Interleukin-33 in systemic sclerosis: correlation with clinical manifestations and disease subset

Magdy A. Zohairy[a], Anna N.A. Raya[a], Akram M. Deghady[b], Riham A. Soliman[a]

[a]Department of Rheumatology Unit, Internal Medicine, [b]Department of Clinical and Chemical Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Correspondence to Riham A. Soliman, MBBCh, Department of Rheumatology Unit, Internal Medicine, Faculty of Medicine, Alexandria University, Alexandria, Egypt
e-mail: rihamaligaberali@yahoo.com

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Background
Systemic sclerosis (SSc) is a generalized connective tissue disorder characterized by sclerotic changes in the skin and internal organs. Interleukin-33 (IL-33) is a newly reported cytokine of the IL-1 family.

Aim of the work
The aim of this study was to determine serum levels of IL-33 in SSc patients and evaluate its association with clinical manifestations and disease subset.

Patients and methods
The patients in this study were divided into group A and group B. Group A included 30 adult patients with SSc, which was subdivided into diffuse systemic sclerosis (dSSc) and limited systemic sclerosis (lSSc). All cases were diagnosed according to the American College of Rheumatology criteria for SSc. Group B included 15 healthy adults (age and sex matched) who served as controls. Serum IL-33 levels were examined by means of enzyme-linked immunosorbent assay in 30 patients with SSc and in 15 healthy individuals. Skin assessment was done using the modified Rodnan skin score.

Results
IL-33 was increased in all SSc patients compared with controls. The levels of IL-33 were significantly higher in the dSSc subset compared with the lSSc subset. IL-33 is highly correlated to the presence of pulmonary fibrosis, Raynaud's phenomenon, pitting scars and ulcers, pulmonary hypertension, joint contracture, and modified Rodnan skin score. Thus, IL-33 levels were increased in SSc patients and correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis. Therefore, IL-33 possibly plays a role in cutaneous and pulmonary fibrosis in SSc patients.

Conclusion
IL-33 may have a significant role in the pathogenesis of SSc. IL-33 serum levels paralleled the severity of the disease subset. Understanding of IL-33 functions is important for the development of new therapeutic approaches including IL-33 inhibitors and IL-33 receptor blockers as a therapeutic target.

Keywords:
diffuse systemic sclerosis, limited systemic sclerosis, modified Rodnan skin score

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Introduction

Systemic sclerosis (SSc) is a generalized connective tissue disorder characterized by sclerotic changes in the skin and internal organs. In addition, SSc is generally regarded as an autoimmune disorder because of the presence of antinuclear antibodies. Although the pathogenesis of SSc remains unclear, previous studies have suggested that some cytokines or growth factors regulate SSc induction by stimulating the synthesis of extracellular matrix components, injuring the endothelial cells and modulating the function of leukocytes [1]. These cytokines or growth factors are produced partly by inflammatory cells infiltrating the affected tissues, such as skin or lungs, of SSc patients [1,2].

Differentiation of the major subset of SSc into limited or diffuse cutaneous forms is based on the maximum extent of skin involvement [3].

The American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) established a committee to provide a joint proposal for new classification criteria for SSc.

The new classification criteria show that skin thickening of the fingers extending proximal to the metacarpophalangeal joints is sufficient for the patient to be classified as having SSc; if that is not present, seven additive items apply, with varying weights for each: skin thickening of the fingers, fingertip lesions, telangiectasia, abnormal nail-fold capillaries, interstitial lung disease or pulmonary

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arterial hypertension, Raynaud's phenomenon, and SSc-related autoantibodies [4].

Epidemiology

Similar to other connective tissue diseases, SSc is more frequent in women than in men, with the most common age range of disease onset being 30–50 years.

Pathophysiology

Endothelial alterations may lead to a cascade of stimulatory changes that involve many cells, including fibroblasts, T lymphocytes, macrophages, and mast cells. In turn, the activated cells secrete a variety of substances, including cytokines and their soluble receptors and enzymes and their inhibitors. These substances lead to changes in the extracellular matrix compounds, including fibronectin, proteoglycans, and collagen types I, III, V, and VII. Increased collagen deposition in tissues is a characteristic feature of SSc. Increased collagen production or disturbances in its degradation can cause excessive collagen deposition in tissues.

