Thymic stromal lymphopoietin in systemic sclerosis interstitial lung disease: a pilot study

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Abstract

Objective

Systemic sclerosis (SSc) is an autoimmune disease, characterised by microvascular alterations, dysregulation of immune system and fibrosis. The most important complication is interstitial lung disease (ILD). The aim of this study was to evaluate serum levels of thymic stromal lymphopoietin (TSLP) in SSc patients and healthy controls (HC). The secondary aim was to correlate TSLP with skin fibrosis and extension of ILD.

Methods

75 SSc patients and 20 HC were enrolled and serum TSLP levels were measured in both cohorts. Pulmonary function tests (PFTs), high-resolution computed tomography (HRCT) and exhaled fraction of nitric oxide (FeNO) were assessed in SSc patients. A visual semi-quantitative staging system, tomographic fibrosis score (TFS), was used to assess SSc-ILD.

Results

Serum levels of TSLP were higher in SSc patients than HC. A positive correlation between TSLP and mRSS was observed ($r=0.409$, $p<0.001$) and a negative correlation was found between TSLP and FVC ($r=-0.356$, $p<0.01$). Serum TSLP was significantly higher in SSc patients with Type 2 inflammation than patients without Type 2 inflammation [172 pg/ml (IQR 154.67;224.67) vs. 150 pg/ml (IQR 110;210.33), $p<0.05$]. The median value of serum TSLP was significantly higher in SSc patients with TFS ≥ 5% than SSc patients with TFS <5% [216.67 pg/ml (IQR 172;298.67) vs 140.67 pg/ml (IQR 122;166.67), $p<0.001$).

Conclusion

In conclusion, TSLP might have a key role in ILD and skin fibrosis.

Key words

thymic stromal lymphopoietin, systemic sclerosis, interstitial lung disease, type 2 inflammation

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Received on September 11, 2024; accepted in revised form on December 28, 2024.

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Competing interests: none declared.

Introduction

Interstitial lung disease (ILD) is the leading cause of death in systemic sclerosis (SSc) (1-3). Diagnosis of SSc-associated ILD (SSc-ILD) is performed using pulmonary function tests (PFTs) and high-resolution computed tomography (HRCT) (4). The pathogenesis of fibrosis in SSc-ILD involves the alveolar epithelial and vascular endothelial injury that promotes the activation of the immune system, the production of profibrotic mediators and the recruitment of fibroblasts and myofibroblasts (5). In SSc has been described activation of both type 1 and type 2 immune response; since these subsets potentially exert opposing roles in regulating tissue remodelling and fibrogenesis, the extreme immune polarisation toward a Th1 or Th2 response can be associated with different disease manifestations and mediate distinct outcomes (6, 7). Type 1 inflammation is characterised by production of interferon-γ (IFNγ) and interleukin-2 (IL 2) with consequent suppression of fibroblast activity, whilst in type 2 inflammation IL 4, IL 5, IL 10, and IL 13 are predominant and activate fibroblasts and collagen production directly or indirectly by inducing secretion of profibrotic cytokines such as transforming growth factor β (TGF-β) (7). The relevance of a type 2-polarised immune response in the pathogenesis and progression of SSc-ILD has been hypothesised (8), whilst a type 1 microenvironment is functionally relevant for generation of the vascular disease seen in scleroderma (7).

Thymic stromal lymphopoietin (TSLP) is a pleiotropic cytokine that binds to a high-affinity heteromeric complex composed of thymic stromal lymphopoietin receptor (TSLPR) chain and interleukin 7 receptor-α (IL-7Rα) (9). TSLP is produced by airway epithelial cells after stimulation by allergens or infective agents, is involved in the induction and perpetuation of airway inflammation and it activates immune cells and inflammatory pathways related to type 2 and non-type 2 inflammation (10). TSLP activates dendritic cells (DCs), group 2 innate lymphoid cells (ILC2s) and mast cells, which promotes type 2 inflammation (10, 11). TSLP has an important role in the airway remodelling, increasing collagen production from fibroblasts and the growth of airway smooth muscle cells (10) (Fig. 1). A Th2-polarised T cell response has been demonstrated to cause tissue damage and fibrosis in SSc-ILD because Th2 cytokines active alternative inflammatory pathways and the transcription of TGFβ, involved in induction and progression of fibrosis (7, 8). TSLP has implicated in bronchial asthma, atopic rhinitis, atopic dermatitis, eosinophilic esophagitis, eosinophilic granulomatosis with polyangiitis, and obstructive pulmonary disease (12-15). TSLP has an important proinflammatory and profibrotic role in SSc. TSLP is significantly elevated in the skin of SSc-like mice models and SSc patients (16). It has been demonstrated that TSLP was overexpressed by epithelial cells, mast cells and fibroblasts in human SSc skin and lung fibrosis, and in the bleomycin model of scleroderma (17, 18).

