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Elevated serum levels of interleukin-1β and interleukin-33 in patients with systemic sclerosis in Chinese population

Introduction

Systemic sclerosis (SSc) is a connective tissue disease which is characterized by the deposition of excess collagen in skin and multiple internal organs [1]. Symptoms of this disease include diffuse vasculopathy, immune activation, low-grade inflammation and subsequent tissue fibrosis [2]. Although the pathogenesis of the disease remains incompletely understood, some studies suggested that fibrotic process and vascular damage which were key processes in onset of SSc might be affected by abnormal expression of cytokines [3, 4]. Among these cytokines, we mainly focus on interleukin (IL)-1α, IL-1β, IL-18 and IL-33 which belong to the IL-1 family in this study. IL-1 family members, whose biological properties are typically pro-inflammatory and pro-fibrosis, are the key regulators of inflammatory response [5]. The associations between SSc and IL-1 family have been probed over years.

Previous studies [6, 7] revealed that elevated expression of IL-1α could upregulate proliferation and collagen production in SSc fibroblasts. Moreover, it also could induce production of IL-6, platelet-derived growth factor A and pro-collagen type I protein. Through these processes, IL-1α could eventually contribute to the fibrosis process of SSc. Further investigation suggested that pre-IL-1α could modulate proliferation and collagen expression of SSc fibroblasts through binding necdin (a nuclear protein with growth suppressor activity) [8].

In terms of IL-1β, which is mainly released by macrophages, it can exert inflammatory effects on fibroblasts and the endothelium [4]. In early study, Kawaquchi et al. [9] reported that IL-1β could induce IL-6 and prostaglandin E2 both in SSc fibroblasts and normal fibroblasts, and SSc fibroblasts are more highly sensitive to IL-1β than normal fibroblasts. There was also a study reporting that IL-1β could promote differentiation of T helper cell 17 (Th17) in aged mice, which may contribute to the development of Th17-mediated autoimmune diseases [10]. However, most findings were limited to cell culture experiments while studies on association of serum levels of IL-1α and IL-1β with SSc are seldom reported.

IL-33, which belongs to IL-1β superfamily, is regarded as an early inflammatory mediator targeting Th2 cells and inducing IL-13 production. It has been indicated that IL-33 binds a heteromeric receptor which consisted of two subunits: the orphan IL-1 receptor ST2, which was highly expressed on Th2 polarized cells and mast cells, and IL-1 receptor accessory protein [11]. Recent studies showed that IL-33 plays a role as chemoattractant for Th2 cells and induces Th2 cell differentiation. It also leads to production of IL-4, IL-5 and IL-13 in the fibrotic stages of SSc [11, 12]. Yanaba et al. included 69 patients and 30 healthy subjects in a case–control study and found serum IL-33 level was elevated in SSc patients. They also found the expression of IL-33 was correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis [13]. In addition, some case–control studies suggested that serum IL-33 levels might be associated with early stage of disease and microvascular involvement in SSc cases [14, 15].

IL-18 is an IFN-γ inducing factor and mainly produced by activated macrophage-lineage cells (represented by Kupffer cells). Some studies revealed that IL-18 is associated with autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus [16, 17]. However, its role in the development of SSc is still controversial. Recently, Artlett et al. [18] found IL-18 and IL-1β secreted by fibroblasts were elevated in SSc patients. In addition, they also found the secretion of IL-18, IL-1β and collagens were reduced when caspase-1 was inhibited. Later, in another study, Artlett et al. concluded that IL-18 or IL-1β mediated by NLRP3 inflammasome could promote myofibroblast differentiation and collagen deposition, which may lead to fibrosis [19]. However, Kim

