Research Article
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Markers of the Development of the Reconstruction Area Restenosis after Endovascular Interventions in Lower Limbs Arteries

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INTRODUCTION Restenosis of the reconstruction zone is one of the main postoperative complications of vascular reconstructions, which develops in 18–40% of cases and leads to an increase in the risk of limb loss to 20–25%. The search for new markers for predicting the development of this complication is an urgent problem today.

AIM OF STUDY To assess the dynamics of markers of apoptosis and cell proliferation at different times after endovascular intervention and their role in the development of restenosis of the reconstruction zone in patients with peripheral arterial disease (PAD).

MATERIAL AND METHODS The clinical study included 30 patients with PAD stage III disease. After further examination, the patients underwent endovascular intervention on the arteries of the femoral-popliteal segment. If restenosis developed, the patients were re-operated. In patients before surgery, within the first hour, and then on days 1, 7, 14, 21, 30 after the operation, venous blood was taken to determine the markers Bax, sFas, PDGF BB using enzyme immunoassay.

RESULTS After endovascular intervention, the first wave of apoptosis was triggered with an increase in the amount of proapoptotic protein Bax (p=0,00003) from 1 to 24 hours, followed by a decrease by day 7 (p=0.0008) compared to the amount on day 1. The PDGF BB level increased from day 1 after surgery (p=0.03) with maximum values on day 7 (p=0.0002) compared to the level on day 1. Then the second wave was triggered with a peak decrease in the level of the apoptosis inhibitor sFas on day 21 (p=0.002). After 9-12 months, restenosis of the intervention zone with a return of limb ischemia developed in 10 patients. During the first hour (p=0.004) in patients with restenosis, the level of Bax protein was significantly increased, with an increase in the level of PDGF BB by day 7 (p=0.011), and sFas by day 21 (p=0.0001), PDGF BB by the end of 1 month (p=0.004) compared to values in patients without this complication.

CONCLUSION 1. Endovascular intervention causes two waves of apoptosis in the postoperative period. The first wave is associated with an increase in Bax protein in the first hours, followed by an increase in PDGF BB on day 7. The second wave of apoptosis is due to a decrease in the inhibitor of apoptosis — sFas for 21 days against the background of the shift of the PDGF BB to the initial level. 2. An increase in Bax protein within the first hours after surgery in the course of PDGF BB growth on day 7 with an increased amount of sFas on day 21 leads and PDGF BB by the end of 1 month leads to the development of restenosis of the intervention area.

Keywords: restenosis, Bax, PDGF BB, sFas, atherosclerosis

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Bax – Bcl-2 associated X protein
EC – endothelial cells
NH – neointimal hyperplasia
AOLE – atherosclerosis obliterans of the lower extremities
PDGF BB – platelet-derived growth factor bb
PTBA – percutaneous transluminal balloon angioplasty
sFas – soluble Fas receptor
SMC – smooth muscle cells

INTRODUCTION

Atherosclerosis obliterans of the arteries of the lower extremities (AOLE) is a widespread disease that occurs in 20% of the world's population. Endovascular intervention is one of the leading methods of treating patients with AOLE, which allows obtaining satisfactory results. However, the development of restenosis of the reconstruction zone, which is based on the formation of neointimal hyperplasia (NH), levels the success of the operation and occurs in 30–40% of cases [1].

The development of restenosis in the postoperative period leads to thrombosis of the reconstruction zone and the return of limb ischemia, which increases the risk of limb loss to 20–25% with an increase in mortality up to 30%. Despite numerous studies devoted to the study of the pathogenesis of this complication, it remains not completely understood [2–4].

Thus, in the vascular wall there is a delicate balance between cell proliferation and their apoptosis. Shifts in this ratio promote the spread of smooth muscle cells (SMCs) from the media to the intima and the synthesis of the extracellular matrix, which is the main sign of NH [2, 3]. Much less work is devoted to the study of the role of cell death in the development of restenosis. Experimental studies have shown that the apoptosis system is triggered in the first hours after an operative injury, correlating with the formation of NH in the postoperative period [4].

The apoptosis cascade is triggered in two main ways: through the internal one, the Bcl2 protein family, and through the external receptor way, Fas-Fas ligand (FasL) [5].

Fas is a member of the tumor necrosis factor receptor family that is expressed in all cell types, while FasL is found predominantly in activated T lymphocytes, natural killer cells, macrophages, endothelial cells (ECs) and SMCs. When FasL binds to Fas, soluble Fas (sFas), which is a natural inhibitor of apoptosis, is activated by blocking this interaction [6]. It has been shown in animal models that the vector delivery of FasL to the area of the damaged artery leads to a decrease in the formation of NH [7].

