

Original Article

Changes of serum IL-6, IL-10 and TNF- α levels in patients with systemic lupus erythematosus and their clinical value

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Abstract: The levels of serum IL-6, IL-10, and TNF- α in patients with systemic lupus erythematosus (SLE) and their value in clinical practice were studied. A total of 68 patients with active SLE treated in our hospital between March 2015 and January 2018 were enrolled into the active SLE group, and they were divided into three groups according to the mild, moderate, and heavy active periods, and also divided into two groups according to the positive and negative anti dsDNA. A total of 60 healthy individuals in the same period were included in the control group (con group). The levels of serum IL-6, IL-10, and TNF- α in all participants were detected via an enzyme-linked immunosorbent assay (ELISA), and the correlation of these values with SLE activity was analyzed. The independent prognostic factors were analyzed through multivariate logistic regression. It was found that the levels of serum IL-6, IL-10, and TNF- α in the SLE groups were all higher than those in the control group; the levels of the inflammatory markers in the severe active SLE group were higher than those in the mild and moderate active SLE groups, and the levels in the moderate active SLE group were higher than those in the mild active SLE group. Additionally, the anti dsDNA positive group showed much higher levels of these than the anti dsDNA negative group. Pearson correlation analysis revealed a positive correlation between anti dsDNA antibody and IL-6, IL-10, and TNF- α levels. The multivariate logistic regression results, the mean course of disease and IL-10 were independent prognostic factors of SLE. The abnormal secretion of peripheral blood cytokines in SLE patients can affect the prognosis of the disease. Monitoring serum cytokines is helpful to understand the activity and prognosis of patients with lupus and guide clinical treatment.

Keywords: Systemic lupus erythematosus, IL-6, IL-10, TNF- α , clinical value

Introduction

At present, the incidence of systemic lupus erythematosus (SLE) is rising annually worldwide. SLE is more common in females, and it is concentrated during youth and middle-aged [1, 2]. Clinically, the etiology of SLE is not clear, and there is evidence that different environments, genetics and estrogen have different degrees of influence on the disease [3]. SLE is an autoimmune disease with long-term attack. The deposition of antibodies and immune complex, the necrosis of blood vessel walls and a series of inflammatory reactions are caused by the increase of autoantibodies such as anti-nuclear antibody, which eventually leads to damage

of important organs in the body [4, 5]. SLE is usually treated through hormone drug therapy. After treatment, the condition of SLE patients can be significantly improved. However, due to the long-term use of hormone drugs, many complications occur, and the immune ability of the body is also affected, resulting in infections, renal failure and early-onset vascular sclerosis, and cardiovascular and cerebrovascular damage; leading to poor prognosis and increased mortality of SLE patients [6, 7]. Research has found that the pathogenesis and treatment of SLE were closely related to the body's immune function. The change of immune function in the body greatly controls the disease trends of SLE patients.

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Interleukin-6 (IL-6) is a cell factor secreted by monocyte macrophages, which promotes the differentiation of B cells. It can induce B cells to produce immunoglobulins and participate in the development of inflammatory responses [8]. Interleukin-10 (IL-10) is a pleiotropic cytokine with double mechanisms of immune-stimulation and inhibition. With different degrees of expression, it plays different roles in SLE patients, mainly by affecting B cell differentiation, apoptosis and production of autoantibodies. Tumor necrosis factor- α (TNF- α) can stimulate inflammatory factors such as interleukins to secrete by acting on immunocompetent cells. TNF- α can also induce expression of intercellular adhesion molecule-1 (ICAM-1) in vascular endothelial cells to complete adhesion, causing leukocyte aggregation and adhesion to damage vascular endothelial cells [10]. The above three kinds of factors play their respective functions in the body's immune function and regulate the development of the disease and prognosis of patients with abnormal immune function. Therefore, this study explored the changes in serum level IL-6, IL-10, and TNF- α in SLE patients and their clinical value.