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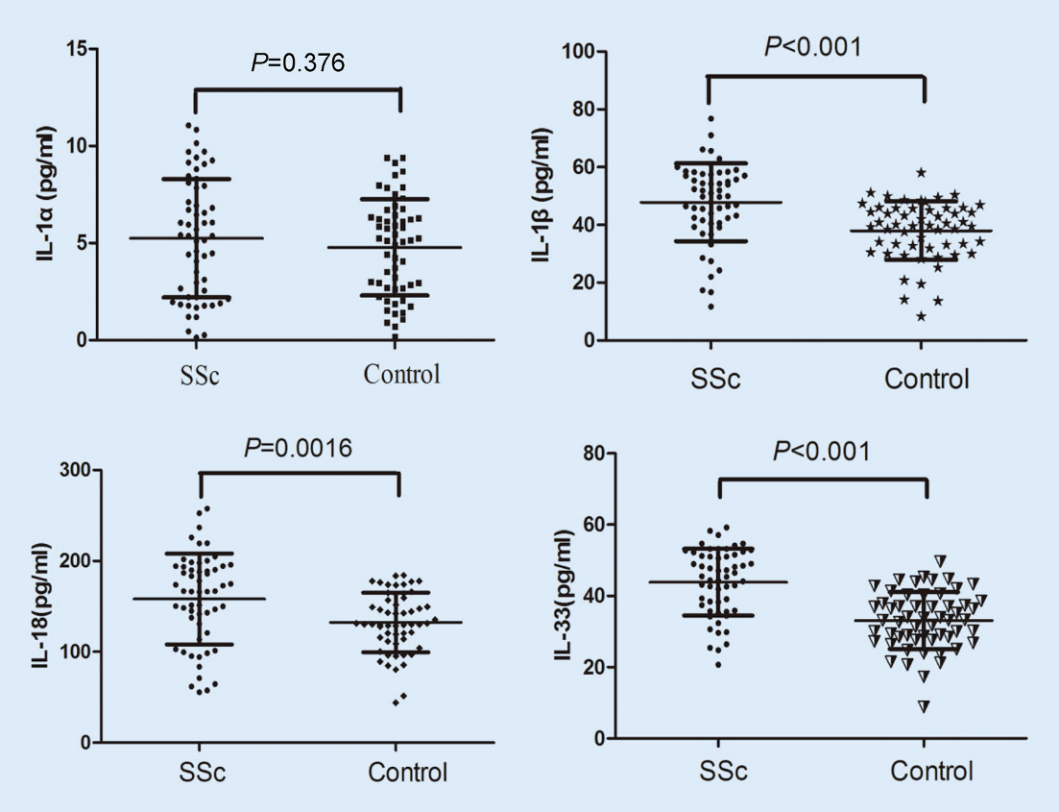

Fig. 1 ◀ Comparisons of IL-1α, IL-1β, IL-18 and IL-33 expression levels in peripheral blood of systemic sclerosis (*SSc*) patients and healthy controls

et al. [20] showed that IL-18 could downregulate collagen production in human dermal fibroblasts directly via the E26 transformation-specific-1 and the ERK pathway, which suggested IL-18 might exert antifibrotic activities in dermal fibroblasts in scleroderma patients. These controversial results drive us to conduct further studies to reveal the role of IL-18 in SSc.

As shown above, IL-1α, IL-1β, IL-18 and IL-33 have important influences on the development of SSc. However, there is little relevant research and the findings are less consistent. Studies about serum levels of these cytokines in SSc patients in Chinese population have not been reported. In this case, this study aimed to reveal serum levels of interleukin (IL)-1α, IL-1β, IL-18 and IL-33 and their associations with clinical manifestations in SSc patients.

Materials and methods

Study participants

From August 2012 to November 2014, 56 independent SSc patients (8 male/48 female; age 48.48 ± 12.82 years) were recruited from Anhui Provincial Hospital. Every patient was diagnosed by the clinicians from the department of rheumatism and immunology according to the 1980 revision of the American College of Rheumatology SSc criteria [21]. None of the SSc patients were receiving any treatment when we collected blood samples. During this period, a group of 56 healthy subjects (8 male/48 female; age 52.38 ± 10.57 years) were selected as normal controls. These controls met the following requirements: (1) They did not meet any item of the diagnostic criteria of SSc; (2) They as well as their direct relatives do not have the history of autoimmune diseases; (3) They were in good physical shape in the past month; (4) They have not used hormone and immunosuppressive drugs. This study was approved by the ethics committee of Anhui Medical University and written informed consent was obtained from subjects before the present study.