Proteins of the Bcl 2 family are key regulators of programmed cell death, which is critical for the development of an organism and the maintenance of tissue homeostasis [8]. One of the main members of this family is the proapoptotic Bax protein. An increased amount of this protein is observed in atherosclerotic plaques (mainly in SMCs and macrophages). The antiapoptotic protein Bcl-XL is abundantly expressed in normal SMC media, but is suppressed after surgery [9].

Platelet growth factor BB (PDGF BB) is one of the main mitogens and chemoattractants of vascular wall cells, which is secreted by platelets, ECs, SMCs, and macrophages. A few seconds after the surgical injury, platelets begin to adhere to the damaged arterial wall and degranulate with the release of PDGF BB, which, by binding to its receptors, triggers the proliferation and migration of SMCs, followed by the formation of NH [10]. C. Rutherford et al. showed that the use of antibodies against PDGF BB and fibroblast growth factor leads to a decrease in the thickness of the NH by 83.8% after surgical interventions [11].

However, the relation between cell death, proliferation, and their role in the formation of NH after arterial reconstruction remains unclear. Identifying biomarkers that could potentially help identify patients at high risk of developing restenosis will provide new ways to prevent this complication.

Aim of study is to assess the level of dynamics and the relationship between markers of apoptosis and cell proliferation at different times after endovascular intervention and their role in the development of restenosis of the reconstruction area

MATERIAL AND METHODS

A prospective, open, non-randomized study included 30 patients with AOLE who were treated in the Department of Vascular Surgery in 2019–2021. The study was approved by the local ethics committee of the I.P. Pavlov Ryazan State Medical University. All patients were with stage III of the disease according to the classification of A.V. Pokrovsky-Fontein. The mean age was 64.3±3.4 years, the number of men was 24 (80%) (Table 1). To compare the studied parameters, 20 healthy volunteers were additionally included as a control group: the average age was 62.3±2.5 years, the number of men was 15 (75%), (p = 0.243). Exclusion criteria: decompensated somatic pathology, diabetes mellitus, active cancer, or a remission period of less than 5 years.

Table 1

Clinical characteristics of patients

	Patients with obliterating atherosclerosis of lower limb arteries, n (%)
Hypertensive disease	14 (46.6%)
Coronary artery disease	12 (40%)
Family history	14 (46.6%)
Smoking	16 (53.3%)
Body mass index	27.3±3.3

All patients underwent endovascular intervention on the arteries of the femoropopliteal segment (Table 2). Percutaneous transluminal balloon angioplasty (PTBA) was performed by experienced specialists using standard methods. The decision to implant a stent was left to the discretion of the operating surgeon. Antiplatelet and anticoagulant therapy in the perioperative period were prescribed in accordance with the National Guidelines for the Diagnosis and Treatment of Lower Extremity Arterial Disease [12].

Table 2

Angiographic characteristics of patients

Type of lesion A / B / C / D (according to TASC II classification)	10/10/10/–
Angioplasty of the femoral-popliteal segment	16 (53.4%)
Angioplasty followed by stenting of the femoral-popliteal segment	14 (46.6%)

After obtaining informed consent, blood samples were taken under baseline conditions (immediately before the intervention), in the first hours, on the 1st, 7th, 14th, 21st, and 30th days after the operation. Determination of the amount of proteins Bax, PDGF BB, sFas in blood serum was performed using enzyme immunoassay using commercial kits. The amount of protein Bcl 2 Associated X Protein (BAX) - using the set "Cloud-Clone Corporation" (China), sFas - using the set "Invitrogene Thermo Fisher" (USA), the amount of PDGF BB - using the set "Invitrogen Thermo Fisher" (USA) according to the manufacturer's instructions.

Patients were followed up 3, 6 and 12 months later, or earlier in case of restenosis of the intervention zone (n = 10) in the presence of objective signs of ischemia.

Statistical data analysis was performed using the STATISTICA 10.0 statistical software package. Due to the normal distribution of data (the Shapiro–Wilk test was used, p >0.05), parametric tests were used for further analysis. To assess the statistical significance of differences within groups, repeated measures analysis of variance (ANOVA) was used, pairwise comparisons were performed using the Newman–Keuls test. Intergroup differences (between patients in the control and experimental groups, patients with restenosis and without this complication) were assessed using Student's t -test.

