

## Original Article

# Relation between serum inflammatory factors and lower-limb arteriosclerosis obliterans

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**Abstract:** Background: Lower-limb arteriosclerosis obliterans (LASO) is an extensive multi-segment vascular lesion which causes the severe ischemia in the lower limbs. This study aimed to investigate the changes of serum inflammatory factor interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and high-sensitivity C-reactive protein (hs-CRP) level in patients with LASO, and discuss their relations with the severity of LASO as well as the complicated diabetes, hypertension and hyperlipidemia. Methods: Sixty-five LASO patients and 30 healthy subjects (control group) were enrolled. The ankle-brachial index (ABI) of the subjects was measured using Doppler blood flow detector. The serum levels of IL-6, TNF- $\alpha$  and hs-CRP were determined using ELISA. Results: The serum IL-6, TNF- $\alpha$  and hs-CRP levels in LASO group were significantly higher than those in control group, respectively ( $P < 0.01$ ). The ABI in LASO group was significantly lower than that in control group ( $P < 0.01$ ). There were significant differences of IL-6, TNF- $\alpha$  and hs-CRP level between LASO with gangrene group and other LASO groups, and between LASO patients with and without complicated diabetes, hypertension and hyperlipidemia, respectively ( $P < 0.05$ ). In LASO patients, the IL-6 and TNF- $\alpha$  levels were positively correlated ( $r = 0.7457$ ,  $P < 0.05$ ), and they were negatively correlated with ABI, respectively (IL-6 with ABI:  $r = -0.5623$ ,  $P < 0.05$ ; TNF- $\alpha$  with ABI:  $r = -0.7129$ ,  $P < 0.05$ ). Conclusion: The serum IL-6, TNF- $\alpha$  and hs-CRP levels are involved in the occurrence and development of LASO, and they are the reliable indicators for judging the LASO severity and the related complications.

**Keywords:** Arteriosclerosis obliterans, IL-6, TNF- $\alpha$ , hs-CRP, relation

## Introduction

Arteriosclerosis obliterans is a disease which involves the peripheral arteries and causes the chronic occlusion [1]. Lower-limb arteriosclerosis obliterans (LASO) is the local performance of arteriosclerosis obliterans at the lower limbs. LASO will cause extensive multi-segment vascular lesions, which is the main reason of severe ischemia in the lower limbs, and is also a difficult and hot spot in clinical treatment [2, 3]. The etiology of LASO is complicated, and it is believed to be the lesion resulting from a variety of factors. The formation of LASO is considered as a slow accumulation process of lipids and celluloses in blood vessels [4]. It is believed that, the local and systemic inflammation plays an important role in the occurrence and development of atherosclerosis and the production of its complications [5]. With the development of experimental techniques, it is found that the inflammation runs through the whole process of arteriosclerosis

obliterans [6], but the specific mechanism is not very clear. Previous studies [7-9] find that, a variety of proteins, cytokines and adhesion molecules are involved in the occurrence and development of atherosclerotic vascular inflammation, among which the roles of interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and high-sensitivity C-reactive protein (hs-CRP) are the most important. This study investigated the changes of serum IL-6, TNF- $\alpha$  and hs-CRP level in patients with LASO, and discussed the relations between these inflammatory factors and the severity of LASO as well as the complicated diabetes, hypertension and hyperlipidemia. The objective was to provide a basis for further studying the mechanism of LASO and its prevention and treatment.

## Subjects and methods

### Subjects

Sixty-five LASO patients treated in Gansu Provincial Hospital from January 2009 to January

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**Table 1.** Comparison of serum inflammatory factor levels and ABI between LASO group and control group (mean  $\pm$  SD)

Group	n	IL-6 (ng/L)	TNF- $\alpha$ (ng/L)	Hs-CRP (mg/L)	ABI
LASO	65	12.27 $\pm$ 2.45 <sup>a</sup>	30.83 $\pm$ 4.14 <sup>a</sup>	10.06 $\pm$ 2.63 <sup>a</sup>	0.35 $\pm$ 0.05 <sup>a</sup>
Control	30	3.93 $\pm$ 0.88	7.95 $\pm$ 1.21	1.23 $\pm$ 0.43	1.16 $\pm$ 0.28

<sup>a</sup>P < 0.01 compared with control group. ABI, Ankle-brachial index; LASO, Lower-limb arteriosclerosis obliterans; IL-6, Interleukin-6; TNF- $\alpha$ , Tumor necrosis factor- $\alpha$ ; hs-CRP, High-sensitivity C-reactive protein.