Study variables

Data of demography (age, gender, body mass index), clinical, laboratory and lifestyle were collected from hospital records or specific questionnaires and reviewed by experienced physicians. Body mass index (BMI) was defined as thin (BMI < 18.5 kg/m^2), normal (BMI: 18.5–23.9 kg/m^2) and overweight (BMI ≥ 24 kg/m^2).

The items of lifestyle were comprised of smoking, drinking and cooking oil in diet. Smokers were defined as those who had smoked during the last month and had smoked ≥1 cigarettes per day for longer than a year [22]. Drinkers were those who had drank ≥50 g per month during the last three months [23]. Cooking oil was divided into two categories: animal oil and vegetable oil.

Clinical features of SSc patients, such as limitation of mouth opening, dyspnea, Raynaud's phenomenon (RP), joint involvement were recorded. Joint involvement was defined as swelling and/or tenderness of joints. Limitation of mouth opening was defined as the space between upper incisor and lower incisor

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Elevated serum levels of interleukin-1β and interleukin-33 in patients with systemic sclerosis in Chinese population

Abstract

Purpose of the study. Systemic sclerosis (SSc) is a multisystem autoimmune disease. Although the pathogenesis of the disease remains incompletely understood, some cytokines or growth factors which regulate SSc induction may be involved in the injury of endothelial cells and the modulation of leukocyte function. We aimed to perform this case–control study to determine serum levels of interleukin (IL)-1α, IL-1β, IL-18 and IL-33 and their associations with clinical manifestations in SSc patients.

Materials and methods. There were 56 patients with SSc and 56 healthy individuals who were recruited from local hospital between 2012 and 2014. Serum IL-1α, IL-1β, IL-18 and IL-33 levels were measured with specific enzyme-linked immunosorbent assay kits.

Results. Univariate analysis revealed that serum IL-1β, IL-18 and IL-33 levels in SSc patients were significantly higher than that in healthy controls. After adjusting possible confounding factors (sex, age, smoking and drinking) by multivariable analyses, serum IL-1β levels (OR = 1.082; 95 % CI: 1.013–1.155) and serum IL-33 levels (OR = 1.100; 95 %CI: 1.022–1.185) were still related factors. There were interrelationships among the serum levels of IL-1α, IL-1β, IL-18 and IL-33 and these associations were not consistent in SSc patients and controls. No associations of serum IL-1α, IL-1β, IL-18 and IL-33 levels with clinical parameters were found.

Conclusion. IL-1β and IL-33 may contribute to the development of SSc. While there were no direct associations between these cytokines and disease manifestations, they still could be considered as serum markers of development of SSc. Further studies are required to validate this incipient data.

Keywords

Systemic sclerosis · Autoimmunity · Cytokines · Interleukin 1 · Inflammatory response

Erhöhte Interleukin-1β- und Interleukin-33-Serumwerte bei Patienten mit systemischer Sklerose in einer chinesischen Population

Zusammenfassung

Zweck der Studie. Die systemische Sklerose (SSc) ist eine multisystemische Autoimmunerkrankung. Obwohl die Pathogenese der Erkrankung nicht vollständig verstanden ist, könnten einige Zytokine oder Wachstumsfaktoren, welche die Induktion der SSc regulieren, an der Verletzung von Endothelzellen und der Modulation der Leukozytenfunktion beteiligt sein. Das Ziel dieser Fall-Kontroll-Studie war es, die Serumwerte von Interleukin(IL)-1α, IL-1β, IL-18 und IL-33 sowie deren Assoziation mit den Krankheitsbildern von SSc-Patienten zu bestimmen.

Material und Methoden. Es wurden 56 Patienten mit SSc und 56 gesunde Personen eingeschlossen, die zwischen 2012 und 2014 von einem lokalen Krankenhaus rekrutiert worden waren. Die IL-1α-, IL-1β-, IL-18- und IL-33-Serumwerte wurden mit speziellen ELISA-Kits („enzyme-linked immunosorbent assay") gemessen.