Aim of this study was to evaluate serum levels of TSLP in SSc patients and healthy controls (HC). Secondary aim was to correlate serum level of TSLP with skin fibrosis and extension of ILD.

Materials and methods

Subjects

Seventy-five SSc patients, fulfilling the ACR/EULAR for SSc (19) and twenty HC, recruited among healthcare workers and matched for sex and age, were enrolled in this study. Cardiopulmonary diseases not related to SSc, pulmonary arterial hypertension (PAH), heart and hepatic failure, end stage renal diseases (ESRD), malignancies, allergic diseases, smoke and breastfeeding were exclusion criteria. Patients treated in the last 6 months with immunosuppressive agents and corticosteroids at an equivalent dose of prednisone ≥10 mg/day were also excluded.

Clinical assessment

The modified Rodnan skin score (mRSS) was used to value skin involvement and the disease subset type was defined as diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc) (20). Disease activity index (DAI) and disease severity scale (DSS) were used

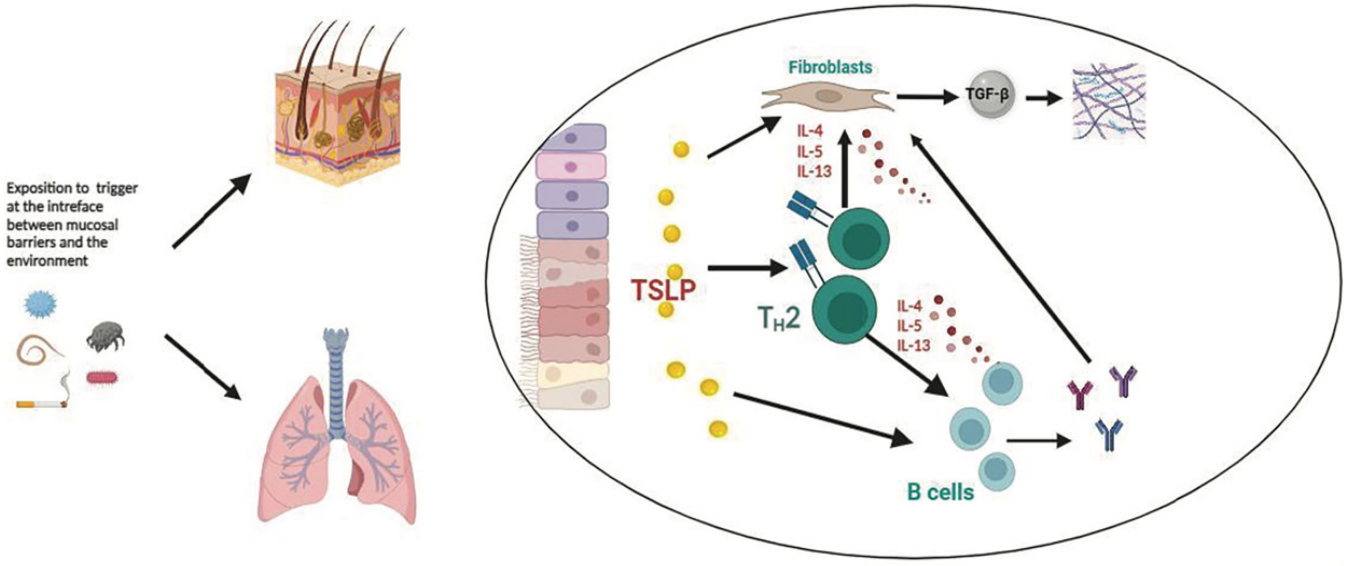

Fig. 1. Role of thymic stromal lymphopoietin (TSLP) in skin and lung fibrosis. TSLP is produced by airway epithelial cells after stimulation by allergens or infective agents. TSLP is involved in the induction and perpetuation of airway inflammation and it activates immune cells and inflammatory pathways related to type 2 and non-type 2 inflammation. TSLP activates inflammatory cells, promotes type 2 inflammation by increasing type 2 cytokine production (*i.e.* IL 4, IL 5, IL 13). TSLP has an important role in the airway remodelling, increasing transforming growth factor (TGF)-β and collagen production from fibroblasts and the growth of airway smooth muscle cells. Th2 cytokines active alternative inflammatory pathways and the transcription of TGF-β, involved in induction and progression of fibrosis.
TSLP: thymic stromal lymphopoietin; IL: interleukin; TGF-β: transforming growth factor β; Th2: T helper 2.

to assess disease activity and severity (21, 22). In all patients nailfold videocapillaroscopy (NVC) was performed according to Cutolo *et al*. (23).

