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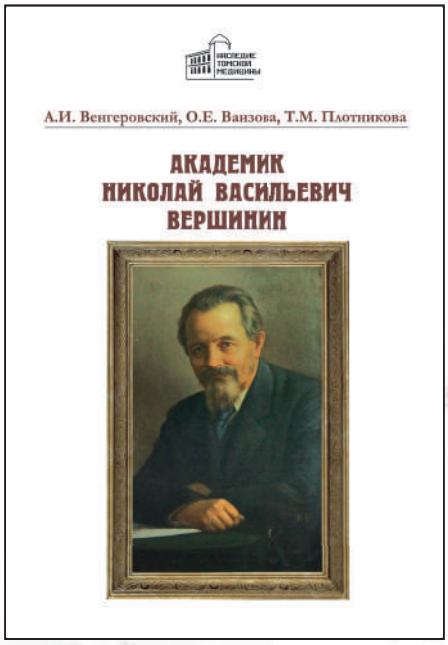

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Effect of Probiotic Strains, L-Arginine and Carvedilol on Myocardial Infarction Size in Systemic Inflammation in Rats

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ABSTRACT

Aim. To determine the cardioprotective effect of a mixture of probiotic strains of *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis subsp*. *Lactis* (BB-12) in rats with systemic inflammatory response syndrome (SIRS) in comparison with the use of α- and β-adrenoblocker carvedilol and L-arginine, the precursor of nitric oxide (NO).

Materials and methods. Experiments were conducted on male Wistar rats in a model of SIRS including obesity and chemically induced colitis. Probiotic strains (PRK), L-arginine (ARG), and the α- and β-adrenoblocker carvedilol (ADB) were intragastrically administered to animals of the corresponding groups. Myocardial ischemia-reperfusion injury was reproduced in an isolated heart perfusion model. The size of the necrosis zone (SNZ) was determined using histochemistry. The concentration of cytokines in blood plasma was measured using an immunoenzyme technique.

Results. Myocardial SNZ in the group with SIRS modeling was significantly higher than in the control group (45 (38; 48)% and 30 (26; 31)%, $p < 0.05$). In the PRK and ARG groups, the SNZ was 32 (28; 35)% and 35 (26; 36)%, respectively, which was significantly lower compared to the SIRS group ($p < 0.05$). In the ADB group, the SNZ was 40 (31; 48)%, similar to the value in the SIRS group ($p > 0.05$). Hemodynamic parameters in isolated heart did not differ between the groups. The concentration of proinflammatory cytokines and transforming growth factor-β in plasma was significantly higher in the SIRS group than in the control. However,, in the PRK and ARG groups a significant decrease in the levels of some of some cytokines was noted, confirming the presence of an anti-inflammatory effect.

Conclusion. Administration of PRK to rats using the SIRS model caused a decrease in the SNZ. However, of α- and β-adrenoreceptors was not accompanied by a decrease in the SNZ in this model. The amino acid L-arginine had a cardioprotective and anti-inflammatory effect similar to that of the PRK group, which may indicate the unidirectionality of the tested effects.

Keywords: myocardium, ischemia-reperfusion, cardioprotection, systemic inflammatory response syndrome, cytokines, probiotics, nitric oxide

Conflict of interest. The authors declare the absence of obvious and potential conflict of interest related to the publication of this article.

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Влияние пробиотических штаммов, L-аргинина и карведилола на размер инфаркта миокарда у крыс в условиях системного воспаления

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РЕЗЮМЕ

Цель. Определение выраженности кардиопротективного эффекта смеси пробиотических штаммов *Lactobacillus acidophilus* (LA-5) и *Bifidobacterium animalis* subsp. *Lactis* (BB-12) у крыс с синдромом системного воспалительного ответа (ССВО) в сравнении с применением α- и β-адреноблокатора карведилола и предшественника оксида азота (NO) L-аргинина.

Материалы и методы. Эксперименты выполнены на самцах крыс стока Вистар на модели ССВО, включающей ожирение и химически индуцированный колит. Животным соответствующих групп внутрижелудочно вводили пробиотические штаммы (ПРК), L-аргинин (АРГ) и α- и β-адреноблокатор карведилол (АДБ). Ишемическое-реперфузионное повреждение миокарда воспроизводили на модели перфузии изолированного сердца. Размер зоны некроза (РЗН) определяли гистохимически. Концентрацию цитокинов в плазме крови оценивали иммуноферментным методом.

Результаты. Размер зоны некроза миокарда в группе с моделированием ССВО был значимо выше, чем в контрольной группе (45 (38;48)% и 30 (26;31)%, $p < 0,05$). В группах ПРК и АРГ РЗН составил 32 (28;35)% и 35 (26;36)%, что значимо меньше по сравнению с группой ССВО ($p < 0,05$). В группе АДБ РЗН составил 40 (31;48)%, не отличаясь от значения в группе ССВО ($p > 0,05$). Гемодинамические показатели изолированного сердца не отличались между группами. В группе ССВО концентрация провоспалительных цитокинов и трансформирующего фактора роста-β в плазме крови была значимо выше, чем в контроле. При этом в группах ПРК и АРГ отмечено значимое уменьшение уровней некоторых цитокинов, подтверждающее наличие противовоспалительного эффекта.

Заключение. Введение ПРК у крыс на модели ССВО вызвало уменьшение РЗН. При этом блокада α- и β-адренорецепторов не сопровождалась уменьшением РЗН на данной модели. Аналогичным группе ПРК кардиопротективным и противовоспалительным действием обладала аминокислота L-аргинин, что может свидетельствовать об однонаправленности эффекта протестированных воздействий.

Ключевые слова: миокард, ишемия-реперфузия, кардиопротекция, синдром системного воспалительного ответа, цитокины, пробиотики, оксид азота

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией данной статьи.

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INTRODUCTION

The increased risk of cardiovascular diseases in metabolic syndrome (MS) and its individual components is confirmed by large-scale clinical observations. According to the World Health Organization, in 2022, every 8th person in the world was obese, and 2.5 billion people over over the age of 18 were overweight. The prevalence of MS in the general population varies from 14 to 24%, depending on the socioeconomic status of the country and region, with the highest prevalence in the United States.

According to 2022 data, the number of overweight children under 5 years of age in Africa has increased by 23% since 2000, while in Asian countries almost half of children of the same age are overweight or obese [1]. These data clearly indicate that experimental research in the field of cardioprotection, i.e. the development of ways to protect the myocardium from ischemia-reperfusion injury (IRI), should be conducted on clinically relevant models that include pathology in the form of visceral obesity, type 2 diabetes mellitus, and arterial hypertension [2].

Considering that systemic inflammation is the pathogenic factor that unifies various MS components, we have developed and validated an experimental model of systemic inflammatory response syndrome (SIRS) in rats, which includes the formation of obesity caused by a high-fat diet chemically induced colitis and the administration of antimicrobial drugs [3, 4]. Using this SIRS model in experiments on an isolated perfused heart and on a model of coronary occlusion myocardial infarction, the literature data were confirmed that systemic inflammation is accompanied by significant changes in the qualitative composition of the intestinal microbiota and a decrease in myocardial resistance to IRI [5, 6].

The effect of proinflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1 β (IL-1β), on the heart is the most important mechanism contributing to the increase in infarction size in SIRS. These cytokines negatively affect the state of cardiac microcirculatory bloodstream, increasing microvascular permeability and inducing the expression of adhesion molecules on endothelium, increase the manifestations of myocardial oxidative, and trigger apoptosis of cardiac myocytes [7]. In this regard, an urgent task is to search for new safe and effective ways to affect the mechanisms of systemic inflammation in order to reduce its negative effects on various processes, including myocardial IRI.

One of such ways is the modulation of the intestinal microbiota composition through various interventions including the use of antibiotics and probiotics, as well as the transplantation of transplantation of intestinal microflora. In recent years, a number of studies have shown that changes in the intestinal microbiota composition are accompanied by an increase in myocardial resistance to IRI both in healthy animals and in those with a concomitant pathology [8, 9]. The studies of our research group have demonstrated that administration of a mixture of probiotic strains of lactobacilli and bifidobacteria to rats with SIRS leads to a decrease in the concentration of proinflammatory cytokines in the blood, is also accompanied by a decrease in the infarction size in the model of global ischemia-reperfusion of an isolated heart [10].

The possible mechanisms of anti-inflammatory and cardioprotective effects of the intestinal microbiota are of particular interest, the study of which is currently at the very initial stage. Some data suggest that optimization of the intestinal microflora composition leads to a decrease in paracellular permeability of intestinal epithelium and a decrease in the effect of bacterial translocation, which, in turn, reduces the stimulation

of innate and adaptive immunity. The direct effect of metabolites produced by certain representatives of the intestinal microbiocenosis on cardiomyocytes with the activation of intracellular signaling pathways of cardioprotection is not excluded [5].

Nitrogen monoxide (NO) is of great importance in the regulation of the inflammatory response; which has an anti-inflammatory effect in physiological concentrations concentrations, but demonstrates pronounced pro-inflammatory properties when its production is locally enhanced by inducible isoforms of NO synthase [11]. It is important to note that the general enhancement of NO production during administration of its pharmacological donors and its precursor L-arginine has a cardioprotective effect effect in healthy animals [12], although blockade of endogenous NO synthesis with the methyl ester L^{ω} -nitro-N-arginine does not eliminate the effect of myocardial ischemic preconditioning [13]. The influence of the sympathetic nervous system is also significant, as its prolonged activation has a pro-inflammatory effect and is considered as one of the leading mechanisms of chronic heart failure progression [14].

Based on these data, it was assumedthat the use of adrenoblockers and substances that enhance NO production may have a cardioprotective effect in in SIRS conditions and have a unidirectional effect with the use of PRK. The aim of the present study was to determine the cardioprotective effect of a mixture of probiotic strains *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis subsp. Lactis* (BB-12) in rats with SIRS in comparison to the use of α- and β-adrenoblocker carvedilol and NO precursor L-arginine.

MATERIALS AND METHODS

The experiments were performed on male Wistar rats of improved conventional status weighing 322 ± 25 g in accordance with the. The for the use of animals was reviewed and approved by the Committee for the Control of the Care and Use of Laboratory Animals of the Almazov National Medical Research Center of the of SIRS was described in detail earlier [3].

Animals were randomly assigned to one of five groups (n = 9 in each group): 1) control (CTR) group, in which which rats received standard food and drinking water *ad libitum*; 2) systemic inflammatory response syndrome (SIRS) group included animals with primary visceral obesity induced by a high fat and carbohydrate diet, under combined anesthesia (zoletil 20 mg/kg i/m, isoflurane 1.5%), 1 ml of a mixture of 3% acetic acid solution and 3% ethanol was injected once rectally to induce acute colitis. Starting that day, the same animals were intragastrically administered a mixture of antimicrobial drugs (AMD) (amoxicillin, metronidazole, and clarithromycin) in 1 ml normal saline solution (NS) at a daily dose of 15 mg of each AMD per rat for 3 days and 1 ml of NS for 5 days; 3) PRK group in which rats that underwent the procedures described for the previous group were additionally intragastrically administered 1 ml of a solution of a mixture of probiotic strains *Lactobacillus acidophilus (LA-5)* and *Bifidobacterium animalis subsp. lactis (BB-12)* at a dose of 10^8 CFU per animal during the last 8 days of the experiment; 4) ARG group – during the last 8 days of the experiment, instead of the probiotic mixture, 50 mg of L-arginine in 1 ml of NS per animal administered intragastrically; 5) ADB group – during the last 8 days of the experiment, 2 mg of α- and β-adrenoreceptor blocker carvedilol orally administered in 1 ml of NS per animal. The doses and modes of administration to rats in the ARG and ADB groups were selected on the basis of the literature [15, 16].

One day before the end of the experiment, whole blood (1.5 ml) was drawn from the large saphenous vein for hematological and immunological analysis in rats under short-term isoflurane anesthesia. The number of erythrocytes, leukocytes, and platelets in blood was determined on an automated veterinary hematology 3-differential analyzer (URIT-3000 Vet Plus, URIT Medical Electronic, China). The levels of TNF-α, IL-1β, IL-6 and transforming growth factor-β (TGF-β) in blood plasma were evaluated by immunoenzyme method (MR-96A, Mindray, China).

Global ischemia-reperfusion of the isolated heart was modeled on the Langendorff apparatus. For this purpose, the animals were anesthetized with isoflurane, fixed on the operating table, and a wide bilateral transdiaphragmatic incision was made to provide access to the heart. The heart was removed and its through the aorta with a Krebs-Henseleit-Henseleit buffer with a hydrostatic pressure of 80 mm Hg at 37°C. Immediately after the start of perfusion, a polyethylene balloon connected by a flexible cannula to a pressure sensor and a software and hardware complex for recording hemodynamics (PhysExp Gold, Cardioprotekt, Russia) was placed into the left ventricle through the mitral valve.

The duration of global ischemia was 30 min, reperfusion – 90 min. After 15 minutes of stabilization

at baseline, as well as at 15, 30, 45, 60, 75, and 90 minutes of reperfusion, the following values were recorded: left ventricular systolic pressure (LVSP, mm Hg), heart rate (HR, bpm), coronary flow (CF, ml/min). After completion of reperfusion, the heart was cut into into five 2-mm-thick transverse sections which were incubated at 37°C in 1% triphenyltetrazolium chloride (TTC) solution for 15 minutes. The sections were then photographed with a digital camera connected to a stereomicroscope, and the images were coded. The researcher, who was not familiar with image coding procedure, performed a planimetric assessment of the nectrotic zone size zone size (NZS) using ImageJ program. The NZS was expressed as the percentage of the area area of TTC-negative areas averaged over five slices.

Statistical processing of experimental data was performed using the STATISTICA 12.0 software package. The Kolmogorov–Smirnov test was used to determine the distribution pattern. Due to the different distribution pattern in the groups, preliminary statistical analysis was performed on the basis of the nonparametric Kruskal–Wallis (H test) to detect statistically significant differences between samples, followed by pairwise comparison using the Mann–Whitney *U* test and appropriate adjustments in the multiple comparison program. Differences determined by the program for each dimension were considered statistically significant. The tables show the the values of the median (*Me*), as well as the lower and upper quartiles (25%;75%).

RESULTS

Clinical blood counts showed a significant increase in leukocytes, platelets, including large platelets in the SIRS group compared to the CTR group (SSD, Table 1). There was a trend toward a decrease in leukocyte count in the PRK group compared to the SIRS group, and there was a significant decrease in large platelets (SSD, Table 1). In the ARG group, there was a significant decrease in the number of large platelets (SSD, Table 1). The parameters of clinical blood count in the ADB group did not differ from those in the SIRS group.

Table 1

Hematologic Parameters, *Me* (25%;75%)

Analyte	Group				
	CTR	SIRS	PRK	ARG	ADB
Leukocytes, ×109 /L, $p = 0.001$	3.4# (3.1;3.8)	5.7* (4.8;6.5)	4.0 (3.5;4.2)	4.0 (3.8;5.5)	5.9* (4.8;8.3)
Erythrocytes, $\times 10^{12}$ /L, $p = 0.2679$	8.0 (7.1;8.7)	8.2 (7.8;9.2)	7.4 (7.1;9.2)	9.8 (8.0;10.1)	8.5 (7.7;9.3)
Platelets, $\times 10^{9}$ /L, $p = 0.0344$	546# (515;596)	781* (693;925)	582 (548;770)	631 (608;806)	752 (503;899)
Large platelets, $\times 10^{9}$ /L, $p = 0.0008$	39# (35;44)	67* (62;69)	45# (40;51)	47# (44;50)	55 (40;64)

Here and in Table 2: CTR – control; SIRS – systemic inflammatory response syndrome; PRK – SIRS and *LA-5* and *BB-12* mixture; ARG – SIRS and L-arginine; ADB – SIRS and carvedilol; *p* – the level of significance.
* statistically significant difference (SSD) in relation to CTR group; # SSD in relation to SIRS group.

When analyzing the level of cytokines in the blood plasma, it was found that the concentrations of TNF-α were significantly higher and IL-1β, IL-6, and TGF-β in the SIRS group were statistically significantly higher (by 64, 41, 43, and 50%, respectively) than in the control (Table 2). The administration of probiotics to the animals was accompanied by a significant decrease in the levels of IL-1β and IL-6 compared to the SIRS group (by 50 and 36%, respectively, Table 2).

The IL-1β concentration was lower in the ARG group than in the SIRS group (SSD, Table 2). No statistically significant changes were observed in the ADB group in relation to the SIRS group.

Table 2

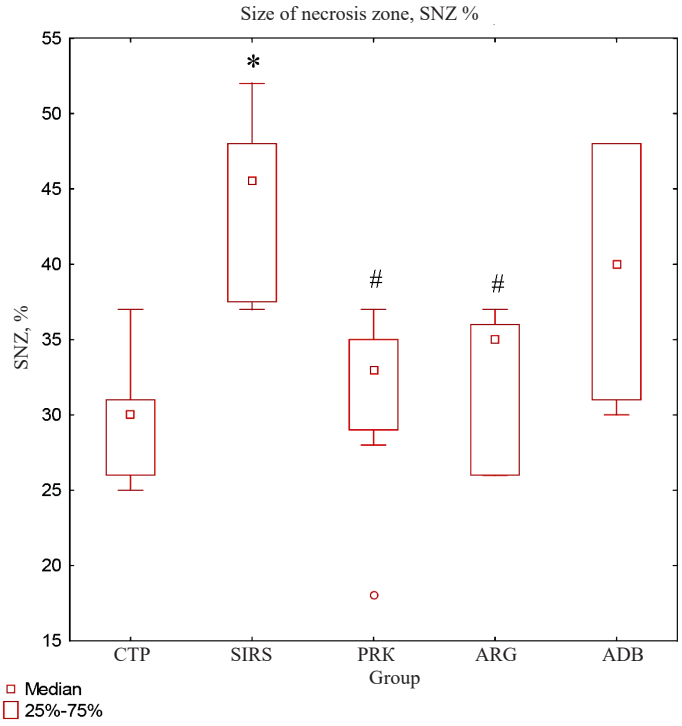
Levels of Cytokines in Plasma, *Me* (25%;75%)

Analyte	Group				
	CTR	SIRS	PRK	ARG	ADB
TNF-α, pg/mL, $p = 0.0393$	1.3 (1.0;1.6)	1.8 (1.5; 3.0)	1.2# (0.4; 1.5)	0.6 (0.5; 1.9)	1.0 (0.9; 3.1)
IL-1β, pg/mL, $p = 0.0004$	80# (70; 90)	120* (113; 150)	80# (62; 95)	77# (66; 78)	90 (80; 100)
IL-6, pg/mL, $p = 0.0007$	5.6# (4.7; 6.0)	8.0* (7.5; 8.6)	5.8# (4.9; 6.2)	6.2 (4.7; 7.0)	7.0 (5.3; 7.3)
TGF-β, pg/mL, $p = 0.0013$	26# (21; 30)	39* (39; 43)	30 (25; 41)	31 (30; 49)	48* (35; 54)

* SSD in relation to the CTR group; # SSD in relation to the SIRS group.

Hemodynamic parameters (left ventricular systolic pressure, heart rate, and coronary flow) at baseline, 15, 30, 45, 60, 90, and 120 minutes after 30 minutes of global ischemia were not statistically different between groups.

The myocardial SNZ in the SIRS modeling group was significantly higher than in the control group (45 (38; 48)% and 30 (26; 31)%, (SSD, Fig.). In the PRK and ARG groups, the SNZ was 32 (28; 35)%, and 35 (26; 36)%, which was significantly lower compared with the SIRS group (SSD, Fig.), whereas in the ADB group, the SNZ was 40 (31; 48)%, which was not significantly different from its value in the SIRS group (Fig.).



SNZ %: F(4; 35) = 8,2786; p = 0,00008; KW-H(4; 40) = 18,5426; p = 0,0010

Figure. Size of myocardial necrosis zone, *Me* (25%;75%): CTR – control; SIRS – systemic inflammatory response syndrome; PRK – SIRS and a mixture of *LA-5* and *BB-12*; ARG – SIRS and L-arginine; ADB – SIRS and carvedilol; p – the level of significance. * SSD in relation to CTR group, # SSD in relation to SIRS group

DISCUSSION

The study confirmed the fact of an increased myocardial infarction size in SIRS caused by primary obesity in combination with chemically induced colitis. The presence of SIRS was verified by increased leukocyte and platelet counts and a significant increase in the concentration of pro-inflammatory cytokines in the blood. Recently, new experimental evidence has been obtained that the presence of acute inflammation with hypercytokinemia leads to a decreased myocardial resistance to IRI. In particular, in the model of dextran sulfate-induced inflammatory bowel disease in mice, it was shown that the severity of myocardial IRI is significantly increased in the presence of systemic inflammation, with IL-6 being one of the mediators of this effect [17]. It is hypercytokinemia that is considered as the leading mechanism of myocardial IRI resistance reduction in SIRS, since TNF-α and IL-1β provoke a number of cardionegative effects, including initiation of cardiomyocyte apoptosis and enhancement of cardiomyocyte oxidative damage.

The conducted experiments demonstrated the infarct-limiting effect of probiotic therapy with *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis subsp. Lactis* (BB-12) in animals with SIRS. However, the infarction size in the group of animals receiving probiotics was not significantly different from the corresponding value in the control. On the other hand, probiotics administration was accompanied by a decrease in the level of TNF-α and IL-1β, which allows us to associate the effect of probiotic cardioprotection with the anti-inflammatory effect of the introduced bacteria and/or the secondary changes in the general microbial landscape of the intestine induced by them. It is known that a number of probiotic strains have the ability to normalize the increased permeability of the intestinal mucosa

by increasing mucus production, preventing the degradation of tight junctions between enterocytes, stimulation of anti-inflammatory mechanisms in the form of activation of T-regulators, etc. [18].

Although these effects have been most fully studied in relation to the manifestations of inflammation directly in the intestinal wall in colitis, in the context of this study, the reduction of manifestations of systemic inflammation is equally important. Mechanisms of probiotic cardioprotection may include other signaling pathways associated with direct activation of cardiomyocyte receptors by bioactive substances produced by intestinal microflora and entering the systemic bloodstream. This assumption is supported by data showing that cardioprotection in SIRS is formed only when live bacteria of *Lactobacillus delbrueckii D5* strain are used and is lost when pasteurized culture is administered to animals [19]. Short-chain fatty acids (acetic, propionic and butyric acids) interacting with free fatty acid receptors 3 (FFAR3) found in the mammalian heart [20], as well as bile acids and their derivatives signaling through nuclear farnesoid X receptor (FXR) and G-protein-coupled membrane receptor of bile acids 1 (TGR5) [21] are claimed to play the role of such cardioprotective signals.

Obviously, the study of the mechanisms of probiotic-mediated cardioprotection is at the initial stage and requires the use of both pharmacological agents blocking certain targets and animal biomodels not expressing or hyperexpressing the corresponding proteins. At the same time, when using appropriate pharmacological tools, it is necessary to evaluate the presence of their own effect on myocardial IRI and its targeting. In the present study, the effects of two substances with anti-inflammatory and cardioprotective effects, the NO precursor L-arginine and the α- and β-adrenoreceptor blocker carvedilol, were tested in a.

