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The effect of the geometry of the proximal anastomosis, markers of apoptosis and cell proliferation on the long-term patency following reconstructive interventions on the femoropopliteal arterial segment

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Abstract

Aim. To study changes in the topography of the orifice of the deep femoral artery (DFA), markers of proliferation, and apoptosis in patients after open interventions on the femoropopliteal arterial segment.
Methods. The study included 35 patients with atherosclerotic peripheral arterial disease (PAD), femoral-popliteal occlusion, stage IIB–III of the disease according to the classification of A.V. Pokrovsky–Fontaine, who underwent open surgery. The average age of the patients was 69±4.6 years. These patients included 26 men. Patients were divided into two groups: group A included 18 patients who underwent femoral-popliteal prosthetics (distal End-To-End bypass anastomoses), group B included 17 patients with femoral-popliteal bypass surgery (distal End-To-Side bypass anastomoses). The groups were comparable in terms of age and disease severity (p >0.05). Determination of serum platelet-derived growth factor BB (PDGF BB) and soluble form Fas (sFas) levels was carried out immediately before the intervention, on the 1st, 7th days, and 1 month after the operation. Duplex scanning (DS) was performed on day 7, after 1 and 18 months. Statistica 10.0 software was used for statistical data processing. The significance of differences between unrelated samples was assessed using the Student's t-test. The correlations between variables were analyzed by using Pearson's method.
Results. On 1st day, there was a decrease in soluble Fas in patients of group A compared with group B (0.41 ng/ml vs 0.78 ng/ml, p=0.01). On the 7th day, the levels of serum platelet-derived growth factor BB were increased in patients of group A compared with group B (35.2 ng/ml vs 23.2 ng/ml, p=0.00001). After 1 month, the level of serum platelet-derived growth factor BB in patients of group A remained elevated compared with those in patients of group B (22.8 ng/ml vs 14.4 ng/ml, p=0.0003).
Conclusion. Femoropopliteal prosthetics leads to a change in branching angle of the deep femoral artery up to 70–80%, accompanied by changing dynamics of apoptotic markers and cell proliferation, leading to an increase in the thickness of neointimal hyperplasia and the progression of atherosclerosis.
Keywords: atherosclerosis, sFas, PDGF BB, deep femoral artery, bifurcation angle, intimal hyperplasia, restenosis.

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Background. Obliterating atherosclerosis of the arteries of the lower extremities represents a significant part in the cardiovascular diseases having population of the different countries worldwide [1]. The steadily progressing course of this pathology leads to the development of a critical limb ischemia, which causes a poor prognosis in relation to its preservation and the patient's quality of life [2].

The femoropopliteal segment of arteries of the lower extremities is one of the most common localizations of impairment, and in such cases of occlusion of the superficial femoral artery >25 cm, an open surgery is recommended [3, 4].

According to the type of formation of the proximal anastomosis of the femoropopliteal shunts, there are two types of interventions namely, the bypass grafting and prosthetics. During the bypass surgery, the proximal anastomosis is formed at an acute angle of up to 60° above the mouth of the deep femoral artery (DFA), which creates better conditions for the blood flow due to a decrease in the hemodynamic resistance and, accordingly, the

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Table 1. Clinical characteristics of the patients.

Stage of chronic ischemia of the lower extremities	Group A	Group B	p
IIB, n (%)	8 (44.4)	7 (41.2)	0.97
III, n (%)	10 (55.6)	10 (58.8)	
Comorbidity			
Ischemic heart disease, n (%)	3 (16.7)	2 (11.8)	0.487
Arterial hypertension, n (%)	6 (33.3)	5 (29.4)	0.539
Postinfarction cardiosclerosis, n (%)	3 (16.7)	2 (11.8)	0.487
The history of ischemic stroke in the carotid system, n (%)	2 (11.1)	2 (11.8)	0.602

energy losses during the passage of the blood from the artery to the shunt [5].