The presence of type 2 inflammation was evaluated (24, 25). Type 2 inflammation was defined by the presence of peripheral eosinophils ≥150 cells/μl and the presence of at least a condition between immunoglobulin E (IgE) >100 KU/L or IL 5 > mean value of HC + 2 standard deviation (SD) or fraction of exhaled nitric oxide (FeNO) >25 parts per billion (PPB).

PFTs parameters [forced expiratory volume in the 1st second (FEV1), forced vital capacity (FVC)] and single-breath diffusing capacity of lung for carbon monoxide (DLco) were performed at the enrolment according to standards of American/European Respiratory Society (26).

Chest HRCT was performed at enrolment or in the previous 3 months of enrolment. A visual semi-quantitative staging system, tomographic fibrosis score (TFS), was used to assess SSc-ILD in our group of study. TFS is the sum of scores for ground glass opacity, reticulation, and honeycombing, valued at 6 anatomical levels: (i) the aortic arch, (ii) the carina, (iii) the pulmonary venous confluence, (iv) the middle of the third and fifth levels, (v) 1 cm above the dome of the right hemidiaphragm, and (vi) 2 cm below the dome of the right hemidiaphragm). Patients with more than 5% of lung parenchyma involved by fibrosis according to TFS score were considered affected by SSc-ILD (27). SSc patients have been divided into 4 distinct groups: group I, SSc patients without Th2 inflammation and TFS ≥5%; group II, SSc patients with type 2 inflammation and TFS ≥5%; group III, SSc patients with type 2 inflammation and TFS <5%; group IV, SSc patients without type 2 inflammation and TFS <5%.

Each patient enrolled in this study had at least 3 measurements of the FeNO. FeNO tests were executed by means of Bosch's handheld Vivatmo-me device (Bosch Healthcare Solutions, Waiblingen, Germany) for FeNO measurements. The Vivatmo-me is a handheld device utilising a single-use mouthpiece and a chemical field-effect transistor, which allows the measurement of FeNO in the range of 5–300 PPB with an accuracy of ± 5 PPB for values <50 PPB (28).

Laboratory assays

Serum levels of TSLP were performed on peripheral venous blood. Serum samples were collected in tubes and centrifuged at 3000 × g for 15 min at 19°C, allotted into 1.5 mL Eppendorf tubes and stored at -80°C until the time of assay. All specimens were measured using ELISA kits specific for human TSLP. The assay was performed according to the manufacturer's instructions.

Ethics

The study was conducted according the Declaration of Helsinki and was approved by the ethics committee of Sapienza University of Rome (IRB 0304).

Statistics

SPSS version 25.0 software (Bioz, Los Altos, CA) was used for statistical analysis. After an evaluation of normality by using the Shapiro-Wilk test, continuous variables were expressed as median and interquartile range (IQR) and categorical variables were expressed as absolute frequencies and percentages (%). Mann-Whitney U-test or Kruskal-Wallis test were performed to evaluate differences between groups. Differences between categorical variables were evaluated by the chi-square or Fisher's exact test, as appropriate. The 2-tails Pearson or Spearman correlation test

was used for bivariate correlations. Stepwise logistic regression analysis was used to evaluate the association between a dependent dichotomous variable (TFS≥5%) and continuous [TSLP (pg/ml), FVC (% of the predicted), DAI] or categorical (dcSSc) independent variables which were significant at the univariable analysis. Results were expressed as odds ratio (OR) and 95% confidence interval (95% CI). A *p*-value<0.05 was considered significant.

Results

Sixty-four (85.3%) patients were females. Median age was 57 years (IQR 48;67) and median diseases' duration was 14 years (IQR 8;20). Thirty-nine (52%) patients had dcSSc and 36 (48%) lcSSc. Median DAI was 2.84 (IQR 1.5;3.26). Twenty-four (32%) patients had a NVC early pattern, 10 (13.3%) had an active pattern and 41 (54.7%) had a late pattern. Thirty-five (46.7%) patients had a Scl70 antibody positivity and 18 (24%) had an anticentromere pattern. Demographic and clinical features of SSc patients are shown in Table I.

Forty-three (57.3%) patients had a type 2 inflammation profile: 46 (61.3%) patients had a value of circulating eosinophils ≥150 cells/μl, 15 (20%) patients had a value of serum IgE >100 KU/l. Thirty-eight (50.7%) patients had TFS ≥5%. These results are shown in Table II.

SSc patients had a statistically significant higher median value of TSLP than HC [171.33 pg/ml (IQR 136;218.67) *vs*. 88.21 pg/ml (IQR 82.87;93.23), *p*<0.001].