2013 were enrolled in this study. There were 26 (40.00%) males and 39 (60.00%) females. The age of patients was 44-71 years, with mean age of 62.23 $\pm$ 9.11 years. The body mass index (BMI) of patients was 24.31 $\pm$ 3.32 kg/m<sup>2</sup>. All cases met the LASO diagnostic criteria [10]. The patients who were combined with heart disease, thyroid disease, severe liver or kidney disease, autoimmune disease or malignant tumor and who were taking medicine that might affect the test results (e.g., methotrexate, anti-epileptic drugs) were excluded. In the same period, 30 healthy subjects undergoing physical examination in our hospital were selected as control, in which the LASO was excluded by disease history inquiry, physical examination, ankle-brachial index (ABI) measurement and color Doppler examination. There were 13 (43.33%) males and 17 (56.67%) females. The age of control participants was 42-69 years, with mean age of 61.88 $\pm$ 7.38 years, and the BMI of them was 25.89 $\pm$ 5.11 kg/m<sup>2</sup>. There was no significant difference in age, gender or BMI between two groups (P > 0.05). This study was approved by the ethics committee of Gansu Provincial Hospital. Written informed consent was obtained from all participants.

### Determination of ABI

ABI of patients was determined using the method recommended by American Heart Association (AHA) [11]. The patients had supine rest for at least 5 min, and the ultrasound examination was performed using BV-660P portable Doppler blood flow detector (Shenzhen Bestman Instrument Co., Ltd., Shenzhen, China). The ABI was calculated by the ratio of lateral ankle artery systolic pressure to maximum bilateral brachial artery systolic pressure. The lower value of bilateral measurement results was used as the final ABI.

### Determination of serum IL-6, TNF- $\alpha$ and hs-CRP levels

Fasting peripheral venous blood (5 ml) was taken on the morning. After centrifugation at

2000 rpm for 10 min, the serum was obtained and stored at 2-8°C. The serum levels of IL-6, TNF- $\alpha$  and hs-CRP were determined using ELISA. The operation was in accordance with the manufacturer's instructions of kits (R&D Systems, Inc., MN, USA).

### Statistical analysis

All statistical analysis was carried out using SPSS17.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as mean  $\pm$  SD. Comparisons between two groups were performed using *t* test. The correlation of continuous variables was investigated using Pearson correlation analysis. P < 0.05 and P < 0.01 were considered as statistically significant and highly statistically significant, respectively.

## Results

### General data of LASO patients

In 65 LASO patients, 18 (27.69%) patients had smoking history. According to the severity of LASO, the patients were divided into gangrene group (10 cases, 15.38%), resting pain group (20 cases, 30.76%) and intermittent claudication group (35 cases, 53.86%). In addition, according to the diagnosis standards of diabetes, hypertension and hyperlipidemia, there were 16 (24.62%), 11 (16.92%) and 12 (18.46%) cases with complicated diabetes, hypertension and hyperlipidemia, respectively. The vascular scanning and arteriography examination showed that, there were 10 (15.38%), 28 (43.08%), 20 (30.76%) and 7 (10.78%) cases with LASO in abdominal aortic-iliac artery, iliac-femoral artery, femoral artery and abdominal aortic artery, respectively.