The literature provides a well enough clarification of the issues of the influence of the geometry of anastomoses on the blood flow generation [5]. To reduce the energy losses due to the blood flow instability, the transition from the artery to prosthesis should be very smooth. For this reason, it is believed that an end-to-end anastomosis is nearly ideal. Decrease in the anastomosis angle reduces the blood flow disturbances in the antegrade direction, but it does not eliminate them completely, since it is impossible to reduce the angle between the shunt and the donor artery to <30° without increasing the length of the anastomosis [6].

However, a natural question arises that, "whether the type of a proximal anastomosis formation affects the changes in topography of the DFA mouth and, accordingly, hemodynamics?" We did not find information on this issue in our literature search.

Several studies have shown that changes in the hemodynamic factors may affect the proliferation and apoptosis of the vascular wall cells [7]. Thus, a decrease in the laminar shear stress increases the expression and subsequent release of the fibroblast growth factor, the proapoptotic protein Bax, with the unchanged values of the antiapoptotic protein Bcl-2 (markers of the internal pathway of the apoptosis) [8, 9].

There were no studies focused on the analysis of the effect of changes in the hemodynamics on the markers of the external pathway of apoptosis found in the literature. One of their main representatives is Fas ligand (FasL) which induces the apoptosis by interacting with the transmembrane receptor Fas. The soluble form of the Fas receptor (sFas) lacks a transmembrane region and blocks the interaction of the FasL with Fas.

Fas is expressed on many types of the vascular wall cells and plays an important role in controlling the extravasation of the inflammatory cells. When expressed endogenously, FasL functions as an inhibitor of the inflammatory responses, causing a Fas-mediated death of the immune cells, as they attempt to invade the vessel wall cells. Expression of Fas and FasL was revealed both in the normal and affected vessel wall, and the activation of this apoptosis pathway is a sign of atherogenesis [10].

Changes of hemodynamics in the blood flow affects the proliferation and migration of the vascular wall cells [11]. Platelet derived growth factor BB (PDGF BB) is one of the most powerful mitogens and chemo attractants for fibroblasts, smooth muscle, and the endothelial cells [12]. In an experimental study, the effect of a laminar blood flow for the duration of 24 hours on the smooth muscle cells of the aortic wall of animals led to a decrease in their proliferation by 54% [7].

The study aimed to analyze the changes in topography of the DFA mouth, markers of the proliferation, and apoptosis in patients after open revascularizing interventions on the arteries of the femoropopliteal segment, as well as the influence of these factors on the results of a surgical treatment after 18 months.

Materials and Methods. The cohort study included 35 patients with a peripheral arterial obliterating atherosclerosis, femoropopliteal occlusion, stage IIB–III disease according to the A.V. Pokrovsky–Fontaine. The study was approved by the local Ethics committee of the I.P. Pavlov Ryazan State Medical University (protocol No. 7, dated 03/03/2020). The average age of the patients was 69 ± 4.6 years. There were 26 (74.3%) male patients in the study.

The patients underwent an open reconstructive surgery using an 8 mm synthetic PTFE prosthesis, followed by a distribution into the two groups, where group A included 18 patients who underwent a femoropopliteal prosthetics above the knee joint cleft (proximal end-to-end anastomosis configuration), and the group B included 17 patients with a femoropopliteal bypass grafting above the knee joint cleft (the end-to-side configuration of the proximal anastomosis). All the patients were operated on in the department of vascular surgery of the Regional Clinical Hospital (Ryazan) in the period from 2020−2021. Table 1 presents the clinical cha-

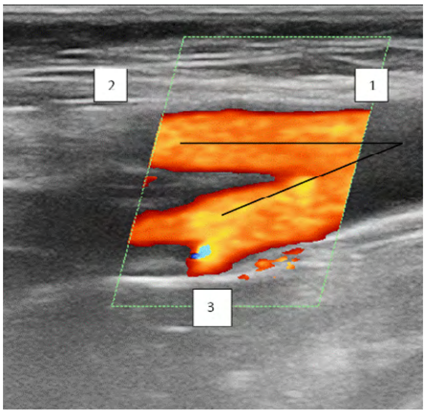

Fig. 1. Duplex scanning image of the bifurcation area of a common femoral artery in a healthy volunteer: 1- common femoral artery, 2- superficial femoral artery, 3- deep femoral artery (angle of its branching is 20°).