Ergebnisse. Eine univariate Analyse zeigte, dass die IL-1β-, IL-18- und IL-33-Serumwerte bei SSc-Patienten signifikant höher waren als die der gesunden Kontrollpersonen. Auch nach Anpassung möglicher Confounding-Faktoren (Geschlecht, Alter, Rauchen und Trinken) mittels multivariabler Analysen stellten die IL-1β-Werte (OR = 1,082; 95 % CI 1,013–1,155) und die IL-33-Werte (OR = 1,100; 95 % CI 1,022–1,185) noch zusammenhängende Faktoren dar. Es bestanden Wechselbeziehungen zwischen den IL-1α-, IL-1β-, IL-18- und IL-33-Serumwerten, die jedoch bei den SSc-Patienten und den gesunden Kontrollpersonen inkonsistent waren. Assoziationen zwischen IL-1α-, IL-1β-, IL-18- und IL-33-Serumwerten und klinischen Parametern wurden nicht gefunden.

Schlussfolgerung. IL-1β und IL-33 könnten zu der Entwicklung einer SSc beitragen. Während es keine direkten Assoziationen zwischen diesen Zytokinen und den Krankheitsbildern gab, könnten sie dennoch als Serummarker für die Entwicklung einer SSc in Betracht gezogen werden. Um diese ersten Daten zu validieren, bedarf es weiterer Studien.

Schlüsselwörter

Systemische Sklerose · Autoimmunität · Zytokine · Interleukin 1 · Inflammatorische Antwort

can not accommodate the horizontally erected index finger, middle finger and ring finger. Dyspnoea was defined as a subjective experience of breathing discomfort. RP was defined as history of sudden onset of cold fingers (or toes) in association with sharply demarcated colour changes of skin pallor (white attack) and/or cyanotic skin (blue attack), followed by erythema upon warming and reperfusion [24].

The laboratory data included elevated erythrocyte sedimentation rate (male: >15 mm/h; female: >20 mm/h); the presence of antinuclear antibody, anti-Scl-70 antibody, anti-SSA antibody (by indirect immunofluorescence) were also reviewed.

Measurement of serum IL-1α, IL-1β, IL-18 and IL-33 levels

Serum was obtained from 5 ml whole blood. The whole blood from SSc patients and controls was collected in the tube without anticoagulant, separated by centrifugation (3000 rpm for 10 min), extracted into Eppendorf tube and stored at −80 °C for subsequent analysis.

Serum IL-1α, IL-1β, IL-18 and IL-33 levels were measured with specific

Table 1 Univariate and multivariate binary logistic regression assessing the relationship between potential factors and systemic sclerosis

Group	Crude OR (95 % CI)	*P*	Adjusted OR (95 % CI)	*P*
IL-1α	1.037 (0.907–1.186)	0.592	0.815 (0.638–1.042)	0.103
IL-1β	1.075 (1.035–1.116)	0.000	1.082 (1.008–1.161)	0.029
IL-18	1.015 (1.005–1.025)	0.002	1.013 (0.997–1.130)	0.121
IL-33	1.136 (1.078–1.197)	0.000	1.114 (1.023–1.212)	0.012
Sex (female vs. male)	0.767 (0.278–2.113)	0.607	1.807 (0.309–10.573)	0.512
Age				
24–44 age	1	–	1	–
45–59 age	0.755 (0.323–1.766)	0.517	1.739 (0.390–7.746)	0.468
60–78 age	0.842 (0.289–2.450)	0.752	1.666 (0.301–9.232)	0.559
Vitamin D deficiency	4.920 (2.199–11.008)	0.000	6.176 (1.823–20.923)	0.003
Smoking	1.306 (0.483–3.529)	0.599	2.065 (0.341–13.605)	0.451
Drinking	0.870 (0.342–2.212)	0.771	0.759 (0.153–3.753)	0.735
Cooking oil (vegetable oil vs. animal oil)	2.688 (0.657–11.000)	0.169	6.231 (0.947–40.987)	0.057
BMI				
Normal	1	–	1	–
Thin	2.194 (0.969–6.917)	0.180	3.272 (0.609–17.569)	0.167
Overweight	0.443 (0.171–1.094)	0.077	0.556 (0.138–2.246)	0.410