Pathogenesis

Activation of T cells is evident in lesional tissues and in peripheral blood, and seems to play a direct role in tissue injury.

Vascular injury and activation are the earliest and possibly primary events in the pathogenesis of SSc.

Clinical features

Skin manifestations

Cutaneous telangiectasia, dilations of dermal blood vessels, occurs in limited cutaneous SSc and diffuse cutaneous SSc, but is more extensive in limited cutaneous SSc, particularly in the subgroup previously designated as having CREST (calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly, and telangiectasia) syndrome.

Gastrointestinal tract manifestations

Esophageal involvement is frequent with dysmotility and lower esophageal sphincter dysfunction, and consequent gastroesophageal reflux is almost universal in SSc [5].

Musculoskeletal involvement

The fibrotic process of SSc commonly affects the tendons (causing tendon friction rubs), ligaments, and joint capsules, restricting movement.

Contraction of the fingers is a hallmark of SSc. It may develop rapidly, and has a significant impact on hand function.

Pulmonary involvement

The most common forms of interstitial lung disease in SSc are histologically classified as usual interstitial pneumonia and nonspecific interstitial pneumonitis.

Pulmonary artery hypertension, defined as an elevation in the mean pulmonary artery pressure greater than 25 mmHg at rest, occurs in limited and diffuse cutaneous forms of SSc and is a leading cause of mortality.

Renal manifestations

Scleroderma renal crisis occurs in 10–15% of patients with diffuse cutaneous SSc and only rarely (1–2%) in limited cutaneous SSc.

Neurologic manifestations

In early-stage diffuse cutaneous SSc, patients commonly report symptoms of median nerve compression, and many patients undergo surgical treatment for carpal tunnel syndrome before the diagnosis of SSc is established.

Interleukin-33

Interleukin-33 (IL-33) is a newly reported cytokine of the IL-1 family.

Recent evidence suggests a role for IL-33/ST2 in several rheumatological diseases, including SSc, rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and systemic lupus erythematosus [6–8].

In SSc, the combination of vascular abnormalities, collagen deposition, and autoimmunity leads to widespread tissue and organ fibrosis [9]. It has been found that, compared with healthy controls, IL-33 expression was significantly increased in SSc patients. Meanwhile, the serum level of IL-33 was correlated with early disease stage and microvascular involvement [10]. Moreover, some other investigators reported the same observations recently [11]. These data led us to believe that IL-33 may be involved in the SSc pathogenesis, and the mechanism may be correlated with the role of IL-33 in promoting fibrosis [12].

Patients and methods

The study included two groups

Group A included 30 adult patients with SSc, who were subdivided into diffuse systemic sclerosis (dSSc) and limited systemic sclerosis (lSSc) groups.

All patients were diagnosed according to the ACR criteria for SSc [13].

Group B included 15 healthy adult persons (age and sex matched) as the control group.

All patients were selected from the Internal Medicine Department, Rheumatology Unit, Main Alexandria University Hospital.

Informed consent was taken from all patients before the beginning of the study.

Methods

This study was conducted on 30 patients with SSc who were admitted to the Rheumatology Unit at Alexandria Main University Hospital, and on 15 healthy adult persons as controls.

The study population was subjected to the following:

(1) Complete history taking, including
   (a) demographic data,
   (b) history of the presenting complaints.
(2) Thorough clinical examination, including
   (a) vital signs,
   (b) head and neck examination,
   (c) cardiovascular examination,
   (d) chest examination,
   (e) abdominal examination,
   (f) skin and extremities.
(3) Skin assessment using the modified Rodnan skin score (mRss) [14].

A semiquantitative estimation of the degree of skin thickening was made.

(4) Laboratory investigations, including
   (a) complete blood count, routine blood chemistry,
   (b) urine analysis,
   (c) anti-Scl-70,
   (d) anti-centromere,
   (e) determination of serum levels of IL-33 measured by human enzyme-linked immunosorbent assay.