To assess the relationship among the studied parameters, the Pearson correlation coefficient was calculated. The accepted level of statistical significance is p <0.05. Numerical data are presented as arithmetic mean and standard deviation.

RESULTS

During the study, it was shown that of the initial indicators, only the amount of Bax protein was increased ($p = 0.041$), and the values of PDGF BB ($p = 0.613$), sFas ($p = 0.479$) were comparable with their values in healthy volunteers (Table 3).

Table 3

Baseline Bax, PDGF BB, sFas in patients with lower limbs obliterating atherosclerosis in comparison with healthy volunteers

Indicators, M± σ	Bax, ng/ml	sFas, ng/ml	PDGF BB, ng/ml
Healthy volunteers	14.9±1.5	1.1±0.34	8.5±6.1
Patients with AOLE	20.4±4.9	0.96±0.4	10.2±4.8
R	0.041*	0.479	0.613

Note: * - statistically significant difference. AOLE – atherosclerosis obliterans of lower extremities

After endovascular intervention, apoptosis rates began to increase immediately after surgery. Thus, the highest value of the pro-aptotic Bax protein ($p = 0.00003$) was obtained in the first hour after the operation. Until the end of the first day, the amount of Bax protein remained elevated ($p = 0.196$) compared with the first hour, followed by a decrease by the 7th day compared to the 1st day ($p = 0.0008$). The level of the apoptosis receptor pathway inhibitor sFas did not differ significantly in the indicated time intervals: by the first hour ($p = 0.128$), by the 1st ($p = 0.640$) and 7th ($p = 0.719$) days.

Starting from the 14th day, the second wave of apoptosis was launched: there was a decrease in sFas ($p = 0.233$) against the background of an increase in the amount of Bax protein ($p = 0.198$) compared with the amount on the 7th day. On the 21st day, sFas significantly decreased ($p = 0.002$) with a further increase in the level of Bax protein ($p = 0.128$) compared with the level on the 14th day. By the end of the first month, the amount of Bax protein decreased ($p = 0.02$) with an increase in sFas values ($p = 0.0001$) (Fig. 1, 2) compared with their amount on the 21st day.

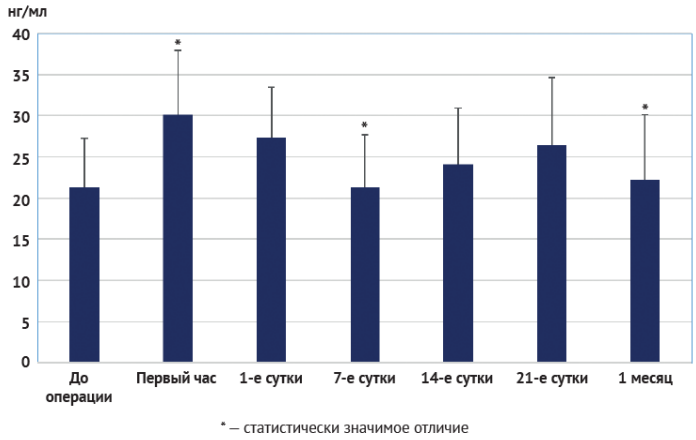

Fig. 1. The dynamics of changes in the proapoptotic protein Bax, different periods of the postoperative period

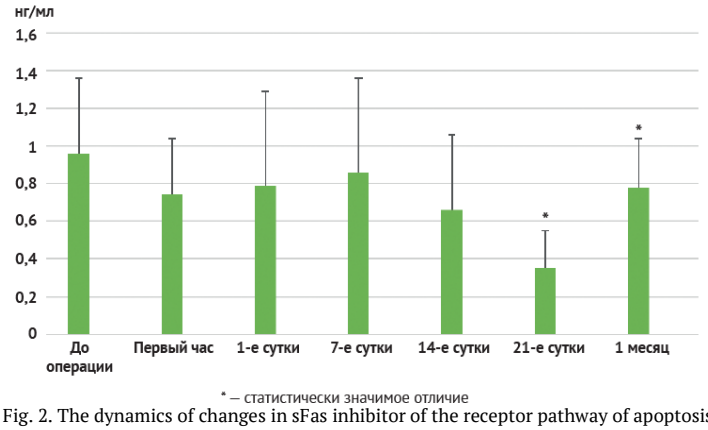

Fig. 2. The dynamics of changes in sFas inhibitor of the receptor pathway of apoptosis in different terms of the postoperative period

In the first hours after surgery, the level of PDGF BB (p = 0.609) did not change, with a subsequent increase by the end of the 1st day (p = 0.03) compared to the first hour and a maximum level on the 7th day (p = 0.0002) compared to day 1. Until the 14th day, the value of this protein did not change (p = 0.541) with a statistically significant decrease in its value by the 21st day (p = 0.002). By the end of the 1st month, the level of PDGF BB did not change statistically significantly compared to the initial values (p = 0.199) (Fig. 3).