### Comparison of serum inflammatory factor levels and ABI between LASO group and control group

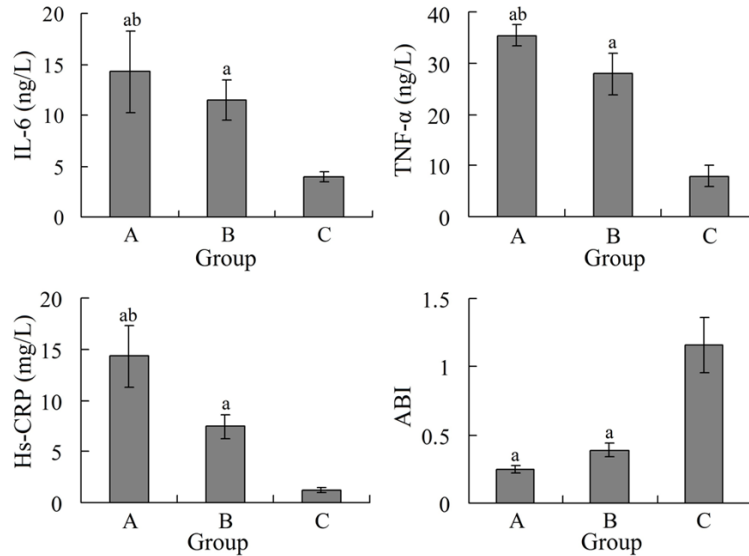
As shown in **Table 1**, the serum IL-6, TNF- $\alpha$  and hs-CRP levels in LASO group were 12.27 $\pm$ 2.45 ng/L, 30.83 $\pm$ 4.14 ng/L and 10.06 $\pm$ 2.63 mg/L, respectively, which were significantly higher than 12.27 $\pm$ 2.45 ng/L, 30.83 $\pm$ 4.14 ng/L and 10.06 $\pm$ 2.63 mg/L in control group, respectively (P < 0.01). The ABI in LASO group was 0.35 $\pm$ 0.05, which was significantly lower than 1.16 $\pm$ 0.28 in control group (P < 0.01).

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**Table 2.** Relations of LASO severity with serum inflammatory factor levels and ABI (mean  $\pm$  SD)

Group	n	IL-6 (ng/L)	TNF- $\alpha$ (ng/L)	Hs-CRP (mg/L)	ABI
Gangrene	10	15.56 $\pm$ 2.03 <sup>a,b,c</sup>	35.51 $\pm$ 3.35 <sup>a,b,c</sup>	15.62 $\pm$ 2.98 <sup>a,b,c</sup>	0.15 $\pm$ 0.04 <sup>a,b,c</sup>
Rest pain	20	12.66 $\pm$ 4.22 <sup>a</sup>	31.26 $\pm$ 4.83 <sup>a</sup>	9.93 $\pm$ 3.03 <sup>a,b</sup>	0.30 $\pm$ 0.12 <sup>a</sup>
Intermittent claudication	35	11.74 $\pm$ 2.02 <sup>a</sup>	28.88 $\pm$ 4.07 <sup>a</sup>	7.02 $\pm$ 3.11 <sup>a</sup>	0.37 $\pm$ 0.06 <sup>a</sup>
Control	30	3.93 $\pm$ 0.88	7.95 $\pm$ 1.21	1.23 $\pm$ 0.43	1.16 $\pm$ 0.28

<sup>a</sup>P < 0.01 compared with control group; <sup>b</sup>P < 0.01 compared with intermittent claudication group; <sup>c</sup>P < 0.01 compared with rest pain group. ABI, Ankle-brachial index; LASO, Lower-limb arteriosclerosis obliterans; IL-6, Interleukin-6; TNF- $\alpha$ , Tumor necrosis factor- $\alpha$ ; hs-CRP, High-sensitivity C-reactive protein.



**Figure 1.** Relations of complicated diabetes with serum inflammatory factor levels and ABI. A. LASO with complicated diabetes; B. LASO with no complicated diabetes; C. Control. <sup>a</sup>P < 0.01 compared with control group; <sup>b</sup>P < 0.05 compared with LASO with no complicated diabetes group. LASO, lower-limb arteriosclerosis obliterans; ABI, ankle-brachial index; IL-6, interleukin-6; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; hs-CRP, high-sensitivity C-reactive protein.