Results

The present study was conducted on two groups:

(1) Group A (30 patients), which was subdivided into dSSc (14 patients) and lSSc (16 patients).
(2) Group B (15 healthy persons).

Table 1 shows IL-33 in the two studied groups. IL-33 in dSSc patients (group A) ranged from 75 to 220 pg/ml, with a mean value of 103.93 ± 35.27 pg/ml; IL-33 in lSSc patients (group A) ranged from 62 to 98 pg/ml, with a mean value of 83.00 ± 10.28 pg/ml; and IL-33 in control patients (group B) ranged from 50 to 84 pg/ml, with a mean value of 65.40 ± 711.26 pg/ml. Values higher than the mean+2 SD (87.8 pg/ml) of the control serum samples were considered to be elevated in this study. There was statistically significant difference regarding IL-33 in the two studied groups ($P < 0.05$). Serum levels were significantly higher in SSc patients than in healthy controls and were significantly higher in the dSSc subset than in the lSSc subset (Fig. 1).

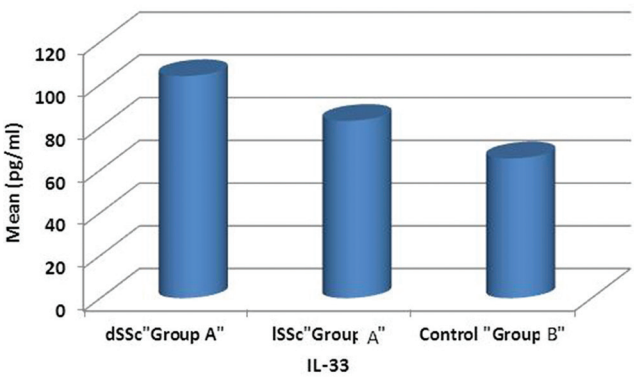
Table 2 shows the correlation between IL-33 and mRss in dSSc and lSSc patients. Mild mRss in dSSc patients ranged from 88 to 100, with a mean value of 94.00 ± 8.49, and that in lSSc patients ranged from 62 to 86, with a mean value of 79.67 ± 9.05. Moderate mRss in dSSc patients ranged from 97 to 100, with a mean value of 98.50 ± 2.12, and that in lSSc patients ranged from 80 to 92, with a mean value of 80.50 ± 6.12. Severe mRss in dSSc patients ranged from 75 to 220, with a mean value of 107.0 ± 41.83, and that in lSSc patients ranged from 65 to 95, with a mean value of 85.67 ±

Table 1 IL-33 in the studied groups

IL-33 (pg/ml)	Minimum	Maximum	Mean	SD	*F*	*P*
Group A						
dSSc	75.00	220.00	103.93	35.27	11.567	0.0001
lSSc	62.00	98.00	83.00	10.28		
Group B						
Control	50.00	84.00	65.40	11.26		

dSSc, diffuse systemic sclerosis; IL, interleukin; lSSc, limited systemic sclerosis.

Figure 1



Interleukin-33 (IL-33) in the studied groups.

14.36. It was found that all dSSc and lSSc patients had a positive correlation between the presence of mRss and IL-33 ($P < 0.05$). Thus, IL-33 levels in dSSc and lSSc patients correlated positively with the extent of skin sclerosis (Fig. 2).