Data and methods

General information

A total of 68 active SLE patients admitted to Hangzhou Jianggan District People's Hospital from March 2015 to January 2018 were selected as the SLE active group. They were divided into three groups according to mild, moderate, and severe active periods and two groups according to anti dsDNA positive and negative. A total of 60 healthy subjects in the same period were taken as control group (con group). On the basis of the criteria of systemic lupus erythematosus disease activity score (SLEDAI) [11], SLE patients were divided into inactive stage according to ≤ 5 points; SLE patients with ≥ 5 points were divided into active stage according to the criteria. They were then divided into 5 points for mild, 6-7 points for moderate and 8 points for severe. According to the double stranded DNA (DS DNA) antibody, SLE patients were divided into Anti DS DNA antibody positive and anti DS DNA antibody negative groups. The active SLE group consisted of 25 cases in the mild active SLE group, 19 cases

in the moderate active SLE group, 24 cases in the severe active SLE group, 32 cases in the anti DS DNA positive group, and 36 cases in the anti DS DNA negative group, including 56 males and 72 females, with a median age of (48.40 ± 10.67) years. No significant difference was seen in age and sex between SLE patients and con group.

Inclusion exclusion criteria

Inclusion criteria: Patients meeting the diagnostic criteria of American College Rheumatology SLE [12]. *Exclusion criteria:* (1) patients with liver or kidney dysfunction; (2) patients with other connective tissue diseases; (3) patients with coagulation dysfunction; (4) patients with cognitive or communication disorders; and (5) those with poor compliance. All patients and their families signed an informed consent after understanding the study, and the experiment was carried out with approval from the medical ethics committee of Hangzhou Jianggan District People's Hospital.

Experimental reagents and materials

EDTA anticoagulant tubes were purchased from Guangzhou Bangbiao Medical Equipment Co., Ltd.; serum separator was purchased from Beckman Company in the United States; ELISA kit was purchased from Shanghai Jingkang Bioengineering Co., Ltd.

Test method

Fasting elbow venous blood (3 ml) was sampled from SLE patients and controls in the early morning. After 2 hours of standing at room temperature in EDTA anticoagulation, the upper serum was subjected to 3000 R/min centrifugation for 10 min and saved in a -80°C freezer. Serum IL-6, IL-10 and TNF- α were detected under the instructions of the ELISA kit. The detection of anti DS DNA antibody, lupus erythematosus activity index, as well as other related laboratory tests in SLE patients were completed by the clinical laboratory and the Department of Microbial Immunology of our hospital.

Observation indicators

(1) Comparison of serum cytokine levels in SLE patients of different severities; (2) comparison of the levels in anti DS DNA positive and nega-

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Table 1. Comparison of serum cytokine levels in SLE patients in different severity

Groups	Cases/n	IL-6 (ng/L)	IL-10 (ng/L)	TNF- α (ng/L)
SLE mild activity group	25	9.34 \pm 2.13*	26.25 \pm 5.24*	14.35 \pm 5.38*
SLE moderate activity group	19	13.04 \pm 2.77*,**	31.35 \pm 6.83*,**	20.92 \pm 5.57*,**
SLE severe activity group	24	16.15 \pm 3.37*,**,***	38.65 \pm 7.11*,**,***	28.45 \pm 6.01*,**,***
SLE activity group	68	13.63 \pm 5.74*	32.53 \pm 12.36*	21.43 \pm 11.26*
con group	60	5.44 \pm 1.48	17.39 \pm 4.27	7.45 \pm 3.59

Note: * indicates $P < 0.05$ vs. the con group; ** indicates $P < 0.05$ vs. the SLE mild activity group; *** represents $P < 0.05$ vs. the SLE severe activity group.

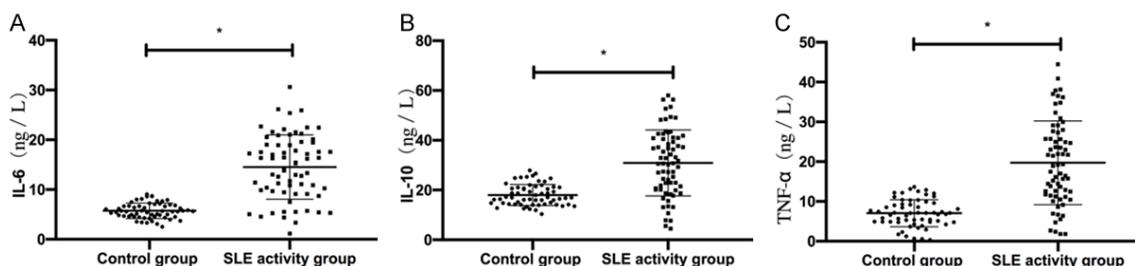


Figure 1. Comparison of serum cytokine levels between patients in active SLE group and con group. A. SLE patients presented higher serum IL-6 levels than the con group. B. SLE patients presented higher serum IL-10 levels than the con group. C. SLE patients showed higher serum TNF- α levels than the con group. Note: * represents $P < 0.05$.

