

## Original Article

# Cytokine expression in patients with interstitial lung disease in primary Sjogren's syndrome and its clinical significance

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**Abstract:** Purpose: To explore the cytokine expression level of interstitial lung disease in primary Sjogren's syndrome (ILD-pSS) patients and its clinical significance. Methods: 50 pSS patients in our hospital from Jan 2017 to Dec 2019 were selected as pSS group and separated into pSS-ILD (15 cases) and pSS-non-ILD (35 cases) based on whether patients had ILD or not. Then 20 healthy people who underwent physical examination at the same time were matched as a control group. The levels of IL-6, IL-8, IL-10 and TNF- $\alpha$  between the pSS-ILD group and the pSS-non-ILD group were compared. The efficacy of ILD in pSS patients based on the analysis of the IL-6, IL-8, IL-10, and TNF- $\alpha$  levels with ROC curves was evaluated. Results: The IL-6, IL-8, and TNF- $\alpha$  levels of patients in the pSS group were significantly higher than those in the control group. The IL-10 expression level in the pSS group was significantly lower than that in the control group ( $P < 0.05$ ). The IL-6, IL-8 and TNF- $\alpha$  expression levels of patients in the pSS-ILD group were higher than these in the pSS-non-ILD group. The IL-10 expression level in the pSS-ILD group was significantly lower than that in the pSS-non-ILD ( $P < 0.05$ ). The result of the ROC curve showed that the AUC of IL-6, IL-8, IL-10, and TNF- $\alpha$  were 0.667, 0.712, 0.894 and 0.733 respectively. Among them, the AUC of IL-10 was the largest (0.894, the sensitivity was 87.54%, the specificity was 78.63%, and cut off value was 5.162 pg/ml. Conclusion: The serum IL-6, IL-8, IL-10 and the cytokine TNF- $\alpha$  are closely related to the ILD in pSS patients. They may participate in the occurrence and development of the pSS-ILD in patients.

**Keywords:** Primary Sjogren's syndrome, the interstitial lung disease, cytokines

## Introduction

As a chronic inflammatory autoimmune disease, primary Sjogren's syndrome (pSS) is caused by reduced secretion from the lacrimal and salivary glands. The main symptoms are dryness in the mouth and eyes and would affect the main organs such as lungs, kidneys and liver [1, 2]. The interstitial lung disease (ILD) is the main manifestation of lung issues in pSS patients. It is also one of the most commonly seen complications among patients with pSS [3]. The interstitial lung disease in pSS-ILD can cause infection and respiratory failure, which is the leading cause of death among pSS patients [4, 5]. The cause of pSS-ILD is currently unclear, but relevant reports have discovered that it may be related to the lung inflammation caused by the release of various inflammatory cytokines [6]. It is known

that inflammatory cytokines such as IL-6, IL-8, IL-10 and TNF- $\alpha$  are related to a variety of autoimmune diseases [7], but report indicating its relation to pSS and pSS-ILD is scarce. This study compared the cytokine, expression levels including IL-6, IL-8, IL-10 and TNF- $\alpha$  between pSS patients and pSS-ILD patients, aiming to explore how they relate to pSS-ILD patients.

## Resources and methods

### General resources

A total of 50 patients presenting to our hospital from Jan 2017 to Dec 2019 were selected as research subjects. Inclusion criteria: (1) Clinically diagnosed with pSS [8]; (2) Aged over 18 years old; (3) No other autoimmune diseases. Exclusion criteria: (1) Malignant tumor in their lungs; (2) Severe lung injury diseases

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**Table 1.** General data of the pSS-ILD group, pSS-non-ILD group, and the healthy group

	pSS-ILD group (n=15)	pSS-non-ILD group (n=35)	Healthy group (n=20)	F/ $\chi^2$	P
Age (years)	59.62±10.46	57.17±9.84	56.95±11.04	0.357	0.701
Gender (Male/Female)	9 (60.0)/6 (40.0)	25 (71.43)/10 (28.57)	13 (65)/7 (35)	0.680	0.712
Average disease course (years)	13.51±2.16	12.73±2.07	11.96±2.42	2.161	0.123
IgG (g/L)	19.61±3.94	18.26±4.27	18.84±4.06	0.570	0.568
IgA (g/L)	3.82±1.04	3.64±1.01	3.17±0.95	2.132	0.127
IgM (g/L)	1.72±0.27	1.85±0.21	1.78±0.22	1.186	0.163
Smoking (Yes/No)	5 (33.33)/10 (66.66)	17 (48.57)/18 (51.43)	12 (60)/8 (40)	2.440	0.295