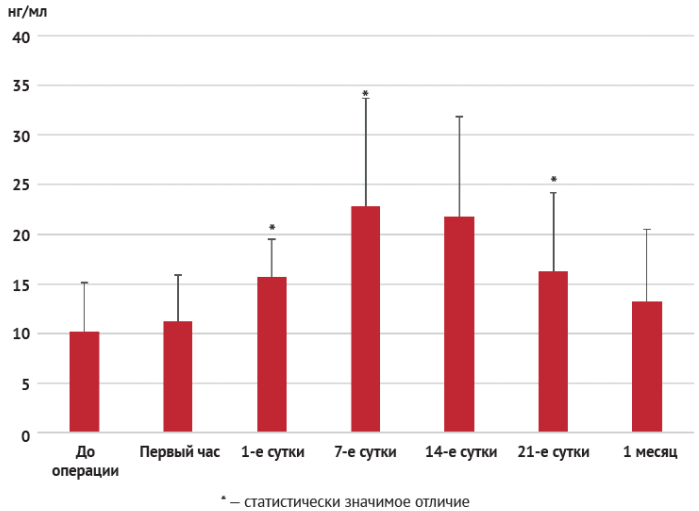

Fig. 3. The dynamics of platelet-derived growth factor BB at different times in the postoperative period

During the statistical processing of the study results, correlations were obtained between the markers Bax and PDGF BB on the 1st day (r = +0.639, p = 0.0002), PDGF BB and Bax on the 7th day (r = - 0.501, p =0.006), PDGF BB and sFas (r =-0.423, p =0.022) on day 14, PDGF BB and sFas (r =-0.488, p =0.007) on day 21 after surgery.

In 10 patients (33%) after 9–12 months, restenosis of the intervention area developed with the return of limb ischemia, which was confirmed by aortoarteriography. According to the initial type of lesion (p = 0.308) according to the TASC II classification and the type of endovascular intervention performed (p = 0.871), these patients were comparable to patients without restenosis. They were reoperated (5 patients underwent femoropopliteal shunting, 5 patients underwent repeated endovascular intervention) with compensation of blood circulation in the lower extremities.

In patients with restenosis of the reconstruction area, the initial values of PDGF BB (p = 0.944), sFas (p = 0.227), and Bax (p = 0.413) did not differ significantly from their values in patients without restenosis. However, in the first hour (p = 0.004) in patients with restenosis, the level of the Bax marker was statistically significantly increased, with an increase in the values of PDGF BB on the 7th day (p = 0.011), and sFas on the 21st day (p = 0.0001), PDGF BB in 1 month (p = 0.004) compared with the values in patients without this complication (Fig. 4).

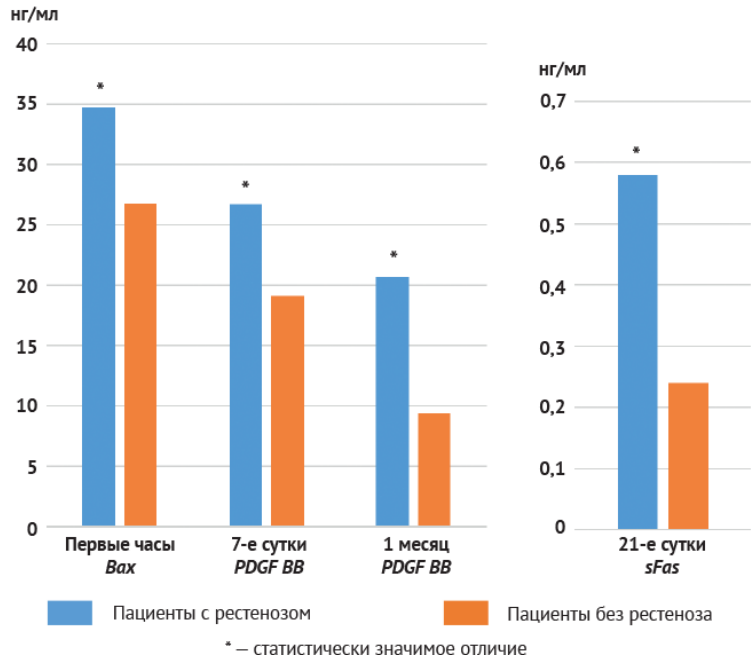

Fig. 4. The comparison of Bax, PDGF BB, sFas indices in patients with and without restenosis of the reconstruction area

DISCUSSION

Apoptosis is an active programmed process that leads to phagocytosis of dying cells without the development of an inflammatory reaction, aimed at maintaining the cellular structure of the tissue [13].