The median value of TSLP was similar (*p*>0.05) in female [171.33 pg/ml (IQR 136;214.67)] and in male [172.67 pg/ml (IQR 135.33;298.67)] SSc patients. We found a statistically significant positive correlation between TSLP and mRSS (r=0.409, *p*<0.001) (Fig. 2A). Moreover, TSLP serum levels were significantly higher in dcSSc patients compared to lcSSc patients [172.67 pg/ml (IQR 136;218.67) *vs*. 152.33 pg/ml (IQR 126;218) *p*<0.05] (Fig. 2B).

No significant (p>0.05) differences of serum TSLP were observed in SSc patients with Scl70 antibody [171.33 pg/ml (IQR 140.67;205.33)], SSc patients with anticentromeric pattern [217 pg/ml (IQR 133.33;272)], SSc patients with RNA pol III antibody [171.33 pg/ml (IQR 166.67;180), SSc patients with ANA [157 pg/ml (IQR 119.67;208] and in SSc patients with Th/To antibody [150 pg/ml (IQR 150;150)].

We did not find significant (*p*>0.05) differences of median serum level of TSLP between SSc patients with NVC late pattern [172 pg/ml (IQR 138;209.33)], SSc patients with early pattern [168 pg/ml (IQR 150;194.67)] and SSc patients with active pattern [170.33 pg/ml (IQR 132;265.66)]. We showed a statistically significant positive correlation between TSLP and DAI (r=0.374, *p*<0.001) (Fig. 2C). A statistically significant negative correlation was found between TSLP and FVC (r= -0.356, *p*<0.01) (Fig. 2D). The median value of TSLP was significantly higher in SSc patients with FVC ≤70% than SSc patients with FVC >70% [218 pg/ml (IQR 172; 386.67) *vs*. 170.33 (IQR 135.33;215.33) *p*<0.05]. We did not find any statistically significant correlation between TSLP and age (r=0.074;

Table I. Clinical and demographic features of systemic sclerosis (SSc) patients.

Age, years	57 (48;67)
F/M	64 (85.3) / 11 (14.7)
BMI, kg/m^2	23.11 (21.23;24.34)
Duration of disease, years	14 (8;20)
dcSSc/lcSSc	39 (52) / 36 (48)
DAI	2.84 (1.5;3.26)
DSS	4 (3;7)
mRSS	16 (9;23)
NVC	
early	24 (32)
active	10 (13.3)
late	41 (54.7)
Autoantibodies	
Scl70	35 (46.7)
ACA	18 (24)
ANA	16 (21.3)
RNApol III	5 (6.7)
Th/To	1 (1.3)
FVC, % of the predicted	91 (82;98)
DLco, % of the predicted	74 (67;88)

All results are reported as median and interquartile range (IQR) or absolute frequency and percentage (%).
SSc: systemic sclerosis; BMI: body mass index; dcSSc: diffuse cutaneous systemic sclerosis; lcSSc: limited cutaneous systemic sclerosis; mRSS: modified Rodnan skin score; DAI: disease activity index; DSS: disease severity scale; NVC: nailfold videocapillaroscopy; ANA: antinuclear antibodies; Scl70: anti-topoisomerase I antibodies; ACA: anticentromeric antibodies; RNApol III: antiRNA polymerase III antibodies; Th/To: anti Th/To antibodies; FVC: forced vital capacity; DLco: diffusing capacity of lung for carbon monoxide.

Table II. Laboratory and radiological features of systemic sclerosis (SSc) patients.

Serum IgE, KU/l	18 (6;81.8)
IgE >100 KU/l	15 (20)
IL 5, ng/ml	15.44 (11.74;18.23)
IL 5 > 9.67 pg/ml	36 (48)
Eosinophils, cells/μl	170 (100;250)
Eosinophils ≥ 150 cells/μl	46 (61.3)
FeNO, PPB	8 (6;12)
FeNO>25 PPB	3 (4)
Th2 inflammation	43 (57.3)
TFS ≥ 5%	38 (50.7)
Group I (TFS ≥ 5%, no Th2)	9 (12)
Group II (TFS ≥ 5% and Th2)	29 (38.7)
Group III (TFS < 5% and Th2)	14 (18.7)
Group IV (TFS < 5%, no Th2)	23 (30.7)

All results are reported as median and interquartile range (IQR) or absolute frequency and percentage (%).
SSc: systemic sclerosis; IgE: immunoglobulin E; FeNO: fraction exhaled nitric oxide; TFS: tomographic fibrotic score.