tive groups; (3) analysis on the correlation between the anti DS DNA antibody levels of SLE patients with anti DS DNA positive and the IL-6, IL-10, and TNF- α levels; (4) based on the best cut-off values of IL-6, IL-10, and TNF- α in SLE patients in each activity group, they were assigned to high and low expression groups. Based on the 3-month prognosis, the patients were assigned to good prognosis and unfavorable prognosis groups. The independent prognostic factors were analyzed by multivariate logistic regression.

Statistical methods

In this experiment, SPSS 19.0 statistical software (net times Technology Co., Ltd., Beijing, China) was employed for analysis on the experimental data, and chi square test was applied for data counting. The quantitative data were represented as the mean \pm standard deviation. T test was adopted for inter-group comparison, and repeated measurement analysis was used for multi-group comparison. Multivariate logistic regression was carried out for analysis on the independent prognostic factors of SLE patients. In this experiment, we used Graphpad prism 8 to illustrate figures, and $P < 0.05$ indicated a significant difference.

Results

Comparison of serum cytokine levels in SLE patients with different severities

SLE patients showed higher levels of serum IL-6, IL-10, and TNF- α levels than the con group ($P < 0.05$). Patients with severe SLE activity showed higher levels of the cytokines than patients with mild or moderate LSE activity; patients with moderate SLE activity showed higher levels than patients with mild SLE activity ($P < 0.05$) **Table 1** and **Figures 1** and **2**.

Comparison of serum cytokines between the positive and negative anti DS DNA groups in SLE patients

The anti DS DNA positive group showed higher IL-6, IL-10 and TNF- α levels than the anti DS DNA negative group ($P < 0.05$) **Table 2** and **Figure 3**.

Correlation analysis of serum cytokines and anti DS DNA antibody

Pearson correlation analysis revealed that there was a positive relationship between anti DS DNA antibodies and IL-6, IL-10, and TNF- α levels ($r = 0.5633$, $r = 0.5589$, $r = 0.6379$, all $P < 0.001$) **Figure 4**.

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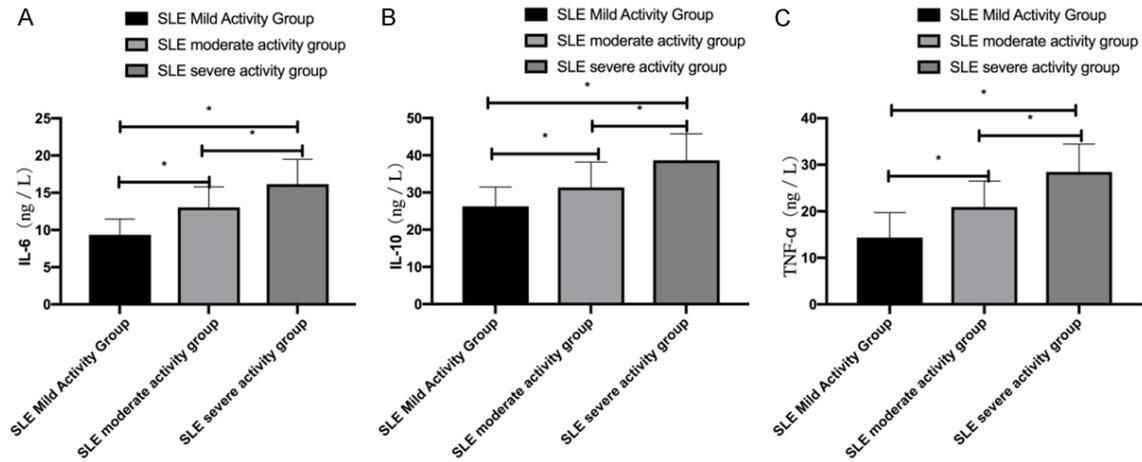


Figure 2. Comparison of serum cytokine levels in SLE patients of different severity. A. Patients with severe SLE activity showed higher serum IL-6 levels than patients with moderate or mild SLE activity, and patients with moderate SLE activity showed higher serum IL-6 levels than patients with mild SLE activity. B. Patients with severe SLE activity showed higher IL-10 levels than patients with moderate or mild SLE activity, and patients with moderate SLE activity showed higher IL-10 levels than patients with mild SLE activity. C. Patients with severe SLE activity showed higher serum TNF- α levels than patients with moderate or mild SLE activity, and patients with moderate SLE activity showed higher serum TNF- α levels than those with mild SLE activity. Note: * represents $P < 0.05$.