### Relations of LASO severity with serum inflammatory factor levels and ABI

**Table 2** showed that, the serum IL-6, TNF- $\alpha$ , hs-CRP and ABI in LASO with gangrene group were 15.56 $\pm$ 2.03 ng/L, 35.51 $\pm$ 3.35 ng/L, 15.62 $\pm$ 2.98 mg/L and 0.15 $\pm$ 0.04, respectively, those in LASO with rest pain group were 12.66 $\pm$ 4.22 ng/L, 31.26 $\pm$ 4.83 ng/L, 9.93 $\pm$ 3.03 mg/L and 0.30 $\pm$ 0.12, respectively, and those in LASO with intermittent claudication group were 11.74 $\pm$ 2.02 ng/L, 28.88 $\pm$ 4.07 ng/L, 7.02 $\pm$ 3.11 mg/L and 0.37 $\pm$ 0.06, respectively. The IL-6, TNF- $\alpha$  and hs-CRP levels in 3 LASO groups with different severity were significantly higher than those in control group (P < 0.01), and those in LASO with gangrene group were significantly higher than those in LASO with intermittent claudication group and LASO

with rest pain group, respectively (P < 0.05). The ABI in 3 LASO groups with different severity were significantly lower than that in control group (P < 0.01), and that in LASO with gangrene group was significantly lower than that in LASO with intermittent claudication and rest pain groups, respectively (P < 0.05).

### Relations of complicated diabetes with serum inflammatory factor levels and ABI

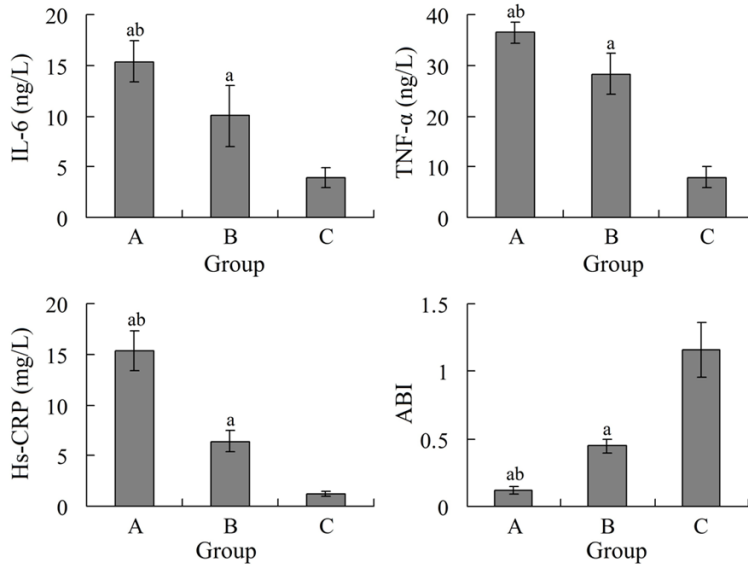
Serum IL-6 levels in LASO patients with and without complicated diabetes were 14.28 $\pm$ 4.23 and 11.46 $\pm$ 2.08 ng/L, respectively; the TNF- $\alpha$  levels in two groups were 35.46 $\pm$ 2.83 and 27.89 $\pm$ 4.36 ng/L, respectively; the hs-CRP levels in two groups were

14.34 $\pm$ 3.12 and 7.44 $\pm$ 1.21 mg/L, respectively; the ABI in two groups was 0.25 $\pm$ 0.03 and 0.39 $\pm$ 0.05, respectively. The IL-6, TNF- $\alpha$  and hs-CRP levels in LASO patients with and without complicated diabetes were significantly higher than those in control group, respectively (P < 0.01), and the levels in LASO patients with complicated diabetes group were significantly higher than those without, respectively (P < 0.05). ABI in LASO patients with and without complicated diabetes was significantly lower than that in control group, respectively (P < 0.01), with no significant difference between two LASO groups (P > 0.05) (**Figure 1**).