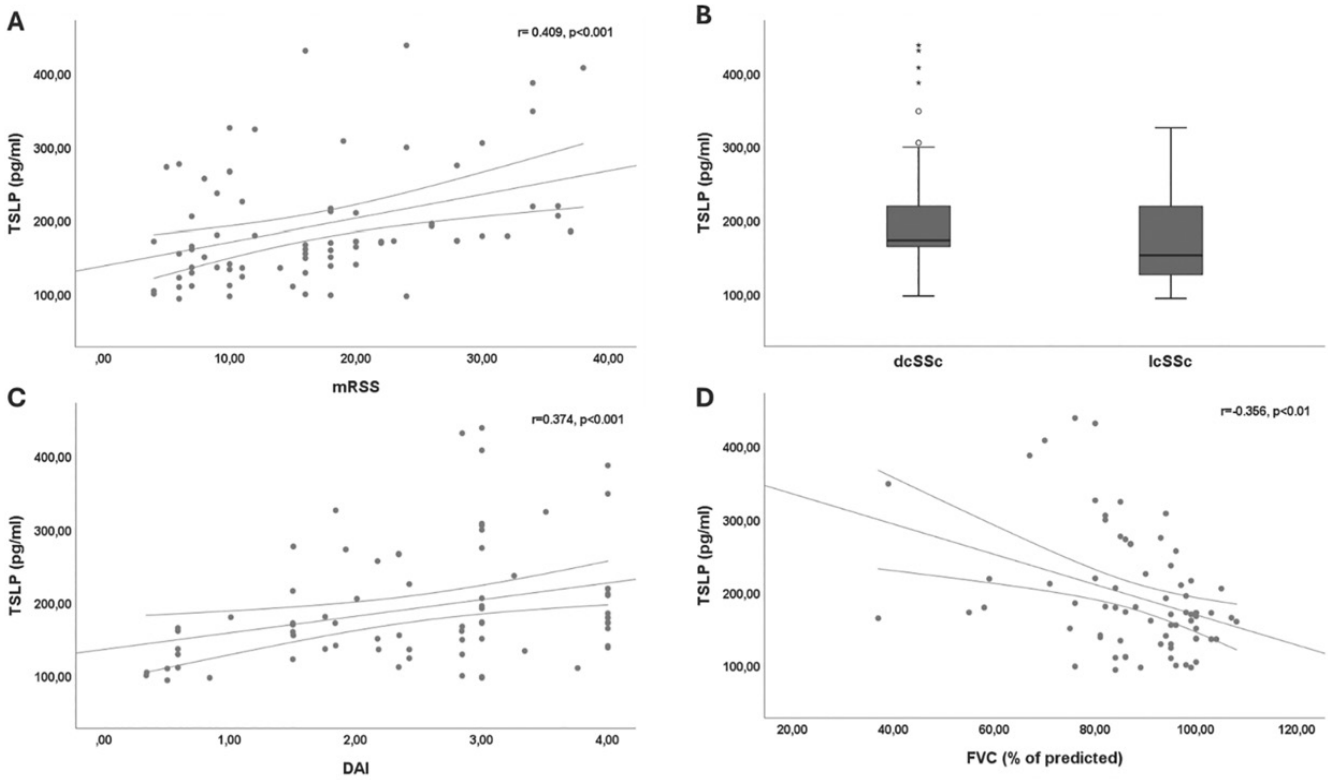

Fig. 2. Serum thymic stromal lymphopoietin (TSLP) in systemic sclerosis (SSc) patients.
A: Positive linear correlation between serum TSLP and modified Rodnan skin score (mRSS); **B**: Median serum TSLP in diffuse cutaneous SSc (dcSSc) patients and limited cutaneous SSc (lcSSc) patients; **C**: Positive linear correlation between TSLP and disease activity index (DAI); **D**: Negative linear correlation between TSLP and forced vital capacity (FVC).