Unlike carvedilol, L-arginine was found to have a significant cardioprotective effect in animals with SIRS, which was associated with a decrease in in the level of IL-1β in the blood plasma and the number of large platelets. Exogenous administration of L-arginine can lead to an increase in NO formation in the body, although the presence of this effect was not shown in all studies and seems to depend on the mode of administration, dose, and other factors [22]. This study did not measure the level of NO metabolites in the blood, which is one of the limitations. Nevertheless, there is reason to believe that the observed cardioprotective effect of L-arginine is associated with an increase in NO production because previously a number of studies on healthy animals showed the administration of L-arginine, as well as NO donators sodium nitroprusside and S-nitroso-N-acetylpenicillamine, was accompanied by an improvement in postischemic recovery of left ventricular contractility and a decrease in the size of myocardial infarction [12, 23]. The mechanism of cardioprotective effect of NO is associated with stimulation of guanylate cyclase, increased production of cGMP in cardiomyocytes and activation of protein kinase G, which, in turn, provides opening of ATP-sensitive mitochondrial potassium channels [24].

Activation of the sympathetic nervous system is traditionally regarded as an important pro-inflammatory mechanism, which is realized both by direct activation of immune cells through stimulation of adrenoreceptors and indirectly through activation of the renin-angiotensin system. In this regard, the use of β-adrenoblockers represents the basis of modern therapy of chronic cardiac insufficiency, aimed at blockade of maladaptive activation of neurohumoral systems. The question about the presence and severity of infarct-limiting effect of β-adrenoblockers is more complicated because most studies have not shown a significant effect of this group of drugs on the infarction size and LV ejection fraction [25].

However, there is evidence that adenoblockers acting on both β- and α-adrenoreceptors may have advantages over selective β-adrenoblockers. For example, the CAPRICORN study found that the α- and β-adrenoreceptor blocker carvedilol improved outcomes in patients with acute myocardial infarction and LV dysfunction compared with a selective β_1 -adrenoreceptor blocker [26]. It is noteworthy that carvedilol has a number of additional pleiotropic properties that enhance its cardio- and vasoprotective potential. Such properties include a pronounced antioxidant effect of carvedilol, realized due to the presence of carbazole group in its composition, and endothelioprotective effect.

In particular, antihypertensive and antiremodeling effects of carvedilol are eliminated by administration of NO synthase blocker nitro-N-arginine, which indicates the important role of NO in the mechanism of action of the drug [27, 28]. These prerequisites allowed us to consider carvedilol as a potential cardioprotective agent having common mechanisms of action with L-arginine. Nevertheless, carvedilol in the used dosage did not have cardioprotective effect in animals with SIRS.

CONCLUSION

Unlike carvedilol, the α- and β-adrenoreceptor blocker, L-arginine, the precursor of NO, has a cardioprotective effect in the presence of systemic inflammation. The infarct-limiting and anti-inflammatory effect of probiotics based on lacto- and bifidobacteria has a similar direction to L-arginine.

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Coronary Calcium Associated with Changes in Instrumental and Humoral Markers of Sympathetic Activity in Patients with Non-Obstructive Coronary Atherosclerosis

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Abstract

Aim. To study the associations between sequential factors of the 10-year coronary heart disease (CHD) risk index MESA, heart rate variability (HRV), molecular markers of sympathetic activity and the presence or absence of calcium in the coronary arteries (CA) in patients with non-occlusive coronary atherosclerosis.

Materials and methods. A total of 30 patients with suspected CHD, as a result of which at least one CA stenosis < 70% with a left ventricular ejection fraction ≥ 50% according to transthoracic echocardiography was identified using coronary computed tomography angiography. HRV was studied by means of daily monitoring of electrocardiograms, analyzing the parameters of time and spectral analysis. All patients had blood samples taken to measure copeptin, catestatin, high-sensitivity C-reactive protein (hsCRP) and amino-terminal pro-brain natriuretic peptide (NT-proBNP). Statistical analysis was performed after dividing the distribution into two subgroups depending on the value of the coronary calcium index (coronary calcium Agatston score; CCI): group 1 (CCI 0, $n = 11$) and group 2 (CCI > 0, $n = 19$).

Results. Statistically significant ($p < 0.05$) correlations of CCI with lipid damage indices were established regarding total cholesterol and low-density lipoprotein cholesterol (LDL-C) ($r = –0.36$ and $r = –0.40$, respectively), coronary artery age ($r = 0.77$), 10-year coronary heart disease risk index MESA ($r = 0.78$) and 10-year prognosis of adverse cardiovascular events ($r = 0.39$). Multivariate regression analysis showed that the presence of coronary artery indices (CCI > 0) in patients with non-obstructive coronary artery lesions is independently associated with a family history of coronary heart disease [odds ratio (OR) 1.92, $p = 0.0011$]; HRV indices [NN (OR 1.75, $p = 0.0001$); SDANN (OR 1.43, $p = 0.0136$); pNN50 (OR 1.34; $p = 0.0153$); rMSSD (OR 1.88; $p = 0.0793$)] and high-density lipoprotein cholesterol (OR 1.09; $p = 0.0111$) were determined. The study determined threshold values of LDL-C (≤ 1.82 mmol/L; AUC = 0.72; $p = 0.002$) and copeptin (≤ 0.485 ngm/L; AUS = 0.672; $p = 0.021$) and hsCRP with catestatin (hsCRP ≤ 1.21 g/L and catestatin ≤ 138.1 μg/ml; AUC = 0.674; sensitivity 56.2%; $p = 0.021$), which in such patients can be used as markers associated with the presence of coronary calcium.

Conclusion. The presence of calcium in the coronary arteries in patients with non-obstructive lesions of the coronary arteries associated with an aggravated family history of CHD, disintegration of the autonomic heart regulation, which is expressed in the suppression of the activity of the parasympathetic division of the autonomic nervous system and the levels of reduction of LDL-C.

Keywords: coronary calcium, non-obstructive coronary atherosclerosis, heart rate variability, markers of sympathetic activity, myocardial blood flow reserve; microvascular dysfunction

Conflict of interest. The authors declare no obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed a voluntary informed consent to participate in the study. The study was approved by the Committee on Biomedical Ethics of Cardiology Research Institute of the Tomsk National Research Medical Center (Protocol No. 177 of 30.10.2018).

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Наличие коронарного кальция ассоциировано с изменением инструментальных и гуморальных маркеров симпатической активности у больных с неокклюзирующим коронарным атеросклерозом

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РЕЗЮМЕ

Цель. Изучить наличие ассоциаций между традиционными факторами 10-летнего индекса риска ишемической болезни сердца (ИБС) MESA, вариабельностью ритма сердца (ВРС), молекулярными маркерами симпатической активности и наличием или отсутствием кальция в коронарных артериях (КА) у пациентов с неокклюзирующим коронарным атеросклерозом.

Материалы и методы. В настоящее исследование включены 30 пациентов с подозрением на ИБС, которым посредством коронарной компьютерной томографической ангиографии был идентифицирован как минимум один стеноз КА менее 70% с фракцией выброса левого желудочка ≥ 50% по данным трансторакальной эхокардиографии. Вариабельность ритма сердца исследовали посредством суточного мониторирования электрокардиограммы, анализируя показатели временного и спектрального анализа. У всех пациентов были взяты образцы крови для измерения копептина, катестатина, высокочувствительного С-реактивного белка (вчСРБ) и аминоконцевого промозгового натрийуретического пептида (NT-proBNP). Статистический анализ проводился после разделения исследуемой популяции на две подгруппы в зависимости от величины индекса коронарного кальция (coronary calcium (Agatston) score; ИКК): 1-я группа (ИКК 0, $n = 11$) и 2-я группа (ИКК >0, $n = 19$).

Результаты. Установлены статистически значимые ($p < 0,05$) корреляции ИКК с показателями липидного спектра: общим холестерином и холестерином липопротеидов низкой плотности (ХС ЛПНП) ($r = –0,36$ и $r = –0,40$ соответственно), возрастом коронарных артерий ($r = 0,77$), индексом 10-летнего риска ИБС MESA ($r = 0,78$) и с 10-летней вероятностью наступления неблагоприятных сердечно-сосудистых событий ($r = 0,39$). Многофакторный регрессионный анализ позволил установить, что наличие кальция в коронарных артериях (ИКК > 0) у пациентов с необструктивным поражением КА независимо связано с отягощенным семейным анамнезом ИБС [отношение шансов (ОШ) 1,92, $p = 0,0011$]; показателями ВРС [NN (ОШ 1,75, $p = 0,0001$); SDANN (ОШ 1,43, $p = 0,0136$); pNN50 (ОШ 1,34; $p = 0,0153$); rMSSD (ОШ 1,88; $p = 0,0793$)] и холестерина липопротеидов высокой плотности (ОШ 1,09; $p = 0,0111$). Определены пороговые значения ХС ЛПНП (≤1,82 ммоль/л; AUC = 0,72; $p = 0,002$) и копептина (≤0,485 нгм/л; AUC = 0,672; $p = 0,021$) и комбинации вчСРБ с катестатином (вчСРБ ≤ 1,21 г/л и катестатин ≤ 138,1 нг/мл; AUC = 0,674; чувствительность 56,2%; специфичность 82,2%; $p = 0,021$), которые у таких пациентов могут использоваться в качестве маркеров, ассоциированных с наличием коронарного кальция.

Заключение. Наличие кальция в коронарных артериях у пациентов с необструктивным поражением КА ассоциировано с отягощенным семейным анамнезом преждевременной ИБС, дезинтеграцией вегетативной регуляции работы сердца, выражающейся в подавлении активности парасимпатического отдела вегетативной нервной системы, и снижением уровней ХС ЛПНП.

Ключевые слова: коронарный кальций, неокклюзирующий коронарный атеросклероз, вариабельность ритма сердца, маркеры симпатической активности, резерв миокардиального кровотока, микроваскулярная дисфункция

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование выполнено в рамках прикладного научного исследования «Новые интегративные высокотехнологичные методы диагностики и лечения ишемической болезни сердца» № 123051500131-6.

Соответствие принципам этики. Все пациенты подписали добровольное информированное согласие на участие в исследовании. Исследование одобрено комитетом по биомедицинской этике НИИ кардиологии Томского НИМЦ (протокол № 177 от 30.10.2018).

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INTRODUCTION

In the age of personalized medicine, the timely detection of cardiovascular risk in people is the decisive factor for ensuring adequate prevention of cardiovascular pathology, which is the leading cause of mortality and morbidity among the working-age population. It is interesting that the absolute risk is often significantly lower than the risk predicted, for example, by the Framingham risk score for non-fatal myocardial infarction or cardiac death in the next 10 years [1–4]. Undoubtedly, the problem of early risk assessment and risk stratification tools is highly relevant in the Russian Federation, since high mortality among people of working age who do not have proven cardiovascular diseases (CVD) remains quite high.

Certain progress has been made in this direction, in particular, Boitsov et al. [1] provide information that the National Medical Research Center for Therapy and Preventive Medicine developed a Russian scale to assess the risk of fatal cardiovascular complications over the next 10 years, based on the SCORE system and designed for the first time for the Russian population; researchers from Saratov State Medical University proposed an automated system for non-invasive monitoring of the degree of risk of developing CVD and its complications [5]. Researchers from Siberia (Barnaul and Tyumen) also made certain advances in this area [5, 6].

As one of the risk modifiers in assessing the risk of CVD, according to the 2018 ESC guidelines for the diagnosis and treatment of chronic coronary syndromes with certain assumptions (no data on the impact on prognosis, unreasonable costs for coronary computed tomography angiography and functional imaging tests to examine asymptomatic patients at low risk, without diabetes mellitus, with no family history of early coronary heart disease (CHD) or smoking history), it was proposed to consider the coronary calcium index (coronary calcium index, CCI), since it provides a 66% improvement in reclassification compared to traditional risk factors [3, 7].

There is another point of view on the appropriateness of using CCI [8]. Thus, in contrast to the 2018 ESC and 2019/2018 AHA/ACC guidelines, experts from the US Preventive Services Task Force (USPSTF) provide data that, compared with non-traditional CVD risk factors, taking into account CCI leads to an unreliable increase in the quality of reclassification. Despite this, it should not be denied that the assessment of coronary calcium of the heart contributes greatly to the reclassification of risk for a particular patient. In particular, according to R.A. Groen et al. (2024), patients' knowledge of their calcium level correlates with improved compliance with the treatment regimen and more effective lifestyle modification [9].

In recent years, there has been some evidence that high atherosclerotic load is closely associated with cardiac vegetative dysfunction through the innervation of vascular walls (endothelial dysfunction), which is mediated by the autonomic nervous system (ANS), the activity of its sympathetic and parasympathetic branches [10]. There is very little evidence of a dose-dependent effect of statins on baroreceptor sensitivity, sympathetic activity, and regulation of cardiovascular reflexes [11]. However, the pathways that mediate the effect of the autonomic nervous system on the structure and function of blood vessels and are complex and poorly understood, as evidenced by scarce data in the scientific literature of recent years.

It is known that endothelial dysfunction, a pathological vascular phenotype of all systemic arteries characterized by the damaging effect of vasoconstrictor, pro-inflammatory and prothrombotic mediators, which can be assessed by the level of humoral biomarkers, on the endothelial vascular membrane and leads to a violation of the endothelium ability to recover, is reasonably considered an independent pathobiological driver of atherosclerosis and related pathogenetic pathways of cardiovascular diseases [12, 13]. Meanwhile, data on the presence of integrative pathophysiological cross-interactions of humoral biomarkers, branches of the autonomic nervous system in relation to the state of myocardial blood flow in the early stages of atherosclerosis development depending on its severity, assessed by the CCI, are clearly insufficient [2]. Interest in studying this problem is also easily explained from the point of view of its practical focus, bearing in mind the search for therapeutic targets and opportunities for effective lifestyle modification and patient adherence to treatment.

A aim of the study was to evaluate the presence of association between traditional factors of the 10-year MESA coronary heart disease risk index, heart rate variability (HRV), and humoral markers of sympathetic activity depending on the presence or absence of calcium in the coronary arteries (CA) in patients with non-occlusive coronary atherosclerosis.

MATERIALS AND METHODS

The prospective, single-center, non-randomized, clinically controlled study to assess the relationship between traditional risk factors, heart rate variability, and molecular and instrumental markers of sympathetic activity and cardiovascular risk and their contribution to the assessment of the 10-year MESA coronary heart disease risk index. Data from patients with suspected coronary heart disease (complaints of pain in the heart and/or shortness of breath during exercise) were collected and analyzed at Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences (Tomsk, Russia) over the period from 2022 to 2023. The study was conducted in accordance with the principles of Declaration of Helsinki and was approved by the Ethics Committee, dated October 30, 2018. Written informed consent was obtained from all patients at the time of their inclusion in the study.

Exclusion criteria were as follows: age under 18 years, left ventricular ejection fraction (LVEF) < 50%, inflammatory myocardial diseases, storage diseases, the presence of congenital or acquired valvular pathology – diagnosed moderate to severe heart disease, history of myocardial revascularization, active infection or serious hematological, metabolic or endocrine dysfunction; chronic kidney disease stages 4–5 (glomerular filtration rate (CKD-EPI) < 30 ml/min/1.73 m^2), condition after pacemaker installation, hypertrophic or dilated cardiomyopathy, ventricular extrasystole grade III–IV (Lown grade), the presence of chronic heart failure (CHF) with preserved LVEF (symptoms and/or signs of CHF; LVEF ≥ 50%; NT-proBNP level more than 125 pg/ml; structural heart change (LV hypertrophy and/or left atrial enlargement) and/or diastolic dysfunction), stroke, transient ischemic attack, history of pulmonary embolism of any duration, acute condition at the time of the study (acute coronary syndrome, acute cerebrovascular accident, pulmonary embolism, acute myocarditis, acute pericarditis, dissecting aortic aneurysm, acute heart failure), obstructive atherosclerotic lesion of the coronary arteries (≥ 70%), identified according to multislice computed tomography coronary angiography (MCTCA), poor quality of MCTCA, the presence of objective signs of previous myocardial infarction, pregnancy, and breastfeeding. . Exclusion criteria from the study also included patient's refusal to further participate in the study.

All patients underwent a general examination, determination of the level of coronary calcium according to MCTCA, and assessment of markers of autonomic dysfunction.

The study included patients (n = 30) aged 59.7 [54.1; 67.2] years (63.3% men) with suspected coronary heart disease, with symptoms of stable angina pectoris and/or dyspnea, who were identified with at least one coronary artery stenosis <70% according to

MCTCA data, LVEF ≥ 50% according to transthoracic echocardiography. Group 1 included patients with CCI = 0, patients with CCI > 0 constituted group 2. There were no statistically significant differences in the number of individuals with cardiovascular risk (SCORE2 scale) in the categories of "low, moderate, high, and very high risk": in group 1 – 18.2, 18.2, 36.4 and 27.3%, in group 2 – 5, 15, 35 and 45%, respectively.

Biochemical studies of markers of autonomic dysfunction. Blood samples were collected, frozen and stored for further analysis of sympathetic activity and cardiovascular risk markers (natriuretic peptide, high-sensitivity C-reactive protein, catestatin, and copeptin).

Blood samples were obtained in the morning on an empty stomach after a 16-hour fast by venipuncture; adequate centrifuged serum samples were stored at -26 °C with one freeze-thaw cycle. Determination of serum biomarkers in vitro was performed by enzyme immunoassay (catestatin, RayBio, USA; copeptin (human), Phoenix Pharmaceutical, Inc., USA; high-sensitivity C-reactive protein, Biomedica immunoassays, Austria; NT-proBNP, Biomedica immunoassays, Austria). Photometric detection of the immunochemical reaction was performed on an Infinite F50 microplate reader (Tecan, Australia).

Heart rate variability (HRV) was analyzed using 24-hour electrocardiogram monitoring (24-hour ECG). At least 12 hours before and during 24-hour ECG, patients were prohibited from drinking caffeinated beverages, and 24 hours before 24-hour ECG, patients who received β-blockers were discontinued. Temporal (SDNN; SDANN; SDNNidx; RMSSD; NN50, 100, 200; pNN50, 100, 200) and spectral parameters of HRV (VLF; LF; HF; LF/HF) were assessed. A detailed description of the HRV parameters and their interpretation are presented in our other work [14].

Echocardiography (EchoCG) was performed in all patients according to the standard protocol using the EPIQ device (Philips Ultrasound, Inc., USA). The cardiac structures were visualized using B- and M-scanning according to the generally accepted method. All studies were performed by a highly qualified specialist.

MCTCA and quantitative assessment of coronary artery calcification. All patients underwent MCTCA on a 64-row tomograph Revolution Evo tomograph (GE HealthCare, USA). The day before MCTCA of the heart, patients were advised to exclude caffeinated beverages, metformin, sildenafil, and painkillers from their diet. To determine the CCI, non-contrast computed tomography of the heart region was performed with prospective ECG synchronization and subsequent reconstruction in 75% of the R-R phase of the cardiac cycle interval. Recording was performed from the level of the tracheal bifurcation to the diaphragm with breath holding (6–8 s) in a step-by-step mode with a slice thickness of 2.5 mm and a tube rotation time of 0.4 s. The voltage in the tube was 120 kV, the current was 200–435 mA. The CI analysis was performed using the Agatston method on the Advantage Workstations 4.7 workstation (GE Healthcare, Milwaukee, WI, USA) in the SmartScore 4.0 software (in Agatston units) [15].

As a result of processing, data were obtained on coronary artery calcification by vascular regions (left coronary artery trunk, anterior descending artery, circumflex artery, and right coronary artery) and in total for the coronary bed [16]. Additionally, based on the MCTCA data, the 10-year risk of coronary heart disease according to MESA and the age of the coronary arteries were estimated using the corresponding calculators developed, tested, and described by Robyn et al. (2015) and Blaha et al. (2021) [17, 18].

To perform MCTCA, patients were administered 70–90 ml of non-ionized, low-osmolar radiopaque contrast agent Iopromide (Ultravis 370) at a rate of 5 ml/s, followed by the introduction of 40 ml of normal saline at the same rate. The recording was made in a spiral ECG (prospective or retrospective) synchronized mode with an X-ray tube rotation speed of 0.35 m and a slice thickness of 0.625 mm. The voltage in the tube was 120 kV, the current was 450–550 mA. CardIQ Xpress 2.0 software was used to plot and analyze the coronary arteries. The analysis collected data on the presence of atherosclerotic plaques and the degree of coronary artery stenosis. The criterion for obstructive lesion was a narrowing of ≥ 70%.

Dynamic single-photon emission computed tomography (SPECT) of the heart and myocardial perfusion scintigraphy (MPS). The study was performed using a 2-day rest/exercise protocol using 99mTc-methoxy-isobutyl-isonitrile; adenosine triphosphate (160 μg/kg/min) was administered as a stress agent during the exercise test. Myocardial perfusion and blood flow parameters were analyzed using 4DM Reserve v.2015 and Corridor 4DM SPECT software (INVIA, Ann Arbor, USA). The standard MPS data were used to assess global indices (SSS, SRS, SDS), and the dynamic SPECT data were used for quantitative global indices such as stress and rest myocardial blood flow (sMBF, rMBF), and myocardial blood flow reserve (MBFR).

Statistical data processing, as in our previous work, was performed using the Statistica 10.0 (StatSoft, Inc., USA). The distribution of features was assessed using the Shapiro–Wilk test, and the homogeneity of general variances was assessed using Levene's test. Quantitative data were presented as median and interquartile range *Me* [Q_{25}; Q_{75}]. The Mann–Whitney *U* test was used to test statistical hypotheses when comparing two independent groups. Correlation analysis was used with the calculation of Spearman's rank correlation coefficients to measure association between variables. When analyzing qualitative features, we analyzed contingency tables using the Pearson's $\chi 2$ test or Fisher's exact test when the mathematical expectation of values in any of the table cells with the specified boundaries was below 10. To assess the sensitivity and specificity of the models and select the cutoff threshold, we used ROC analysis with the construction of characteristic curves and calculation of the area under the curve (AUC, area under curve). The AUC value exceeding 0.70 was considered significant. To identify factors that have a significant impact on the course and prognosis of the disease, we calculated the odds ratio (OR) with a 95% confidence interval (CI). The critical significance level *p* for all statistical analysis procedures used was 0.05.

RESULTS

The clinical baseline characteristics of the study population are shown in Table 1.