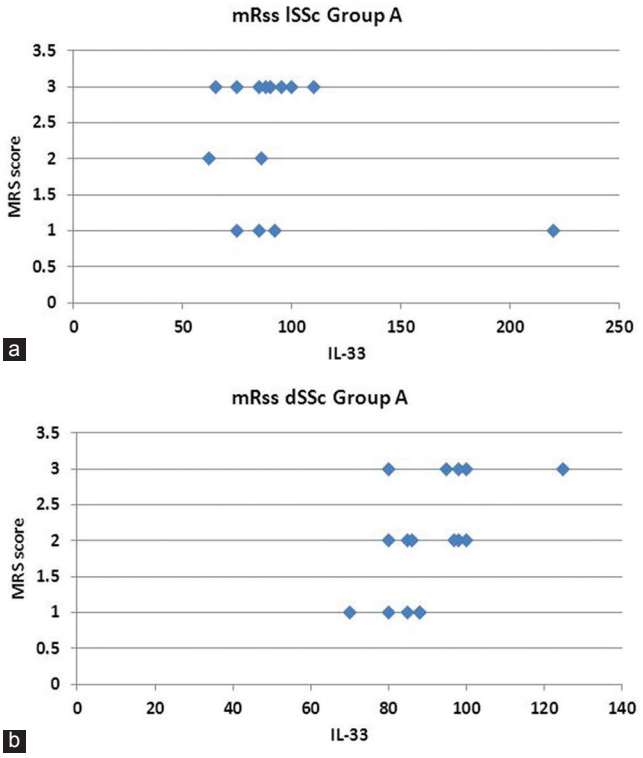
Table 3 shows the correlation between IL-33 and pulmonary fibrosis in the dSSc and lSSc patients.

Table 2 Correlation between IL-33 and mRss in the dSSc and lSSc patients

Modified Rodnan skin score	Group A	
	dSSc	lSSc
Mild		
Range	88.0–100.0	62.0–86.0
Mean	94.00	79.67
SD	8.49	9.05
Moderate		
Range	97.0–100.0	80.0–92.0
Mean	98.50	80.50
SD	2.12	6.12
Severe		
Range	75.0–220.0	65.0–95.0
Mean	107.00	85.67
SD	41.83	14.36
R	0.425	0.51
P	0.031	0.011

dSSc, diffuse systemic sclerosis; IL, interleukin; lSSc, limited systemic sclerosis; mRss, modified Rodnan skin score.

Figure 2



(a, b) Correlation between interleukin-33 (IL-33) and modified Rodnan skin score in the diffuse systemic sclerosis (dSSc) and limited systemic sclerosis (lSSc) patients.

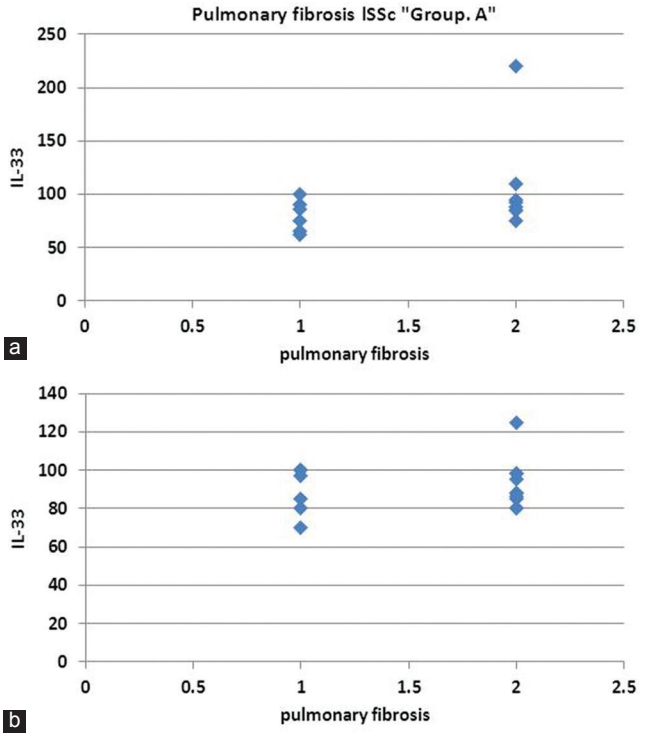
IL-33 levels in dSSc cases without pulmonary fibrosis ranged from 75 to 188, with a mean of 85.2 ± 23.9, and those in lSSc cases without pulmonary fibrosis ranged from 60 to 90.2, with a mean of 74.3 ± 12.11. IL-33 levels in dSSc cases with pulmonary fibrosis ranged from 88.19 to 220, with a mean of 109.71 ± 29.8, and those in lSSc cases with pulmonary fibrosis ranged from 71.2 to 98, with a mean of 82.45 ± 11.3. It was found that all dSSc and lSSc patients had a positive correlation between the presence of pulmonary fibrosis and IL-33 ($P < 0.05$) (Fig. 3).