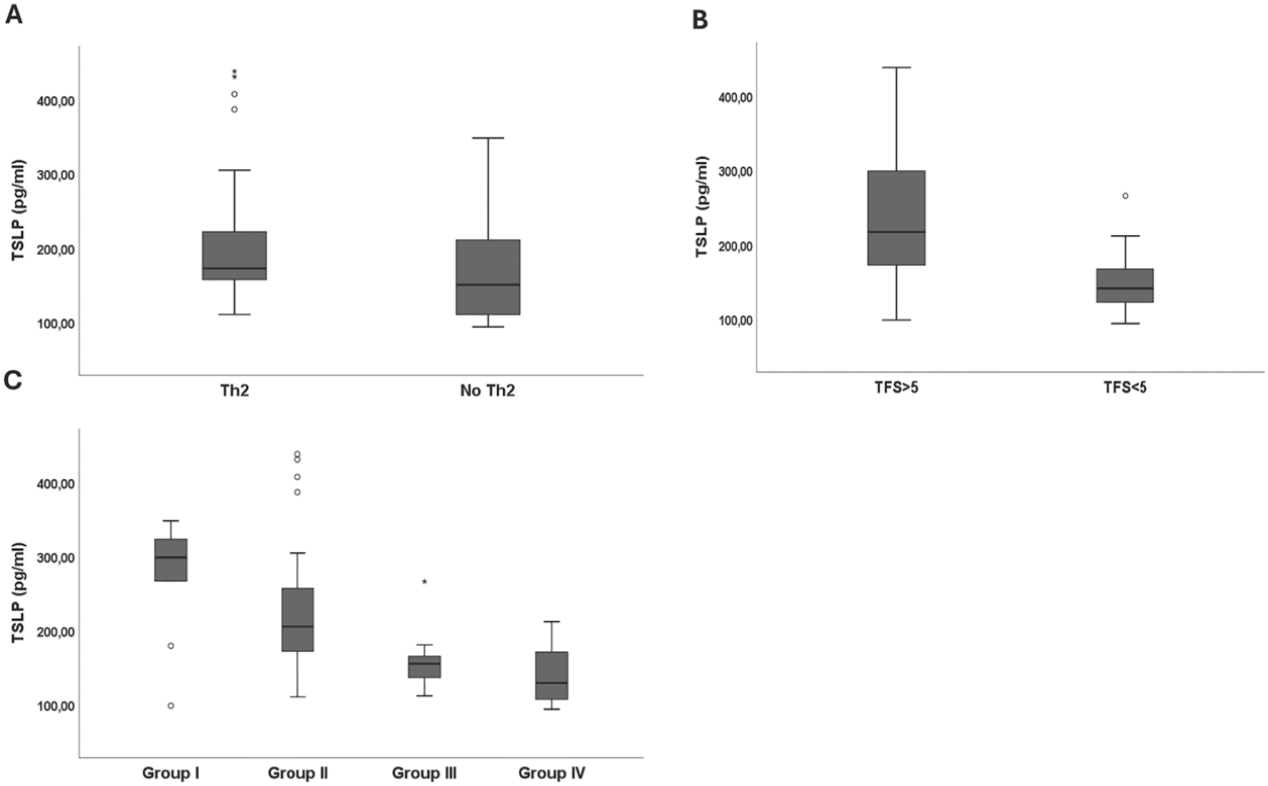

Fig. 3. Serum thymic stromal lymphopoietin (TSLP) in systemic sclerosis (SSc) patients.
A: Median serum TSLP in SSc patients with or without Th2 inflammation; **B**: Median serum TSLP in SSc patients with tomographic fibrotic score (TFS) ≥5% and SSc patients with TFS <5%; **C**: Median serum TSLP in group I, II, III e IV.

$p>0.05$), BMI (r=0.219; $p>0.05$), disease duration (r=0.067, $p>0.05$), DSS (r=0.118, $p>0.05$), DLco (r= -0.136, $p>0.05$) or FEV 1 (r=-0.102, $p>0.05$).

The median value of TSLP was significantly higher in SSc patients with type 2 inflammation than patients without type 2 inflammation [172 pg/ml (IQR 154.67;224.67) *vs*. 150 pg/ml (IQR 110;210.33), $p<0.05$] (Fig. 3A). We did not find significant ($p>0.05$) differences of DLco [73% (IQR 65;85) *vs*. 78.5% (IQR 71;91)] and FVC [91% (IQR 81; 99) *vs*. 91% (IQR 84; 97)] between SSc patients with type 2 inflammation and without type 2 inflammation. We did not find significant ($p>0.05$) differences of FEV1 between SSc patients with type 2 inflammation and without inflammation [87% (IQR 78;96) *vs*. 84% (IQR 77; 91)].

The median value of TSLP in SSc patients with TFS ≥5% was significantly higher than SSc patients withTFS <5% [216.67 pg/ml (IQR 172;298.67) *vs*. 140.67 pg/ml (IQR 122;166.67), $p<0.001$) (Fig. 3B). The median value of DLco was significantly lower in SSc with TFS ≥5% than SSc patients with TFS <5% [71.5% (IQR 59;82) *vs*. 77% (IQR 71;94) $p<0.01$]. The median value of FVC was significantly lower in SSc with TFS ≥5% than SSc patients withTFS <5% [84.5% (IQR 76;95) *vs*. 95% (IQR 87;100) $p<0.01$]. We did not find significant ($p>0.05$) differences of FEV1 between SSc with TFS ≥5% than SSc patients with TFS <5% [81.5% (IQR 76;93) *vs*. 88% (IQR 78;96)].

We did not find significant ($p>0.05$) differences of DLco between group I [71% (IQR 58; 82)], group II [72% (IQR 60; 80)], group III [75% (IQR67; 102)] group IV [87(IQR 72;91)]. The median value of FVC was significantly lower in group I than group III [82% (IQR 76;85) *vs*. 97.5% (IQR 87; 100), $p<0.01$], and group IV [82% (IQR 76;85) *vs*. 95 % (IQR 88;99), $p<0.001$]; we did not find a difference in FVC between group I and II [71% (IQR 58 ;82) *vs*. 86% (IQR 80;96), $p>0.05$].