Our study showed that endovascular intervention triggers the apoptosis system of arterial wall cells by activating the Bcl 2 protein family and the apoptosis receptor pathway (sFas), which proceeds in two waves.

The first wave is a rapid burst of apoptosis with a maximum increase in Bax protein values by the first hour. Our data are consistent with the results of experimental studies on animal models, in which PTBA led to the induction of media SMC apoptosis with maximum expression of markers after 30 minutes [2]. The consequences of early apoptosis provoke a strong proliferative response to overcome the cellular deficit. This is due to the fact that dying cells release cytokines that enhance the proliferation and migration of SMCs from the media to the intima in response to injury [14].

In the course of our study, after the peak of apoptosis, a proliferative response followed with maximum PDGF BB values by the end of the first week and maintaining elevated values up to 14 days. It is known that the accumulation of SMCs in the NH of damaged rat arteries reaches its maximum level after 2 weeks with continued cell proliferation up to 12 weeks. At the same time, the total number of cells in the walls of the artery does not increase by the 12th week. The mechanism of this phenomenon has not been fully determined [15]. We assume that the activation of the second wave of apoptosis limits this process. It occurs much later after injury and with less frequency. In our study, from the 14th day, a decrease in the level of the apoptosis receptor pathway inhibitor sFas was observed, maximum on the 21st day, which led to a significant decrease in the amount of PDGF BB. This was confirmed by the conducted correlation analysis. By the end of the 1st month, the studied parameters tended to return to the initial level. The end of the second wave may be due to endothelialization of the intervention zone. Thus, M.L. Bochaton-Piallat et al. proved that on the 45th day re-endothelialization of the intervention area occurs and further cell death stops [16].

Activation of the predominantly receptor pathway in the second wave is due to the fact that SMCs of the developing NH are sensitive to the receptor pathway of apoptosis. In contrast to SMCs, ECs are usually resistant to Fas -mediated apoptosis due to the synthesis of the FLIP protein (a protein that inhibits caspase 8). This is the uniqueness of this system, which, while inducing apoptosis in the SMC of the developing NH, does not interfere with the process of reendothelialization of the reconstruction zone. In addition, Fas-FasL suppresses the inflammatory response by killing T cells and macrophages, stimulating the production of anti-inflammatory

interleukin-10, and also prevents extravasation of leukocytes into the vascular wall. Considering all the possibilities of this system, its activation in the second wave helps limit the growth of NH [17–19].

In the present study, it was possible to demonstrate the role of apoptosis markers in the development of restenosis in the reconstruction zone after endovascular interventions.

Patients who developed restenosis had a statistically significant increase in the amount of Bax protein in the first hours after surgery, which led to a pronounced increase in PDGF BB on the 7th day. In turn, an increased amount of sFas, on the one hand, could not compensate for the enhanced proliferative response on the 7th day, and on the other hand, enhance the inflammatory response by blocking the action of FasL. Subsequently, this led to an increase in the amount of PDGF BB by the end of the 1st month, followed by the development of restenosis after 9–12 months. A more significant cell death in the first wave of apoptosis leads to an enhanced proliferative response. And without sufficient apoptotic cell death in the second wave, continued proliferation led to the development of NH.

The limitation of our work is that so far the dynamics of only one of the representatives of each apoptosis pathway has been studied, but we continue to study a larger number of indicators that perform opposite functions. Another limitation is the small sample size and the relatively small number of patients with restenosis. Therefore, our findings should be viewed as generating hypotheses that need to be confirmed by larger studies.

CONCLUSIONS

1. Endovascular intervention causes two waves of apoptosis in the postoperative period. The first wave is associated with an increase in the blood level of the Bax protein within the first hours, followed by an increase in PDGF BB on the 7th day. The second wave of apoptosis was caused by a decrease in the level of the apoptosis inhibitor, sFas, on the 21st day against the background of a shift in PDGF BB to the initial level.

2. An increase in the blood level of the Bax protein in the first hours after surgery against the background of an increase in PDGF BB on the 7th day with an increased value of sFas on the 21st day, PDGF BB for 1 month can lead to the development of restenosis of the intervention zone.

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