Discussion

One of the proinflamatory cytokines believed to be involved in the pathology of SSc is IL-33. Recent evidence suggests a role for IL-33/ST2 in several rheumatological diseases, including SSc, rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and systemic lupus erythematosus [6–8].

The present study was conducted on 30 patients with SSc fulfilling the ACR criteria for diagnosis of the disease and 15 age-matched and sex-matched healthy individuals.

Figure 3



(a, b) Correlation between interleukin-33 (IL-33) and pulmonary fibrosis in the diffuse systemic sclerosis (dSSc) and limited systemic sclerosis (lSSc) patients.

Table 3 Correlation between IL-33 and pulmonary fibrosis in the dSSc and lSSc patients

Pulmonary fibrosis	Group A	
	dSSc	lSSc
Without		
Range	75.0–188.0	60.0–90.2
Mean	85.2	74.3
SD	23.9	12.11
With		
Range	88.19–220.00	71.2–98.00
Mean	109.71	82.45
SD	29.8	11.3
R	0.339	0.403
P	0.0125	0.009

dSSc, diffuse systemic sclerosis; IL, interleukin; lSSc, limited systemic sclerosis.

IL-33 levels in dSSc patients (group A) ranged from 75 to 220 pg/ml, those in lSSc patients (group A) ranged from 62 to 98 pg/ml, and those in controls (group B) ranged from 50 to 84 pg/ml. There was a statistically significant difference regarding IL-33 in the two studied groups ($P < 0.5$).

In the present study IL-33 was increased in all SSc patients as compared with controls.

Furthermore the levels of IL-33 were significantly higher in the dSSc subset compared with the lSSc subset.

Thus, IL-33 serum levels paralleled the severity of the disease.

Similar results have been reported by several studies, including that by Wagner *et al*. in 2015 [15].

Wagner and colleagues suggested that endothelial, T cell, and fibroblast activation can be present in patients with early SSc, suggesting new routes of investigation into cell–cell dynamics in target tissues predating overt disease manifestations, thus opening new therapeutic approaches.

Furthermore in the study by Yanaba *et al.* (2011) [16] serum samples were obtained from 69 Japanese patients with SSc [56 women and 13 men; 13–73 years of age (mean, 47 years)]. All patients fulfilled the criteria for SSc proposed by the ACR. Twenty-seven patients had limited cutaneous SSc (lSSc) and 42 had diffuse cutaneous SSc (dSSc). All patients were 13–73 years of age (mean, 47 years). The median disease duration was 1.9 years. Serum IL-33 levels were measured using specific enzyme-linked immunosorbent assay kits.

As in our study, Yanaba found that serum IL-33 levels were significantly higher in SSc patients than in healthy individuals. IL-33 levels in dSSc patients were significantly higher than those in lSSc patients or healthy individuals. There was no significant difference in serum IL-33 levels between lSSc patients and healthy individuals.

Clinical and laboratory parameters at the first evaluation were compared between SSc patients with elevated IL-33 levels and those with normal IL-33 levels. Elevated IL-33 levels were observed in 33% of all SSc patients, in 45% of dSSc patients, and in 15% of lSSc patients. SSc patients with elevated IL-33 levels more frequently had dSSc than those with normal IL-33 levels.

In our study we studied the correlation of IL-33 levels in SSc patients with clinical manifestations.

We found a significant positive correlation between IL-33 levels and the presence of pulmonary fibrosis, Raynaud's phenomenon, pitting scars and ulcers, pulmonary hypertension, joint contracture, and mRss.

Similar results were reported by Yanaba and colleagues. They found that SSc patients with elevated IL-33 levels had a significantly higher modified Rodnan Total skin score (TSS) compared with those with normal IL-33 levels. Furthermore, IL-33 levels correlated positively with modified Rodnan TSS.

Also Yanaba found the prevalence of PF and decreased percent predicted FVC in SSc patients with elevated IL-33 levels to be significantly higher than those in patients with normal IL-33 levels Moreover, IL-33 levels correlated inversely with percent predicted FVC in SSc patients.