### Relations of complicated hypertension with serum inflammatory factor levels and ABI

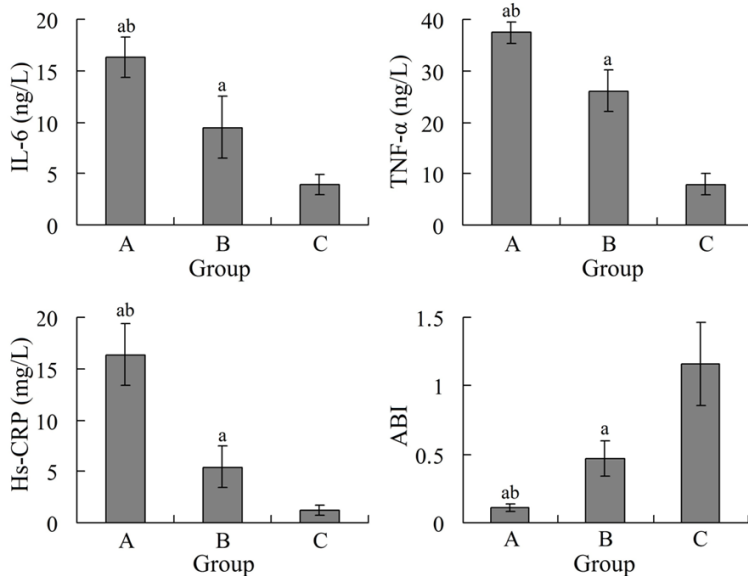
As shown in **Figure 2**, the serum IL-6 levels in LASO patients with and without complicated

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**Figure 2.** Relations of complicated hypertension with inflammatory factor levels and ABI. A. LASO with complicated diabetes; B. LASO with no complicated diabetes; C. Control. <sup>a</sup>P < 0.01 compared with control group; <sup>b</sup>P < 0.05 compared with LASO with no complicated diabetes group. LASO, lower-limb arteriosclerosis obliterans; ABI, ankle-brachial index; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; hs-CRP, high-sensitivity C-reactive protein.

hs-CRP levels in two groups were  $36.46 \pm 2.34$  and  $28.33 \pm 4.83$  ng/L,  $15.34 \pm 2.14$  and  $6.44 \pm 1.06$  mg/L, respectively; the ABI in two groups was  $0.12 \pm 0.04$  and  $0.45 \pm 0.05$ , respectively. The IL-6, TNF-α and hs-CRP levels in LASO patients with and without complicated hypertension were significantly higher than those in control group, respectively (P < 0.01), and those levels in LASO patients with complicated hypertension were significantly higher than those in LASO patients with no complicated hypertension, respectively (P < 0.05). ABI in LASO patients with and without complicated hypertension was significantly lower than that in control group, respectively (P < 0.01), and that in LASO patients with complicated hypertension was significantly lower than that in LASO patients with no complicated hypertension (P < 0.05).



**Figure 3.** Relations of complicated hyperlipidemia with serum inflammatory factor levels and ABI. A. LASO with complicated diabetes; B. LASO with no complicated diabetes; C. Control. <sup>a</sup>P < 0.01 compared with control group; <sup>b</sup>P < 0.05 compared with LASO with no complicated diabetes group. LASO, lower-limb arteriosclerosis obliterans; ABI, ankle-brachial index; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; hs-CRP, high-sensitivity C-reactive protein.

### Relations of complicated hyperlipidemia with serum inflammatory factor levels and ABI

Figure 3 showed that, the serum IL-6 levels in LASO patients with and without complicated hypertension were  $15.33 \pm 2.11$  and  $9.46 \pm 3.83$  ng/L, respectively; the TNF-α levels in two groups were  $37.44 \pm 2.16$  and  $26.11 \pm 4.01$  ng/L, respectively; the hs-CRP levels in two groups were  $16.33 \pm 3.10$  and  $5.24 \pm 2.01$  mg/L, respectively; the ABI in two groups was  $0.17 \pm 0.03$  and  $0.47 \pm 0.13$ , respectively. The IL-6, TNF-α and hs-CRP levels in LASO patients with and without complicated

hyperlipidemia were significantly higher than those in control group, respectively (P < 0.01), and those levels in LASO patients with complicated hyperlipidemia were significantly higher than those in LASO patients with no complicated hyperlipidemia, respectively (P < 0.05). ABI in LASO patients with and without complicated hyperlipidemia was significantly lower than that in control group, respectively (P < 0.01), and that in LASO patients with complicated hyperlipidemia was significantly lower than that in LASO patients with no complicated hyperlipidemia (P < 0.05).

hyperlipidemia were significantly higher than those in control group, respectively (P < 0.01), and those levels in LASO patients with complicated hyperlipidemia were significantly higher than those in LASO patients with no complicated hyperlipidemia, respectively (P < 0.05). ABI in LASO patients with and without complicated hyperlipidemia was significantly lower than that in control group, respectively (P < 0.01), and that in LASO patients with complicated hyperlipidemia was significantly lower than that in LASO patients with no complicated hyperlipidemia (P < 0.05).