The median value of FVC was significantly lower in group II than group III [86%(IQR 80;96) *vs*. 97.5% (IQR 87; 100), $p<0.05$]; we did not find significant ($p>0.05$) differences of FVC between group II and IV [86% (IQR 80;96) *vs*. 95% (IQR 88;99)]. We did not find significant ($p>0.05$) differences of FVC between group III and IV [97.5% (IQR 87; 100) *vs*. 95% (IQR 88;99)].

The median value of TSLP was significantly higher in group I than HC [298.67 pg/ml (IQR 266;323.33 *vs*. 88.21 pg/ml (IQR 82.87;93.23) $p<0.001$], in group II than HC [204.67 pg/ml (IQR 171.33;256 *vs*. 88.21 pg/ml (IQR 82.87;93.23) $p<0.001$], in group III vs HC [154.67 pg/ml (IQR 136;164.67) *vs*. 88.21 pg/ml (IQR 82.87;93.23) $p<0.001$] and in group IV than HC [128.67 pg/ml (IQR 104;171.33) *vs*. 88.21 pg/ml (IQR 82.87;93.23) $p<0.001$]. The median value of TSLP was significantly ($p<0.001$) higher in group I than group II, III and IV, in group II than group III and IV but was similar between group III and IV (Table III and Fig. 3C).

In the logistic regression analysis, only dcSSc [OR 15.496 (CI 95%: 2.338;102.687), $p<0.01$] and TSLP [OR 1.033 (CI 95%: 1.015;1.051), $p<0.001$] were variables associated with SSc-ILD (Table IV).

Table III. Comparative analysis of serum of thymic stromal lymphopoietin (TSLP) in systemic sclerosis (SSc) patients divided according to presence of type 2 inflammation and tomographic fibrotic score (TFS) into group I, SSc patients without type 2 inflammation and TFS ≥5%; group II, SSc patients with type 2 inflammation and TFS ≥5%; group III, SSc patients with type 2 inflammation and TFS <5%; group IV, SSc patients without type 2 inflammation and TFS <5%.

	Group I 298.67 pg/ml (IQR 266;323.33)	Group II 204.67 pg/ml (IQR 171.33;256)	Group III 154.67 pg/ml (IQR 136;164.67)	Group IV 128.67 pg/ml (IQR 104;171.33)
Group I 298.67 pg/ml (IQR 266;323.33)	-	$p<0.05$	$p<0.01$	$p<0.001$
Group II 204.67 pg/ml (IQR 171.33;256)	$p<0.05$	-	$p<0.001$	$p<0.001$
Group III 154.67 pg/ml (IQR 136;164.67)	$p<0.01$	$p<0.001$	-	$p>0.05$
Group IV 128.67 pg/ml (IQR 104;171.33)	$p<0.001$	$p<0.001$	$p>0.05$	-

Table IV. Logistic regression analysis showing the association between systemic sclerosis (SSc) associated interstitial lung disease (ILD) defined as tomographic fibrotic score (TSF) >5% and independent variables.

	TFS ≥ 5%	
	OR (CI 95%)	*p*
TSLP (pg/ml)	1.033 (1.015;1.051)	<0.001
FVC (% of predicted)	0.972 (0.900;1.051)	>0.05
dcSSc	15.496 (2.338;102.687)	<0.01
DAI	1.862 (0.762; 4.552)	>0.05

TFS: tomographic fibrotic score; TSLP: thymic stromal lymphopoietin; FVC: forced vital capacity; dcSSc: diffuse cutaneous systemic sclerosis; DAI: disease activity index; OR: odds ratio; CI 95%: 95% confidence interval.

Discussion

We demonstrated that serum level of TSLP is increased in SSc patients compared to HC and serum level of TSLP is increased in dcSSc patients and in SSc patients with ILD.

TSLP was found to be highly expressed by lung epithelial cells and epidermal keratinocytes and is recognised to be a critical mediator driving type 2 responses in the airways and skin at the interface between mucosal barriers

and the environment (29). In our study, we demonstrated a significant positive linear correlation between TSLP and mRSS. Serum TSLP levels are higher in dcSSc patients than lcSSc patients. Previous studies have shown that TSLP plays a role in the development of skin fibrosis in SSc patients (16, 17). TSLP was overexpressed by epithelial cells, mast cells and fibroblasts in human SSc skin and in the bleomycin-induced scleroderma in TSLPR deficient mice model of scleroderma. TSLP expression was inducible by activation of TLR, particularly TLR3. In TSLPR-deficient mice, bleomycin-induced fibrosis was significantly reduced in parallel with significantly reduced local expression of IL 13 (17). Previous studies have demonstrated that TSLP is overexpressed in the skin of dcSSc patients. Subcutaneous stimulation of skin by TSLP activates Smad2 phosphorylation and leads to alterations in gene expression that overlap significantly with gene expression induced by TGF-β and IL 13 (16). Our data confirm the previous results of other studies, which have shown a pro-fibrotic effect of TSLP on the skin of patients with SSc.

