactive protein

\*significant p<0.05.

other hand, CD163 might have an anti-fibrotic function through TWEAK inhibition, which can induce activation of fibroblast (33, 34), but it remains unclear, which role predominates in SSc.

A limitation of our results lies in the relative small number of patients included in the study. Another limitation is the use of Doppler echocardiography as a reliable tool for the evaluation of pulmonary hypertension instead of right heart catheterization, which is more invasive. Further longitudinal studies are recommended with a larger number of patients to define the exact role of sCD163 in the vascular involvement and development of pulmonary hypertension in patients with SSc.

In conclusion, the elevated serum sCD163 serum levels in patients with SSc and its association with pulmonary hypertension suggest a role of macrophages in the pathogenesis and vascular involvement of systemic sclerosis.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Benha University School of Medicine.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - W.A.S.E.H., E.A.E.B.; Design - W.A.S.E.H.; Supervision - W.A.S.E.H., T.M.G.;

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