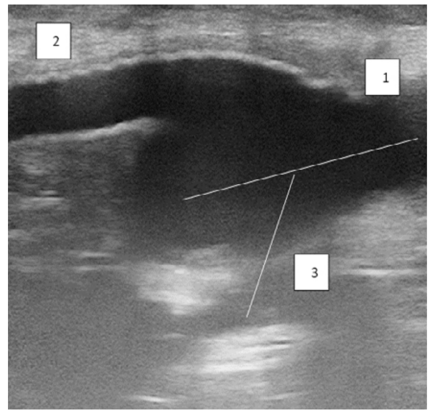

Fig. 2. Duplex scanning image of the area of bifurcation of a common femoral artery in a patient from group B after 1 month: 1- common femoral artery, 2- synthetic prosthesis, 3- deep femoral artery (branching angle 60°).

racteristics of the patients. The comparison group was represented by the 30 healthy volunteers (all men, mean age 64 ± 2.3 years).

After obtaining the informed consent from the participants, all the blood samples were taken from the cubital vein immediately before the intervention, on day-one, day-seven, and one-month after the surgery.

The amount of the PDGF BB and sFas proteins in the blood serum was determined using an enzyme-linked immunosorbent assay with the commercial kits Invitrogen Thermo Fisher (USA) in accordance with the manufacturer's instructions.

A duplex scanning of the lower limb arteries was performed on the day-7 and after 1 and 18 months, assessing the patency of the shunts and geometry of the anastomoses with a calculation of the resistive index (RI). The study was performed on the Esaote My Lab Alfa apparatus and having a linear probe with a frequency of 3–12 MHz and convex probe with a frequency of 3–5 MHz were used, respectively. The modes of color doppler mapping, spectral doppler sonography, X-flow, B-mode were used.

Statistical analysis of the data was performed using the statistical software package Statistica 10.0. Normality of the data distribution was tested using the Shapiro–Wilk test ($p > 0.05$). As a result, for further work with the hypotheses, parametric statistical methods were used; for pairwise comparisons of the mean values, the student's t-test was used for the cases with the unrelated samples. To test the presence and closeness of the relationship between the indicators, the Pearson correlation test was used. Numerical data were presented as an arithmetic mean value and as a standard deviation. The accepted level of statistical significance p was <0.05.

Results. During the duplex scanning of the arteries of the lower extremities, the attention was drawn to the change in the angle of DFA branching in the patients after a surgical treatment compared with the healthy volunteers. Normally, the angle of DFA branching relative to the common femoral artery in all the cases did not exceed 30°, so that it corresponded to 20° in 90% of the cases (27 patients) and 30° in 10% (3 patients). RI was 1.0 ± 0.2 (Fig. 1).

In the patients of group B, the angle of DFA branching was 40−0°, namely it corresponded to 40° in 10 (58.8%) patients and 50° in 7 (41.2%) patients. On the day-seven, the RI was 0.84 ± 0.18, and by the end of the month-one, it was 1.04 ± 0.11 (Fig. 2).

In the patients of group A, the angle of DFA branching was 70−80°, namely it corresponded to 70° in 13 (72.2%) patients, 75° in 3 (16.7%) patients, and 80° in 2 (11.1%) patients. The RI was 1.02 ± 0.2 ($p = 0.009$). By the end of the month-one, the RI values reached 1.24 ± 0.1, and were significantly higher than in the group B patients ($p = 0.000003$) (Fig. 3).

Before the surgery, the values of markers sFas (0.89 ± 2.4 ng/mL; $p = 0.677$) and PDGF BB (12.8 ± 1.7 ng/mL; $p = 0.46$) in patients of the group A were comparable with the values in patients of the group B (0.93 ± 0.18 and 13.3 ± 2.3 ng/mL, respectively).