VD vitamin D, *BMI* body mass index, *OR* odds ratio

enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's guidance. The lower detection limits of each of these assays were as follows: IL-1α: 0.1 pg/ml; IL-1β: 1.0 pg/ml; IL-18: 1.0 pg/ml; IL-33: 1.0 pg/ml. Briefly, standards and specimen were attached to the microporous plate which contain antibodies to IL-1α (IL-1β, IL-18 or IL-33). Then, sample diluent should be added, and finally, the detected antibodies marked by horseradish peroxidase were added. Color was developed with 3,3',5,5'-tetramethylbenzidine substrate and the absorbance at 450 nm was measured. The concentration of IL-1α (IL-1β, IL-18 or IL-33) in each sample was determined by interpolation from a standard curve. The results were expressed as picograms per milliliter (pg/ml).

Statistical analysis

The data were analyzed with SPSS version 10.01 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± standard deviation or median (interquartile range) if they were not in normal distribution. Mann–Whitney U test was used to compare serum concentration among different groups. Simple binary logistic regressions were used to examine the independent roles of cytokines (IL-1α, IL-1β, IL-18 and IL-33) and multivariate binary logistic regression was then used to adjust potential risk factors of SSc (full model). The relationship between two continuous variables was estimated by Spearman's rank correlation. *P* values ≤0.05 on the two-side tests were considered statistically significant.

Results

Difference in serum levels of IL-1 four members between SSc cases and healthy controls

There were 56 patients with SSc (8 male/48 female; age range 24–78 years) and 56 healthy control subjects (8 male/48 female; age range 25–76 years) included in this study. The serum levels of IL-1a, IL-1β, IL-18 and IL-33 in SSc patients and healthy controls were shown in **Fig. 1**.

The results showed that serum IL-1β levels in SSc patients were significantly higher than that in healthy controls (49.88 [40.89, 57.60] pg/ml vs. 39.64 [32.14, 45.77] pg/ml, $P < 0.001$); serum IL-18 levels were significantly higher in SSc patients (166.86 [123.43, 194.13] pg/ml) than in healthy controls (130.31 [112.24, 158.81] pg/ml, $P < 0.001$); serum IL-33 levels in these two groups were significantly different (45.42 [35.73, 51.45] pg/ml vs. 33.60 [27.87, 39.86] pg/ml, $P < 0.001$). While serum IL-1α levels in patients with SSc and healthy individuals were 5.26 (2.20, 7.67) pg/ml and 5.20 (2.69, 6.65) pg/ml, respectively, the difference was not statistically significant ($Z = 0.889$, $P = 0.699$).

Univariate and multivariate logistic regression of the onset of SSc with the serum levels of IL-1 four members

Univariate binary logistic regression was used to analyze the influence of serum levels of IL-1 four members on SSc (**Table 1**). Besides, in order to control potential confounding factors (sex, age, smoking, drinking, cooking oil and BMI), we also performed multivariate binary logistic regression. The results showed that after adjusting for confounders, the association between serum IL-1β levels and SSc as well as the association between serum IL-33 levels and SSc were still significant (IL-1β: OR = 1.082, 95 % CI: 1.013–1.155; IL-33: OR = 1.100; 95 % CI: 1.022–1.185). However, the association of serum IL-18 levels with the onset of SSc was meaningless after adjusting potential confounding factors (**Table 1**).

The interrelationships among the serum levels of IL-1α, IL-1β, IL-18 and IL-33

Spearman's correlation was used for correlation analysis between different cytokines. The results, shown in **Table 2**, revealed that relationships between IL-1β and IL-18 (Control: $r_s = 0.504$, $P < 0.001$; Case: $r_s = 0.267$, $P = 0.046$), IL-1β and IL-33 (Control: $r_s = 0.554$, $P < 0.001$; Case: $r_s = 0.357$, $P = 0.007$), IL-18 and IL-33 (Control: $r_s = 0.405$, $P = 0.002$; Case: $r_s = 0.372$, $P = 0.005$) were significant between SSc cases and healthy individu-

Table 2 The correlation of different cytokines

		IL-1α		IL-1β		IL-18	
		Case	Control	Case	Control	Case	Control
IL-1β	*rs*	0.301	0.199	–	–	–	–
	P	0.024	0.142	–	–	–	–
IL-18	*rs*	0.139	0.293	0.333	0.519	–	–
	P	0.306	0.029	0.012	0.000	–	–
IL-33	*rs*	0.440	0.200	0.395	0.628	0.330	0.449
	P	0.001	0.140	0.003	0.000	0.013	0.001

rs Spearman's rank correlation coefficient

als. In addition, the associations between IL-1α and IL-1β, IL-1α and IL-33 were significant among patients with SSc; the association between IL-18 and IL-1α was significant among healthy controls.