Table 1

Basic Clinical and Laboratory Characteristics of Patients with Non-Obstructive Coronary Atherosclerosis

Parameters	Group 1, *n* = 11 CCI = 0	Group 2, *n* = 19 CCI > 0	*p*
Age, years, *Me* [Q_{25}; Q_{75}]	57.1 [40.9; 68.8]	62.9 [48.1; 76.5]	0.1853
Female, *n* (%)	4 (36.4)	7 (36.8)	0.7162
Diabetes mellitus, *n* (%)	0 (0)	2 (10.5%)	0.1379
Atrial fibrillation, *n* (%)	1 (9.1)	5 (26.3)	0.1577
Arterial hypertension, *n* (%)	11 (100.0)	19 (100.0)	1.0000
Current smoking, *n* (%)	1 (9.09)	8 (42.11)	0.0142
Family history of premature CHD, *n* (%)	11 (100)	12 (63.16)	0.0021
Systolic blood pressure, mm Hg, *Me* [Q_{25}; Q_{75}]	125.0 [120.0; 130.0]	125.0 [120.0; 130.0]	0.9178
Diastolic blood pressure, mm Hg, *Me* [Q_{25}; Q_{75}]	80.0 [72.0; 90.0]	80.0 [70.0; 85.0]	0.6623
Heart rate, beats per minute, *Me* [Q_{25}; Q_{75}]	73.0 [70.0; 77.0]	68.0 [65.0; 72.0]	0.0048
Glucose, mmol/L, *Me* [Q_{25}; Q_{75}]	5.65 [4.47; 6.54]	5.40 [4.12; 5.90]	0.6484
Cathestatin, μg/ml, *Me* [Q_{25}; Q_{75}]	222.23 [136.7; 294.1]	172.40 [100.2; 210.4]	0.1467
Copeptin, ng/mL, *Me* [Q_{25}; Q_{75}]	0.439 [0.334; 0.689]	0.441 [0.374; 0.485]	0.5801
hsCRP, g/L, *Me* [Q_{25}; Q_{75}]	2,50 [1,20; 4,70]	3,1 [1,70; 11,90]	0,0491
Total cholesterol, mmol/l, *Me* [Q_{25}; Q_{75}]	5,18 [4.10; 6.12]	4.3 [3.41; 5.52]	0.0292
HDL-C, mmol/L, *Me* [Q_{25}; Q_{75}]	1.3 [1.02; 1.59]	1.20 [1.03; 1.59]	0.4910
LDL-C, mmol/L, *Me* [Q_{25}; Q_{75}]	2.81 [2.11; 3.9]	1.82 [1.49; 2.50]	0.0014
Achievement of target LDL-C levels, n (%)	1 (9.09%)	4 (21.05%)	0.2760
Therapy, *n* (%)			
Statins	7 (63.64%)	15 (78.94%)	0.1116
Beta-blockers	5 (45.45%)	10 (52.63%)	0.4114
ACE inhibitors	2 (18.18%)	8 (42.11%)	0.1063
Angiotensin II receptor blockers	3 (27.27%)	4 (21.05%)	0.4175
Calcium channel antagonists	2 (18.18%)	6 (31.58%)	0.3784
Diuretic	3 (27.27%)	4 (21.05%)	0.4175

Note. Here and in Table 5:NT-proBNP – N-terminal propeptide of natriuretic hormone (B-type); ACE – angiotensin-converting enzyme; hsCRP – high-sensitivity C-reactive protein; CCI – coronary calcium index; CHD – coronary heart disease; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol.

A total of 30 patients with an average age of 59.7 years [54.1; 67.2] were included in the cohort. Two-thirds of the examined patients were men (men 63.3%, *n* = 19). All patients were taking antihypertensive drugs and had a history of arterial hypertension, and dyslipidemia was diagnosed in 100% of cases, for which three out of four patients were taking statins. At the same time, judging by the number of patients

who achieved target LDL-C levels, the drug doses were clearly not optimal. Another predominant cardiovascular risk factor (76.7%, n = 23) was a family history of premature coronary heart disease. Significantly less common among other risk factors were smoking (30%), atrial fibrillation (20%), and type 2 diabetes mellitus (6.7%). According to MSCT-CAG data, all patients were found to have stenosis of up to 70% of the vessel lumen in at least one coronary artery, with stenosis of 50–70% in almost half of those examined (46.7%, n = 14).

The patients were divided into groups depending on the CCI value: group 1 (CCI 0, n = 11) and group 2 (CCI > 0, n = 19). Family history of premature coronary heart disease was found in all patients of group 1 and significantly less frequently (63.2%, p = 0.0021) in group 2. Moreover, among patients with CCI > 0 there were more (p = 0.0142) smokers, they were diagnosed with 50–70% coronary artery stenosis and damage to more than one epicardial artery twice more often (p = 0.0077). The catestatin level in patients with CCI > 0 was 22.4% (p = 0.1467) lower than in individuals with CCI = 0, but the hsCRP content, on the contrary, was higher in those examined in group 2 (2.50 vs. 3.1; p = 0.049).

The levels of cholesterol and LDL-C in patients with CCI = 0 were higher than similar indicators in individuals with CCI > 0, while the frequency of achieving the target level of LDL-C in group 1 was clearly lower than in group 2, despite a comparable number of patients taking statins.

The MESA 10-year coronary heart disease risk index, taking into account coronary calcium in patients of group 2, was 2.5 (p < 0.0343) times higher than the same indicator in group 1 (Fig. 1). Statistically significant differences also concerned such an indicator as the difference between coronary age and chronological age taking into account the CCI, in particular, such in the group of patients with CCI > 0 was 2.5 times higher than the same indicator in patients of group 1 (CCI = 0).

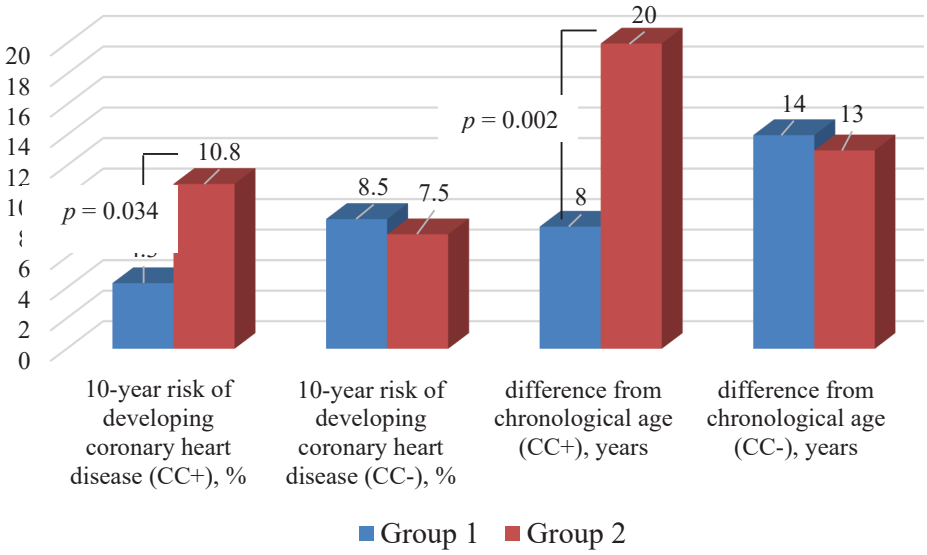


Fig. 1. The risk index for developing coronary heart disease over 10 years and the difference between coronary age and chronological age in groups of patients with non-occlusive coronary atherosclerosis depending on the presence or absence of coronary calcium

The analysis of global myocardial SPECT data is presented in Table 2. Standard parameters of SCF, myocardial blood flow reserve, and myocardial blood flow parameters at rest and under stress test conditions did not differ between the groups. At the same time, statistically significant (p < 0.05) differences were observed in the number of coronary arteries with atherosclerotic plaques and the number of patients with 50–70% stenosis.

Table 3 presents the temporal parameters of HRV, but the spectral indicators did not have significant differences and are not presented in the table.

When analyzing the results of the HRV study, signs of disintegration of the autonomic regulation of cardiac function were revealed, expressed in the suppression of the activity of the parasympathetic division of the autonomic nervous system, which reflects one of the HRV indicators – rMSSD [19]. In particular, compared with group 1, in patients with CCI > 0, rMSSD was 1.6 times lower (p = 0.044).

Analysis of the ROC curve characteristics showed that copeptin levels ≤0.485 ng/ml allow identification of patients whose CCI exceeds zero, although they showed low but statistically significant discriminatory ability of the model (AUC = 0.672; sensitivity 88%, specificity 60%; p = 0.021) (Fig. 2, *a*).

Table 2

Data of MCTCA and Dynamic SPECT in Patients with Non-Obstructive Coronary Atherosclerosis

Parameters	Group 1, n = 11 CCI = 0	Group 2, n = 19 CCI > 0	p
Calcium index, Agatston units, *Me* [Q_{25}; Q_{75}]	0 [0.0; 0.0]	191.00 [68.0; 367.0]	0.000
CA stenosis 50–70%, n (%)	3 (27.27)	11 (57.90)	0.008
Number of CA with plaques, n (%)	1.0 [1.0; 2.0]	3.0 [2.0; 3.0]	0.000
Standard semiquantitative indices of myocardial perfusion impairment, Me [Q_{25}; Q_{75}]			
SSS	2.0 [0.0; 3,0]	2.0 [0.0; 3.0]	0.450
SRS	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]	0.568
SDS	2.0 [0.0; 3.0]	2.0 [0.0; 2.0]	0.217
Dynamic SPECT parameters, Me [Q_{25}; Q_{75}]			
sMBF, ml/min/g	1.33 [0.81; 1.79]	1.29 [0.98; 1.75]	0.840
rMBF, ml/min/g	0.94 [0.59; 1.23]	0.76 [0.56; 1.08]	0.367
MBFR	1.41 [1.22; 1.61]	1.47 [1.23; 2.09]	0.429

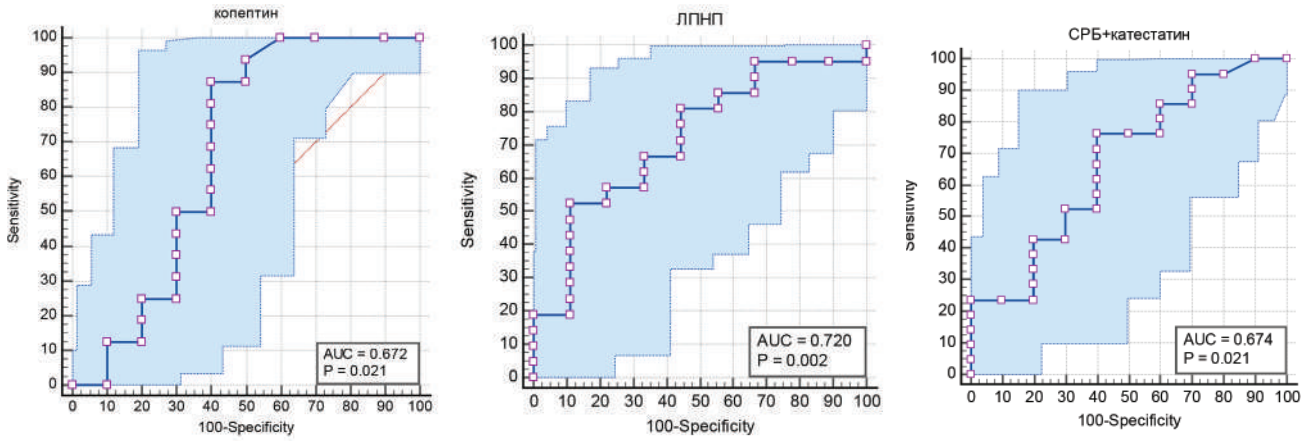
Note. Here and in Table 4: SDS – stress-rest difference; SRS – rest score; SSS – stress score; CA – coronary artery; MCTCA – multislice computed tomography coronary angiography; SPECT – single photon emission computed tomography; rMBF – rest myocardial blood flow; MBFR – myocardial blood flow reserve; sMBF – stress myocardial blood flow.

Table 3

Data from Daily Holter ECG Monitoring (Indicators of Heart Rate Variability in Patient Groups), *Me* [Q_{25}; Q_{75}]

Parameters	Group 1, n = 11 CCI = 0	Group 2, n = 19 CCI > 0	p
SDNN, ms	123.5 [90.0; 169.0]	115.00 [99.0; 130.0]	0.5946
SDANN, ms	92.5 [70.0; 118.0]	93.00 [79.0; 102.0]	0.8801
SDNNidx, ms	63 [53.0; 73.0]	59 [53.0; 72.0]	0.3948
NN50, ms	4,132.5 [1,731.0; 12,890.0]	6,189.00 [3578.0; 16,397.0]	0.2049
NN100, ms	885.5 [369.0; 1,499.0]	438.00 [269.0; 6,363.0]	0.2049
pNN50, %	7.9 [4.0; 22.7]	5.75 [4.5; 18.0]	0.5946
rMSSD, %	57.0 [30.0; 72.0]	35.00 [27.0; 59.0]	0.0438

Note. Here and in Table 5: SDNN is the standard deviation of the full array of RR cardiointervals; SDANN is the standard deviation of the averaged normal R-R sinus intervals of all 5-minute periods for the entire observation period; SDNNidx is the average value of standard deviations of NN intervals calculated for 5-minute intervals in the specified recording period; rMSSD is the square root of the average sum of squares of differences between adjacent NN intervals; NN50 (100, 200) is the number of pairs of adjacent NN intervals that differ by more than 50 (100, 200) ms; pNN50 (100, 200)% is the NN50 (100, 200) value divided by the total number of NN intervals in the analyzed monitoring period (norm = 6.3 ± 0.8%); p is the statistical significance of intergroup differences.



a *b* *c*

Fig. 2. ROC curve characteristics of humoral biomarkers for optimal binary classification of the presence or absence of coronary calcium in patients with suspected CHD (ROC analysis): a) copeptin; b) LDL-C; c) hsCRP+catestatin. Note. The ordinate axis shows sensitivity (%), the abscissa axis shows 100 minus specificity (%). The rectangles show the sensitivity (Sensitivity) and specificity (Specificity) estimates for the corresponding decision rule threshold (Criterion), as well as the area under curve (AUC) values together with the significance level (p) estimates.

The best characteristics of the binary classification of the presence or absence of coronary calcium in patients with suspected coronary heart disease among humoral markers were shown by LDL-C ≤1.82 mmol/L (AUC = 0.72; sensitivity 52%, specificity 89%; $p = 0.002$) (Fig. 2B) and a combination of hsCRP with catestatin (hsCRP ≤ 1.21 g/L and catestatin ≤ 138.1 μg/ml; AUC=0.674; sensitivity 56.2%; specificity 82.2%; $p = 0.021$) (Fig. 2, *c*).

According to the correlation analysis, statistically significant (the *p* parameters for this analysis ranged from 0.001 to 0.02) relationships were established between the 10-year risk of developing coronary heart disease (taking into account the CCI) and the difference between coronary and chronological age with humoral biomarkers characterizing neurohumoral activation and reflecting the severity of endogenous neurohormonal stress, indices of myocardial perfusion impairment and indicators of myocardial blood flow and reserve (Table 4). In particular, the 10-year risk of developing coronary heart disease (taking into account the CCI) was directly associated with the volume of myocardial perfusion impairment at rest (SRS) ($r = 0.60$), and the myocardial blood flow indices at rest and under stress ($r = –0.45$ and $r = –0.35$, respectively) and the copeptin level ($r = –0.31$) were inversely associated. The difference between the coronary and chronological ages was negatively correlated with the levels of both biomarkers (copeptin – $r = –0.36$ and catestatin – $r = -0.44$) and was directly associated with SRS ($r = 0.66$). In turn, there was a moderate degree of association between the 10-year risk of developing coronary heart disease (taking into account the CCI) and the difference between the coronary and chronological ages ($r = 0.69$).

Table 4

"Heat Map" of Correlations between the Analyzed Biomarkers

Parameter	Copeptin	Cate-statin	sMBF	rMBF	MBFR	SRS	SDS	10-year risk	Difference from ChA
Copeptin		*				*		*	*
Catestatin	*								*
sMBF				*				*	
rMBF			*		*			*	
CFR				*		*	*		
SRS	*				*			*	*
SDS					*				
10-year risk	*		*	*		*			*
Difference from ChA	*	*				*		*	

Note. ChA – chronological age. * *p* for this correlation analysis were between 0.001 and 0.05.

Multivariate regression analysis revealed that the presence of coronary artery calcification (CCI = 0) in patients with non-obstructive coronary atherosclerosis was independently associated with the levels of lipid spectrum parameters, catestatin, and copeptin, a family history of coronary heart disease, smoking, diabetes mellitus, and the HRV parameter associated with the regulation of parasympathetic activity of the autonomic nervous system (rMSSD) (Table 5).

Table 5

Multivariable Linear Regression Model to Assess the Association between the Presence of Coronary Calcium and Clinical Characteristics, Heart Rate Variability Parameters, and Laboratory Biomarkers

Parameter	OR	95% CI
LDL-C decrease ≤ 1.82 mmol/L	10.83	2.13–23.12
Copeptin level decrease ≤ 0.485 ng/mL	2.67	1.09–5.89
Early heredity of coronary heart disease	1.15	1.01–2.98
rMSSD ≤ 42, ms	1.11	0.99–2.17
HDL-C increase ≥ 1.12 mmol/L	6.73	4.87–11.65
Total cholesterol, mmol/L	4.27	2.19–6.12
Type 2 diabetes mellitus	3.59	1.98–7.18
Catestatin concentration decrease ≤ 138.1 μg/mL	2.12	1.98–3.19

End of table 5

Parameter	OR	95% CI
Statin use	2.10	1.16–5.98
SDNNidx ≤ 60, ms	1.97	1.13–5.14
Smoking	1.10	0.98–3.09

Note. OR – odds ratio; CI – 95% confidence interval; $p < 0.0001$.

Other clinical, instrumental, including myocardial blood flow indicators, and laboratory parameters did not show statistical significance.

DISCUSSION

An increasing number of evidence has shown that the coronary calcium index is more useful as a predictor of cardiovascular disease and a tool for cardiovascular risk stratification than the conventional Framingham risk score, C-reactive protein, or carotid intima-media thickness, especially in intermediate-risk cohort [2; 4, 20]. In a prospective cohort study of 6,814 people followed up for 3.8 years, compared with patients with a CCI of 0 ($p < 0.001$), the hazard ratios for developing a coronary event were 7.73 (CCI 101–300) and 9.67 (CCI > 300), and the AUC of the model was significantly higher (0.82 vs 0.77; $p < 0.001$) when CCI was added to standard risk factors [21].

Vonder et al. (2020) presented data that coronary calcium assessment may be of additional value in patients with stable chest pain to exclude CHD in case of a zero score or to stratify patients with increased risk who may require more intensive treatment [3]. Gottlieb et al. (2010) showed that in symptomatic patients referred for traditional coronary angiography, the absence of coronary calcification does not exclude obstructive CHD or the need for revascularization. [22]. Detection and characterization of coronary atherosclerosis using imaging tools are key key to determining the management of patients with known or suspected CHD [23].

According to the Consensus of the Quantitative Cardiovascular Imaging Study Group, among non-invasive diagnostic methods, MCTCA is the leading method for analyzing coronary atherosclerosis [24]. It has been demonstrated that the absence of coronary calcification is not a reliable indicator of the absence of functionally significant narrowing of the lumen of the coronary artery [21, 25]; in particular, in 3.5% of symptomatic patients, according to the CONFIRM study (n = 10,037), 50–70% coronary artery stenosis is detected [26].

In our study in patients with non-occlusive coronary atherosclerosis, depending on the presence or absence of calcium in the coronary arteries, we examined the presence of associations between traditional factors of the 10-year MESA coronary heart disease risk index, heart rate variability, and humoral markers of sympathetic activity. We showed that in patients with CCI > 0, the 10-year risk of developing coronary heart disease is 2.5 times higher ($p = 0.034$) compared to patients without coronary artery calcification. The coronary age in individuals of group 2 is also 2.5 times ($p = 0.02$) higher than the corresponding indicator in group 1. At the same time, the 10-year risk of developing coronary heart disease was associated with the sum of points at rest according to myocardial SPECT data and inversely correlated with myocardial blood flow indices at rest and under stress conditions. It is possible that the weak strength of the relationship is caused by both the small number of observations and the influence of a combination of clinical risk factors for CVD on the dynamics of myocardial blood flow and reserve indicators, which does not contradict the opinion of other researchers [27].

It was established that stenosis in at least one coronary artery of 50–70% was found in 27.3 and 57.9% of patients with CCI = 0 and CCI > 0, respectively, and all of them had moderate, high, or very high risk. At the same time, according to the data on the assessment of myocardial blood flow and perfusion, we did not obtain statistically significant differences. However, it is noteworthy that in the group of patients without coronary atherosclerosis, there was a tendency to decrease myocardial blood flow at rest, although stress MBF was practically at the same level. This caused a tendency to decrease the reserve of myocardial blood flow in this group compared to group 2. It should be noted that there is clearly insufficient data in the scientific literature on the assessment of myocardial ischemia in myocardial perfusion studies in patients with CCI = 0. Neves et al. (2017) presented the summary results of 8 studies (n = 3,717) that performed myocardial perfusion imaging under stress test conditions: on average, myocardial ischemia was detected in 7% of patients with CCI = 0 and in 13% of patients with CCI > 0 [25].

According to the correlation analysis of the associations of heart rate variability parameters with clinical and laboratory parameters and parameters of dynamic SPECT of the heart and MCTCA, no associations were found, but at the same time, a decrease in the rMSSD level was noted in patients with CCI > 0, which indicates the disintegration of the autonomic regulation of the heart, manifested in the suppression of the parasympathetic activity of the HRV, and is confirmed by the data of Hoshi et al. (2023), from which it follows that the presence of coronary artery calcification is associated with a worse cardiac vegetative profile [10; 28]. It should also be noted that, according to multivariate analysis, several HRV parameters characterizing the parameters of sympathetic activity in patients with non-occlusive atherosclerosis were associated with coronary calcification: rMSSD OR 1.105 (95% CI 0.99–2.17; $p < 0.000$) and SDNNidx OR 2.52 (95% CI 1.13–3.94; $p < 0.000$).

In addition to the listed indicators, the following clinical factors acted as independent markers of coronary calcification: the presence of diabetes mellitus OR 3.59 (95% CI 1.98–7.18; $p < 0.000$), a history of smoking OR 1.1 (95% CI 0.98–3.09; $p < 0.000$), and a family history of premature coronary heart disease OR 1.15 (95% CI 1.01–2.98; $p < 0.000$), the fact of taking statins OR 2.10 (95% CI 1.16–5.98; $p < 0.000$), as well as humoral biomarkers of sympathetic activity and CVD risk – catestatin OR 2.12 (95% CI 1.98–3.19; $p < 0.000$) and copeptin OR 2.67 (95% CI 1.09–5.89; $p < 0.000$), and lipid spectrum parameters – LDL-C OR 10.83 (95% CI 2.13–23.12; $p < 0.000$), HDL-C OR 6.73 (95% CI 4.87–11.65; $p < 0.000$), total cholesterol OR 4.27 (95% CI 2.19–6.12; $p < 0.000$).

When assessing the levels of lipid spectrum parameters, it was noted that in patients with CCI 0, the content of total cholesterol and LDL-C is clearly higher than in individuals with CCI > 0. However, the number of patients taking statins and, particularly, achieving target levels of LDL-C, in group 1 is lower (63.6 vs. 78.9% and 9.1 vs. 21.1%) compared to group 2. We did not obtain statistical significance for these parameters, but we should note a clear trend. This is not contradicted by the results of studies indicating that statin therapy increases coronary plaque calcification, and therefore researchers pay attention to the fact that when preventive therapy and effective treatment of risk factors begin, the degree of coronary calcification can increase, and the risk of cardiovascular events can decrease [9].

The data from the multicenter PARADIGM study also indicate an increase in the total calcium content in the structure of atherosclerotic plaques, as well as the absence of an effect on the degree of coronary artery stenosis. In particular, it was found that patients who were prescribed statin therapy (rosuvastatin and atorvastatin) showed a statistically significant slowdown in the progression of the total plaque volume (1.76 ± 2.40% versus 2.04 ± 2.37% per year) and no increase in the volume of the soft tissue component (0.49 ± 2.39% versus 1.06 ± 2.42% per year) was observed according to MSCT-CG data compared to individuals who did not receive these drugs [29; 30]. The NOTIFY-1 study ($n = 173$) found that patients who started statin prophylaxis earlier and were more adherent to treatment had significantly lower LDL-C levels compared with the standard treatment group (97.2 vs 115.3 mg/dL; $p = 0.005$, respectively) [31].