On day-one, in the group A patients, the sFas index (0.41 ± 0.33 ng/mL; $p = 0.01$) was lower compared to the values in the group B patients (0.78 ± 0.49 ng/mL). The value of the PDGF BB was 19 ± 1.5 ng/mL in the group A patients, 17.9 ± 1.6 ng/mL in the group B patients and did not differ statistically between the groups ($p = 0.097$).

On the day-seven, the amount of sFas in the group A patients (0.57 ± 0.41 ng/mL) were reduced compared to its amount in the group B (1.1 ± 0.74 ng/mL; $p = 0.007$). The PDGF BB values were increased in the group A patients (35.2 ± 5.1 ng/mL) compared with the group B (23.2 ± 5.8 ng/mL; $p = 0.00001$).

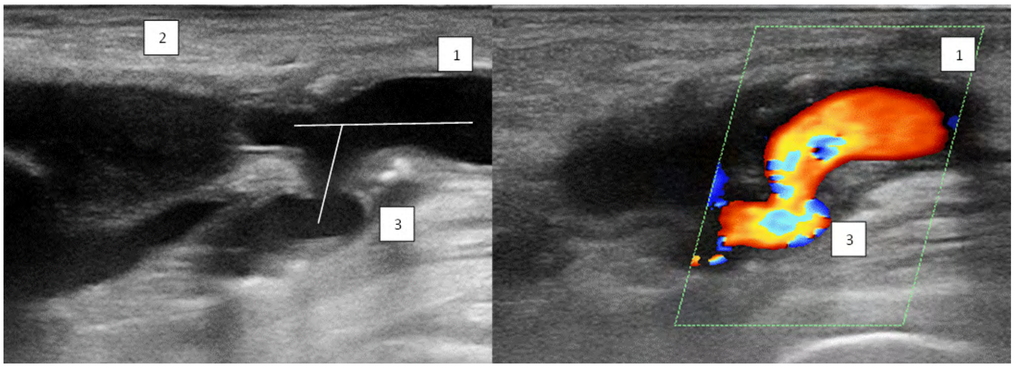

Fig. 3. Duplex scanning image of the area of bifurcation of a common femoral artery in a group A patient after 1 month: 1- common femoral artery, 2- synthetic prosthesis, 3- deep femoral artery (branching angle 75°).

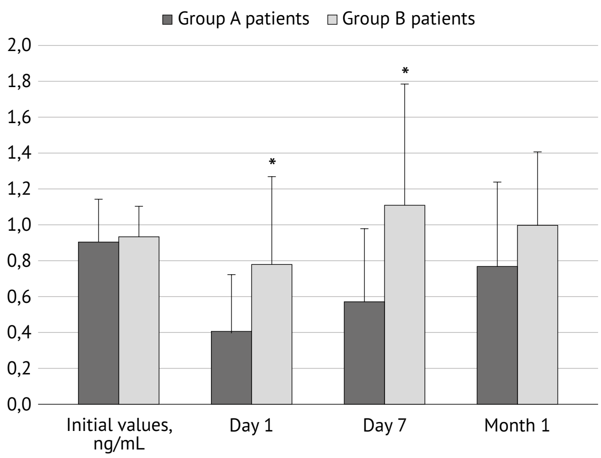

Fig. 4. Comparison of the dynamics of changes in the concentration of the sFas marker in the postoperative period in patients of the studied groups; *statistically significant difference (p < 0.05) between the groups A and B.

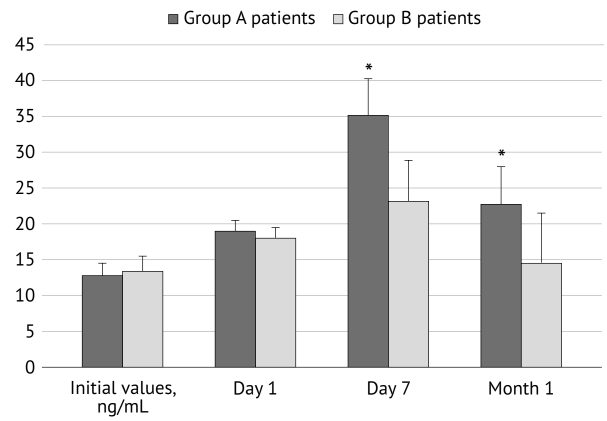

Fig. 5. Comparison of the dynamics of changes in the concentration of platelet growth factor BB in the postoperative period in patients of the study groups; *statistically significant difference (p < 0.05) between the groups A and B.

