

Predictive value of P-selectin and endothelin-1 for vascular restenosis after interventional procedures for peripheral artery disease

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Abstract. Peripheral artery disease (PAD) is caused by the building up of plaques in the arteries that carry blood to the lower limbs. The present study aimed to assess the predictive value of the plasma levels of P-selectin (Ps) or endothelin-1 (ET-1) regarding the occurrence of vascular restenosis after endovascular therapy for PAD. Patients with or without vascular restenosis confirmed by computed tomography angiography after endovascular therapy between March and December 2015 (n=20 per group) were enrolled. The serum levels of Ps and ET-1 prior to the operation and at 1 h, as well as 1, 2 and 3 weeks after the operation were compared between the two groups. At 1 h after the operation, the serum levels of Ps and ET-1 were significantly increased as compared with the pre-operative levels ($P<0.05$). The serum levels of Ps and ET-1 at 1 h, as well as 1, 2 and 3 weeks after the operation in the restenosis group were significantly higher as compared with those in the non-stenosis group ($P<0.05$). However, for the non-stenosis group, the serum levels of Ps and ET-1 at 1, 2 and 3 weeks after the operation did not significantly differ from the pre-operative levels ($P>0.05$). The diagnostic sensitivity and specificity of the serum ET-1 levels at 1 h after the operation for predicting post-operative restenosis in PAD patients with a cut-off of 0.1089 pg/ml were 85 and 85%, respectively. In conclusion, the serum levels of Ps and ET-1 have a high predictive value for post-operative vascular restenosis after endovascular therapy for PAD patients.

Introduction

Peripheral artery disease (PAD) is caused by the formation of atherosclerotic plaques in the lower extremities, resulting in arterial stenosis and occlusion to finally lead to chronic ischemia of the extremities. Epidemiological surveys have indicated that PAD is positively correlated with age, and hypertension, hyperlipidemia, smoking and a family history of PAD are all risk factors for PAD (1,2). In China, the incidence of patients with PAD and arteriosclerosis obliterans of the lower extremities is increasing annually, and this is attributed to an aging population due to improved medical care and overall living standards (3). Minimally invasive endovascular therapies, including stent placement (4) and percutaneous transluminal angioplasty (PTA) (5), provide a safer and more reliable treatment for PAD. However, these therapies may cause damage to the vascular intima, leading to post-operative vascular restenosis (6). Furthermore, interventional procedures severely affect post-operative recovery and the quality of life of elderly PAD patients (7). Therefore, early prediction and treatment of post-operative vascular restenosis in PAD patients may greatly improve their prognosis (8). P-selectin (Ps) (9) and endothelin-1 (ET-1) (10) are important proteins that reflect the damage, activation and synthesis of vascular endothelial cells (VECs). In the present study, the serum levels of Ps and ET-1 were monitored in PAD patients after endovascular therapy and their correlation with post-operative vascular restenosis was assessed in order to determine their early predictive value regarding this adverse event.

Patients and methods

Patients. A total of 20 PAD cases with restenosis and another 20 PAD cases or without restenosis after endovascular therapy were enrolled in the present study between March 2015 and December 2015 from Taizhou Hospital Affiliated to Nanjing University of Chinese Medicine. The restenosis and non-restenosis groups were comparable in age, sex, incidence of concurrent hypertension or hyperlipidemia, location of the lesion, Rutherford classification and family history of PAD ($P>0.05$). The demographic and clinicopathological characteristics of the subjects at baseline are presented in Table I. All

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Table I. Comparison of the demographic and clinicopathological characteristics of the patients in the restenosis vs. non-restenosis group (n=20 per group).