Correlation of serum cytokines levels with clinical and laboratory data in SSc patients

The association of serum IL-1α, IL-1β, IL-18 and IL-33 levels with the clinical and laboratory features in SSc patients are shown in **Table 3**. The clinical and laboratory data were compared between SSc patients with different features, but no correlation was found.

Discussion

This is the first study to explore associations among serum levels of IL-1α, IL-1β, IL-18 and IL-33 with disease manifestations in SSc patients in Chinese population. Among 4 interleukins included in this study, serum levels of IL-1β and IL-33 showed significant differences between healthy controls and SSc cases while others were not found different after adjustment for confounders. Each of them is discussed in detail in the following text.

Our findings about IL-1α were consistent with the previous study performed by Needleman et al. [25]; we found that the expressions of IL-1α in SSc patients and healthy controls were of equal frequency. However, in a recent Japanese study, Maekwa et al. [26] reported that there was significant difference in serum IL-1α levels between 66 SSc patients and 19 healthy controls. This was the first report of elevated serum IL-1α levels in SSc patients, but similar to our results there was no statistically significant difference in incidence of most clinical or laboratory features (including RP and joint involvement) between patients and healthy controls. Although IL-1 could stimulate the expression of a range of profibrotic growth factors and cytokines both in vitro and vivo, the direct evidence about the effect of IL-1 on SSc was still controversial and lacking [26]. The contradiction about IL-1α between previous study and our results may partly be caused by the reason that population groups from different countries were distinct. The observed discrepancies may also result from differences in the treatment (whether and how many treated with immunosuppressive agents) or the dissimilarities in disease duration. In addition, the limited sample size in this study might also result in inconsistent results.

In 2005, Hussein et al. [4] indicated that IL-1β concentrations in the serum and the bronchoalveolar lavage fluid in SSc patients were higher than that in healthy control subjects, which was consistent with our results. Several studies have indicated increased expression of IL-1β in fibroblasts, cultured peripheral blood mononuclear cells and blood T lymphocytes among SSc patients [9, 27, 28]. Furthermore, with the development of biotechnology, anti-cytokine therapy has been studied for the treatment of autoimmune and inflammatory diseases. Anakinra, rilonacept and canakinumab, which reduce the activity of IL-1α and/or IL-1β through blocking the IL-1 receptor, can inhibit the inflammatory reaction effectively [29]. The latest study pointed that boswellic acids could reduce Th17 differentiation via blockade of IL-1β-mediated IRAK1 signalling. This was beneficial for reducing the response of inflammation and immunization [30]. But IL-1β was not detected in any sera from 78 SSc patients in Needleman et al. study [25], and the associations between serum IL-1β levels and disease manifestations in our study were not significant. Research on IL-1β is required to be performed on a more general level.

Our study showed that serum IL-18 levels in SSc patients were increased when compared with healthy subjects, but the difference was not statistically significant. Naturally, our results were opposite to the previous study which reported that serum IL-18 levels were significantly associated with the risk and clinical grades of SSc [31]. The role of IL-18 in the development of SSc has aroused a heated debate in recent years. In 2011, Pan et al. [32] reviewed relevant studies and suggested that IL-18 might function both as a profibrotic and antifibrotic mediator of fibrosis based on the induction of Th1 or Th2 cytokine profiles. Another review concluded that in autoimmune diseases, pleiotropic effects of IL-18 (especially the induction of interferon γ) is essential for immunity against invading pathogens, but improper regulation of IL-18 can potentially lead to inflammation and self-destruction [33]. A recent study suggested that the inhibition of IL-18 signalling by IL-18 binding protein isoform a (IL-18 BPa is a soluble decoy receptor for IL-18) may be involved in the development of pulmonary vascular involvement which leads to pulmonary hypertension and modulate the systemic inflammation in SSc [34]. However, these results have not reached the consistent conclusion.