After a month, the level of PDGF BB in the group A patients (22.8 ± 5.3 ng/mL) remained elevated compared with its value in the group B patients (14.4 ± 7.1 ng/mL; p = 0.0003). The sFas values in the group A patients (0.77 ± 0.48 ng/mL) did not differ significantly from the values of the group B patients (0.99 ± 0.41 ng/mL; p = 0.133) in the indicated time interval (Fig. 4, 5).

Correlation analysis in the group A patients revealed an inverse correlation on the day-seven between the values of sFas and PDGF BB ($r = -0.867$, $p = 0.0001$), RI and sFas ($r = -0.726$, $p = 0.001$), and a direct correlation between the values of a PDGF BB and RI by the end of month-one ($r = +0.676$, $p = 0.002$). The group B patients showed a direct relationship between the sFas and PDGF BB values ($r = 0.69$, $p = 0.002$) on the day-seven.

According to the data of a duplex scanning, in the patients of group A, after 18 months, stenosis at the DFA mouth of 50–70% was detected, which was regarded as the progression of atherosclerosis. They also had an intimal hyperplasia in the prosthesis, which was 2.3 ± 0.51 mm in the indicated period.

In the group B patients, after 18 months, stenosis at the DFA mouth did not exceed 30–40%, the neointima thickness was 0.97 ± 0.3 mm and was 46.7% less than in patients of group A ($p = 0.000003$).

Discussion. Despite the significant advances in the modern vascular surgery, the problem of primary patency after a surgery remains unresolved. The progression of the atherosclerosis and neointimal hyperplasia are the main causes of the unsatisfactory results. Hyperplasia is a poorly organized and structured proliferative reaction of the intima to a surgical trauma [3].

In the animal experimental models, it was demonstrated that a trauma to the arterial wall during a surgery causes the proliferation of the smooth muscle cells from the media to the intima, which is usually self-limiting and does not lead to the development of a hemodynamically significant stenosis and occlusions [13]. At the same time, the surgical interventions in the patients with an atherosclerosis create new geometric conditions that could contribute to the significant changes in a blood flow and shear stress.

In our study, it was possible to show that the type of formation of the proximal anastomosis affects the change in a geometry of the angle of DFA branching and, accordingly, an increase in the resistance of a blood flow for the first time. Thus, the angle of DFA branching from the common femoral artery among all the healthy volunteers did not exceed 30°. In the patients with a femoropopliteal bypass grafting with a synthetic prosthesis, the angle of DFA branching increased to 40−60°, and with the prosthetics, changes in the angle of DFA branching were the greatest from 70−80°. We assume that this was due to a prosthesis tension during the distal anastomosis formation, when the DFA mouth is mechanically displaced.

Even with the physiological parameters of the angle of DFA branching, the arterial bifurcation region leads to the formation of a complex system of the blood flow [14]. The blood flow is divided along the anterior or outer wall of the branching artery. This results in a displacement of the velocity profile toward the posterior or inner wall, contributing to the formation of the areas with a low shear stress, and increases the time of interaction between the blood and endothelium.

It has been revealed *in vitro* that the vascular wall cells respond to a decrease in the shear stress by changing the orientation, morphology, and structure of the cytoskeleton by secreting the mitogens, thereby stimulating the proliferation and migration of the smooth muscle cells from the intima to the media [3]. However, the underlying cellular mechanisms are not fully understood.

Our results confirm the hypothesis of the scientists that the signaling through the external pathway of apoptosis plays an important role in a regulation of the vital activity of the vascular wall cells [15]. Surgical intervention leads to the apoptosis markers activation on the day-one after the intervention. This may be due the trauma of the arterial wall itself and the development of oxidative stress in response to a reperfusion, or both, which is one of the main inducers of the cell apoptosis.