Characteristics	Restenosis group	Non-restenosis group	t/ χ^2	P-value
Average age, years	63.20±6.39	62.70±6.18	0.252	0.803
Male, n (%)	12 (60)	11 (55)	0.102	0.794
Comorbidities				
Hypertension, n (%)	18 (90)	19 (95)	0.360	0.548
Hyperlipidemia, n (%)	16 (80)	13 (65)	1.129	0.288
Family history of PAD, n (%)	15 (75)	13 (65)	0.476	0.490
Smoking, n (%)	12 (60)	8 (40)	1.600	0.206
Rutherford stage, n (%)			0.702	0.873
III	3 (15)	5 (25)		
IV	9 (45)	8 (40)		
V	4 (20)	4 (20)		
VI	4 (20)	3 (15)		
Lesion site, n (%)			0.567	0.904
Unilateral iliac artery	6 (30)	5 (25)		
Simple superficial femoral artery	5 (25)	7 (35)		
Ipsilateral iliac and femoral artery	5 (25)	5 (25)		
Femoral popliteal artery	4 (20)	3 (15)		

Values are expressed as the mean \pm standard deviation or n (%).

patients provided written informed consent. All of the experiments were approved by the Ethics Committee of Nanjing University of Chinese Medicine (Nanjing, China).

Diagnostic methods and comparisons. Restenosis was diagnosed by post-operative examination using computed tomography angiography (CTA). Cases with and without restenosis were compared regarding age, sex, incidence of concurrent hypertension or hyperlipidemia, location of the lesion, Rutherford classification, family history of diseases and smoking status. The serum levels of Ps and ET-1 prior to the operation and at 1 h, 1, 2 and 3 weeks after the operation were compared within and between groups.

Processing and detection of blood samples. From each subject, 5 ml blood was drawn on the morning prior to the operation and at 1 h, 1, 2 and 3 weeks after the operation. The blood samples were collected in tubes containing Na²EDTA anti-coagulant and centrifuged at 1,500 x g for 10 min. The supernatant was collected and preserved at -80°C or directly used for the detection of Ps and ET-1. Serum Ps was detected using a human Ps detection kit (cat. no. P1038; Shanghai Airui Biotechnology Co., Ltd., Shanghai, China) and serum ET-1 was detected using a human ET-1 detection kit (cat. no. E2567; Shanghai Yu Bo Biotech Co., Ltd., Shanghai, China), which were performed in triplicate.

Statistical analysis. Data were analyzed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). The patient age and serum levels of Ps and ET-1 were expressed as the mean \pm standard deviation. An independent-samples t-test was performed to determine the significance of differences

between the two groups. Count data were expressed as n (%) and compared between the two groups using the Chi-squared test. The predictive value of Ps and ET-1 for restenosis was determined by generating receiver operating characteristics (ROC) curves and determining the area under the ROC curve (AUC), as well as the sensitivity and specificity at optimal cut-off values. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of pre- and post-operative CTA observations between the two groups. The position of the lesions was confirmed by CTA. The two representative cases in Fig. 1A and B had stenosis of the iliac artery and received PTA combined with stent placement. They were followed up for 1 year post-operatively. The first representative case had post-operative restenosis (Fig. 1C), while the second one had no restenosis (Fig. 1D).

Comparison of serum Ps levels at different time-points. Compared with the pre-operative level, the level of serum Ps 1 h after the operation was significantly increased in the PAD cases (P<0.05), and then declined over time until stabilizing 2 weeks post-operatively. The serum Ps levels at 1 h, 1, 2 and 3 weeks after the operation in the restenosis group were significantly higher than those in the non-restenosis group (P<0.05). Within the restenosis group, the post-operative Ps levels were also significantly higher than the pre-operative ones (P<0.05). However, for the non-restenosis group, the serum Ps levels at 1, 2 and 3 weeks post-operatively were not significantly different from the pre-operative levels (P>0.05; Table II).

Table II. Comparison of serum P-selectin levels at different time-points (ng/ml).

Groups/time	Restenosis group (n=20)	Non-restenosis group (n=20)	t	P-value
Pre-operative	20.54±2.40	20.97±2.13	0.594	0.556
Post-operative				
1 h	41.67±3.89 ^a	34.82±3.16 ^a	6.123	<0.001
1 week	34.19±3.76 ^a	22.63±3.69	9.817	<0.001
2 weeks	32.15±2.56 ^a	19.95±2.05	16.616	<0.001
3 weeks	32.00±2.77 ^a	19.88±1.76	16.504	<0.001

^aP<0.05, compared with the pre-operative level for the same group.