Thus, IL-33 levels correlated not only with the extent of skin sclerosis but also with the severity of PF in SSc.

Elevation of IL-33 levels was consistently associated with the greater extent of skin fibrosis and greater frequency and severity of PF.

These results suggest that IL-33 plays a role in cutaneous and pulmonary fibrosis in SSc patients.

In SSc patients, $CD4^+$ T-cell infiltration is observed around small vessels in the dermis of early skin lesions. Furthermore, the degree of cell infiltration correlates with both degree and progression of skin thickening. In this study, serum IL-33 levels were associated with the extent of skin sclerosis and severity of PF. IL-33 induces migration of Th2 lymphocytes and enhances Th2 cytokine production. Consistent with this, serum concentrations of Th2 cytokines such as IL-4 and

IL-13 in SSc patients are increased, whereas SSc patients exhibit substantial Th2 cytokine production in cultures of $CD4^+$ T lymphocytes isolated from their affected skin. Furthermore, Th2 cytokines such as IL-4 enhance collagen production by fibroblasts. Thus, IL-33 is likely to contribute to the Th2 lymphocyte infiltration and promote Th2 cytokine production, leading to skin fibrosis in SSc.

It has recently been reported that IL-33 and its receptor ST2 are abnormally expressed in patients with SSc. In lesional skin from patients with early SSc, IL-33 protein is downregulated or absent in endothelial cells and the epidermis, whereas IL-33 mRNA is upregulated. Moreover, endothelial cells, perivascular infiltrating mast cells, macrophages, T cells, B cells, and activated fibroblasts/myofibroblasts exhibit strong ST2 expression. By contrast, in lesional skin from patients with late SSc, IL-33 protein is constitutively found in endothelial cells, whereas IL-33 mRNA expression is normal. Furthermore, ST2 expression in the skin of patients with late SSc is weaker than that in the skin of those with early SSc. In early skin lesions of patients with SSc, mononuclear cells infiltrate around small vessels in the dermis and promote endothelial cell damage. These results suggest that the damage of endothelial cells in patients with early SSc enhances IL-33 mRNA expression and the release of IL-33 protein into circulation. Consistent with these findings, serum IL-33 levels were elevated in patients with early SSc but significantly decreased during the follow-up. Thus, serum IL-33 levels are likely to reflect the degree of endothelial damage in patients with early SSc. Further studies are needed to address the role of IL-33 in the pathogenesis of SSc. Nonetheless, measurement of serum IL-33 in patients with early SSc may offer an important means for further evaluation of SSc disease severity because no specific serum marker for SSc has been found to date.

Finally, as in our study Yanaba and colleagues reported that serum IL-33 levels were elevated in SSc patients compared with healthy individuals. Patients with diffuse cutaneous SSc had higher levels of IL-33 than did those with limited cutaneous SSc, and IL-33 levels correlated positively with the extent of skin fibrosis and pulmonary fibrosis, thus suggesting that IL-33 may possibly play a role in organ fibrosis in SSc patients.

Another study by Zhao and Chen in 2014 [17] found that IL-33 and ST2 play an important role in allergic diseases and various mucosal immune responses, suggesting the involvement of the IL-33/ST2 pathway in the pathogenesis of a large number of diseases, especially in the responses based on the Th2 type.

Conclusion

The following conclusions can be drawn from the current study:

IL-33 was increased in all SSc patients as compared with controls.

It may be concluded that IL33 may has a significant role in the pathogenesis of SSc.

Furthermore the levels of IL-33 were significantly higher in the dSSc subset compared with the lSSc subset.

Thus, IL-33 serum levels paralleled the severity of the disease subset.

IL-33 is highly correlated to the presence of pulmonary fibrosis, Raynaud's phenomenon, pitting scars and ulcers, pulmonary hypertension, joint contracture, and mRss.

Understanding of IL-33 functions is important for the development of new therapeutic approaches including IL-33 inhibitors and IL-33 receptor blockers as a therapeutic target.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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