Thus, at this research stage, it is scientifically abd practically important to focus to focus on the use of CCI to assess its potential as a personal risk stratification tool and to determine its contribution to individual patient treatment decisions. However, the lack of randomized controlled trials on the cost-intensive use of coronary calcium remains a pressing issue, but the number of new studies in this area and their apparent usefulness mean that integration of coronary calcium into cardiovascular imaging and risk stratification is likely a matter of time rather than opportunity.

CONCLUSION

The presence of calcium in the coronary arteries in patients with non-obstructive coronary atherosclerosis is associated with a family history of premature coronary heart disease, disintegration of the autonomic regulation of cardiac function, expressed in the suppression of the activity of the parasympathetic division of the autonomic nervous system, and the absence of significant levels of humoral markers of sympathetic activity. Threshold values of LDL-C and copeptin have been determined, which in such patients can be used as markers of the presence or absence of coronary calcium. Independent predictors of the absence of coronary artery calcification (CCI = 0) in patients with non-obstructive coronary atherosclerosis are lipid spectrum parameters (total cholesterol, LDL-C, HDL-C) and copeptin levels, the presence of a family history of coronary heart disease, smoking, and HRV parameters associated with the regulation

of parasympathetic activity of the autonomic nervous system (SDANN, SDNNidx, rMSSD).

The limitations of this study included: 1) a small sample size of patients; 2) the absence of a control group of conditionally healthy individuals; 3) this study, did not evaluate the prognostic value of CCI.

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Grakova E.V. – collection and interpretation of clinical data, database compilation, statistical data processing, critical revision for important intellectual content, final approval of the manuscript for publication. Kopeva K.V. – interpretation of clinical data, performing daily ECG monitoring, drafting of the manuscript, final approval of the manuscript for publication. Maltseva A.N., Dasheeva A.S. – conducting scintigraphic studies, collection and interpretation of data, database compilation, final approval of the manuscript for publication. Zavadovsky K.V. – conducting scintigraphic studies, assessing blood flow parameters, drafting of the manuscript, final approval of the manuscript for publication. Gusakova A.M. – determination of serum biomarker levels, data collection and interpretation, database compilation, final approval of the manuscript for publication. Vorozhtsova I.N. – collection and interpretation of clinical data, critical revision for important intellectual content, final approval of the manuscript for publication. Antsiferova E.L., Shadrina Yu.L. – interpretation of clinical data, database filling, final approval of the manuscript for publication.

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Analysis of the Structure of Deaths from Sporadic Metachronous Primary Multiple Malignant Neoplasms in a General Hospital

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ABSTRACT

Aim. To analyze the structure of deaths from sporadic metachronous primary multiple malignant neoplasms in 2017–2023 in a multidisciplinary inpatient facility.

Materials and methods. The study included 2,394 fatal cases of patients hospitalized in a multidisciplinary inpatient facility for emergency medical care. In 2017–2023, 29 metachronous primary multiple malignant neoplasms were identified, which was 1.3% of the total number of fatal outcomes and 11% of malignant neoplasms. The median age of patients was 72.0 (69.0–82.0) years. We examined the protocols of pathological studies of autopsy material of patients hospitalized in the inpatient facility of multidisciplinary clinics of Siberian State Medical University. In patients with metachronous primary multiple malignant neoplasms, the nosological structure, stage of the process, histological form of the neoplasm, the period between the diagnosis of the first and second malignant tumor, and the immediate cause of death were analyzed. Statistical processing of the results was carried out using the Statistica 10.0 software package.

Results. The first tumor was most often caused by squamous cell skin cancer (21%) or invasive ductal carcinoma of the breast (17.5%). The interval between the diagnosis of the first and second tumor was 72.0 (48.0–96.0) months. All patients received definitive treatment for the first tumor without progression. The second metachronous tumors were verified in an advanced stage in 72% of cases and caused the death of patients. Most often (17.5%) these were diffuse gastric cancer.

Conclusion. Metachronous primary multiple malignant tumors can occur long after the first ones (about 6 years), often in advanced forms (in this case, tumors verified exclusively postmortem – 69%), being the cause of death of patients. The most common first tumors in sporadic metachronous primary multiple malignant neoplasms are recurrent squamous cell skin cancer and ductal carcinoma of the mammary gland. The main target organ for the development of oncopathology is the stomach.

Keywords: sporadic, metachronous, primary multiple, malignant neoplasms

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Анализ структуры летальных исходов от спорадических метахронных первично-множественных злокачественных новообразований в стационаре общего профиля

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РЕЗЮМЕ

Цель. Провести анализ структуры летальных исходов от спорадических метахронных первично-множественных злокачественных новообразований (ПМЗНО) за период с 2017 по 2023 г. на примере стационара общего профиля.

Материалы и методы. Исследовались случаи летальных исходов 2 394 больных, поступивших в порядке скорой помощи в стационар общего профиля. В 2017–2023 гг. выявлено 29 метахронных ПМЗНО, что составило 1,3% от общего числа летальных исходов и 11% – от злокачественных новообразований. Медиана возраста больных соответствовала 72,0 (69,0–82,0) годам. Изучались протоколы патологоанатомических исследований аутопсийного материала пациентов, поступивших в стационарное отделение клиник общего профиля СибГМУ. В случаях с метахронными ПМЗНО анализировались нозологическая структура, стадия процесса, гистологическая форма новообразования, срок между диагностикой первой и второй злокачественной опухоли, непосредственная причина смерти. Статистическая обработка результатов проводилась с применением пакета программ Statistica 10.0.

Результаты. Первая опухоль чаще была представлена плоскоклеточным раком кожи (21%) или инвазивной протоковой карциномой молочной железы (17,5%). Временной промежуток между диагностикой первой и второй опухоли составлял 72,0 (48,0–96,0) мес. Все пациенты были радикально пролечены по поводу первой опухоли без прогрессирования. Вторые метахронные опухоли в 72% были верифицированы в запущенной стадии и являлись причиной смерти больных. Чаще (17,5%) они были представлены диффузным раком желудка.

Заключение. Метахронные ПМЗНО могут возникать через длительный временной промежуток (около 6 лет) после первых, часто в запущенных формах (в данном исследовании преимущественно посмертно – 69%), являясь причиной смерти больных. Наиболее частыми первыми опухолями при спорадических метахронных ПМЗНО являются плоскоклеточный рак кожи и протоковая карцинома молочной железы, а основным органом-мишенью для повторного развития онкопатологии – желудок.

Ключевые слова: спорадические, метахронные, первично-множественные, злокачественные новообразования

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

One of the relevant problems of modern oncology is the steady increase in multiple primary malignant neoplasms (MPMN). The prevalence of primary multiple malignant tumors in the world ranges from 2 to 17% [1]. In 2015–2023 in Russia, the number of MPMN diagnosed in the reporting year increased from 39,195 to 77,433, the percentage of MPMN from the number of newly diagnosed malignant neoplasms is from 6.7 to 11.5%, and the MPMN rate per 100,000 people ranges from 26.8 to 52.8. As of the end of 2023, 288,345 patients who were under follow-up monitoring had MPMN, which amounted to 6.9% of the total number of patients registered in cancer institutions [2].

MPMN may be hereditary and non-hereditary [3–7]. Detection of genetic disorders possibly associated with hereditary syndromes is an effective tool for early diagnosis of MPMN and selection of optimal treatment tactics [8]. However, about 70% of MPMN are cases of sporadic metachronous malignant tumors diagnosed after six or more months from the moment of verification of the first neoplasm [8, 9]. The steady increase in their number is explained by the improved methods of early diagnosis and therapy of malignant neoplasms and, as a consequence, an increase in the survival rate of cancer patients, environmental and biological factors, and an increase in the overall life expectancy of the population [10–12]. However, there is still no unified concept in understanding the key mechanisms of development of sporadic metachronous MPMN, and the nosological structure and mortality from sporadic metachronous MPMN are yet to be properly analyzed.

The study of clinical morphological and molecular genetic structures with the aim of implementing these principles, as well as the analysis of the structure of fatal outcomes from sporadic metachronous MPMN can become a platform for the development of a system for early detection of the second tumor with subsequent modern definitive treatment, which will lead to an improvement in the quality of life, overall survival increase and a decrease in mortality in patients with widespread metachronous MPMN.

The aim of the study was to analyze the mortality structure from sporadic metachronous multiple primary malignant neoplasms in 2017–2023 using the example of a multidisciplinary inpatient facility.

MATERIALS AND METHODS

The study included 2,394 fatal cases of patients hospitalized for emergency care in multidisciplinary inpatient facility of Tomsk in the period from 01.01.2017 to 31.12.2023, including 264 cases of malignant neoplasms. We examined the protocols of pathological studies of autopsy material. Histological preparations stained with hematoxylin and eosin were examined by light microscopy using an Axio Lab.A1 microscope (Carl Zeiss, Germany). In cases with metachronous MPMN, the following parameters were analyzed the nosological structure, stage of the process, histological form of the neoplasm, the period between the diagnosis of the first and second malignant tumor, and the immediate cause of death.

Statistical processing was carried out using the Statistica 10.0 software package. Basic statistical data and nonparametric criteria were used. The frequency of detection of features was determined by the descriptive statistics method. Comparison of the frequency of detection of features was carried out using the Student's t-test. Differences were considered statistically significant at $p < 0.05$.

The study complies with the standards of the Declaration of Helsinki and was approved by an independent Ethics Committee of Siberian State Medical University of the Ministry of Healthcare of the Russian Federation, protocol No. 5600 dated 23.10.2017.

RESULTS

In 2017–2023, 29 sporadic metachronous MPMN were identified, accounting for 1.3% of all deaths and 11% of deaths from malignant neoplasms. The median age of patients with MPMN was 72.0 (69.0-82.0) years. The most common type was squamous cell carcinoma of the skin (21.0%) or invasive ductal breast cancer (17.5%). Other localizations were less common (Table 1).

Table 1

Localization and Morphological Type of the First Tumors of Deceased Patients with Multiple Primary Malignant Neoplasms, *n* = 29

Localization and histotype of the first tumor	Number of patients abs. (%)
Skin, squamous cell carcinoma of moderate differentiation	6 (21.0%), $p_{3,4,5,10,12} = 0.017$, $p_{8,9,11} = 0.047$
Breast, invasive ductal carcinoma	5 (17.5%), $p_{3,4,5,10,12} = 0.037$
Stomach, moderately differentiated adenocarcinoma	1 (3.5%)
Thyroid gland, papillary cancer	1 (3.5%)
Rectum, moderately differentiated adenocarcinoma	1 (3.5%)
Prostate gland, acinar adenocarcinoma	4 (14%)
Lung, invasive adenocarcinoma	3 (10.5%)
Colon, moderately differentiated adenocarcinoma	2 (6.5%)
Kidney, clear cell renal cell carcinoma	2 (6.5%)
Uterine body, endometrioid adenocarcinoma	1 (3.5%)
Bladder, high-grade urothelial carcinoma	2 (6%)
Salivary glands, adenoid cystic cancer	1 (3.5%)
Total	29 (100%)

Note. Here and in Tables 2, 3: abs. – absolute number.

Diffuse gastric cancer was most often detected as the second tumor in metachronous MPMN (17.5%) (Table 2).

Table 2

Localization and Morphological Type of the Second Tumor in Deceased Patients with Multiple Primary Malignant Neoplasms, *n* = 29

Localization and histotype of the second tumor	Number of patients abs. (%)
Breast, invasive lobular carcinoma	3 (11%)
Lung, small cell carcinoma	4 (14%)
Stomach, diffuse cancer	5 (17.5%) $p_{5,6,8,12} = 0.031$
Pancreas, low grade ductal adenocarcinoma	2 (6. 5%)
Thyroid gland, papillary cancer	1 (3.5 %)

End of table 2

Localization and histotype of the second tumor	Number of patients abs. (%)
Skin, squamous cell carcinoma of moderate differentiation	1 (3.5 %)
Rectum, low grade adenocarcinoma	2 (6.5%)
Liver, cholangiocarcinoma of the intrahepatic bile ducts	1 (3.5 %)
Colon, moderately differentiated adenocarcinoma	2 (6.5%)
Prostate gland, acinar adenocarcinoma	2 (6.5%)
Uterine body, high grade serous carcinoma	3 (11%)
Oropharynx, low grade squamous cell carcinoma	1 (3.5 %)
Ovary, high grade serous carcinoma	2 (6.5%)

Combinations of the first and second tumors were varied. Of the 29 cases of metachronous MPMN examined, 27 different combinations were identified. Only two sequences occurred more than once: skin – stomach and mammary gland – ovary (Table 3).

Table 3

Combinations of Localization of the First and Second Tumors in Deceased Patients with Multiple Primary Malignant Neoplasms, *n* = 29

Combinations of localization of the first and second tumor	Number of patients abs. (%)
Skin – mammary gland	1 (3.5 %)
Mammary gland – mammary gland	1 (3.5 %)
Stomach – lung	1 (3.5 %)
Thyroid gland – stomach	1 (3.5 %)
Mammary gland – pancreas	1 (3.5 %)
Rectum – thyroid gland	1 (3.5 %)
Skin – skin	1 (3.5 %)
Prostate gland - rectum	1 (3.5 %)
Prostate gland – liver	1 (3.5 %)
Skin – colon	1 (3.5 %)
Lung – prostate gland	1 (3.5 %)
Colon – prostate gland	1 (3.5 %)
Lung – uterine body	1 (3.5 %)
Skin – stomach	2 (6.25%)
Lung – colon	1 (3.5 %)
Sigmoid colon – uterine body	1 (3.5 %)
Right kidney – lung	1 (3.5 %)
Prostate gland – stomach	1 (3.5 %)
Kidneys – oropharynx	1 (3.5 %)
Uterine body – mammary gland	1 (3.5 %)
Bladder – rectum	1 (3.5 %)
Salivary gland – pancreas	1 (3.5 %)
Mammary gland – ovary	2 (6.25%)
The prostate gland – lung	1 (3.5 %)
Mammary gland – uterine body	1 (3.5 %)
Bladder – stomach	1 (3.5 %)
Skin – lung	1 (3.5 %)

The period between diagnosis of the first and second tumors was 72.0 (48–96) months (approximately 6 years). All patients received definitive treatment for the first tumor, and no progression was observed.

The second tumor was first detected when patients were admitted to the emergency care unit in a serious condition, often met the T4 criterion (48%) and was accompanied by distant metastases (72%) (Fig. 1). In 69% of cases, sporadic metachronous MPMN were histologically verified only postmortem. Certain difficulties arose when patients had a second (metachronous) tumor in the liver or lung. It was possible to differentiate it from possible progression of the first tumor with distant metastases to the lung or liver using conventional light microscopy on preparations stained with hematoxylin and eosin due to the different histotypes of the first and second tumors.

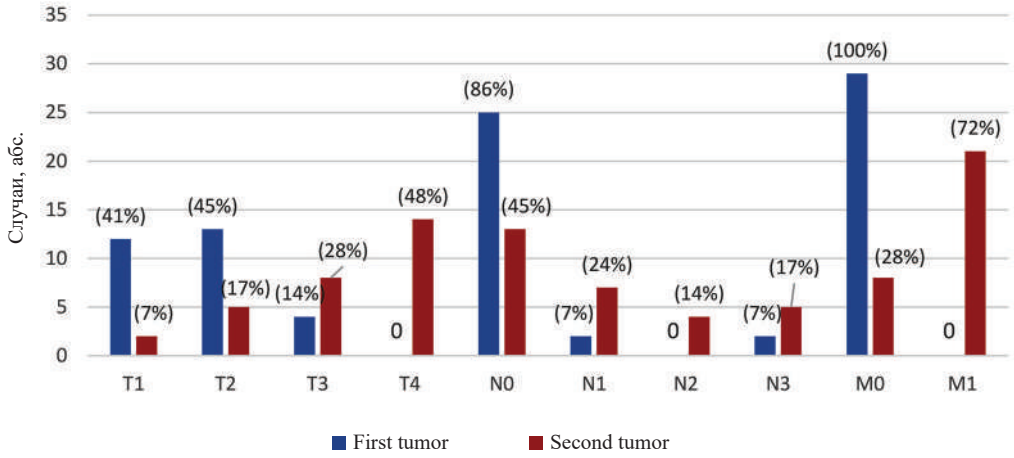

Fig. 1. TNM criteria for the first and second tumor in primary multiple malignant neoplasms, $n = 29$

In other cases, immunohistochemical examination would be necessary to differentiate between metastatic lesions and MPMN. A study of the localization of second tumor distant metastases showed that isolated massive metastatic lesions of the liver were observed more often (39%), isolated massive metastatic lesions of the lungs were observed less often (20%), and even less often – of the brain (4.5%), bones (4.5%) or generalized lesions with the involvement of several organs in the metastatic process in various combinations: lungs and liver (9.5%) and other variants of multiple metastasis – one case each (4.5%) (Fig. 2).

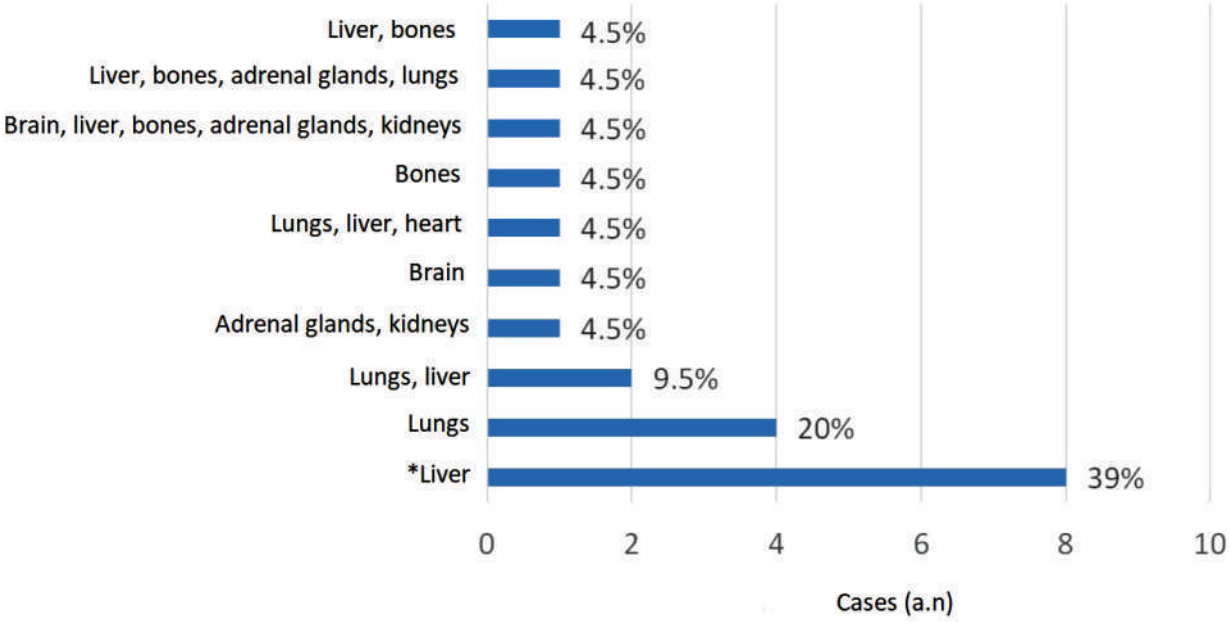

Fig. 2. Localization of distant metastases in primary multiple malignant neoplasms, $n = 21$: * $p_{1,5,6,7,8,9,10} = 0.003$, $p_2 = 0.011$

In metachronous MPMN, the most common causes of death are acute liver failure associated with massive metastatic liver disease (31%), acute terminal failure due to massive metastatic lung disease (21%), and peritonitis complicated by mechanical failure due to tumor stenosis (18%). In addition, in some cases, the following signs of death were diagnosed: a combination of acute liver and left ventricular failure (21%); acute renal failure; edema and dislocation of the brain; a combination of acute liver and renal failure; hemorrhagic shock (Fig. 3).

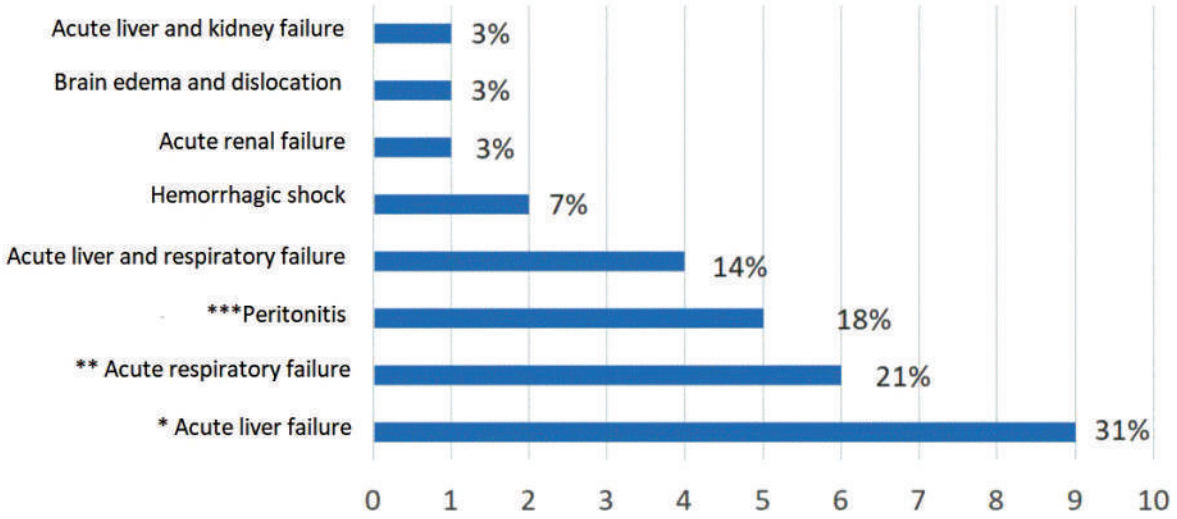

Fig 3. Causes of death in primary multiple malignant neoplasms, $n = 29$: * $p_{4,5,6} = 0.002$, $p_8 = 0.009$; ** $p_{4,5,6} = 0.017$; *** $p_{4,5,6} = 0.031$

DISCUSSION

The problem of multiple neoplasm development is comprehensive and highly complicated. The literature mainly describes cases of multiple neoplasms of certain localizations [13, 14]. Issues of studying the multiple neoplasm development patterns in the population aspect are becoming more and more relevant. In addition to the accumulated hereditary burden, risk factors of the urbanized environment, habitual intoxications (alcohol, smoking), radio-chemotherapy of the first neoplasms, the increase in the incidence of multiple neoplasms is associated with the improvement of medical and diagnostic care, in particular oncological [6], which contributes to an increase in the life expectancy of cancer patients, and, consequently, the risk of tumor recurrence. The incidence of multiple neoplasms, in addition to the territorial features of the diversity of oncopathology, is affected by the quality of diagnostics and monitoring of oncopathology in the region [2].

This study, in which cases of fatal outcomes of patients hospitalized for emergency care were examined, demonstrates the problems of cancer patient follow-up: in 72% of cases, the second tumor was diagnosed in an advanced form with distant metastases, in 69% – postmortem. The high level of neglect [2] is alarming in the case of a newly diagnosed cancer and twice as alarming in the case of recurrent tumors. Under the existing federal rules for lifelong follow-up monitoring at special medical facilities with at least annual medical checkup of cancer patients, the detection of recurrent malignant tumors in an advanced form should be an exception.

The analysis showed that in the absence of topomorphological associations in most cases of MPMN, squamous cell skin cancer and ductal breast cancer should be considered as the most common first malignant neoplasm. Skin cancer was associated with the development of metachronous tumors of the mammary gland, skin, colon, lung and in two cases – stomach; breast cancer was associated with the development of tumors of the other mammary gland, pancreas, ovary and tumor of the uterine body.