In our study, univariate and multivariate analyses both confirmed that serum IL-33 levels in SSc cases were significantly higher than that in control group. This result was consistent with Japanese, German and Italian studies [11, 13–15]. IL-33 is a new member of the IL-1 family; it signals via the IL-1 receptor-related protein ST2 and could induce Th2-associated cytokines [35]. It is likely to be involved in the development of SSc by contributing to the Th2 lymphocyte infiltration and promote Th2 cytokine production [13]. On the other hand, it was sug-

Table 3 Correlation of serum cytokine levels with clinical features and laboratory data in patients with systemic sclerosis

Group	IL-1α				IL-1β			IL-18			IL-33		
	N	Serum level	Z	*P*	Serum level	Z	*P*	Serum level	Z	*P*	Serum level	Z	*P*
Involvement of joints													
Yes	18	5.86 (3.36, 7.53)	−0.868	0.385	53.09 (42.41, 57.76)	−0.912	0.362	180.69 (135.79, 208.50)	−1.404	0.160	49.17 (43.08, 52.64)	−1.412	0.158
No	38	5.12 (1.88, 7.98)	–	–	47.32 (40.18, 57.17)	–	–	151.41 (116.45, 191.30)	–	–	43.07 (35.66, 49.65)	–	–
Dyspnoea													
Yes	30	5.24 (2.05, 7.16)	−1.010	0.312	47.32 (41.94, 54.89)	−1.084	0.278	176.47 (138.97, 193.83)	−0.739	0.460	44.95 (35.83, 52.28)	−0.181	0.857
No	26	5.82 (2.46, 8.65)	–	–	54.29 (40.31, 58.38)	–	–	158.09 (102.47, 195.39)	–	–	45.94 (36.86, 50.73)	–	–
RP													
Yes	32	4.84 (2.32–7.03)	−0.695	0.487	48.76 (40.89–54.80)	−1.275	0.202	166.55 (119.72–193.17)	−0.331	0.741	44.85 (35.27–51.05)	−0.480	0.631
No	24	5.60 (2.14–8.21)	–	–	54.10 (41.80–58.31)	–	–	169.65 (123.43–194.23)	–	–	46.81 (39.16–52.05)	–	–
Limitation of mouth opening													
Yes	30	4.75 (2.13, 7.22)	−0.657	0.511	50.35 (41.36, 57.71)	−0.049	0.961	151.41 (111.02, 194.16)	−0.789	0.430	43.63 (34.98, 48.99)	−1.643	0.100
No	26	5.55 (2.18, 8.03)	–	–	49.86 (40.13, 57.17)	–	–	169.96 (128.31, 194.33)	–	–	48.75 (38.37, 52.89)	–	–
ESR													
Elevated	27	5.10 (1.95, 8.10)	−0.377	0.706	49.77 (36.97, 55.68)	−0.762	0.446	151.41 (102.84, 188.13)	−1.107	0.268	42.71 (34.44, 51.21)	−1.238	0.216
Normal	29	5.69 (2.21, 7.90)	–	–	50.93 (42.39, 57.57)	–	–	174.90 (132.05, 196.80)	–	–	47.12 (42.25, 52.34)	–	–
ANA													
Positive	51	5.37 (2.21, 7.94)	−0.934	0.350	49.99 (42.34, 57.50)	−0.733	0.464	166.94 (130.76, 194.30)	−1.250	0.211	46.51 (37.43, 51.54)	−1.034	0.301
Negative	5	4.33 (1.04, 6.90)	–	–	40.65 (24.97, 65.56)	–	–	143.14 (79.54, 173.47)	–	–	43.17 (27.54, 48.66)	–	–
Anti Scl-70 antibody													
Positive	16	6.00 (3.43, 8.68)	−1.306	0.192	49.16 (41.79, 57.15)	0	1	169.88 (109.82, 192.41)	−0.308	0.754	44.95 (35.27, 50.53)	−0.843	0.399
Negative	40	4.86 (1.99, 7.07)	–	–	49.88 (40.52, 57.38)	–	–	166.04 (125.15, 194.40)	–	–	45.94 (37.64, 52.39)	–	–
Anti-SSA antibody													
Positive	10	6.42 (2.10, 8.07)	−0.587	0.564	52.00 (45.13, 57.12)	−0.642	0.521	172.49 (166.18, 196.90)	−1.262	0.207	46.82 (37.43, 53.22)	−0.364	0.716
Negative	46	5.12 (2.18, 7.91)	–	–	49.88 (40.18, 57.77)	–	–	151.41 (102.47, 193.80)	–	–	44.89 (35.83, 51.29)	–	–