Changes in the angle of DFA branching after a surgery leads to the appearance of the blood flow turbulence (modes of color doppler mapping and X-flow), and an increase in the peripheral resistance index, which is more pronounced in the patients of group A. We assume that this, in turn, leads to a stronger activation of the apoptosis system after a surgery.

The increased level of PDGF BB on the day-seven after a surgery may be due to the following factors,

First, it is the proliferative response of cells to an increased apoptosis on the day-one to overcome the cellular deficit, which was confirmed by the results of a correlation analysis. Damage to the endothelial barrier during a surgery makes smooth muscle cells available for the action of mitogens derived from the blood or blood components such as, PDGF BB. Dying cells release cytokines that enhance the proliferation and migration of the neighboring cells when their phenotype changes from a contractile to synthetic.

Secondly, it is the lack of an inhibitory effect of the laminar blood flow on the proliferation and migration of the smooth muscle cells from the media to the intima of the vascular wall. Responsive proliferation becomes enhanced, and uncontrolled proliferation of the cells may lead to the restriction of the vessel diameter and neointimal hyperplasia, which was revealed in the patients of group A.

All this led to the persistence of the elevated PDGF BB values by the end of the month-one and was expressed in an increased degree of stenosis by an atherosclerotic plaque of the DFA mouth up to 50%–70%, and the neointima thickness in the prosthesis.

In the group B patients, although the apoptosis system was activated in response to a surgical trauma, it was compensated for by the proliferative response and led to the restoration of the dead cells. Less pronounced changes in the angle of DFA branching and the blood flow turbulence in the proximal anastomosis site enabled apoptosis, and cell proliferation to return to their initial values by the end of the month-one.

The limitation of our work included the smaller sample size, a relatively small number of the studied parameters of both the apoptosis system and proliferation. In our opinion, it is required to expand the work both by increasing the number of studied markers of the system of the apoptosis and cell proliferation, and by studying them in patients with the autovenous shunts, xenoprostheses, and allografts.

CONCLUSIONS

1. Femoropopliteal prosthetics leads to a change in the angle of branching of the DFA up to 70%–80% and a change in the dynamics of markers of the apoptosis and cell proliferation in the postoperative period, which is expressed in a neointimal hyperplasia and progression of the atherosclerosis.

2. Reconstructive surgery on the arteries of the femoropopliteal segment using the bypass

technique leads to a more physiological angle of branching of the DFA, and a decrease in the risk of intimal hyperplasia in the anastomotic area.

Author contributions. R.E.K. created the study design, edited the text, and arranged final approval of the article; I.A.S edited the text, arranged final approval of the article; E.A.K. created the study design, collected the data, analyzed, and interpreted the data, wrote the article, performed statistical analysis; I.N.Sh. analyzed and interpreted the data, wrote the article.

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Conflict of interest. The authors declare no conflict of interest.

REFERENCES

1. Shabani Varaki E., Gargiulo G.D., Penkala S., Breen P.P. Peripheral vascular disease assessment in the lower limb: a review of current and emerging non-invasive diagnostic methods. *Biomed. Eng. Online*. 2018; 17 (1): 61. DOI: 10.1186/s12938-018-0494-4.

2. Yudin V.A., Vinogradov S.A., Krylov A.A., Gerasimov A.A. Autovenous revascularization of the lower extremity arteries in patients with variant anatomy of the peripheral blood flow and the progressive atherosclerotic process. *Probl. Sotsialnoi Gig. Zdravookhranenniia i Istor. Med.* 2019; 27 (6): 1093–1097. DOI: 10.32687/0869-866X-2019-27-6-1093-1097.

3. Ascher E. Haimovici's vascular surgery. Blackwell, Massachustts, USA. 2004; 1221 p. Russ ed.: Ascher E. *Sosudistaya hirurgiyapo Hajmovichu.* Vol. 2. M.: Binom. Laboratoriya znanij. 2010; 536 p. (In Russ.)