Table 2. Comparison in detection levels of serum cytokines between positive and negative anti DS DNA groups in SLE patients

Group	Cases/n	IL-6 (ng/L)	IL-10 (ng/L)	TNF- α (ng/L)
Anti DS DNA positive group	32	15.48 \pm 3.47*	36.75 \pm 6.43*	27.32 \pm 5.94*
Anti DS DNA negative group	36	10.36 \pm 2.67	28.48 \pm 5.53	15.93 \pm 5.35

Note: * represents $P < 0.05$ vs. the anti dsDNA negative group.

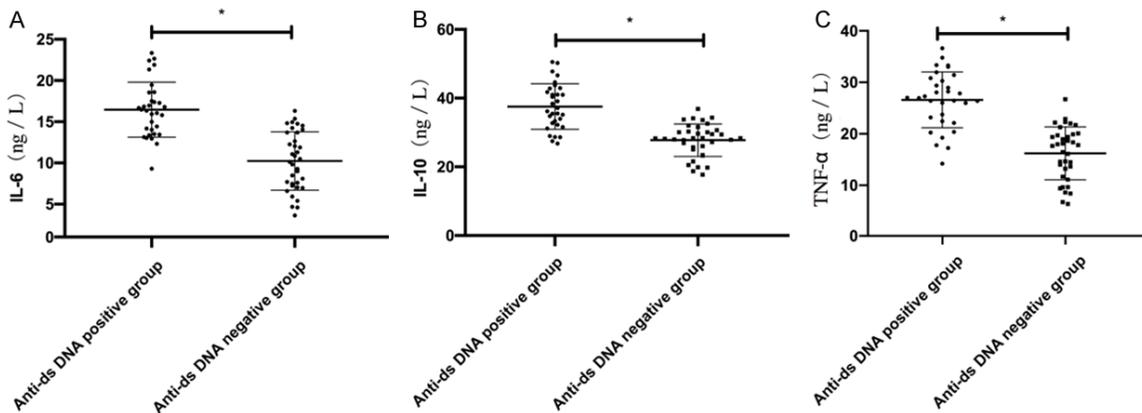


Figure 3. Comparison of serum cytokines between positive and negative anti DS DNA groups in SLE patients. A. The anti DS DNA positive group showed higher IL-6 levels than the anti DS DNA negative group. B. The anti DS DNA positive group showed higher IL-10 levels than that the anti DS DNA negative group. C. The anti DS DNA positive group showed higher TNF- α levels than the anti DS DNA negative group. Note: * represents $P < 0.05$.

Univariate analysis of patients' prognosis

According to the prognosis of SLE patients within 3 months, the patients with fever, rash and

other clinical signs and symptoms that were improved were divided into good prognosis group ($n = 42$). The patients with fever, rash and other clinical symptoms and signs without

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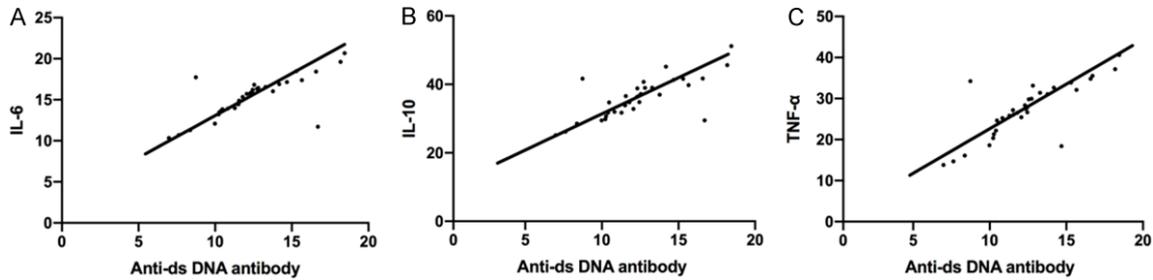