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**Table 3.** Pearson correlation analysis of IL-6, TNF- $\alpha$  and ABI in LASO patients

Index	TNF- $\alpha$		ABI	
	r	P	r	P
IL-6	0.7457	< 0.05	-0.5623	< 0.05
TNF- $\alpha$	-	-	-0.7129	< 0.05

IL-6, Interleukin-6; TNF- $\alpha$ , Tumor necrosis factor- $\alpha$ ; ABI, Ankle-brachial index; LASO, Lower-limb arteriosclerosis obliterans.

and those in LASO patients with complicated hyperlipidemia were significantly higher than LASO patients with no complicated hyperlipidemia, respectively ( $P < 0.05$ ). ABI in LASO patients with and without complicated hyperlipidemia was significantly lower than that in control group, respectively ( $P < 0.01$ ), and that in LASO patients with complicated hyperlipidemia was significantly lower than that in LASO patients with no complicated hyperlipidemia ( $P < 0.05$ ).

### Results of correlation analysis

Pearson correlation analysis showed that, in LASO patients, the serum IL-6 and TNF- $\alpha$  levels were positively correlated ( $r = 0.7457$ ,  $P < 0.05$ ), and the IL-6 and TNF- $\alpha$  levels were negatively correlated with ABI, respectively (IL-6 with ABI:  $r = -0.5623$ ,  $P < 0.05$ ; TNF- $\alpha$  with ABI:  $r = -0.7129$ ,  $P < 0.05$ ) (Table 3).

### Treatment and follow up

In 65 LASO patients, 10, 22 and 11 cases received amputation (toe), bypass operation, and percutaneous transluminal angioplasty, respectively. Other 22 cases obtained the remission after drug therapy. The follow up was performed for 6 months to 2 years. In the reexamination, the mean serum IL-6, TNF- $\alpha$  and hs-CRP levels after treatment were significantly lower than those before treatment, respectively ( $P < 0.01$ ) (Table 4).

### Discussion

There are three types of arteriosclerosis, including atherosclerosis, artery medial calcification and thin artery sclerosis. Arteriosclerosis is a systemic vascular lesion. When arteriosclerosis occurs in the peripheral blood vessels and causes the distal limb ischemia, leading to a series of symptoms, it is known as the arteriosclerosis obliterans [12]. The pathogenesis of arteriosclerosis obliterans is not completely

clear. At present, the damage response theory is generally accepted, which proposes that the arteriosclerosis obliterans is a chronic inflammatory reaction [13]. LASO is the manifestation of systemic atherosclerosis in the local parts of body. It is the degenerative and proliferative change of the arterial intima and media [14]. IL-6, TNF- $\alpha$  and hs-CRP are the common indicators of inflammatory response. This study has explored the relationships of IL-6, TNF- $\alpha$  and hs-CRP with the LASO. It has provided a reference for further studying the mechanism of LASO and its prevention and treatment.

IL-6 is a multifunctional cytokine produced by macrophages, vascular endothelial cells, fibroblasts and activated T cells. It can induce the proliferation and differentiation of B cells, and activate the T cells, playing an important role in the induction and maintenance of inflammatory response [15]. The activated vascular endothelial cells can secrete a large amount of IL-6 which promotes the adhesion of monocytes and vascular endothelial cells, and their transformation into foam cells, so the lipid accumulation is formed, leading to the thickening of vascular intima and decrease of vascular elasticity, which finally causes the occurrence of atherosclerosis formation of plaque [16]. In addition, IL-6 can induce the liver to produce acute-phase C-reactive protein (CRP) and activate NK- $\kappa$ B signaling pathway for inducing the expression of ICAM-1 [17]. Previous study [18] has showed that, the increase of IL-6 level is related to the poor prognosis of arteriosclerosis, suggesting that the inflammatory reaction can promote the arteriosclerosis. Results of this study showed that, the serum IL-6 level in LASO group was significantly higher than control group ( $P < 0.01$ ), and there was significant difference between LASO with gangrene group and other LASO group, and between LASO patients with and without complicated diabetes, hypertension and hyperlipidemia, respectively ( $P < 0.05$ ). This indicates that, all the LASO severity, complicated diabetes, hypertension and hyperlipidemia can affect the serum IL-6 level.