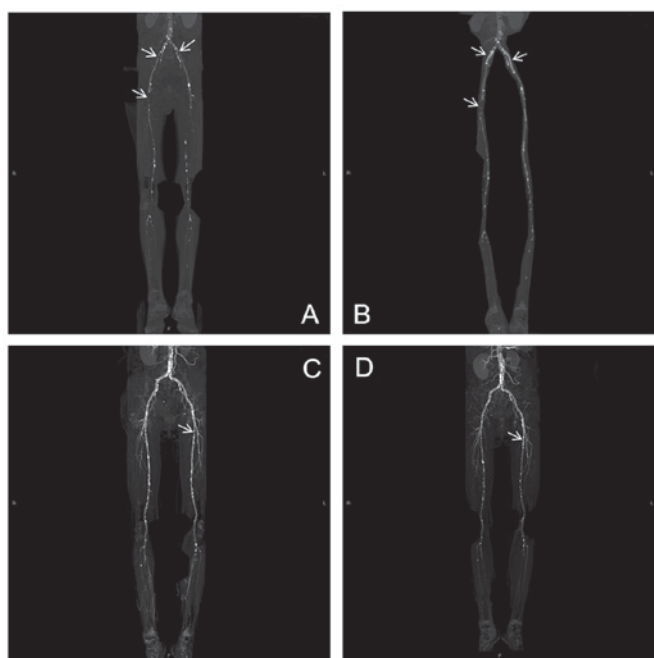


Figure 1. Representative pre- and post-operative CTA images for the two groups. (A) Right stenosis indicated by pre-operative CTA in the restenosis group and (B) right restenosis indicated by post-operative CTA in the restenosis group from a 54-year-old male patient. (C) Left stenosis indicated by pre-operative CTA in the non-restenosis group and (D) no restenosis on the left indicated by post-operative CTA in the non-restenosis group from a 52-year-old male patient. The site of (previous) (re)stenosis is indicated by arrows. CTA, computed tomography-angiography.

Comparison of serum ET-1 levels at different time-points. As compared with the pre-operative level, the serum ET-1 level at 1 h after the operation was significantly increased in all PAD cases ($P<0.05$), and it declined afterwards until stabilizing at 2 weeks post-operatively. The serum ET-1 levels at 1 h, 1, 2 and 3 weeks post-operatively in the restenosis group, were significantly higher than those in the non-restenosis group ($P<0.05$). Within the restenosis group, they were also significantly higher than the pre-operative levels ($P<0.05$). However, in the non-restenosis group, the serum ET-1 levels at 1, 2 and 3 weeks post-operatively were not significantly different from the pre-operative levels ($P>0.05$; Table III).

Predictive value of Ps and ET-1 levels for post-operative restenosis. ROC curves comparing the sensitivity vs.

specificity of the serum levels of Ps or ET-1 for predicting post-operative restenosis were plotted using SPSS 19.0 software (Fig. 2). For the serum levels of Ps as well as ET-1, the AUC was >0.5 at each time-point post-operatively. The AUC increased over time, indicating a better diagnostic value of the later time-points for post-operative restenosis. However, in the clinic, earlier discovery and treatment of post-operative restenosis for PAD cases results in better the outcomes.

The optimal diagnostic threshold at 1 h post-operatively was 38.83 ng/ml for serum Ps levels, as calculated from the ROC curve. The corresponding sensitivity and specificity for predicting restenosis were 75 and 90%, respectively. The optimal diagnostic threshold at 1 h post-operatively was 0.1089 ng/ml for serum ET-1 levels, as calculated from the ROC curve. The corresponding sensitivity and specificity for predicting restenosis were 85 and 85%, respectively.