Figure 4. Correlation analysis between serum cytokine level and anti DS DNA antibody. A. Pearson correlation analysis revealed a positive relation between anti DS DNA antibody and IL-6 expression ($r = 0.5633$, $P < 0.001$). B. Anti DS DNA antibody and IL-10 expression ($r = 0.5589$, $P < 0.001$). C. Anti DS DNA antibody and TNF- α level ($r = 0.6379$, $P < 0.001$).

improvement were divided into poor prognosis group ($n = 26$). The clinical data of SLE patients were gathered and the optimal critical value of each index was selected for single factor analysis. The results showed that there was no difference in sex and smoking and drinking history between the two groups ($P > 0.05$), but there were remarkable difference in age, BMI, average course of disease, IL-6, IL-10 and TNF- α between them (all $P < 0.05$) **Table 3**.

Multivariate analysis on survival

We assignned indexes with differences in univariate analysis (See **Table 4** for the assignment), and then proceeded forward to conduct a multivariate logistic regression analysis. It came out that age, BMI, IL-6, TNF- α were not independent prognostic factors of patients, while the average duration (or: 1.349, 95% CI: 1.102~1.654), and IL-10 (or: 1.794, 95% CI: 1.083~3.164) were independent prognostic factors of patients. See **Table 5**.

Discussion

Early onset of SLE in patients may result in repeated fever or low fever for a long time, pain and soreness of joints and general tiredness after exertion, and patients with different constitutions have different degrees of digestive system symptoms, and the overall performance is similar to cold symptoms [13, 14]. With untimely treatment or improper treatment, patients with aggravation of the disease will have punctate hemorrhaging, ulcers or even erosion of oral mucosa, typical Butterfly erythema on the face, and serious damage to the heart, kidneys and other important organs [15, 16]. With a pathogenesis that is related to the

immune system, SLE can activate the immune complex and complement system, and combine with antigens to form a complex. They are deposited in glomeruli, joints and other different tissue sites, causing acute or chronic inflammatory changes in the body, thus causing multiple organ functional damage. The process of the disease has great influence on the production of autoantibodies and the amount of interleukin [17, 18]. There is no specific plan to cure SLE. People mainly regulate the progress of the disease through conservative treatment, and the main therapeutic effect requires anti-inflammation, relieving acute attacks and improving symptoms [19, 20]. In order to study a more suitable treatment plan for SLE patients, we statistically analyzed serum IL-6, IL-10, TNF- α levels in SLE patients, and analyzed the levels in different active stages of SLE patients to determine whether there is prognostic value.

We found that SLE patients showed significantly higher IL-6, IL-10 and TNF- α levels than the con group, and patients with severe SLE activity showed significantly higher levels than those with moderate or mild SLE activity, and patients with moderate SLE activity showed significantly higher levels than those with mild SLE activity. Studies have shown that [21, 22], SLE is an autoimmune disease, which is usually mediated by Th2 cytokines; and IL-6, IL-10 are important Th2 cytokines, which can affect the secretion and function of a variety of cells. It has been confirmed that [23], endogenous immune complexes stimulate the activity of plasma like dendritic cells, a large number of dendritic cells promote the secretion and synthesis of TNF- α , and regulate the immune system *in vivo* leading to SLE. The higher the activity index is, the worse the prognosis is. The results showed that

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Table 3. Single factor analysis [n (%)]

Clinicopathological features	Good prognosis group (n = 42)	Poor prognosis group (n = 26)	X ² value	P value
Age			5.635	0.018
< 48	25 (59.52)	9 (34.62)		
\geq 48	17 (40.48)	17 (65.38)		
Gender			0.027	0.869
male	17 (40.48)	10 (38.46)		
female	25 (59.52)	16 (61.54)		
BMI (kg/m ²)			4.317	0.038
\leq 23	27 (64.29)	10 (38.46)		
> 23	15 (35.71)	16 (61.54)		
Average duration (month)			4.484	0.034
\leq 42	24 (57.14)	8 (30.77)		
> 42	18 (42.86)	18 (69.23)		
Smoking history (cases)			0.476	0.490
Yes	19 (45.24)	14 (53.85)		
No	23 (54.76)	12 (46.15)		
Drinking history (cases)			0.183	0.669
Yes	22 (52.38)	15 (57.69)		
No	20 (47.62)	11 (42.31)		
IL-6 (ng/L)			4.741	0.030
\leq 13	29 (69.05)	11 (42.31)		
> 13	13 (30.95)	15 (57.69)		
IL-10 (ng/L)			3.985	0.046
\leq 32	25 (59.52)	9 (34.62)		
> 32	17 (40.48)	17 (65.38)		
TNF- α (ng/L)			5.182	0.023
\leq 21	28 (66.67)	10 (38.46)		
> 21	14 (33.33)	16 (61.54)		