caused by other reasons such as COPD, tuberculosis and bronchiectasis; (3) Have been taking drugs which cause lung fibrosis for a long time; (4) Chronic cardiac, liver and kidney insufficiency; (5) Also participated in other research. Patients were divided into the pSS-ILD group and the primary Sjogren's syndrome with non-interstitial lung disease group (pSS-non-ILD group) based on whether combined with ILD or not. In addition, the study selected 20 healthy people who have undergone physical examination in our hospital during the same period as the control group. Their general information such as gender and age were comparable with pSS patients. This study has been permitted by the Medical Ethics Committee in the hospital. All research subjects signed informed consent forms.

### Methods

Cytokine detection method: 3 ml of fasting venous blood in the early morning was taken. Then, the blood was placed into a sterile test tube and centrifuged at 300 r/min for 10 mins. The upper serum was collected and stored at a low temperature of -80°C. The serum IL-6, IL-8, IL-10, and TNF- $\alpha$  expression levels were tested by the enzyme-linked immunoassay assay (ELISA). All the operation steps were strictly in line with the kit instructions. The serum IL-6 >4.38 pg/ml, IL-8 >18.71 pg/ml, IL-10  $\leq$ 5.61 pg/ml and TNF- $\alpha$  >6.01 pg/ml indicate abnormal status. The ELISA kits were provided by MilliporeSigma; the Cat. No. of IL-6, IL-8, IL-10 and TNF- $\alpha$  were RAB0307, RAB0319, RAB0244, and RAB1089-1KT, respectively.

The ILD diagnosis method: The result of high resolution CT (Bright speed 64, Siemens, Germany) of the lungs was used to determine whether the patient had ILD or not. If the

HRCT results had honeycomb lungs, ground-glass shadow or grid-like shadows, it indicated the patient had ILD. We also collected the data such as gender, age, course of disease, immunoglobulin and smoking status of patients between two groups.

### Statistical analysis

The study utilized SPSS 25.0 statistical software to analyze data and draw figures. The quantitative data with normal distribution were given as ( $x \pm s$ ), and examined by t test. The qualitative data were represented by n (%), and examined by  $\chi^2$  test. The ROC curve was used to analyze the predictive ability of IL-6, IL-8, IL-10, and TNF- $\alpha$  expression levels of pSS-ILD patients.  $P < 0.05$  indicated the difference reached statistical significance.

### Results

#### General data of pSS-ILD group, pSS-non-ILD group, and healthy group

Among the 50 pSS patients in the study, patients with ILD accounted for 30.0% (15/50), and patients without ILD were 70.0% (35/50). The comparison of the basic data of patients between two groups such as age, gender, average course of disease, immunoglobulin (IgG, IgA and IgM), and smoking status of patients with and without ILD had no significant difference ( $P < 0.05$ ). See **Table 1**.

#### Comparison of cytokine expression level between the pSS patients and the healthy group

There were notable differences in the IL-6, IL-8, IL-10, and TNF- $\alpha$  expression levels between the two groups ( $P < 0.05$ ). The IL-6, IL-8, and TNF- $\alpha$  expression levels of patients in the pSS group were notably higher than these of the control

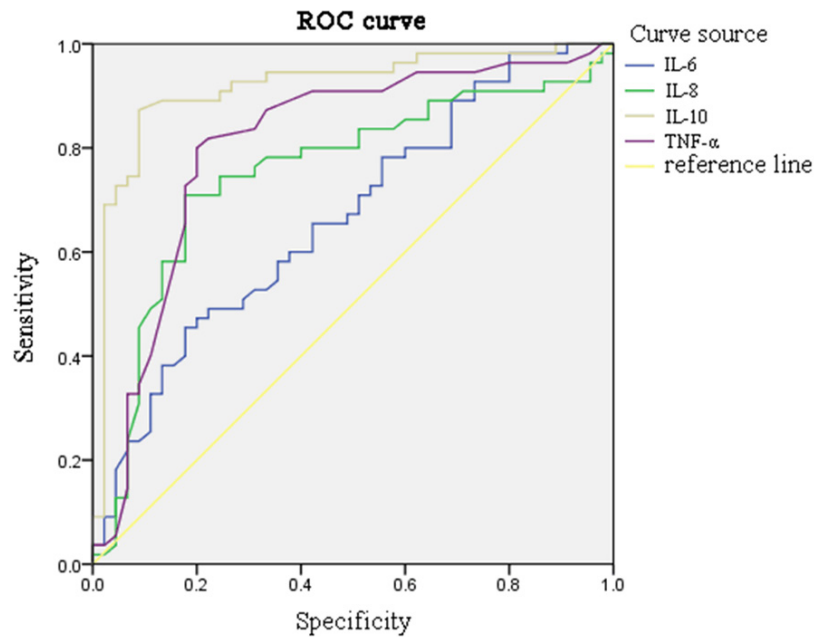
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**Table 2.** Comparison of the IL-6, IL-8, IL-10, and TNF- $\alpha$  expression levels between the pSS group and control group ( $\bar{x} \pm s$ , pg/ml)