This finding is close to the conclusions of some authors [5] based on follow-up examinations of patients who received definitive treatment, indicating the organs in which metachronous tumors most often occur: in skin cancer, these are the mammary gland, body of the uterus, stomach, and colon; in breast

cancer, these are the second mammary gland, body of the uterus, stomach, skin, and ovaries. The literature data [5] also correspond to the obtained data on the more frequent metachronous stage of malignant tumor in the stomach (17.5%). This circumstance identifies the importance studying gastric cancer as the second tumor in sporadic metachronous MPMN: its associative connections with certain cancer types.

The question of the approximate time frame for the development of the second tumor depending on the topomorphological characteristics of the first tumor and other risk factors remains open. In our study, it is about 6 years, but it is worth considering the manifestation of these events in an advanced form with multiple, mainly distant metastases.

CONCLUSION

Metachronous primary multiple malignant tumors can occur after a long period of time (about 6 years) after the first ones, often in advanced forms (in this study, they were diagnosed mainly postmortem – 69%), causing the death of patients. The most common first tumors in sporadic metachronous MPMN are squamous cell skin cancer and ductal breast cancer, and the main target organ for cancer relapse is the stomach.

It seems relevant to continue research into the patterns of MPMN development with the search for solutions aimed at developing methods for predicting second malignant tumors based on a combination of clinical, morphological and molecular genetic factors, as well as a monitoring system for this group of patients to early diagnose the second tumor and increase the effectiveness of antitumor treatment.

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DNA-Containing Extracellular Structures of Tumor Cells Inhibit the Formation of Neutrophil Extracellular Traps in Vitro

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ABSTRACT

Aim. To study the parameters of formed DNA-containing extracellular structures during co-cultivation of neutrophils from healthy donors, HCT116 adenocarcinoma cells and K562 myeloblastoma.

Materials and methods. Erythrocyte sedimentation in EDTA-treated blood was carried out using Dextran 500. The neutrophil-enriched layer of blood plasma was collected. The admixture of mononuclear cells was less than 1%. Platelets were removed using differential centrifugation. Isolated neutrophils in RPMI-1640 medium were used in short-term culture experiments. HCT116 adenocarcinoma and K562 myeloblastoma cells were obtained from the American Type Culture Collection. In the experiments, donor neutrophils and tumor cells were co-cultivated for 3 hours. SYBR Green fluorescence microscopy was used to visualize the DNA-containing extracellular structures formation by cells cultured in RPMI-1640 medium.

Results. Neutrophils recognize tumor cells and respond to contact interactions, forming neutrophil extracellular traps in the form of neutrophil networks. Contacts with HCT116 adenocarcinoma cells cause rapid formation of neutrophil web-like structures – within 1 hour. The opening of neutrophil web-like structures induced by contacts with K562 myeloblastoma cells requires a longer incubation (2 hours). HCT116 cells form large bundles of DNA-containing fibers, which completely inhibit the formation of neutrophil networks. K562 cells suppress neutrophil defense responses by reducing the number and size of neutrophil networks. The effect of inhibition of neutrophil networks by K562 cells is probably due to the action of a soluble factor that suppresses neutrophil functions described earlier.

Conclusion. The study shows that both tumor cell lines are capable of suppressing innate immune cell responses through different mechanisms. Adenocarcinoma cells inhibit neutrophil network formation upon direct contact due to the large size DNA-containing fibers they produce. Myeloblastoma cells produce the same effect, probably acting by secreting humoral factors.

Keywords: HCT116, K562, NETs, DNA-containing fibers, oncology, pathophysiology

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ДНК-содержащие экстраклеточные структуры опухолевых клеток подавляют формирование нейтрофильных экстраклеточных ловушек *in vitro*

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РЕЗЮМЕ

Цель. Исследование параметров формируемых ДНК-содержащих экстраклеточных структур при совместном культивировании нейтрофилов здоровых доноров, клеток аденокарциномы HCT116 и миелобластомы K562.

Материалы и методы. Осаждение эритроцитов в крови, обработанной ЭДТА, проводили, используя декстран 500. Слой плазмы крови, обогащенный нейтрофилами, отбирали. Примеси мононуклеарных клеток составляли менее 1%. С помощью дифференциального центрифугирования освобождались от тромбоцитов. Выделенные нейтрофилы в среде RPMI-1640 использовали в экспериментах по кратковременному культивированию. Клетки аденокарциномы HCT116 и миелобластомы K562 были получены из American Type Culture Collection. В экспериментах проводили совместное культивирование нейтрофилов доноров и опухолевых клеток в течение 3 ч. Для визуализации формируемых ДНК-содержащих внеклеточных структур клетками, культивированными на среде RPMI-1640, использовали флуоресцентную микроскопию с красителем SYBR Green.

Результаты. Нейтрофилы распознают опухолевые клетки и реагируют на контактные взаимодействия, формируя нейтрофильные экстраклеточные ловушки в форме нейтрофильных сетей. Контакты с клетками аденокарциномы HCT116 вызывают быстрое формирование нейтрофильных сетей – в течение 1 ч. Раскрытие нейтрофильных сетей, индуцированное контактами с клетками миелобластомы K562, требует более продолжительной инкубации – в течение 2 ч. Клетки HCT116 формируют пучки ДНК-содержащих волокон значительного размера, которые полностью ингибируют формирование нейтрофильных сетей. Клетки K562 подавляют нейтрофильные защитные реакции, уменьшая количество и размеры нейтрофильных сетей. Эффект ингибирования нейтрофильных сетей со стороны клеток K562 обусловлен, вероятно, действием растворимого фактора, подавляющего функции нейтрофилов, описанного ранее.

Заключение. Исследование показывает, что обе клеточные линии опухолевых клеток способны подавлять реакции клеток врожденного иммунитета с помощью различных механизмов. Клетки аденокарциномы ингибируют формирование нейтрофильных сетей при непосредственных контактах за счет продуцируемых ДНК-содержащих волокон значительного размера. Клетки миелобластомы вызывают тот же эффект действуя, вероятно, путем секреции гуморальных факторов.

Ключевые слова: HCT116, K562, НЭЛ, ДНК-содержащие волокна, онкология, патофизиология

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

One of the basic positions of the modern oncological and immunological concept describing the interaction of tumor cells and neutrophils is that neutrophils forming neutrophil extracellular traps (NETs) are capable of causing tumor recurrence and metastasis [1, 2]. It is suggested that NETs can capture circulating cancer cells and promote their spread. In addition, it has been reported that NETs awaken dormant cancer cells, triggering tumor recurrence and metastasis. Therefore, it is quite natural that researchers propose to inhibit the formation of neutrophil extracellular traps to prevent tumor growth [3].

They also see the reason for the activation of tumor cell growth in the adverse effect of tumor-associated fibroblasts, which are able to support carcinogenesis. The authors acknowledge that the lack of knowledge about the tumor microenvironment (TME) is an obstacle to the introduction of innovative methods of treating tumor diseases.

Many researchers note that both neutrophil extracellular traps and tumor-associated fibroblasts as cellular factors of the tumor microenvironment have not been sufficiently studied [4, 5]. The complex composition of the components of the tumor microenvironment (humoral and cellular components) creates an environment necessary for the growth, proliferation, phenotypic flexibility and variability of tumor cells, which is at the same time rigid and immunosuppressive for the body, with a deficiency of nutrients [6]. NETs may be involved in the process of carcinogenesis and cancer progression. However, it is still difficult to decide whether netosis plays a pro- or antitumor role [7]. At the same time, some researchers have recently noted the dual nature of the effects of NETs regarding antitumor therapy, studying their potential to either neutralize or even improve treatment results [8]. It is also believed that NETs play a key role in the formation of a positive response to chemotherapy and have significant potential to increase the effectiveness of treatment [9].

It is possible to clarify the role of neutrophils and neutrophil extracellular traps formed by them in carcinogenesis only with direct studies of the interaction of tumor cells and cells of the innate immune system. It should be noted that such works are rare, and this circumstance determines the relevance of this study.

Aim. To study the parameters of the formed DNA-containing extracellular structures during the co-cultivation of neutrophils from healthy donors, adenocarcinoma cells HST116 and myeloblastoma K562.

MATERIALS AND METHODS

Obtaining Neutrophil Cell Fractions from Healthy Donors, as well as Adenocarcinoma Cells and Myeloblastoma K562 Cells

The isolation of neutrophils, in order to study the cellular reactions developing in the patient's body, requires the exclusion of exposure to chemical or mechanical stimuli on these cells *in vitro*, therefore, standard methods of isolation in the ficoll density gradient are of little use.

Neutrophils were isolated from the venous blood of volunteers treated with EDTA using Dextran 500. To do this, 1 ml of a 10% Dextran 500 solution (Fluka) prepared in a sodium phosphate buffer solution (50 mM, pH 7.4) was added to 10 ml of peripheral blood and gently mixed. After precipitation of erythrocytes for 30 minutes at +37°C, a layer of blood plasma (200 µl) was taken, closely adjacent (at a distance of 1 mm) to the layer of erythrocytes.

The blood plasma from this layer contains only neutrophils, platelets and a small number of red blood cells. The impurities of mononuclear cells are less than 1%. To get rid of platelets, 10 ml of 50 mM sodium phosphate buffer solution, pH 7.4, was added to 200 µl of blood plasma containing neutrophils and precipitated by centrifugation at 1,200 rpm (400 g), 15 min. The supernatant fluid was removed and the precipitate was resuspended in 1 ml of RPMI-1640 medium. Isolated neutrophils in RPMI-1640 medium were used in short-term cultivation experiments. The viability of the isolated neutrophils was at least 95% (test with 0.1% trypan blue solution).

The human colon adenocarcinoma HCT116 cell line was obtained from the American Type Culture Collection (Manassas, Virginia). Myeloblastoma cells K562 were obtained from the Laboratory of Mechanisms of tumor Cell Death of Blokhin National Medical Research Center of Oncology.

Cell Culture

All the studied cells in RPMI-1640 medium were incubated in an atmosphere of 5% CO_2 at 37°C in all experiments for 3 hours. The final concentration of cells in the culture medium was 2×10^5 cells/ml.

Lipopolysaccharides (LPS) (*Klebsiella pneumoniae*, Sigma, Japan), 25 mcg/ml, were added to some of the samples to activate neutrophils from

healthy donors and acquire the ability to form neutrophil extracellular traps. This technique makes it possible to obtain NETs in a characteristic morphological form – neutrophil networks. Of the four main morphological structures of neutrophil extracellular traps (web-like structures, single filaments, fibers and veils) [10], only the web-like structures have functional activity [11].

Immunofluorescence Staining of Cells Forming Extracellular DNA-Containing Structures

Fluorescence microscopy was used to visualize and determine the parameters (number and size) of extracellular DNA-containing structures of the studied cells (neutrophils of healthy donors, adenocarcinoma cells HST116 and myeloblastoma K562) [12]. The results were expressed as a percentage, as the ratio of the number of DNA-containing extracellular structures to the total number of cells in the field of view. An eyepiece micrometer was used to determine the size of DNA-containing extracellular structures. Extracellular DNA-containing structures were detected using the fluorescent dye SYBR Green (Evrogen; Russia), which specifically interacts with double-stranded DNA. Microscopy, counting and photo registration of cells and extracellular structures were performed at a ×1000 magnification.

The results were processed using Statistica 12.0 software (StatSoft Inc., USA). The data are presented as a mean and the standard error of the mean ($M \pm m$). Quantitative characteristics were compared based on the results of the Student's t-test and analysis of variance. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Observations of the behavior of neutrophil cells from healthy donors, adenocarcinoma HST116 and myeloblastoma K562 cells during their short-term co-cultivation in various combinations for 3 hours demonstrate the active formation of DNA-containing extracellular structures by these cells. The determination of the parameters (number and size) of these DNA-containing extracellular structures makes it possible to identify characteristic dynamic changes in the extracellular structures formed during the entire observation period.

Cells were cultures in the following sample variants: 1) intact neutrophils of healthy donors; 2) neutrophils of donors with the addition of LPS; 3) neutrophils of donors and adenocarcinoma cells HCT116; 4) neutrophils of donors with the addition of LPS and adenocarcinoma cells HCT116; 5) neutrophils of donors and myeloblastoma cells K562; 5) neutrophils of donors with the addition of LPS and myeloblastoma cells K562.

The results of co-cultivation in different combinations for 1 hour (Table 1) indicate that neutrophils of healthy donors quickly respond to contact interactions with adenocarcinoma cells and form neutrophilic networks. However, the reaction of neutrophils to contacts with cells of myeloblastoma K562 in the form of the formation of neutrophilic networks during 1 hour of cultivation is practically absent. A characteristic feature of the studied tumor cells (HCT116 and K562) is the formation of DNA-containing extracellular structures of a considerable size. HCT116 cells form single filaments with a length of 54.92±6.82 microns, and K562 cells form 99.00±8.41 microns. The revealed differences between HCT116 and K562 cells in their contacts with neutrophils from healthy donors consist in the fact that the reaction of neutrophils and K562 cells is clearly slowed down. During 1 hour of co-cultivation of K562 cells with neutrophils, the formation of neutrophil networks does not occur, this reaction manifests itself later.

Cultivation of HCT116 cells with neutrophils in the presence of LPS does not significantly change the parameters of cellular reactions (Table 1). Neither the number of DNA-containing extracellular structures (8.87 ± 1.20% and 7.41 ± 0.39%, respectively) nor their sizes (54.92 ± 6.82 μm and 53.92 ± 6.06 μm, respectively) change.

Cultivation of K562 cells with neutrophils in the presence of LPS shows a sharp increase in the number of DNA-containing extracellular structures (Table 1). The presence of LPS in samples with K562 cells and neutrophils causes an increase in the number of neutrophil networks (from 2.97 ± 0.32% to 10.70 ± 1.81%), i.e. almost 3 times. At the same time, the sizes of DNA-containing single strands from K562 cells are significantly (twofold) reduced (from 99.00 ± 8.41 microns to 57.00 ± 9.34 microns). The decrease in the size of single strands from K562 cells is probably due to spontaneous enzymatic degradation of DNA under the action of DNAases localized on chromatin itself, which can be activated during chromatin despiralization.

After 2 hours of cultivation (Table 2) the number of cells producing DNA-containing structures during the co-cultivation of neutrophils and HCT116 cells is 6.47 ± 0.46%.

Table 1

Parameters of Extracellular Structures during Co-Cultivation in Various Combinations of Neutrophils from Healthy Donors, Adenocarcinoma Cells HCT116 and Myeloblastoma K562 for 1 hour, *M ± m*

Cells studied	Number of extracellular structures, %	Dimensions of extracellular structures, μm	Description of emerging extracellular structures
Neutrophils (N)	0.00	0.00	Do not form extracellular structures, retain a segmented structure
N + HCT116	8.87 ± 1.20	54.92 ± 6.82	Neutrophils form networks, and HCT116 adenocarcinoma cells form single filaments
N + LPS	4.69 ± 0.29	32.25 ± 2.60	Neutrophils form networks
N + LPS + HCT116	7.41 ± 0.39	53.92 ± 6.06	Combination of neutrophil networks with single fibers of adenocarcinoma cells
N + K562	2.97 ± 0.32	99.00 ± 8.41	There are no neutrophil networks. Only single filaments of K562 myeloblastoma cells are observed
N + LPS + K562	10.70±1.81*	57.00 ± 9.34*	Neutrophil networks in combination with single filaments of K562 myeloblastoma cells

* $p < 0.05$ compared to samples without LPS – here and in Tables 2, 3.

Table 2

Parameters of Extracellular Structures during Co-Cultivation in Various Combinations of Neutrophils from Healthy Donors, Adenocarcinoma Cells HCT116 and Myeloblastoma K562 for 2 Hours, *M ± M*

Cells studied	Number of extracellular structures, %	Dimensions of extracellular structures, μm	Description of emerging extracellular structures
Neutrophils (N)	0.00	0.00	Do not form extracellular structures, retain a segmented structure
N + HCT116	6.47 ± 0.46	44.25 ± 2.47	HCT116 adenocarcinoma cells form fiber bundles
N + LPS	23.65 ± 2.35	39.67 ± 2.81	Neutrophils form networks
N + LPS + HCT116	10.75 ± 0.84*	46.67 ± 5.54	Combination of neutrophil networks with bundles of adenocarcinoma cell fibers
N + K562	8.25 ± 0.59	21.58 ± 2.80	Small neutrophil networks
N + LPS + K562	9.64 ± 0.97	18.08 ± 1.08	Small neutrophil networks

Moreover, there is a clear change in the morphology of DNA-containing extracellular structures that form HCT116 cells. Bundles of single fibers are formed, and the number of NETs forming networks decreases sharply (it does not exceed 10% of the total number of DNA-containing extracellular structures). Under the LPS influence, the total number of DNA-containing extracellular structures increases to 10.75±0.84%, due to an increase in structures produced by neutrophils (neutrophil networks).

Co-incubation of neutrophils with HCT116 cells in the presence of LPS leads to the detection of neutrophil networks in the samples together with bundles of DNA-containing fibers of adenocarcinoma cells. The presence of LPS in the samples causes the activation of neutrophils and the formation of NETs. The sizes of fiber bundles produced by HCT116 cells practically do not change with the addition of LPS (44.25 ± 2.47 microns and 46.67 ± 5.54 microns, respectively, without LPS and in the presence of LPS).

Neutrophils cultured with K562 cells for 2 hours form exclusively neutrophil networks (NETs) numbering 8.25 ± 0.59%; in the presence of LPS, their number is 9.64 ± 0.97%, so it practically does not change (Table 2). K562 cells do not form any DNA-containing extracellular structures during this observation period. A characteristic feature of K562 cells is their ability to suppress the activating effect of LPS on neutrophils, which these cells must receive through innate immunity receptors (TLRs). Probably, the K562cells line inhibit the activity of neutrophils through humoral factors. The sizes of neutrophilic networks in the presence of K562 cells are small and amount to 21.58 ± 2.80 μm. In the presence of LPS, even a slight decrease in the size of neutrophilic networks to 18.08 ± 1.08 μm was noted. This circumstance confirms the inhibitory effect of K562 cells on activated neutrophils.

The results of co-cultivation of tumor cells with neutrophils from healthy donors for 3 hours are shown in Table 3.

Table 3

Parameters of Extracellular Structures during Co-Cultivation in Various Combinations of Neutrophils from Healthy Donors, Adenocarcinoma Cells HCT116 and Myeloblastoma K562 for 3 Hours, *M ± M*

Cells studied	Number of extracellular structures, %	Dimensions of extracellular structures, μm	Description of emerging extracellular structures
Neutrophils (N)	0.00	0.00	Do not form extracellular structures, retain a segmented structuree
N + HCT116	20.13 ± 1.71	135.92 ± 12.43	HCT116 adenocarcinoma cells form bundles of fibers. There are no neutrophil networks
N + LPS	21.92 ± 1.41	30.33 ± 3.57	Neutrophils form networks
N + LPS + HCT116	29.46 ± 5.09	> 350	Bundles of adenocarcinoma cell fibers. There are no neutrophil networks
N + K562	8.26 ± 0.32	28.00 ± 2.56	Small neutrophil networks
N + LPS + K562	14.82 ± 1.27*	25.08 ± 2.62	Small neutrophil networks

During this period, the number of cells producing DNA-containing structures during co-cultivation of neutrophils and HCT116 cells is 20.13 ± 1.71%, and these are exclusively DNA-containing extracellular structures that produce adenocarcinoma cells (Fig.). They are represented by bundles of DNA-containing fibers, and neutrophil networks (NETs) are completely absent. The number of DNA-containing extracellular structures in the presence of LPS increases to 29.46 ± 5.09%. Neutrophils of healthy donors cultured for 3 hours together with HCT116 cells practically do not respond to the activating effects of LPS. The addition of LPS to the samples does not cause an increase in the formation of neutrophilic networks. The fiber bundles originating from HCT116 adenocarcinoma cells during this period are long, exceeding 100 μm (135.92 ± 12.43). Under the influence of LPS, the length of fibers from HCT116 cells increases even more (it becomes more than 350 μm), which lies outside the measuring range.

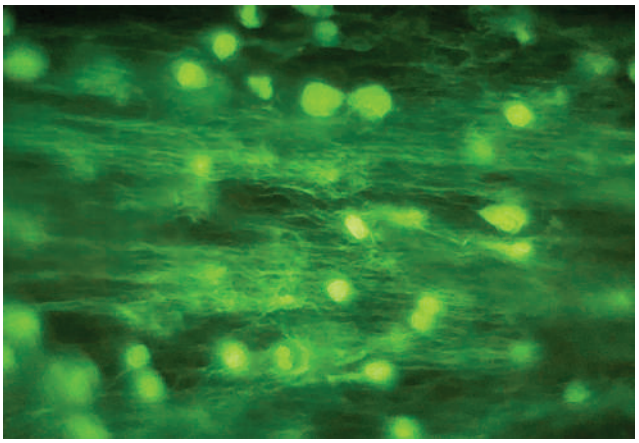

Fig. Adenocarcinoma HCT116 cells spontaneously form bundles of single DNA-containing strands. Neutrophils of a healthy donor present in the preparation do not form neutrophil networks. Incubation time is 3 hours. Staining with CYBR Green. The magnification is x1000.

In studies on experimental animals, data were obtained on the ability of NETs to change the metabolic program of cancer cells and, due to the release of neutrophilic networks, promote the growth of cancer cells [13]. The relationship between the formation of NETs, the frequency of metastasis, and survival rates was also revealed [14]. It should be noted that these results [13, 14] were obtained by indirect methods. These studies did not determine the number of NETs, but only obtained signs of the presence of NETs in the body of experimental animals and patients in the form of soluble factors (neutrophil elastase, myeloperoxidase, citrullinated histone H3). These cited results are in some contradiction with the results of our *in vitro* study. However, it should be noted that the true morphological structure of NETs in cancer patients has not been determined and this issue has not been comprehensively investigated.

Neutrophils cultured with K562 cells form only 8.26 ± 0.32% NETs, and under the influence of LPS their number increases to 14.82 ± 1.27, while filamentous structures from K562 cells are no longer recorded after 3 hours of incubation (Table 3). Possibly single DNA-containing strands, myeloblastomas originating from cells, which we observed during incubation for 1 hour, underwent spontaneous enzymatic degradation and therefore are not detected in later samples. The sizes of neutrophilic networks are small and do not depend on the presence of LPS in the samples. So, without LPS, the sizes of neutrophilic networks are 28.00 ± 2.56 μm, and in the presence of LPS – 25.08 ± 2.62 μm.

The results of the study of co-cultivation of K562 cells with neutrophils from healthy donors for 3 hours indicate a possible inhibitory effect of myeloblastoma cells on the formation of neutrophil networks by neutrophils, which has a protective

nature. Our assumption is confirmed by other data from researchers, where it was determined that K562, a chronic myeloid leukemia cell line, secretes a soluble factor (K562-IF) with a low molecular weight (6–8 kDa), which suppresses the functions of neutrophils during inflammation [15].

CONCLUSION

The results of the study indicate the ability of neutrophils to recognize tumor cells (HCT116 and K562) and respond to interaction with them, forming neutrophil extracellular traps in the morphological form of neutrophil networks. The rate of development of the neutrophil reaction that opens the neutrophil networks varies. Interaction with HCT116 adenocarcinoma cells causes very rapid formation of neutrophil networks – within 1 hour. The opening of neutrophil networks induced by contacts with K562 myeloblastoma cells requires a longer incubation – for 2 hours. The cultivation of tumor cells (HCT116 and K562) for 3 hours demonstrates the ability of these cells to form DNA-containing extracellular structures.