We found an increased serum TSLP in SSc patients with extension of ILD ≥ 5% at TFS score and a negative linear correlation between serum TSLP and FVC. In idiopathic pulmonary fibrosis (IPF), TSLP plays a key role. The epithelium derived triple cytokines (IL 25/IL 33/TSLP) significantly contribute to allergic nasal/airway inflammation and pulmonary fibrosis. Alveolar epithelial cells (AECs) can respond to a variety of pathogen-associated molecular patterns by their constitutively expressed toll-like receptors. When AECs are activated, they can produce IL 25/IL 33/TSLP (29). Datta *et al*. showed that the TSLP/TSLPR axis was remarkably upregulated in patients with IPF, especially in AECs and myofibroblasts. In IPF biopsy material, the authors demonstrated that type 2 polarising cytokines play a role in the pathogenesis of non-allergic diseases characterised by a type 2 phenotype and organ fibrosis. TLSP was highly expressed also in bronchoalveolar lavage fluids of IPF patients and it has been demonstrated an overexpression of TSLP and TSLPR in human IPF lung biopsy material. Lung fibroblasts represent both a novel cellular source and target of TSLP, inducing CCL2 release and subsequent monocyte chemotaxis (29, 31). Although there is no data in scleroderma ILD but only on skin fibrosis, we can suppose that TSLP may play a role in the pathogenesis of SSc ILD.

TSLP plays a key role in the pathogenesis of Th2 diseases (10-12). Allergens, viruses, bacteria, pollutants or environmental stimuli can promote loss of integrity of the airway epithelium, causing the release of the alarmin cytokines (IL 25, IL 33 and TSLP), promoting an increased production of Th2 cells, eosinophils, mast cells, basophils, and ILC2s (32). In this study, serum levels of TSLP are increased in SSc patients with ILD with and without Th2 inflammation, compared to SSc patients without ILD with and without type 2 inflammation. Serum TSLP levels were higher in SSc patients with ILD without type 2 inflammation than in SSc patients with ILD and type 2 inflammation. In SSc patients without ILD, serum TSLP levels are still higher in patients with type 2 inflammation compared to SSc patients without ILD and without type 2 inflammation. Pellicano *et al*. demonstrated that Th2 cytokines serum levels were increased in SSc patients compared to HC (8), Th2 cytokines are increased in the early phase of SSc-ILD. The authors demonstrated a positive linear correlation between serum Th2 cytokines and the extent of ground glass pattern assessed by CALIPER software in SSc patients. IL 4 was associated with DLco ≤60% of the predicted and with fibrotic involvement of≥10% of lung parenchyma in SSc patients (8). In SSc ILD pathogenesis, the role of type 2 inflammation is misunderstood and has long been debated (33). Th2 cytokines, released in the early inflammatory phase of SSc-ILD, lead to the activation of alternative inflammatory pathways and to the transcription of TGF-β, involved in induction and progression of fibrosis (34). IL 4 and IL 13 have gained attention for their important roles in tissue repair and fibrosis. IL 4 is a major profibrotic cytokine that stimulates collagen synthesis by fibroblasts also in human dermal fibroblasts isolated from SSc patients (35). B cells are overactivated and promote fibrosis by autoantibodies that activate fibroblasts or inhibit the degradation of the extracellular matrix (36). We can suppose that TSLP activates both type 2 inflammation and fibroblast. In early phase, IL 4 promotes inflammation with the development of ground glass. Subsequently, type 2 inflammation cytokines activate the transcription of TGF-β with induction and progression of fibrosis (7, 33). In the late phase, TSLP acts directly on the fibroblast and induces Th2 cytokines that activate TGF-β and this causes the development of fibrosis. We can believe that TSLP has a predominant role in inducing fibrosis while Th2 cytokines are responsible for the initial phase of SSc-ILD.

Monocentric design and the small sample size are the main limitations of this study. In addition, the serological data of the TSLP were not confirmed in biopsy material and/or bronchoalveolar lavage fluids. Finally, the selection of patients, excluding patients undergoing immunosuppressants treatment, and the lack of longitudinal data are additional limitations of this study. Further longitudinal and multicentric studies are necessary to confirm and deepen the results of our study.

We can conclude that the serum level of TSLP was higher in SSc patients than HC. A positive correlation between TSLP and mRSS was observed. The median value of serum TSLP was significantly higher in SSc patients with TFS ≥5%. TSLP might have a key role in ILD and skin fibrosis. Future large studies are needed to confirm these findings.

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