Group	Cases	IL-6	IL-8	IL-10	TNF- $\alpha$
pSS group	50	6.95 $\pm$ 1.73	19.14 $\pm$ 5.02	5.06 $\pm$ 1.13	8.62 $\pm$ 2.79
Control group	20	3.94 $\pm$ 1.05	11.95 $\pm$ 3.67	6.88 $\pm$ 1.59	5.12 $\pm$ 1.66
<i>t</i>		7.247	5.804	5.393	5.238
<i>P</i>		<0.001	<0.001	<0.001	<0.001

**Table 3.** Comparison of cytokine levels between the pSS-ILD group and pSS-non-ILD group ( $\bar{x} \pm s$ , pg/ml)

Groups	Cases	IL-6	IL-8	IL-10	TNF- $\alpha$
pSS-ILD group	15	7.62 $\pm$ 1.97	21.65 $\pm$ 5.18	4.12 $\pm$ 1.03	10.12 $\pm$ 3.72
pSS-non-ILD group	35	5.78 $\pm$ 1.64	17.73 $\pm$ 4.82	5.94 $\pm$ 1.17	7.09 $\pm$ 2.13
<i>t</i>		3.412	2.578	5.215	3.646
<i>P</i>		0.001	0.013	<0.001	<0.001



**Figure 1.** ROC curve analysis of IL-6, IL-8, IL-10, and TNF- $\alpha$  expression levels.

group. The IL-10 level in the pSS patient group was significantly lower than that of the control group ( $P < 0.05$ ). See **Table 2**.

### Comparison of cytokine levels between the pSS-ILD group and pSS-non-ILD group

The comparison of IL-6, IL-8, IL-10, and TNF- $\alpha$  expression levels between two groups reached significant differences ( $P < 0.05$ ). The IL-6, IL-8, and TNF- $\alpha$  expression levels of patients in the pSS-ILD group were significantly higher than

these in the pSS-non-ILD group. The IL-10 expression level in the pSS-ILD group was significantly lower than that in the pSS-non-ILD group ( $P < 0.05$ ). See **Table 3**.

### ROC curve analysis of IL-6, IL-8, IL-10, and TNF- $\alpha$ expression levels in pSS patients and its prediction value in ILD

The AUC, sensitivity, and specificity of IL-6 were 0.667, 90.88%, and 62.75, respectively; those of IL-8 were 0.712, 81.16%, and 63.82, respectively; those of IL-10 were 0.894, 87.54%, and 78.63%; those of TNF- $\alpha$  were 0.733, 80.56%, and 73.19, respectively. See **Figure 1** and **Table 4**.

### Discussion

pSS is a global disease and its incidence increases with age. The incidence of pSS among the elderly exceeds 4% in China, and it is one of the main diseases threatening people's health [9]. Besides the obvious reduction of exocrine gland secretion, pSS patients have other symptoms, such as skin lesions, skeletal muscle

pain, and damage to important organs and systems. The lung is the most vulnerable organ outside the glands of pSS. Some epidemiologic studies have shown that about 8% to 75% of pSS patients develop ILD due to lung damage [10]. pSS-ILD patients usually develop obvious breathing difficulties and restrictive pulmonary ventilation dysfunction. What's more, the lung diffusion function decreases, which eventually develops into diffuse pulmonary fibrosis, respiratory failure, and even death. Studies have indicated that the mortality rate of pSS-ILD

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**Table 4.** Analysis result of ROC curves of IL-6, IL-8, IL-10, and TNF- $\alpha$  indicators