HCT116 adenocarcinoma cells form bundles of DNA-containing fibers of a considerable size. These DNA-containing fibers completely inhibit the development of neutrophilic reactions in the form of the formation of neutrophilic networks. These observations allow us to conclude that the cells of the HCT116 tumor line turn off the protective reactions of the innate immune system in the form of the opening of neutrophil networks.

K562 myeloblastoma cells suppress neutrophil defense responses, reducing the number and size of emerging neutrophil networks. The effect of inhibition of neutrophil networks by K562 cells is probably due to the action of a soluble factor that suppresses neutrophil functions, described earlier.

Our study shows that both types of tumor cell lines are capable of suppressing the reactions of innate immune cells using various mechanisms. Adenocarcinoma cells inhibit the formation of neutrophilic networks in direct contact due to the production of DNA-containing fibers of a considerable size. Myeloblastoma cells cause the same effect, probably by secreting humoral factors.

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Author contribution

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Association of Metabolic and Inflammatory Molecule Levels and Post-COVID Syndrome of Varying Severity

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ABSTRACT

Aim. To study the associations of the levels of metabolic and inflammatory molecules and the severity of post-COVID syndrome (PCS) in COVID-19 convalescents.

Materials and methods. The observational cross-sectional study included 270 individuals aged 18–84 who were COVID-19 convalescents, including 191 patients with PCS of whom 97 patients had mild PCS and 94 had moderate PCS. Serum concentrations of metabolic and inflammatory molecules were determined using enzyme-linked immunosorbent assay (ELISA), including: alpha interferon (IFN-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), monocyte chemoattractant protein 1 (MCP-1), insulin, C-peptide, and high-sensitivity C-reactive protein (hs-CRP).

Results. In COVID-19 convalescents with PCS of varying severity, the level of IL-6 was 1.3 times higher than in individuals without PCS. Among men with PCS, the levels of IL-6, MCP-1, and hs-CRP were 1.5, 1.2 and 1.9 times higher, respectively, compared with men without PCS. In men with moderate PCS, the level of IL-6 was 1.9 times higher and hs-CRP was 1.7 times higher than in men without PCS. The risk of having moderate PCS in COVID-19 convalescents was directly associated with the concentration of C-peptide in the blood. In men, the risk of having PCS was directly associated with the concentration of hs-CRP in the blood.

Conclusion. In COVID-19 convalescents, the risk of having moderate PCS is directly associated with the level of C-peptide in the blood. In men, the risk of having PCS is directly associated with the level of hs-CRP in the blood.

Keywords: post-COVID syndrome, interleukin-6, C-peptide, high-sensitivity C-reactive protein

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest associated with the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee of the Research Institute of Internal and Preventive Medicine, a branch of the Institute of Cytology and Genetics (Minutes No. 10 dated November 10, 2020).

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Ассоциации уровней метаболических и воспалительных молекул и постковидного синдрома разной степени тяжести

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РЕЗЮМЕ

Цель. Изучить ассоциации уровней метаболических и воспалительных молекул и постковидного синдрома (ПКС) разной степени тяжести у реконвалесцентов коронавирусной инфекции (COVID-19).

Материалы и методы. В обсервационное одномоментное исследование были включены 270 человек, возраст 18–84 года, являющихся реконвалесцентами COVID-19, в том числе с наличием ПКС – 191 пациент, из которых с легкой степенью тяжести ПКС – 97 человек, а со средней степенью тяжести ПКС – 94 человека. У всех пациентов в сыворотке крови методом иммуноферментного анализа (ИФА) определяли концентрации метаболических и воспалительных молекул: интерферона альфа (ИФН-α), интерлейкина 1бета (ИЛ-1β), интерлейкина 6 (ИЛ-6), интерлейкина 8 (ИЛ-8), моноцитарного хемотаксического фактора 1 (МСР-1), инсулина, С-пептида, высокочувствительного С-реактивного белка (вчСРБ).

Результаты. У реконвалесцентов COVID-19 с ПКС разной степени тяжести уровень ИЛ-6 был выше в 1,3 раза, чем у лиц без ПКС. Среди мужчин с ПКС уровень ИЛ-6 был выше в 1,5 раза, МСР-1 – в 1,2, вчСРБ – в 1,9 раза, чем у мужчин без ПКС. Среди мужчин с ПКС средней степени тяжести уровень ИЛ-6 был выше в 1,9 раза, уровень вчСРБ – в 1,7 раза, чем у мужчин без ПКС. Шанс наличия ПКС средней степени тяжести у реконвалесцентов COVID-19 прямо ассоциирован с концентрацией в крови С-пептида. У мужчин шанс наличия ПКС прямо ассоциирован с концентрацией в крови вчСРБ.

Заключение. У реконвалесцентов COVID-19 шанс наличия ПКС средней степени тяжести прямо ассоциирован с уровнем в крови С-пептида. У мужчин шанс наличия ПКС прямо ассоциирован с уровнем вчСРБ в крови.

Ключевые слова: постковидный синдром, интерлейкин-6, С-пептид, высокочувствительный С-реактивный белок

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией данной статьи.

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИТПМ – филиал ИЦиГ СО РАН (протокол № 10 от 10.11.2020).

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INTRODUCTION

After an acute period of novel coronavirus infection (COVID-19), 41.7% of patients [1] develop various symptoms. In October 2021, the World Health Organization (WHO) developed a definition characterizing this condition, known as post-COVID condition or post-COVID syndrome (PCS): a condition after COVID-19 that occurs in individuals with a history of suspected or confirmed SARS-CoV-2 infection, usually starting 3 months after the initial COVID-19 infection, with symptoms that last at least 2 months that cannot be explained by another diagnosis [2]. PCS is characterized by multiple organ damage of varying severity and can lead to serious complications. In clinical practice, this category of patients is increasingly experiencing both the development of new diseases and the progression of existing chronic diseases after infection.

Chronic inflammation is a hallmark of PCS and is believed to contribute to many symptoms. The study of the exact mechanisms that cause long-term inflammation after COVID-19 is a matter of scientific interest. Currently, it is believed that several factors play a role in the development of chronic inflammation in PCS: immune dysregulation, impaired hemostasis, prolonged persistence of the virus after convalescence, and autoimmune reactions.

Existing data indicate the high prognostic value of certain cytokines, inflammatory and metabolic markers in the acute phase of COVID-19 determining the risk of severe disease and death in patients. It is likely that the mechanisms underlying the development of long-term manifestations of the post-COVID period lead to chronic inflammation due to a long-term increase in the level of pro-inflammatory molecules with aberrant immunity.

Studies of metabolic and inflammatory molecules in the context of PCS are relevant and important, since it is necessary to understand whether they retain their high predictive significance regarding both the development of PCS and the severity of its course. The study of changes in the levels of metabolic and inflammatory molecules and their associations with PCS opens up opportunities for the development of new methods for the prevention and treatment of patients with long-term symptoms in the post-COVID period.

The aim of this study was to investigate the associations of levels of metabolic and inflammatory molecules and PCS of varying severity in COVID-19 convalescents.

MATERIALS AND METHODS

The single-stage observational study was conducted at the Research Institute of Internal and Preventive Medicine in 2020–2021. The study included 270 people (48.1% men, average age 53.2±13.2 years) who were convalescents of COVID-19. Inclusion criteria were as follows: the presence of COVID-19, confirmed by a positive analysis of SARS-CoV-2 coronavirus RNA by polymerase chain reaction (PCR) during the disease and/or the presence of IgG antibodies to SARS-CoV-2 coronavirus; two months after convalescence.

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee of the Research Institute of Internal and Preventive Medicine, a branch of the Institute of Cytology and Genetics (Minutes No. 10 dated November 10, 2020). All patients gave their informed consent to participate in the study.

All patients were divided into four groups based on the presence or absence of PCS and its severity (mild or moderate), which were determined according to the criteria published earlier in a systematic review [3].

Group 1 included 79 people without PCS, group 2 included 191 people with PCS, group 3 included 97 people with mild PCS, and group 4 included 94 people with moderate PCS. The enzyme-linked immunosorbent assay (ELISA) with Vector-Best kits (Russia) was used to determine the levels of metabolic and inflammatory molecules, interferon alpha (IFN-a), interleukin 1beta (IL-1beta), interleukin 6 (IL-6), and interleukin 8 (IL-8), monocyte chemotactic factor 1 (MCP-1), insulin, C-peptide, high-sensitivity C-reactive protein (hs-CRP) in blood serum.

Statistical processing of the obtained results was performed using the SPSS software package. The normality of the distribution of continuous features was checked using the Kolmogorov–Smirnov test. Due to the nonparametric distribution of quantitative data, the median interquartile range $Me\ [Q_{25};\ Q_{75}]$ was used. The statistical significance of the differences in quantitative indicators in the two groups was assessed using the nonparametric Mann–Whitney test. In cases where the number of groups was more than two, the Kruskal–Wallis test and Dunn's test, a nonparametric multiple-comparison procedure, were used. Associative relationships were studied using univariate and multifactorial logistic regression models. The results are presented as the odds ratio (OR) and the 95% confidence interval (CI) for OR. When testing statistical hypotheses, the critical level

of significance was at $p < 0.05$.

RESULTS

A comparative analysis of the concentrations of the studied metabolic and inflammatory molecules in patients of four groups is presented in Table 1. In patients with both mild and moderate PCS, the blood level of IL-6 was 1.3 times higher than in people without PCS.

Among men with PCS, the blood level of IL-6 was 1.5 times higher, MCP-1 was 1.2 times higher, and hs-CRP was 1.9 times higher than in men without PCS (Table 2). In men with moderate PCS, the blood levels of IL-6 and hs-CRP were 1.9 and 1.7 times higher than in men without PCS, respectively. The level of hs-CRP was also statistically significantly 2.1 times higher in men with mild PCS compared with men without PCS.

A similar analysis in women showed no statistically significant differences in the levels of the studied metabolic and inflammatory molecules in these subgroups.

Table 1

Variability of the Levels of Studied Metabolic and Inflammatory Molecules in COVID-19 Convalescents Depending on the PCS Presence and Severity, *Me* [Q_{25}; Q_{75}]

Parameter	Group 1, no PCS $n = 79$	Group 2, with PCS $n = 191$	Group 3, mild PCS $n = 97$	Group 4, moderate PCS $n = 94$	p
IFN-a, pg/mL	0.82 [0.34;4.06]	1.05 [0.27;4.80]	1.09 [0.27;4.08]	1.05 [0.41;5.03]	$p_{1-2} = 0.312$ $p_{1-3} = 0.383$ $p_{1-4} = 0.333$
IL-1b, pg/mL	2.06 [1.28;3.27]	2.42 [1.41;3.51]	2.56 [1.38;3.76]	2.11 [1.51;3.26]	$p_{1-2} = 0.507$ $p_{1-3} = 0.273$ $p_{1-4} = 0.977$
IL-6, pg/mL	2.10 [1.35;3.08]	2.76 [1.73;4.43]	2.80 [1.79;4.87]	2.72 [1.66;4.24]	**$p_{1-2} = 0.016$** **$p_{1-3} = 0.027$** **$p_{1-4} = 0.032$**
IL-8, pg/ml	7.19 [5.26;11.45]	8.81 [5.84;12.15]	8.55 [5.29;12.22]	9.06 [6.44;12.11]	$p_{1-2} = 0.180$ $p_{1-3} = 0.452$ $p_{1-4} = 0.080$
MCP-1, pg/mL	302.55 [211.27;402.67]	342.68 [258.42;433.39]	339.78 [263.15;415.90]	353.24 [256.48;441.55]	$p_{1-2} = 0.085$ $p_{1-3} = 0.134$ $p_{1-4} = 0.101$
Insulin, mME/L	3.34 [0.76;8.25]	3.71 [1.31;9.83]	4.14 [1.34;10.19]	3.62 [1.11;9.10]	$p_{1-2} = 0.136$ $p_{1-3} = 0.138$ $p_{1-4} = 0.230$
C-peptide, pmol/L	98.21 [50.27;280.17]	143.27 [50.80;377.14]	128.85 [54.46;312.57]	172.97 [45.75;411.88]	$p_{1-2} = 0.294$ $p_{1-3} = 0.417$ $p_{1-4} = 0.264$
hs-CRP, mg/L	2.70 [1.36;8.93]	3.76 [1.90;9.31]	3.60 [1.71;9.31]	3.80 [2.42;9.27]	$p_{1-2} = 0.138$ $p_{1-3} = 0.238$ $p_{1-4} = 0.105$

Table 2

Variability of the Levels of the Studied Metabolic and Inflammatory Molecules in Men, Convalescents of COVID-19, Depending on the PCS Presence and Severity, *Me* [Q_{25}; Q_{75}]

Parameter	Group 1, no PCS $n = 29$	Group 2, with PCS $n = 75$	Group 3, mild PCS $n = 46$	Group 4, moderate PCS $n = 29$	p
IFN-a, pg/mL	0.54 [0.00;4.68]	1.28 [0.51;5.03]	2.30 [0.51;5.05]	1.09 [0.14;5.93]	p_{1-2}=0.178 p_{1-3}=0.140 p_{1-4}=0.432
IL-1b, pg/mL	2.63 [1.35;4.15]	2.47 [1.11;3.79]	2.49 [0.99;4.03]	2.31 [1.71;3.58]	p_{1-2}=0.679 p_{1-3}=0.840 p_{1-4}=0.549
IL-6, pg/mL	1,82 [1,35;2,90]	2,77 [1,64;4,59]	2,36 [1,59;4,80]	3,52 [1,98;4.34]	**p_{1-2}=0.018** p_{1-3}=0.089 **p_{1-4}=0.008**
IL-8, pg/ml	7.02 [5.01;10.59]	8.03 [5.57;11.98]	7.67 [5.31;11.66]	8.54 [6.13;12.01]	p_{1-2}=0.413 p_{1-3}=0.652 p_{1-4}=0.266

End of table 2

Parameter	Group 1, no PCS $n = 29$	Group 2, with PCS $n = 75$	Group 3, mild PCS $n = 46$	Group 4, moderate PCS $n = 29$	p
MCP-1, pg/mL	310.19 [215.85;392.50]	361.11 [281.48;420.83]	351.14 [254.65;415.39]	376.39 [288.29;436.45]	**p_{1-2}=0.042** p_{1-3}=0.095 p_{1-4}=0.047
Insulin, mME/L	4.77 [0.88;8.26]	7.19 [1.86;11.86]	7.38 [1.91;11.18]	6.39 [1.70;12.54]	p_{1-2}=0.087 p_{1-3}=0.105 p_{1-4}=0.176
C-peptide, pmol/L	123.08 [29.58;335.04]	198.08 [67.57;434.57]	189.42 [66.89;425.78]	198.08 [66.57;536.64]	p_{1-2}=0.140 p_{1-3}=0.213 p_{1-4}=0.166
hs-CRP, mg/L	2.32 [1.36;7.44]	4.41 [2.59;10.68]	4.78 [2.43;10.65]	4.03 [2.74;11.43]	**p_{1-2}=0.011** **p_{1-3}=0.028** **p_{1-4}=0.020**

At the next stage of statistical processing, the metabolic and inflammatory molecules we studied were sequentially included in a univariate logistic regression analysis model with standardization by gender and age (Table 3). It was found that the chance of moderate PCS in COVID-19 convalescents is directly associated with the concentration of C-peptide in the blood.

Univariate logistic regression analysis in men shows that the chance of PCS and mild PCS is directly associated with the hs-CRP level (Table 4).

Table 3

Univariate Logistic Regression Analysis of the Chance of Moderate-Grade PCS in COVID-19 Convalescents (with Standardization by Gender and Age)

Parameter	Univariate analysis		
	Exp B	95% CI	p
IFN-a, pg/mL	1.004	0.928–1.086	0.921
IL-1b, pg/mL	0.780	0.901–1.081	0.780
IL-6, pg/mL	1.162	0.936 –1.442	0.173
IL-8, pg/ml	1.015	0.942 –1.094	0.701
MCP-1, pg/mL	1.001	0.998 –1.003	0.695
Insulin, mME/L	1.027	0.972 –1.085	0.339
C-peptide, pmol/L	1.001	1.000 –1.003	**0.048**
hs-CRP, mg/L	1.015	0.979 –1.052	0.425

Table 4

Univariate Logistic Regression Analysis of the Chance of Having PCS and Mild PCS in Men with Standardization by Age)

Parameter	Univariate analysis of the chance of PCS			Univariate analysis of the chance of mild PCS		
	Exp B	95% CI	p	Exp B	95% CI	p
IFN-a, pg/mL	1.002	0.901–1.114	0.970	1.012	0.907–1.129	0.834
IL-1b, pg/mL	0.973	0.804–1,178	0.781	1.000	0.822–1.216	0.996
IL-6, pg/mL	1.309	0.982–1.745	0.067	1.257	0.943–1.676	0.118
IL-8, pg/ml	1.020	0.929–1.120	0.679	1.025	0.935–1.123	0.598
MCP-1, pg/mL	1.002	0.998–1.006	0.308	1.001	0.997–1.006	0.511
Insulin, mME/L	1.050	0.971–1.136	0.219	1.051	0.963–1.148	0.262
C-peptide, pmol/L	1.001	0.999–1.003	0.217	1.001	0.999–1.003	0.316
hs-CRP, mg/L	1.113	1.005–1.223	0.040	1.127	1.008–1.260	0.036

When the metabolic and inflammatory molecules were included in the multivariate logistic regression analysis, which showed a statistically significant difference between the subgroups (IL-6, MCP-1, C-peptide, hsCRP), simultaneously, in COVID-19 convalescents and, separately, in men and women, there were no associative links with the chance of having PCS and its severity.

DISCUSSION

The study of pathophysiological changes and the mechanisms that cause the occurrence of both ongoing and *de novo* PCS symptoms continues. Considering the existing data on the presence of higher levels of metabolic and inflammatory molecules (in particular, IL-1b, IL-6, IL-8, MCP-1, insulin, C-peptide, and hs-CRP) in patients infected with SARS-CoV-2 in the acute period and their associations with the risk of severe coronavirus infection course [4–6], we evaluated this profile in the blood serum of COVID-19 convalescents.

In study of Schultheiß et al., it was shown that there were significantly higher serum levels of IL-1b and IL-6 in the group of patients with PCS compared with those without PCS. The authors also demonstrated data reflecting the positive correlation of the molecules both with each other and with the presence of PCS [7]. Zhdanova et al. provided similar data revealing that the median levels of IL-1b and IL-6 were 1.3 and 4.5 times higher, respectively, in the group of patients with PCS compared with healthy individuals [8]. The PHOSP-COVID study demonstrated elevated IL-6 level 5 months after hospitalization for COVID-19 in a group of convalescents with mild cognitive impairments [9]. Our findings are consistent with the literature data: the level of IL-6 was 1.3 times higher in COVID-19 convalescents with PCS compared with those without PCS. We also found an increased IL-6 content in the blood serum of individuals with PCS of varying severity. It is reported that IL-6 is a key inflammatory factor in the immunopathogenesis of the new coronavirus infection, which is reflected in its use as a marker of severity in COVID-19 [10]. Taking into account the hypothesis of long-term persistence of the virus in the post-COVID period [11], an increase in the level of pro-inflammatory molecules, in particular IL-6, may be explained by ongoing immune reactions against viral antigens.

According to research data, MCP-1 (proinflammatory chemokine) is a key mediator involved in the pathogenesis of COVID-19, its level was higher in critically ill patients and correlated with respiratory failure, acute renal failure and death from COVID-19 [5, 12, 13]. In our study, the median MCP-1 level was significantly higher in men only in the group of patients with PCS compared with those without PCS. This may probably be due to the fact that male sex is a risk factor for severe COVID-19 [14, 15].

Of particular interest are the results that demonstrate the bidirectional relationship of carbohydrate metabolism disorders and COVID-19. According to some data, diabetes mellitus was found to be associated with the severity of COVID-19 and mortality [17]. Man et al. study reported that PCS is the main risk factor for changes in the metabolic status of patients, leading to insulin resistance [18]. According to the results of a subanalysis of the joint registers ACTIV and ACTIV 2, it was found that one year after the infection, patients with type 2 diabetes mellitus and newly diagnosed hyperglycemia are more likely to have symptoms characteristic of post-COVID syndrome [19]. Several mechanisms underlie the pathological interaction of the SARS-CoV-2 virus and carbohydrate metabolism disorders. Since diabetes mellitus is characterized by chronic inflammation, this could contribute both to the development of a more severe course of the disease in the acute period and to increased systemic inflammation observed during follow-up. On the other hand, exposure to an acute inflammatory process leads to insulin resistance in the body. Probably, these data altogether can determine our findings regarding the direct association of the chance of moderate severity PCD with the blood level of C-peptide.

The study of the level of hs-CRP has acquired additional significance in the post-COVID period. Monitoring of hs-CRP as one of the inflammatory markers is included in the guidelines "Features of Long-COVID Infection Clinical Course. Therapeutic and Rehabilitation Measures" [20]. Castro et al. compared patients ($n = 277$) who were hospitalized in 2020 during the first wave of COVID-19 in Brazil, depending on the absence or presence of advanced PCS. An extensive profile of biomolecules in the blood serum was examined 6–12 months after discharge, and a higher level of hs-CRP was noted in the group of patients with PCS compared with those without PCS [16].

The study of Maamar et al. reported that men with hs-CRP levels in the range of low intense inflammation (>0.3 mg/dl and <1.0 mg/dl) had a 10–17-fold increased risk of developing post-COVID syndrome [21]. The results obtained regarding the direct association of the chance of the presence of PCS with the hs-CRP level are consistent with the literature data. The advantage of this inflammatory marker over others (for example, ferritin and IL-6) is that it is readily available and widely used in clinical practice.

CONCLUSION

In COVID-19 convalescents, the chance of moderate PCS is directly associated with the blood level of C-peptide. In men, the chance of having

PCS is directly associated with the level of hs-CRP in the blood. The data obtained during the study demonstrate specific changes in the cytokine profile in patients with PCS. It indicates the need for further in-depth research, enabling to develop methods for personalized management of this patients category.

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Author contribution

Karaseva A.A. – collection, analysis, interpretation of data, drafting of the manuscript. Afanaseva A.D., Garbuzova E.V. – verification of intellectual content, translation of the article. Kashtanova E.V., Polonskaya Ya.V., Stakhneva E.M., Shramko V.S. – performing

biochemical studies, analysis of research data. Shcherbakova L.V. – statistical data processing. Logvinenko I.I. – verification of intellectual content, final approval of the manuscript for publication. Ragino Yu.I. – conception and design, final approval of the manuscript for publication.

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Prognostic Value of Acute Kidney Injury in Patients Hospitalized with Acute Decompensation of Chronic Heart Failure

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ABSTRACT

Aim. To study the effect of acute kidney injury in patients hospitalized with acute decompensation of chronic heart failure (ADCHF) in relation to combined renal and cardiovascular outcomes during 1 year of follow-up.