Discussion

VECs provide a barrier between the vascular system and underlying tissues. The nitric oxide, endothelin, angiotensin and growth factors secreted by VECs mediate vascular relaxation and contraction. Arteriosclerosis may induce the secretion of heparin-like substances, smooth muscle relaxant factors and smooth muscle constricting factors by VECs, thus inhibiting the proliferation and migration of VECs (9,10). Abnormal proliferation and migration of the VECs are considered as the major reasons for post-operative restenosis for PAD patients, as they result in intimal hyperplasia and remodeling of elasticity of vascular walls (11). Interventional therapies for PAD patients may cause damage to the vascular endothelium, leading to decreased secretion of factors that inhibit the proliferation and migration of smooth muscle cells (SMCs); this in turn may cause restenosis to occur (6). In addition, damage of VECs may further promote thrombosis and vasoconstriction, leading to restenosis. Therefore, evaluating the damage of VECs in PAD patients after endovascular therapy is of high importance for predicting post-operative restenosis.

Ps is an important member of the selectin family of cell adhesion molecules (12). Under normal physiological conditions, Ps rests on the granule membrane of platelets. Once activated by VEC damage, the platelets release the platelet α granules and Ps is released along with them. Therefore, Ps is considered as an indicator of the activated state of the platelets. For PAD patients who have received interventional therapy,

Table III. Comparison of serum endothelin-1 levels (pg/ml) at different time-points.

Groups/time	Restenosis group (n=20)	Non-restenosis group (n=20)	t	P-value
Pre-operative	75.29±3.62	75.16±3.56	0.110	0.913
Post-operative				
1 h	114.21±4.60 ^a	101.97±5.18 ^a	7.901	<0.001
1 week	98.58±5.16 ^a	80.66±5.46	10.664	<0.001
2 weeks	97.95±5.88 ^a	77.39±4.79	12.114	<0.001
3 weeks	97.71±5.34 ^a	77.04±3.34	14.680	<0.001

^aP<0.05, compared with the pre-operative level for the same group.

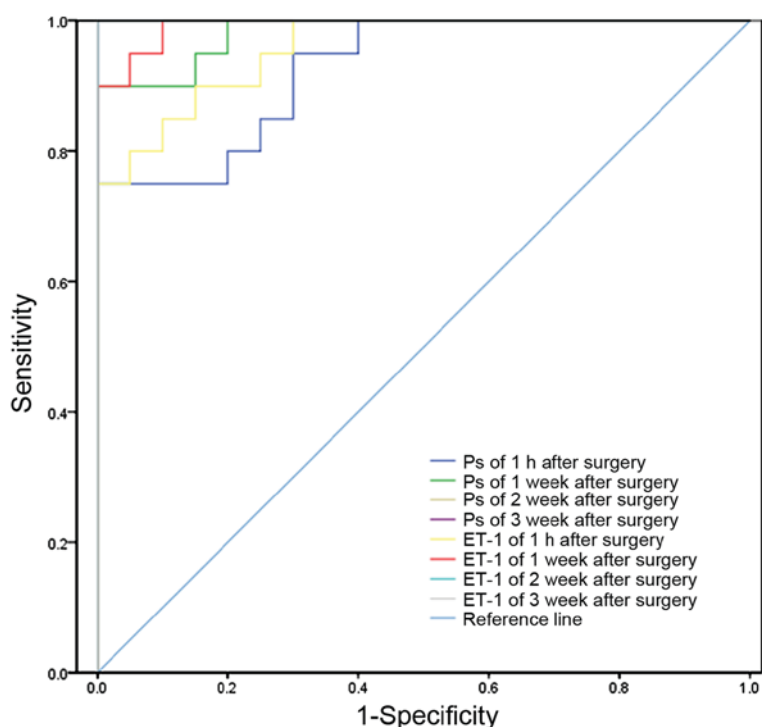


Figure 2. Receiver operating characteristic curves for assessing the utility of serum Ps and ET-1 levels determined at different post-operative time-points in diagnosing restenosis. Ps, P-selectin; ET-1, endothelin-1.

the activated state of the platelets may be used to assess the degree of VEC damage. Therefore, Ps is an indirect indicator of VEC damage. In the present study, the serum Ps levels were significantly increased in PAD patients at 1 h after the operation as compared with the pre-operative levels ($P<0.05$). Thomas *et al* (13) reported a significant correlation between Ps levels and the post-operative onset of cardiovascular events in acute coronary syndrome. According to Myers Jr *et al* (14), oral Ps inhibitor PSI-697 reduced thrombosis in rats with venous stenosis. This means that Ps levels in the blood are not only an indicator of VEC damage, but also associated with thrombosis and restenosis. Inhibiting blood Ps may help reduce restenosis in PAD cases.