Table 4. Assignment table

Factor	Assignment
Age	< 48 = 1, \geq 48 = 0
BMI	< 23 = 1, \geq 23 = 0
Average course of disease	\leq 42 = 1, > 42 = 0
IL-6	< 13 = 1, \geq 13 = 0
IL-10	< 32 = 1, \geq 32 = 0
TNF-alpha	< 21 = 1, \geq 21 = 0
Prognosis	Good prognosis = 1, poor prognosis = 0

with the increase of the activity index, the expression of serum IL-6, IL-10 and TNF- α elevated. With the aim of further exploring the changes of these three factors in SLE patients, we compared the expression of these three factors in anti DS DNA positive and negative environments, finding that the anti DS DNA positive

group presented with significantly higher IL-6, IL-10, and TNF- α levels than the anti DS DNA negative group. Previous studies have revealed that anti dsDNA antibodies are one of the indicators for clinical diagnosis of SLE, which is of great importance for the diagnosis and prognosis evaluation of SLE. From our study, it seems that the level of detection factors in the anti DS DNA positive group is higher, which proves that the severity of SLE is inextricably related to the detection factors in this experiment. The specific observation of Pearson correlation analysis revealed a positive correlation between anti DS DNA antibodies and serum IL-6, IL-10, TNF- α levels in SLE patients ($r = 0.5633$, $r = 0.5589$, $r = 0.6379$, all $P < 0.001$). It has been reported in the literature [24-26] that by detecting serum IL-6 in SLE patients, it was significantly increased, and was positively related to the SLE activity and the anti DS DNA antibody level in serum. It has also been reported in the literature [27, 28] that the IL-10 level in serum and peripheral blood mononuclear cell culture supernatant of

SLE patients was generally elevated, and was related to disease activity. It has also been reported that IFN- α can promote the generation of IL-10 by activated CD4+ T cells and monocytes, and there is an interaction between the IL-10 and IFN- α levels. This further explained that the interaction trend of the detection fac-

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Table 5. Multi factor analysis

Factor	B	Wals	Sig.	Exp (B)	EXP (B) 95% C.I.	
					lower limit	Upper limit
Age	0.921	2.102	0.061	1.023	0.746	4.323
BMI	0.923	1.894	0.067	1.013	0.659	3.747
Average course of disease	0.303	8.904	0.004	1.349	1.102	1.654
IL-6	0.623	3.672	0.055	1.879	0.992	3.542
IL-10	0.582	5.813	0.016	1.794	1.083	3.164
TNF- α	0.462	0.539	0.462	1.588	0.466	5.413

Note: B: constant term, Se: standard deviation, SIG: P value, exp (b): odds ratio, 95% C.I. of exp (b): 95% confidence interval of odds ratio.

tors and anti dsDNA antibodies in SLE patients is positive, and that positive interaction can predict the development of SLE. At last, the influencing factors of SLE prognosis were further explored. Single factor analysis revealed that there were no differences in sex, smoking history and drinking history between the two groups, but remarkable differences were found in age, BMI, average course of disease, IL-6, IL-10 and TNF- α . The multivariate logistic regression analysis revealed that age, BMI, IL-6, TNF- α were not independent prognostic factors, while the mean course of disease (or: 1.349, 95% CI: 1.102-1.654), and IL-10 (or: 1.794, 95% CI: 1.083-3.164) were independent prognostic factors.

To sum up, the abnormal secretion of IL-6, IL-10 and TNF- α in SLE patients' peripheral serum can affect the prognosis of the disease. Monitoring serum cytokines can help to understand the activity and prognosis of lupus patients to guide clinical treatment. However, there are still some deficiencies in this study. First, this study only explores the correlation of anti DS DNA antibodies with the detection factors in this experiment, and does not explore the correlation between other autoantibodies that are detected in SLE disease. Secondly, there is little literature about whether detection factors are prognostic factors for SLE, which needs to be further studied in the follow-up experiments.

Disclosure of conflict of interest

None.

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