Materials and methods. A total of 108 patients hospitalized with ADCHF (mean age 68.3 ± 10.0 years, 60% men) were included in a single-center prospective study. All patients included in the study underwent a standard physical and laboratory instrumental examination, including an assessment of the clinical condition according to the Rating Scale of Clinical State (RSCS) and laboratory tests (including serum creatinine level, glomerular filtration rate (GFR) using the CKD-EPI 2021 equation, albumin to creatinine ratio in urine, natriuretic peptide (NT-proBNP) upon admission and discharge. Acute kidney injury (AKI) was diagnosed based on the KDIGO guidelines (Kidney Disease: Improving Global Outcomes). The total rate of all-cause mortality and repeated hospitalizations from ADCHF was evaluated as cardiovascular outcomes. Renal outcomes included deterioration of renal function in the form of a decrease in GFR >15% of baseline and a decrease in GFR < 30 ml/min/1.73 m^2. Combined renal and cardiovascular outcomes were assessed during outpatient visits 3, 6, 12 months after discharge.

Results. The incidence of AKI during hospitalization in patients with ADCHF was 14% (n = 15). The groups with and without AKI were comparable in terms of clinical and demographic parameters and clinical assessment scale parameters. However, patients in the AKI group had higher baseline values of NT-proBNP and more pronounced impaired renal function, which persisted for 6–12 months of follow-up. There were no differences in clinical and laboratory data during the follow-up period. In patients with ADCHF, the presence of AKI during hospitalization significantly increases the risk of combined renal and cardiovascular outcomes during 1 year of follow-up (HR = 7.6; 95%CI = 2–29; p = 0.003).

Conclusion. The development of AKI during hospitalization in patients with ADCHF is a predictor of an unfavorable prognosis for combined renal and cardiovascular outcomes during 1 year of follow-up.

Keywords: acute decompensation of chronic heart failure, acute kidney injury, prognostic value

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee of RUDN University.

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Прогностическое значение острого повреждения почек у пациентов, госпитализированных с острой декомпенсацией хронической сердечной недостаточности

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РЕЗЮМЕ

Цель: изучить влияние острого повреждения почек (ОПП) на прогноз у пациентов, перенесших госпитализацию по поводу острой декомпенсации хронической сердечной недостаточности (ОДХСН), в отношении комбинированных почечных и сердечно-сосудистых исходов в течение 1 года.

Материалы и методы. Включены 108 пациентов, госпитализированных по поводу ОДХСН. Мужчины составляли 60%, средний возраст 68 ± 11 лет. Проводилось стандартное физическое обследование по шкале оценки клинического состояния (ШОКС), лабораторные исследования (определялся уровень креатинина сыворотки, скорость клубочковой фильтрации (СКФ) CKD-EPI 2021, натрийуретический пептид (NTproBNP), альбумин/креатининурия) при поступлении, выписке. Диагноз ОПП устанавливался согласно критериям KDIGO (Kidney Disease: Improving Global Outcomes, Болезнь почек: улучшение глобальных результатов). В качестве сердечно-сосудистых исходов оценивали суммарный показатель смертности от всех причин и повторных госпитализаций с ОДХСН. Почечные исходы включали ухудшение функции почек в виде снижения СКФ > 15% от исходного, снижение СКФ < 30 мл/мин/1,73м^2. Почечные и сердечно-сосудистые исходы оценивались во время амбулаторных визитов через 3, 6, 12 мес после выписки.

Результаты. Частота развития ОПП во время госпитализации у пациентов с ОДХСН составила 14% (n = 15). Группы с наличием и без ОПП были сопоставимы по клинико-демографическим показателям, клиническому состоянию.

Пациенты в группе ОПП на момент госпитализации имели более высокий уровень NT-proBNP, более выраженные нарушения функционального состояния почек, которые сохранялись в течение 1 года. В течение периода наблюдения не выявлено различий по клиническим и лабораторным данным.

Наличие ОПП в период госпитализации у пациентов с ОДХСН достоверно повышает риск возникновения комбинированных почечных и сердечно-сосудистых исходов в течение 1 года наблюдения (отношение рисков 7,6; 95%-й доверительный интервал: 2–29; p = 0,003).

Заключение. Развитие ОПП у пациентов с ОДХСН является предиктором неблагоприятного прогноза в отношении комбинированных почечных и сердечно-сосудистых исходов в течение 1 года.

Ключевые слова: острая декомпенсация сердечной недостаточности, острое повреждение почек, прогноз

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом РУДН им. Патриса Лумумбы.

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INTRODUCTION

Acute kidney injury (AKI) is a sudden decline in kidney function, which is a common problem in people admitted to hospital. The incidence is estimated between 7 and 18% amongst in-patients with the incidence reaching 70% in intensive care units [1], making AKI one of the most common complications following hospital discharge. AKI is a common concomitant syndrome in patients with heart failure (HF), with an average incidence of 13–20% [2, 3]. According to the meta-analysis, which included 37 studies, it was found that the overall incidence of AKI in patients with HF is even higher and amounts to 33% [4]. The high incidence of AKI in patients with HF may be due to multiple reasons. First, patients with HF are more likely to have reduced renal perfusion due to systolic or diastolic dysfunction. Second, a considerable number of patients may have had underlying chronic HF, a condition associated with chronic renal impairment [4].

A number of studies have shown that AKI is a robust independent predictor of both in-hospital and one-year mortality for HF patients [2, 5]. AKI is independently associated with a higher risk of cardiovascular complications and recurrent hospitalizations due to ADCHF after hospital discharge [6–8]. This relationship is also associated with a higher likelihood of chronic kidney disease (CKD), expedited progression to end-stage renal disease, and a decline in health-related quality of life [3, 9]. Thus, early detection of patients at risk of AKI is essential for improving outcomes [5]. However, most of these studies were conducted mainly in patients with stable HF.

Thus, it is important to determine the prognostic value of AKI in relation to combined renal and cardiovascular outcomes during one year follow-up in patients who were hospitalized for ADCHF.

MATERIALS AND METHODS

The study included 108 patients hospitalized with ADCHF in Vinogradov Clinical Hospital in Moscow. The study did not include patients with chronic kidney disease (CKD) stage 5 receiving renal replacement therapy, acute coronary syndrome, malignant neoplasms receiving active antitumor treatment, and mobility impairment with serious medical state, which makes it impossible to discharge a patient.

The study was performed in accordance with the standards of Good Clinical Practice and the principles of the Helsinki Declaration. The research protocol was approved by the local ethics committee. All patients signed an informed consent prior to the examination procedures.

All patients underwent a standard physical examination and laboratory and instrumental examinations. The clinical condition of the RSCS was assessed, the serum creatinine level was determined with the calculation of GFR according to the formula CKD-EPI 2021, the level of natriuretic peptide (NTproBNP), and the ratio of albumin to creatinine in urine at admission and discharge.

AKI was diagnosed according to the KDIGO criteria (Kidney Disease: Improving Global Outcomes) when serum creatinine increased by 0.3 mg/dl (26.5 mcmol/l) for 2 days or by 50% for 7 days.

Renal and cardiovascular outcomes were assessed during outpatient follow-up 3, 6, and 12 months after discharge. The total mortality and repeated hospitalizations for ADCHF were determined by the combined cardiovascular outcome. Renal outcomes included deterioration of renal function in the form of a decrease in GFR > 15% of baseline and a decrease in GFR < 30 ml/min/1.73 m^2. The clinical and demographic characteristics of the patients who were included in the study are presented in Table 1.

Table 1

Clinical and Demographic Characteristics of Patients Included in the Study, *n* = 108

Parameter	Value
Gender (male), *n* (%)	64 (60%)
Age, years *M*±*SD*	68±11
BMI, kg/m^2, *M*±*SD*	30 ± 6
Left ventricular ejection fraction, %, *M*±*SD*	43 ±12
Left ventricular ejection fraction <40%, *n* (%)	44 (41%)
Arterial hypertension, *n* (%)	99 (92%)
Obesity, *n* (%)	44 (41%)
Coronary heart disease, *n* (%)	55 (51%)
Atrial fibrillation or flutter, *n* (%)	64 (59%)
Chronic kidney disease before hospitalization, *n* (%)	28 (26%)
Diabetes mellitus, *n* (%)	38 (35%)
Anemia, *n* (%)	20 (19%)
Chronic obstructive pulmonary disease or bronchial asthma (without exacerbation), *n* (%)	16 (15%)

The statistical analysis was carried out using the StatTech v. 3.1.8 software (developed by Stattech LLC, Russia). Quantitative variables with a normal distribution were described using arithmetic mean and standard deviations *M*±*SD*. In case of an asymmetric distribution, they were described using the median and the interquartile range *Me* (*Q1; Q3*). Categorical data were described as absolute values and percentages

(*n* (%). In case of a normal distribution of data, the statistical significance of the differences was assessed using the Student's *t*-test, and the Mann–Whitney test was used for a distribution other than normal. Pearson's $\chi 2$ test was used to compare groups by frequency of qualitative variables. Differences were considered statistically significant at $p < 0.05$.

When constructing Kaplan-Meier curves, the frequency of reaching the combined endpoint was estimated. Differences in reaching the primary endpoint were assessed using the likelihood-ratio test. To assess the prognostic significance of AKI in relation to the onset of the combined endpoint, univariate Cox regression analysis models were used, the hazard ratio (HR) and 95% confidence interval (CI) were calculated. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The incidence of AKI during hospitalization in patients with ADCHF was 14% (*n* = 15). Then, clinical, demographic, and laboratory parameters were analyzed depending on the presence of AKI (Table 2). The groups were comparable in terms of the main clinical and demographic characteristics, as well as clinical profile (RSCS). Patients from the AKI group had higher serum creatinine and NT-proBNP levels, as well as albumin/creatinuria.

Table 2

Clinical, Demographic and Laboratory Parameters of Patients with ADCHF Depending on the Presence of AKI at Admission

Parameter	Patients with AKI (*n* = 15)	Patients without AKI (*n* = 93)
Clinical and demographic parameters		
Gender (male), *n* (%)	11 (78.6)	53 (57.0)
Age, years, *M* ± *SD*	67.0±10.0	68.0±11.0
Left ventricular ejection fraction, %, *M* ± *SD*	40,2±13.1	43.1±12.1
Left ventricular ejection fraction <40%, *n* (%)	6 (40.0)	38 (40.8)
RSCS score upon admission, points, *Me* (Q_1; Q_3)	6.5 [6; 8]	7.0 [5; 9]
RSCS score at discharge, points, *Me* (Q_1; Q_3)	4.0 [1; 5]	4.0 [2; 5]
Arterial hypertension, *n* (%)	15 (100)	84 (90.3)
Obesity, *n* (%)	8 (53.3)	36 (38.7)
BMI, kg/m^2, *M* ± *SD*	33.2±7.3	30.0±6.1
Coronary heart disease, *n* (%)	10 (66.7)	45 (48.4)
Atrial fibrillation or flutter, *n* (%)	6 (40.0)	58 (61.14)
Chronic kidney disease before hospitalization, *n* (%)	2 (13.3)	26 (28)
Diabetes mellitus, *n* (%)	8 (53.3)	30 (32.3)
Anemia, *n* (%)	5 (33.3)	15 (16.1)
Chronic obstructive pulmonary disease or bronchial asthma (without exacerbation), *n* (%)	4 (26.7)	12 (12.9)
Laboratory parameters		
Creatinine, mcmol/l	186.73 ± 34.02	99.45 ± 23.70*
GFR-EPI, ml/min/1.73 m^2	29.00 [25.5; 33.5]	63.00 [45.4; 65.6]***
Albumin/creatinine in urine, mg/g	49 [3.5; 128.5]	17 [4; 64]*
NT-proBNP, pg/ml	1,370.5 [996; 1,975]	1,042 [288; 1,675]*

End of table 2

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ when comparing groups.

The dynamics of laboratory parameters during one-year follow-up is shown in Table 3. In the group of patients who developed AKI during hospitalization, significantly higher creatinine values were noted after 3, 6, and 12 months, lower GFR values after 3 months, higher values of the albumin/creatinine ratio in urine after 3 and 6 months of follow-up. There were no significant differences in the clinical condition assessed on the RSCS and the level of NT-proBNP during follow-up. After 3 months of follow-up, 43.5% (*n* = 47) of patients were diagnosed with CKD, while 17.5% (*n* = 19) of patients were diagnosed with *de novo* CKD. After 6 and 12 months of follow-up, no new cases of CKD were detected.

Table 3

Changes in Laboratory Parameters during One-Year Follow-Up, *Me* (Q_1; Q_3)

Parameter	Patients with AKI (*n* = 15)	Patients without AKI (*n* = 93)
Creatinine, mcmol/l		
3 months	121 [102; 132.5]	91 [77; 109]**
6 months	117 [89; 139]	91,5 [77; 114.03]*
12 months	122 [94; 142]	94 [79; 166]**
GFR, ml/min/1.73 m^2		
3 months	59 [41; 71]	70 [54; 89]*
6 months	61 [38.5; 76.5]	69 [52; 90]
12 months	52 [36.5; 70]	66 [48; 86]
Albumin/creatinine in urine, mg/g		
3 months	38 [19; 118]	13 [4; 33]**
6 months	26 [13; 87.5]	11 [4.25; 30]*
12 months	22 [4.5; 51.5]	12 [3; 31]
NT-proBNP, pg/ml		
3 months	425 [85; 933.3]	601 [296.7; 942.2]
6 months	1,067 [510; 1,486]	716 [349.8; 1,440.5]
12 months	1,290 [781.8; 1,400]	1,133 [466.5; 2,400]

End of table 3

Parameter	Patients with AKI (n = 15)	Patients without AKI (n = 93)
RSCS score, points		
3 months	2.5 [2; 4.75]	3.5 [2; 5]
6 months	2 [2; 3.75]	3 [2; 4]
12 months	2.5 [1; 4.25]	3 [2; 4]

*p < 0.05, **p < 0.01, ***p < 0.001 when comparing groups.

The incidence of adverse cardiovascular outcomes for one-year follow-up was 38% (n = 41), including 5 deaths and 36 hospitalizations for ADCHF, and 30.5% (n = 33) had an unfavorable renal outcome in the form of a decrease in GFR by more than 15% per year. At the same time, 14% (n = 15) of patients had both renal and cardiovascular outcomes.

To identify the main predictors of combined renal and cardiovascular outcomes, Cox regression analysis was performed during one-year follow-up. It revealed that the development of AKI during hospitalization was significantly associated with a higher probability of developing combined renal and cardiovascular outcomes during one-year follow-up (HR = 7.6; 95% CI: 2–29; p = 0.003).

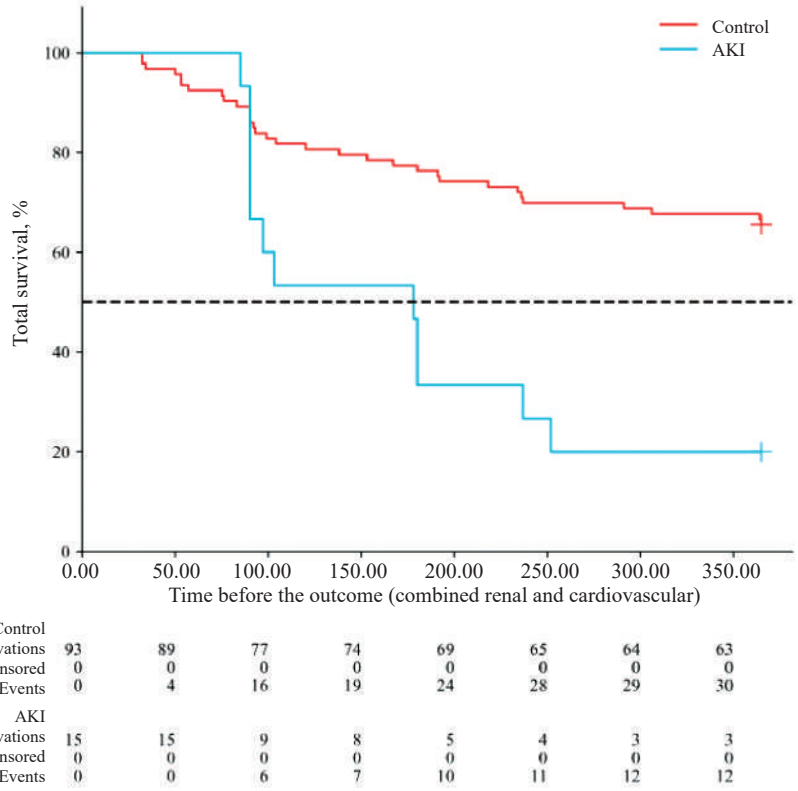

Fig. Kaplan-Meier curves (renal and cardiovascular outcomes) depending on the development of AKI during hospitalization in patients with ADCHF, n = 108

DISCUSSION

AKI is characterized by a sudden decrease in renal function, which is manifested by an increase in serum creatinine or a decrease in the level of diuresis [10]. The presence of AKI is highly common among patients admitted to the hospital for ADCHF. In a cohort of 30,529 patients with acute and chronic HF, the incidence of AKI was 10.4% [2]. In this study, the incidence of AKI in patients hospitalized for ADCHF was consistent with literature data and amounted to 14%.

Congestive phenomena in ADCHF affect pathophysiological regulation of kidney function. Venous hypertension leads to a decrease in perfusion, an increase in interstitial pressure in the kidneys, a decrease in the gradient of arterial and venous renal pressure, a decrease in GFR, inadequate autoregulatory reactions, and other neurohumoral imbalances. Higher renal pressure weakens glomerular filtration, causes tubular collapse and tubulointerstitial fibrosis [11].

A number of studies have shown that AKI is associated with serious long-term problems in patients, including the development or progression of CKD [12, 13], renal failure, cardiovascular complications [14, 15], and decreased survival [16].

AKI is generally associated with the development of CKD in the future [13], although the long-term prognosis after AKI in HF requires clarification. In the cohort of patients admitted with ADCHF, deterioration of renal function was associated with a significant increase in hospital mortality, more frequent complications, and an increase in the duration of hospitalization [17]. The transition of AKI to acute kidney disease is associated with mortality within 1 year and the development of *de novo* CKD [18]. In this study, *de novo* CKD was diagnosed in 18% (n = 19) patients after 3 months of follow-up. The association of acute kidney disease with short-term (90 days) [19] and long-term (5 years) [20] adverse prognosis (risk of mortality and adverse renal events) has been demonstrated.

This study showed that the development of AKI during hospitalization in patients with ADCHF was associated with a higher probability of developing combined renal and cardiovascular outcomes during one-year follow-up (HR = 7.6; 95% CI = 2–29; p = 0.003), which is consistent with the literature data. A meta-analysis of 11 studies showed that hospital mortality is higher in patients with AKI than in patients without AKI (HR = 3.65; 95% CI: 3.04–4.39, $p < 0.001$). Mortality was assessed in five studies, and it was found that the mortality rate remained high during one-year follow-up after AKI (HR = 1.85; 95% CI: 1.54–2.22, $p < 0.001$) [4]. AKI was associated with a higher mortality rate within 30 days after hospitalization for HF (HR = 5.3; 95% CI: 2.2–13.2) [21]. In patients with HF and normal initial renal function admitted with acute renal failure who developed AKI, increased hospital mortality was observed: 4.9% vs. 1.6%, adjusted odds ratio (OR) 3.21; $p \leq 0.001$ [3].

CONCLUSION

The development of AKI during hospitalization in patients with ADCHF is a predictor of an unfavorable prognosis for combined renal and cardiovascular outcomes during one-year follow-up.

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The Relationship of Hormones of the Hypothalamic – Pituitary – Thyroid Axis with Cognitive Impairment in Patients with Schizophrenia

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ABSTRACT

Aim. To study the relationship of serum fT_3, fT_4, and TSH levels with cognitive impairment in patients with schizophrenia.

Materials and methods. The study included 74 patients with schizophrenia. Socio-demographic and clinical data were collected, the severity of psychopathological symptoms was assessed using PANSS, and cognitive functions were evaluated using BACS. Serum levels of fT_3, fT_4, and TSH in patients were determined using enzyme immunoassay kits.

Results. In the group of men with schizophrenia, a negative correlation was found between the concentration of fT_3 and verbal fluency ($r_s = –0.325$; $p = 0.033$), whereas in women, a positive correlation was found between the concentration of fT_4 and motor skills ($r_s = 0.372$; $p = 0.039$).

Conclusion. The study revealed a linear relationship between thyroid hormones and cognitive impairment in patients with schizophrenia, but the nature of the relationship found differed in men and women. The results of the study confirm the need for regular dynamic monitoring of thyroid hormone levels in patients with schizophrenia in order to prevent the progression of cognitive impairment.

Keywords: schizophrenia, thyroid hormones, neurocognitive disorders, verbal fluency, motor skills

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Mental Health Research Institute of Tomsk NRMC (Minutes No. 157 dated November 11, 2022).

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Связь гормонов гипоталамо-гипофизарно-тиреоидной оси с когнитивными нарушениями у больных шизофренией

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РЕЗЮМЕ

Цель. Изучить связь показателей тиреотропного гормона (ТТГ) и свободных фракций T_3, T_4 в сыворотке крови с когнитивными нарушениями у больных шизофренией.

Материалы и методы. В исследование включены 74 пациента с шизофренией. Собраны социально-демографические и клинические данные, проводилась оценка тяжести психопатологической симптоматики с использованием PANSS, а также когнитивных функций с помощью BACS. Уровень свободных фракций T_3, T_4 и ТТГ в сыворотке крови у пациентов определяли с помощью наборов для иммуноферментного анализа.

Результаты. В группе мужчин больных шизофренией выявлена отрицательная корреляция между концентрацией T_3 св. и речевой беглостью (r_s = –0,325; p = 0,033), тогда как у женщин обнаружена положительная корреляция между концентрацией T_4 св. и моторными навыками (r_s = 0,372; p = 0,039).

Заключение. Исследование показало, что существует линейная связь между гормонами щитовидной железы и когнитивными нарушениями у пациентов с шизофренией, однако характер выявленной связи отличается у мужчин и женщин. Результаты данного исследования подтверждают необходимость регулярного динамического мониторинга показателей ТТГ и тиреоидных гормонов у пациентов с шизофренией с целью предотвращения прогрессирования когнитивных нарушений.

Ключевые слова: шизофрения, тиреоидные гормоны, нейрокогнитивные нарушения, речевая беглость, моторные навыки.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование проведено в рамках выполнения государственного задания No 075-01392-23-00 «Персонализированная диагностика и терапия больных полиморбидными расстройствами шизофренического и аффективного спектра», регистрационный номер 123041900006-4.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом и НИИ психического здоровья Томского НИМЦ (протокол № 157 от 18.11.2022).

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INTRODUCTION

Schizophrenia is a mental disorder characterized by the presence of positive and negative symptoms and cognitive impairment, a chronic course, and a functional decline resulting in reduced quality of life and disability [1, 2]. Studies show that cognitive impairment manifests before the onset of the illness and is present in approximately 75–84% of patients with schizophrenia [3, 4]. The etiology and pathogenesis of neurocognitive deficits in schizophrenia are not fully understood, but some studies have shown that neuroendocrine disorders may be associated with cognitive symptoms [4–6].

The thyroid hormones thyroxine (T4) and triiodothyronine (T3) play an important role in brain differentiation and growth and, consequently, in cognitive function. In addition, thyroid hormones maintain normal glucose metabolism, which is essential for brain function [7]. A number of researchers have shown that people with hypothyroidism have troubles in many cognitive functions, such as attention, memory, language, visual perception, and executive functions [8, 9]. Additionally, there are studies

confirming that patients with even mild or subclinical hypothyroidism have reversible cognitive impairment [8, 10]. Moreover, people with thyrotoxicosis have been found to have impaired concentration and executive function [11]. Both increased and decreased thyroid-stimulating hormone (TSH) levels are associated with cognitive impairment [12].