In the present study, the serum Ps levels at 1 h, 1, 2 and 3 weeks post-operatively in the restenosis group were significantly higher than those in the non-restenosis group ($P<0.05$); they were also considerably increased as

compared with the pre-operative level within the restenosis group ($P<0.05$). However, for the non-restenosis group, there was no significant difference in the serum Ps levels at 1 h, 1, 2 and 3 weeks post-operatively as compared with the pre-operative level ($P>0.05$). For the serum Ps levels, the sensitivity and specificity for predicting restenosis in PAD patients were 75 and 90%, respectively, with a cut-off at 38.85 ng/ml. Ps on the platelet α granule membrane not only promotes the expression and secretion of tissue factors by the leukocytes, thus triggering coagulation, but also recruit neutrophil granulocytes, thus aggravating VEC damage. This further promotes thrombosis and increases the risk of restenosis (15). Ps is a member of the selectin family of cell adhesion molecules. It is expressed on stimulated endothelial cells and activated platelets, and mediates leukocyte rolling on stimulated endothelial cells, as well as heterotypic aggregation of activated platelets onto leukocytes (16). The importance of Ps-mediated cell

adhesive interactions in the pathogenesis of inflammation and thrombosis has been demonstrated in Ps-knockout mice (17). Therefore, the post-operative serum Ps levels in PAD cases may provide information on VEC damage and serve in the prediction of restenosis.

ET-1 is a vasoconstrictor secreted mainly by endothelial cells. It activates the Ca²⁺ channel of vascular SMCs by binding to receptors and then induces contraction of vascular SMCs. In the present study, a significant increase in the serum ET-1 levels was identified in PAD patients at 1 h post-operatively (P<0.05). Furthermore, the ET-1 levels at 1 h, 1, 2 and 3 weeks post-operatively in the restenosis group were significantly higher than those in the non-restenosis group (P<0.05); they were also higher than the pre-operative levels within the restenosis group (P<0.05). However, for the non-restenosis group, the serum ET-1 levels at 1 h, 1, 2 and 3 weeks post-operatively were not significantly different from the pre-operative level (P>0.05).

The reasons for the increased ET-1 secretion after endovascular therapy remain to be fully elucidated. Upregulation of ET-1 promotes the proliferation and migration of SMCs, thus leading to intimal hyperplasia and post-operative restenosis (18). According to Biasin *et al* (19) and Jiang *et al* (20) upregulation of ET-1 resulted in the proliferation of pulmonary artery SMCs. Under normal conditions, a dynamic balance between vasoconstrictive factors (e.g., ET-1) and vasodilatory factors (e.g., nitric oxide) prevails. However, upregulation of ET-1 following endovascular therapy disturbs this balance, leading to enhanced platelet adhesion, thrombosis, and abnormal proliferation and migration of SMCs (21,22). This finally results in restenosis. Therefore, the ET-1 levels after endovascular therapy may serve as a predictive marker for restenosis. Based on the present results, the sensitivity and specificity of serum ET-1 with a cut-off at 0.1089 pg/ml at 1 h after the operation for predicting restenosis were 85 and 85%, respectively.

In conclusion, the levels of serum Ps and ET-1 in PAD patients after endovascular surgery are of great clinical value for predicting post-operative vascular restenosis.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Authors' contributions

NC and TL contributed to the concept and design of the study. NC and TL analyzed and interpreted the patient data. LC, SJ and ZW analyzed the data. NC, LC and TL prepared the manuscript. All authors read and approved the final manuscript.

Ethical approval and consent to participate

All of the experiments were approved by the Ethics Committee of Nanjing University of Chinese Medicine (Nanjing, China). All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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