TNF- $\alpha$  is a cytokine produced and released by macrophages, lymphocytes, smooth muscle cells and fibroblasts. Recent study [19] finds that, the positive rate of TNF- $\alpha$  is high in atherosclerotic plaques, which indicates that TNF- $\alpha$  plays an important role in the systemic and local inflammatory reaction in atherosclerosis. The main functions of TNF- $\alpha$  include producing

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**Table 4.** Comparison of serum IL-6, TNF- $\alpha$  and hs-CRP levels in 65 LASO patients between before and after treatment (mean  $\pm$  SD)

Group	n	IL-6 (ng/L)	TNF- $\alpha$ (ng/L)	Hs-CRP (mg/L)
Before treatment	65	12.27 $\pm$ 2.45	30.83 $\pm$ 4.14	10.06 $\pm$ 2.63
After treatment	65	5.68 $\pm$ 1.34 <sup>a</sup>	12.29 $\pm$ 3.04 <sup>a</sup>	3.17 $\pm$ 0.84 <sup>a</sup>

<sup>a</sup>P < 0.01 compared with before treatment. IL-6, Interleukin-6; TNF- $\alpha$ , Tumor necrosis factor- $\alpha$ ; hs-CRP, High-sensitivity C-reactive protein; LASO, Lower-limb arteriosclerosis obliterans.

the toxicity to endothelial cells, promoting adhesion of monocytes to endothelial cells, stimulating the proliferation of vascular smooth muscle cells, promoting the coagulation, and inhibiting the fibrinolysis [20]. Results of this study showed that, the serum TNF- $\alpha$  level had the change rule the same with IL-6. This suggests that, all the LASO severity, complicated diabetes, hypertension and hyperlipidemia can also affect the serum TNF- $\alpha$  level.

C-reactive protein (CRP) is found to be a kind of acute-phase protein associated with inflammation, which is involved in acute injury, infection or other inflammatory stimulation [21]. CRP is a  $\gamma$ -globulin synthesized by liver. When the tissue injury or inflammation occurs, the rate of synthesis of CRP in liver is increased, and its serum concentration is increased significantly [22]. Recent study [23] shows that, CRP is not only the inflammatory marker in the development of arteriosclerosis, but plays a direct role in the process of vascular injury. Because the reference range of CRP level in the healthy population is relatively wide (< 3 mg/L), it is difficult to accurately determine the its change in the body. Hs-CRP is a highly sensitive index of inflammatory response, and can be detected with a very low level (0.001 mg/L). Hs-CRP can be used to monitor the change of cardiovascular disease and predict the disease prognosis. In addition, it can be used to forecast the risk of cardiovascular disease in sub-healthy population [24]. In the present study, the serum hs-CRP level has the same change rule with IL-6 and TNF- $\alpha$ , which suggests that, the hs-CRP level is also affected by the LASO severity and the complicated diabetes, hypertension and hyperlipidemia.

There are certain correlations among IL-6, TNF- $\alpha$  and hs-CRP in the body. D'Auria et al [25] find that, in patients with bullous pemphigoid, the serum level of IL-6 is positively correlated with

that of CRP (P < 0.05). Stannus et al [26] find that, IL-6 and TNF- $\alpha$  are positively correlated in older adults with knee radiographic osteoarthritis and knee cartilage loss. Results of Rahmani et al's study find that, TNF- $\alpha$  and hs-CRP are positively correlated in chronic gastritis patients [27]. In the present study, the correlation analysis showed that, in LASO patients, the serum IL-6 and TNF- $\alpha$

level were positively correlated, and the IL-6 and TNF- $\alpha$  level were negatively correlated with ABI, respectively. This is basically consistent with the above results. In conclusion, IL-6, TNF- $\alpha$  and hs-CRP are involved in the occurrence and development of LASO, and they are the reliable indicators for judging the LASO severity as well as the related complications. This study still has some limitations. The sample size of this study is relatively small, which may affect the results. In the next studies, the sample size should be further increased for making the results more convincing.

### Disclosure of conflict of interest

None.

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