4. Sapelkin S.V., Kuznetsov M.R., Kalashnikov V.Yu., Ostroumova O.D., Galstyan G.R., Chupin A.V., Chernyavskiy M.A. *Proekt natsional'nykh rekomendatsiy po diagnostike i lecheniyu zabolevaniyu arteriy nizhnikh konechnostey.* (National guidelines for the diagnosis and treatment of lower limb artery disease.) M.: Rossijskij soglasitel'nyj dokument. 2018; 111 p. (In Russ.)

5. Cronenwett J.L., Johnston K.W. *Rutherford's vascular surgery.* 8th ed. Elsevier. 2014; 2784 p.

6. Amiri M.H., Keshavarzi A., Karimipour A., Bahiraei M., Goodarzi M., Esfahani J.A. A 3-D numerical simulation of non-Newtonian blood flow through femoral artery bifurcation with a moderate arteriosclerosis: investigating Newtonian/non-Newtonian flow and its effects on elastic vessel walls. *Heat Mass. Transf.* 2019; 55 (7): 2037–2047. DOI: 10.1007/s00231-019-02583-4.

7. Fitzgerald T.N., Shepherd B.R., Asada H. Laminar shear stress stimulates vascular smooth muscle cell apoptosis via the Akt pathway. *J. Cell. Physiol.* 2008; 216 (2): 389–395. DOI: 10.1002/jcp.21404.

8. Gosgnach W., Messika-Zeitoun D., Gonzalez W., Philipe M., Michel J.B. Shear stress induces iNOS expression in cultured smooth muscle cells: Role of oxidative stress. *Am. J. Physiol. Cell. Physiol.* 2000; 279 (6): 1880–1888.

9. Kalinin R.E., Suchkov I.A., Klimentova E.A., Egorov A.A., Povarov V.O. Apoptosis in vascular pathology: present and future. *I.P. Pavlov Russian Medical Biological Herald.* 2020; 28 (1): 79–87. (In Russ.) DOI: 10.23888/PAVLOVJ202028179-87.

10. Klimentova E.A., Suchkov I.A., Egorov A.A., Kalinin R.E. Apoptosis and cell proliferation markers in inflammatory-fibroproliferative diseases of the vessel wall (review). *Sovremennye tekhnologii v meditsine.* 2020; 12 (4): 119–128. (In Russ.) DOI: 10.17691/stm2020.12.4.13.

11. Haga M., Yamashita A., Paszkowiak J., Sumpio B.E., Dardik A. Oscillatory shear stress increases smooth muscle cell proliferation and Akt phosphorylation. *J. Vasc. Surg.* 2003; 37 (6): 1277–1284. DOI: 10.1016/s0741-5214(03)00329-x.

12. Papadopoulos N., Lennartsson J. The PDGF/PDGFR pathway as a drug target. *Mol. Aspects Med*. 2018; 62: 75–88. DOI: 10.1016/j.mam.2017.11.007.

13. Kalinin R.E., Abalenikhina Yu.V., Pshennikov A.S., Suchkov I.A., Isakov S.A. Interrelation between oxidative carbonylation of proteins and lysosomal proteolysis of plasma in experimentally modelled ischemia and ischemia-reperfusion. *Nauka molodykh (Eruditio Juvenium)*. 2017; (3): 338–351. (In Russ). DOI: 10.23888/HMJ20173338-351.

14. Spanos K., Petrocheilou G., Karathanos C., Labropoulos N., Mikhailidis D., Giannoukas A. Carotid bifurcation geometry and atherosclerosis. *Angiology*. 2017; 68 (9): 757–764. DOI: 10.1177/0003319716678741.

15. Apenberg S., Freyberg M.A., Friedl P. Shear stress induces apoptosis in vascular smooth muscle cells via an autocrine Fas/FasL pathway. *Biochem. Biophys. Res. Commun*. 2003; 310 (2): 355–359. DOI: 10.1016/j.bbrc.2003.09.025.