N number, *RP* Raynaud's phenomenon, *ESR* erythrocyte sedimentation rate, *ANA* anti-nuclear antibody

gested that IL-33 might mediate the very early pathogenic events of SSc because the damage of endothelial cells in early SSc patients could enhance the expression of IL-33 mRNA and the release of IL-33 protein into circulation [11, 13]. A new research reported that rs7044343 polymorphism of IL-33 gene is associated with the susceptibility to the SSc in Turkish population [36]. All of these studies revealed that increased level of IL-33 might have an association with onset of SSc. However, serum IL-33 levels in SSc patients among Japanese population (mean: 261.7 ± 141.9 pg/ml), Italian population (median 148.08 pg/ml, range: 0–4791.0 pg/ml) and German population (mean: 2550.3 ± 1578.2 pg/ml) were greatly higher than that in our patients with SSc (mean: 43.62 ± 9.56 pg/ml, range 20.67–59.23 pg/ml) [13–15]. In addition, these previous case–control studies reported IL-33 levels were associated with microvascular involvement, the extent of skin sclerosis, the severity of pulmonary fibrosis and the presence of digital ulcers. However, our results show that there was no correlation between serum IL-33 concentration and clinical parameters, including involvement of joints, limita-

tion of mouth opening, dyspnoea and RP. Moreover, the correlation between IL-33 and other important clinical parameters (e.g. PAH and ILD) is unclear. How about the interaction between IL-33 and clinical parameters of SSc patients? The exact mechanism is still unclear. Therefore, further studies are still needed to clarity the relationship between IL-33 levels and clinical features in SSc.

On the other hand, our results also showed significant associations among different cytokines. IL-1α, IL-1β, IL-18 and IL-33 are four inflammatory agonists of IL-1 family. There may be direct or indirect correlation among expressions of them. For example, IL-33 could induce IL-18 and IL-1β [35]. However, these interrelations were not consistent in the cases and controls, which suggested that these associations may be affected by the disease of SSc. As we know, IL-33 mainly localizes to the nucleus. When stimulated by appropriate signal (such as inflammation), it would be in response processed and passively released from necrotic cells or actively secreted into the extracellular milieu. Then it could act as a proinflammatory cytokine, which is involved in the development of many autoimmune diseases, by binding to its receptor ST2 [37].

In summary, we found significant differences in serum levels of IL-1β and IL-33 between SSc patients and healthy controls. But due to the limited cases, all patients in this study belong to diffuse cutaneous SSc, we failed to perform further analysis of whether there are differences in serum IL-1β and IL-33 levels between limited cutaneous SSc and diffuse cutaneous SSc. Moreover, one study [13] showed that dcSSc had higher levels of IL-33 than lcSSc ($P < 0.05$). This suggests that IL-33 may play different roles in the pathogenesis of two SSc groups. But the clear mechanism is still unclear. Therefore, larger sample sizes of studies are needed to further clarify the effect of IL-1β and IL-33 in the pathogenesis of multi-type SSc patients. Nonetheless, they still could be considered as important serum markers of development of SSc.

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Compliance with ethical guidelines

Conflict of interest. Y.-J. Zhang, Q. Zhang, G.-J. Yang, J.-H. Tao, G.-C. Wu, X.-L. Huang, Y. Duan, X.-P. Li, D.-Q. Ye and J. Wang state that they have no competing interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

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