Indicator	AUC	Cut-off value (pg/ml)	Sensitivity (%)	Specificity (%)	P value	95% CI	
						Upper limit	Lower limit
IL-6	0.667	7.109	90.88	62.75	0.012	0.560	0.772
IL-8	0.712	20.094	81.16	63.82	0.009	0.641	0.813
IL-10	0.894	5.162	87.54	78.63	0.003	0.796	0.917
TNF- $\alpha$	0.733	9.116	80.56	73.19	0.005	0.692	0.866

patients is over 60% [10]. The causes of pSS-ILD are currently elusive. It is clinically believed that it is related to a variety of factors such as genetics, viral infections, and abnormal sex hormones [11]. However, recent studies suggest that chronic inflammation is closely related to pSS-ILD. Therefore, the current treatment of pSS-ILD starts from the reduction of inflammatory response, which helps to prevent or improve the progression of pulmonary fibrosis [12, 13]. However, the relationship between pSS-ILD and inflammatory cytokines still needs adequate research for further confirmation.

We observed in this study that the IL-6, IL-8, IL-10, and TNF- $\alpha$  expression levels in the pSS patients and the healthy group were significantly different. The study also found that the expression levels of serum IL-6, IL-8, TNF- $\alpha$ , and other inflammatory factors in patients with ILD were greatly higher than these without ILD. The IL-10 expression level was lower than that of patients without ILD. The possible reason is that pSS patients with ILD may have alveolar inflammation caused by excessive secretion of various inflammatory factors in the body. IL-6 is a core part of inflammatory cytokines. It is a type of proinflammatory factor produced by activated T cells, fibroblasts, and macrophages when exposed to external stimuli. It can effectively respond to the inflammation in the body, highlighting an essential cause of a variety of immune diseases [14, 15]. IL-8 is an inflammatory factor mainly produced by monocytes, that can promote the chemotaxis of neutrophils. It has been proven that it plays an important role in the pathogenesis of bronchitis and cystic fibrosis [16, 17]. TNF- $\alpha$  is tumor necrosis factor, and can act on T cells to produce a variety of inflammatory factors and induce the inflammation in the body. Also, the terminal carboxyl repeat structure can enhance the activity of inflammatory factors through combining the glycoprotein of inflammation factors such as IL-8, which can further aggravate the inflamma-

tory response [18]. The serum IL-6, IL-8, and TNF- $\alpha$  of pSS patients with ILD were significantly higher than these of pSS patients without ILD, which indicates the severe inflammation in pSS-ILD patients. Many inflammatory factors infiltrate the lungs over a long time, and these inflammatory factors may promote the spread of lung fibroblasts and the construction of an extracellular pattern, which leads to the occurrence and development of pulmonary fibrosis.

IL-10 is a cytokine that inhibits the inflammatory response, and participates in the inflammatory and immune responses. Additionally, it is closely related to blood, digestion, and cardiovascular diseases, and it can be synthesized by almost all lymphocytes. The mechanism of inhibiting inflammation is to inhibit the release of inflammatory mediators by mononuclear macrophages, thereby inhibiting the secretion of proinflammatory factors such as IL-6, IL-8, and TNF- $\alpha$  caused by LPS and IFN- $\gamma$ , and reducing the inflammatory response in the body [19]. IL-10 can inhibit the secretion of inflammatory factors, and also reduce the expression of the stimulating factor CD86 and the adhesion factor CD54. The serum IL-10 expression level of pSS-ILD patients is significantly reduced. This may be because Treg cells are suppressed under the disease state, which leads to a reduced secretion of IL-10 [20]. ROC curve analysis has found that IL-10 has the highest efficacy in predicting pSS patients with ILD. Also, when the IL-10 of pSS is below 5.162 pg/ml, the possibility of occurrence of ILD significantly increases. This may be because IL-10 cannot fight the inflammatory response in the body when its level is too low. This causes lung damage due to the large number of inflammatory factors infiltrating in the lungs. Moreover, we analyzed the relationship between the expression level of serum IL-6, IL-8, IL-10, and TNF- $\alpha$  in pSS patients and ILD. It revealed that inflammatory cytokines such as IL-10 may play an essential part in the lung interstitium in pSS

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patients. Also, this provides new ideas to clarify the pathogenesis of pSS-ILD and the treatment of pSS-ILD. However, there may be some bias in the result due to the small number of samples. We hope more samples for more comprehensive studies can be included in the future.

In summary, the serum IL-6, IL-8, IL-10, and TNF- $\alpha$  cytokines are correlated to findings in pSS-ILD patients. The IL-10 expression level has the highest efficacy in predicting pSS patients with ILD.

### Disclosure of conflict of interest

None.

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