Neurohormonal imbalance plays an essential role in the pathogenesis of many mental disorders. Several previous studies in patients with schizophrenia have reported abnormalities in the hypothalamic–pituitary–thyroid axis in the form of decreased T_3 and T_4, and increased thyroid autoantibody production [13, 14]. Relatively recent studies show that in female patients with schizophrenia, free T_3 ($T_{3\ free}$) and T_4 ($T_{4\ free}$) levels are decreased, and TSH levels are elevated [14]. Few studies have also investigated the relationship between thyroid hormones and cognitive processes in different groups of diseases due to the critical role of thyroid dysfunction in neurodegeneration and development of the nervous system [15, 16]. For example, Kapaki et al. [16] found that the direct effect of thyroxine on cholinergic neurons increases the risk of developing Alzheimer's disease. In a study of patients with early psychosis, higher levels of $T_{4\ free}$ (but not TSH or thyroid autoantibodies) were associated with better cognitive performance, such as attention and thinking [15, 17]. Another study of 93 patients with schizophrenia found that $T_{3\ free}$ levels were associated with better performance on the Mini-Mental State Examination (MMSE), but not with positive, negative, or general psychopathological symptoms [18].

The aim of the study was to investigate the association of serum fT_3, fT_4, and TSH levels with cognitive impairment in patients with schizophrenia.

MATERIALS AND METHODS

The study was conducted according to the protocol approved by the local Ethics Committee at Mental Health Research Institute of Tomsk NRMC (Minutes No.157 dated November 18, 2022). The study included 74 patients with an established diagnosis of schizophrenia receiving treatment at Mental Health Research Institute clinics. Inclusion criteria: age 18–55 years, verified diagnosis of schizophrenia according to ICD-10 criteria, patient's consent to participate in the study. Non-inclusion criteria: dependence on psychoactive substances other than tobacco, mental retardation, or dementia, neurologic history (brain injury, stroke). After obtaining patients consent, socio-demographic and clinical data were collected, the severity of psychopathological symptoms was assessed using the Positive and Negative Syndrome Scale (PANSS) [19], and cognitive functions were assessed by the Brief Assessment of Cognition in Schizophrenia (BACS) using normative indicators calculated for the Tomsk population [20]. Data collection and psychometric assessment of patients' condition were performed by psychiatrists.

Blood samples to determine the hypothalamic–pituitary–thyroid axis hormone levels were collected in the morning on an empty stomach from the ulnar vein into vacuum Vascette tubes. Blood serum was obtained by centrifugation at 2,000 rpm for 30 minutes. Concentrations of TSH, $T_{3\ free,}$ and $T_{4\ free}$ in patients' serum were determined by the solid-phase enzyme-linked immunosorbent assay using reagent kits from Vector-Best JSC (Novosibirsk, Russia).

Statistical analysis was performed using Statistica software, version 12.0 for Windows (StatSoft, Inc.). Descriptive statistics were presented as the median and the interquartile range *Me* [$Q_{1;}$ Q_3], and the normality of variable distribution was assessed using the Shapiro–Wilk test. The Mann–Whitney *U* test and the factorial ANOVA were used to compare independent samples. The correlation analysis with the Spearman's rank correlation coefficient (r_s) was used to determine the linear relationship between the variables. Multiple linear regression was used to assess the effect of variables on cognitive function scores. The critical significance level *p* was 0.05.

RESULTS

The study included 74 patients with schizophrenia: 31 women and 43 men. The mean age of the patients was 34 [26; 43] years. Twenty-five (33.8%) patients had higher education, 8 (10.8%) – incomplete higher education, 28 (37.8%) – vocational secondary education, and 13 (17.6%) – secondary education. The duration of the disease was 9 [5; 21] years, and the age of onset was 22 [19; 27] years. The PANSS total score was 96 [88; 105], the positive symptom subscale score – 18 [16; 23], the negative symptom subscale score – 24 [22; 28], and the general psychopathological symptom subscale score – 51 [46; 56].

All patients included in the study received baseline antipsychotic therapy with atypical antipsychotics (risperidone, quetiapine, olanzapine, clozapine) – 58 (78.4%) people and conventional antipsychotics (haloperidol, chlorprothixene, trifluoperazine) – 16 (21.6%) individuals in therapeutic doses approved by the Ministry of Health of the Russian Federation.

The median total antipsychotic exposure (expressed as chlorpromazine equivalent (CPZeq)) was 360 [199; 500] mg/day, and the duration of baseline therapy was 8 [3; 20] years.

Serum $T_{3\ free}$ and $T_{4\ free}$ concentrations in patients were 5.51 [4.75; 6.65] pmol/l and 17.05 [14.67; 18.9] pmol/l, respectively, and TSH level was 2.15 [1.38; 3.16] mIU/l. $T_{3\ free}$, $T_{4\ free,}$ and TSH concentrations were comparable in men and women ($p > 0.05$), whereas men and women were also comparable in age and clinical, therapeutic, and neuropsychological parameters ($p > 0.05$). A negative correlation was found between the PANSS positive symptom score and $T_{3\ free}$ ($r_s = –0.335$; $p = 0.005$) and $T4_{\ free}$ ($r_s = –0.444$; $p < 0.001$). A correlation was also revealed between the PANSS total score and the TSH concentration ($r_s = 0.245$; $p = 0.045$).

Scores on the verbal and working memory subscales were 37 [32; 43] and 18 [16; 21], respectively. The scores on the motor skill, verbal fluency, attention, and executive function subscales were 54 [44; 64], 39 [32; 49], 41 [34; 47], and 17 [15; 19], respectively. Based on the BACS [20] conducted earlier, a significant decrease in performance was observed for all subtests ($p < 0.001$) in the studied group of patients with schizophrenia. Gender had a significant effect on all cognitive domains examined (F (6.67) = 3.237; $p = 0.007$). In addition, a statistically significant relationship was found in men between working memory and PANSS positive symptom scores ($r_s = –0.326$; $p = 0.048$), and between verbal fluency and PANSS negative symptom scores ($r_s = –0.547$; $p < 0.001$). In women, however, the only association found was between the duration of baseline therapy and motor skills ($r_s = 0.434$; $p = 0.023$).

We did not find statistically significant correlations between cognitive tests and the functioning of the hypothalamic–pituitary–thyroid axis in the total sample of patients with schizophrenia ($p > 0.05$). However, taking into account the patients gender, we obtained significant relationships: in the group of male patients with schizophrenia, we found a negative correlation between $T_{3\ free}$ concentration and verbal fluency ($r_s = –0.325$; $p = 0.033$), while in the group of females, we found a positive correlation between $T_{4\ free}$ concentration and motor skills ($r_s = 0.372$; $p = 0.039$) (Table 1).

Table 1

Correlations between TSH, Thyroid Hormones, and Cognitive Function in Patients with Schizophrenia, R_s

Parameter		I	II	III	IV	V	VI
Total sample of patients with schizophrenia, $n = 74$							
TSH	r_s	–0.033	–0.032	0.029	–0.138	0.027	0.026
	p	0.777	0.788	0.808	0.242	0.817	0.826
$T_{3\ free}$	r_s	–0.036	–0.024	–0.013	–0.228	–0.167	0.064
	p	0.763	0.841	0.911	0.051	0.156	0.589
$T_{4\ free}$	r_s	0.035	0.148	0.148	–0.004	0.009	0.099
	p	0.764	0.209	0.209	0.974	0.936	0.401
Men with schizophrenia, $n = 43$							
TSH	r_s	–0.122	0.143	–0.084	–0.199	0.062	–0.127
	p	0.434	0.360	0.592	0.200	0.692	0.419
$T_{3\ free}$	r_s	–0.075	–0.108	–0.158	–0.325*	–0.266	0.029
	p	0.633	0.492	0.310	0.033*	0.085	0.852
$T_{4\ free}$	r_s	–0.080	0.219	–0.046	–0.126	–0.117	0.044
	p	0.609	0.158	0.772	0.420	0.456	0.781
Women with schizophrenia, $n = 31$							
TSH	r_s	0.148	–0.254	0.143	–0.034	–0.028	0.152
	p	0.426	0.168	0.442	0.854	0.883	0.413
$T_{3\ free}$	r_s	0.058	0.134	0.124	0.018	0.011	–0.058
	p	0.755	0.472	0.505	0.921	0.954	0.757
$T_{4\ free}$	r_s	0.147	0.040	0.372*	0.150	0.210	0.194
	p	0.429	0.830	0.039*	0.421	0.258	0.295

Note: I – memorization of a list of words, II – a sequence of numbers, III – a motor test with chips, IV – verbal fluency, V – "Cipher", VI – "Tower of London". * – statistically significant differences.

Based on the correlation analysis data, in order to determine the influence of clinical and biochemical parameters on cognitive function in male and female patients with schizophrenia, a series of separate regressions were additionally conducted, where the following were used as independent variables: scores

on the PANSS positive (1) and negative (2) symptom subscales, and $T_{3\ free}$ concentration (3) for men; duration of baseline therapy (1) and $T_{4\ free}$ concentration (2) for women. In the male group, the predictive model for the verbal fluency parameter was statistically significant (F (3.33) = 4.714; p = 0.007). The R^2 value was 0.301, indicating that scores on the PANSS positive and negative symptom subscales and serum $T_{3\ free}$ concentration explained approximately 30% of the variability in verbal fluency in male patients with schizophrenia. Statistically significant predictors were the scores on the PANSS negative symptom subscale (t = -2.515; p = 0.016) and the $T_{3\ free}$ concentration (t = –2.985; p = 0.011) (Table 2).

Table 2

Coefficients of the Multiple Linear Regression Model for the Relationship of Verbal Fluency with Clinical and Biochemical Parameters in Men with Schizophrenia

Parameter	Coefficient B	Standard Error	Significance (t)	Significance (p)
Constant	75.997	12.539	6.061	0.001
$PANSS_p$ (score)	–0.104	0.428	–0.243	0.808
$PANSS_n$ (score)	–1.118	0.444	–2.515	0.016
$T_{3\ free}$ (pmol / l)	–1.348	0.369	–2.985	0.011
R = 0.547; R^2 = 0.301				

Note: $PANSS_p$ – scores on the positive symptom subscale, $PANSS_n$ – scores on the negative symptom subscale.

In the female group, the model for predicting the motor skill scores was statistically significant (F (2.34) = 3.545; p = 0.039). R^2 was 0.272, indicating that the duration of therapy and the concentration of $T_{4\ free}$ in the blood serum explain approximately 27% of the variability in the motor skill scores in female patients with schizophrenia. Both predictors were statistically significant: the duration of baseline therapy (t = 2.038; p = 0.049) and the $T_{4\ free}$ concentration (t = 2.816; p = 0.032) (Table 3).

Table 3

Coefficients of the Multiple Linear Regression Model for the Relationship of Motor Skills with Clinical and Biochemical Parameters in Women with Schizophrenia

Parameter	Coefficient B	Standard Error	Significance (t)	Significance (p)
Constant	24.281	11.038	2.199	0.034
Duration of therapy (years)	0.534	0.262	2.038	0.049
$T_{4\ free}$ (pmol / l)	1.164	0.641	2.816	0.032
R = 0.415; R^2 = 0.272				

DISCUSSION

The present study investigated the relationship between indices of cognitive function and hormones of the hypothalamic–pituitary–thyroid axis, as well as some clinical and dynamic parameters in patients with schizophrenia. The results of the study revealed a linear relationship between thyroid hormones and cognitive functions, but the direction of this relationship differed between male and female patients.. In males, high levels of $T_{3\ free}$ in the blood serum were associated with lower indices of verbal fluency, whereas in females, the concentration of $T_{4\ free}$ correlated directly with motor skill scores. The study also found that factors influencing cognitive function differed between males and females with schizophrenia. In the resulting regression model, the significant predictors of verbal fluency in males were the $T_{3\ free}$ concentration and the severity of negative symptoms. In females, the predictors of the motor skill score were the duration of baseline therapy and the $T_{4\ free}$ concentration.

Thyroid hormone levels are known to be important for cognitive abilities, including motor function and language. Studies investigating the relationship between thyroid hormone levels and cognition in patients with mental disorders are limited. One study of patients with psychosis found that changes in $T_{3\ free}$ within the normal range (reference values) had a significant effect primarily on the attention [15]. Another study in patients with schizophrenia showed that higher $T_{3\ free}$ levels were associated with higher MMSE scores [18]. The same study emphasized that T_3 replacement therapy could improve cognitive function in patients with schizophrenia. However, in our study, inverse relationships were found for $T_{3\ free}$ only in males, suggesting that $T_{3\ free}$ levels are more strongly associated with cognitive symptoms in males with schizophrenia than $T_{3\ free}$ concentrations.

Some studies have shown that changes in $T_{4\ free}$ in patients with psychosis affect some cognitive functions [15, 17]. Increased $T_{4\ free}$ levels in patients with psychosis are associated with improved attention, but no such relationship was found in the healthy control group [15]. In our study, we found a linear relationship between $T_{4\ free}$ levels and motor function scores only in females, suggesting that $T_{4\ free}$ levels play a more important role in cognitive function in patients with schizophrenia than $T_{3\ free}$.

We also found significant relationships between thyroid hormone levels and positive symptoms on the PANSS, although some authors have previously reported no such relationships [5, 18]. The study

it confirms the effect of thyroid hormones not only on cognitive functions, but also on the severity of psychopathological symptoms of schizophrenia.

However, our study study has some limitations. Although the study used a comprehensive neurocognitive test battery, the number of patients was relatively small, which could have a negative effect on statistical significance. Moreover, due to the cross-sectional nature of the study, it was impossible to establish a causal relationship between the changes found. In addition, we studied a group of chronic patients with schizophrenia who were receiving long-term antipsychotic therapy, and we cannot exclude the influence of therapy on our results, nor can we be sure that patients adhered to the treatment regimen in the long term.

CONCLUSION

The study showed that there is a linear relationship between thyroid hormones and cognitive impairment in patients with schizophrenia, however, the nature of the relationship identified differs between male and female patients.. The results of this study confirm the need for regular dynamic monitoring of TSH and free thyroid hormone levels in patients with schizophrenia to prevent progression of cognitive impairment. Further studies with larger numbers of patients are needed to better understand the relationship between thyroid dysfunction and cognitive symptoms and to consider the possibility of including hormone replacement therapy in the treatment strategy for patients with schizophrenia.

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Author contribution

Kornetova E.G. – conception and design, clinical, psychopathological, and psychometric examination of the sample, review of publications on the topic of the article, critical revision of the manuscript for important intellectual content. Galkin S.A. – drafting of the manuscript, statistical analysis of the data. Lobacheva O.A. – laboratory analysis of the parameters, review of publications on the topic of the article. Tiguntsev V.V. – preparation of the sample, maintenance of the database. Mednova I.A. – examination of the sample, maintenance of the database. Kornetov A.N. – study design, editing of the manuscript, final approval of the manuscript for publication.

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Anti-Inflammatory Effect and a Possible Mechanism of Action of Ethowurtzine from the Class of Hexaazaizowurtzitane Derivatives

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ABSTRACT

Aim. To assess the anti-inflammatory and gastroprotective effects of ethowurtzine compared to the reference drug diclofenac in a rat model of chronic inflammation; to evaluate the influence of ethowurtzine on nitric oxide production as a possible mechanism of its anti-inflammatory effects.

Materials and methods. The object of the study was a new patented ethowurtzine from the class of hexaazaizowurtzitane derivatives with an acceptable safety profile.

The gastroprotective and anti-inflammatory effects of ethowurtzine compared to diclofenac were studied in a model of chronic inflammation using 69 female SD rats. The compound (12.5–100 mg / kg) and a non-selective COX inhibitor diclofenac (5 mg / kg) were administered intragastrically for 7 days, 1 hour before subcutaneous implantation of a cotton swab. On day 8, the proliferative response (%), the exudative response (%), and the ulcerogenic effect of the compounds were assessed.

Nitric oxide (NO) synthesis by macrophages obtained from peritoneal cavity of 25 C57Bl/6 mice (*in vitro* inflammation model) was evaluated by the concentration of nitrites in the cell supernatant after incubating cells in the presence of ethowurtzine and / or lipopolysaccharide (LPS) for 48 hours. The classical MTT test (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA)) was used to assess the effects of ethowurtzine on macrophage proliferation.

Results. A comparative study of ethowurtzine and diclofenac in a rat model of chronic inflammation revealed the predominant anti-exudative effect of the new substance and a suppressive effect on granulation tissue proliferation comparable to that of NSAID.

The macroscopic examination of the gastric mucosa in rats receiving ethowurtzine did not reveal any ulcer damage. On the contrary, in 30% of the rats receiving diclofenac, the severity score of ulcer was 2. In the *in vitro* inflammation model, the addition of LPS to the macrophage culture resulted in a significant increase in NO synthesis. The introduction of ethowurtzine at different concentrations together with LPS dose-dependently reduced the NO production. A statistically significant decrease in the NO synthesis was noted at high doses of the test substance compared to the group of isolated LPS use. However, the introduction of ethowurtzine at different concentrations together with LPS did not cause statistically significant changes in the proliferation of macrophages compared to the group with the isolated LPS use.

Conclusion. The newly synthesized ethowurtzine had a pronounced anti-inflammatory effect and caused a significant decrease in granulomatous infiltration and exudative edema in the chronic inflammation model. Suppression of the NO synthesis is one of the possible mechanisms in the anti-inflammatory effect of ethowurtzine. The obtained data allow to suggest possible administration of the new patented analgetic ethowurtzine in chronic pain treatment associated with inflammation.

Keywords: hexaazaisowurtzitane, ethowurtzine, inflammation, gastric toxicity, nitric oxide

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Противовоспалительный эффект и возможный механизм действия анальгетика этовюрцина из класса гексаазаизовюрцитанов

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РЕЗЮМЕ

Цель: изучение противовоспалительного и гастропротективного действия этовюрцина в сравнении с диклофенаком на модели хронического воспаления; исследование его влияния на продукцию оксида азота.

Материалы и методы. Объект исследования – впервые синтезированный этовюрцин из класса гексаазаизовюрцитанов с приемлемым профилем безопасности.

Гастрозащитное и противовоспалительное действие этовюрцина в сравнении с диклофенаком исследовали на модели хронического воспаления у 69 самок крыс стока SD. Вещество (12,5–100 мг/кг) и нестероидное противовоспалительное средство (НПВС) (5 мг/кг) вводили *per os* в течение 7 сут, начиная за 1 ч до подкожной имплантации ватного тампона. На 8-й сут эксперимента оценивали пролиферативную (%) и экссудативную реакцию (%), ульцерогенное действие веществ.

На модели воспаления *in vitro* продукцию оксида азота (NO) макрофагами (МФ), полученную из перитониальной полости 25 мышей линии C57Bl/6, оценивали по концентрации нитритов в супернатанте клеток после 48-часового культивирования в присутствии различных концентраций этовюрцина и (или) липолисахарида (ЛПС). Влияние этовюрцина на пролиферацию макрофагов определяли в тесте MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma, США)) в лизате МФ.

Результаты. Сравнительное исследование этовюрцина и диклофенака на модели хронического воспаления у крыс выявило преимущественное антиэкссудативное действие вещества при сравнимом с НПВС подавляющем влиянии на пролиферацию грануляционной ткани.

В слизистой оболочке стенки желудка у крыс, получавших этовюрцин, не обнаружено язвенных деструкций. Напротив, у 30% животных, получавших диклофенак, тяжесть ульцерогенного повреждения составила 2 балла. На модели воспаления *in vitro* внесение этовюрцина в различных концентрациях совместно с ЛПС снижало выработку оксида азота, а при использовании высоких доз вещества наблюдалось статистически значимое уменьшение продукции NO по сравнению с группой применения ЛПС. Однако исполь-

зование этовюрцина совместно с ЛПС не оказывало статистически значимого влияния на пролиферацию макрофагов относительно группы применения ЛПС.

Заключение. Сравнительное исследование противовоспалительного действия впервые синтезированного этовюрцина на модели хронического воспаления у крыс выявило его преимущественную активность относительно диклофенака и отсутствие гастротоксичности. Полученные результаты свидетельствует об его альтернативном механизме противовоспалительного действия, не связанном с ингибированием циклооксигеназы. Подавление выработки оксида азота может быть одним из механизмов его противовоспалительного действия.

Ключевые слова: гексаазаизовюрцитан, этовюрцин, воспаление, гастротоксичность, оксид азота

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Inflammation is a universal pathological process that underlies many nosologies, varies in clinical manifestations, and is one of the central problems in the treatment of pain syndromes of various etiologies [1–4]. The modern concept of analgesic therapy involves an integrated approach with the use of drugs and treatment methods and affects different chains of the pain pathogenesis [1–6]. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) as first-line therapy is associated with a big role of inflammation in the development of pain responses [1, 3, 6]. According to the literature, hundreds of substances containing steroidal and non-steroidal anti-inflammatory agents have been synthesized worldwide over more than 140 years to control the inflammatory process [3–6].

Despite the fact that drugs from other pharmaceutical groups, in particular some psychotropic, neurotropic drugs, antihistamines, alpha- and beta-receptor agonists, and others, somewhat resemble NSAID action [4, 5], NSAIDs still remain the main drugs for suppressing inflammation, pain, and fever [1, 6]. Nonetheless, the risk of developing dangerous adverse effects, primarily in the gastrointestinal tract [2] and the cardiovascular system (gastroduodenopathy, nephro- hepato-, and hematotoxicity, cardiovascular diseases, etc.) significantly limits the use of NSAIDs even with high therapeutic efficacy [1–3]. Searching for and designing new analgesics with different action mechanisms and combined anti-inflammatory and analgesic effects, which can become a safe alternative to NSAIDs, remain urgent.

The development of a prototype of the efficacious non-toxic analgesic thiowurtzine (120 mg capsules) based on a first-in-class molecule from the hexaazaisowurtzitane class has marked the beginning of a priority national research area – modeling pharmacologically active candidate molecules from a pharmacophore – a high-energy compound 2,4,6,8,10,12 hexaazatetracyclo[5,5,0,$0^{3,11}$,$0^{5,9}$] dodecane (hexaazaizowurtzitane) [7, 8]. Within this research area, the compound 4,10-di(ethoxyacetyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,$0^{3,11}$,$0^{5,9}$] dodecane (ethowurtzine) from the class of hexaazaizowurtzitanes was first synthesized at the Institute for Problems of Chemical and Energetic Technologies [9]. The analgesic effect of the substance is comparable and superior in some parameters to the activity of the reference agent tramadol in models of the somatogenic pain of various origin [8, 9]. The statistically significant anti-exudative activity of ethowurtzine was revealed to be comparable to that of diclofenac only in carrageenan-

induced edema when evaluating the anti-inflammatory effect of ethowurtzine in models of carrageenan- and histamine-induced inflammation [9, 10]. In a model of chronic inflammation, the substance is not inferior to the selective cyclooxygenase (COX) inhibitor meloxicam in terms of the studied parameters of anti-inflammatory action, while demonstrating no ulcerative damage to the gastrointestinal mucosa secondary to the death of animals in the NSAID group due to severe gastrotoxicity [10]. It is advisable to examine experimentally the anti-inflammatory and ulcerogenic effects of ethowurtzine compared to the non-selective COX inhibitor diclofenac and a possible mechanism of the specific activity of the substance.

The aim of this study was to assess the anti-inflammatory and gastroprotective effects of ethowurtzine compared to the reference drug diclofenac in a rat model of chronic inflammation, as well as to evaluate the influence of ethowurtzine on nitric oxide production as a possible mechanism of its anti-inflammatory effects.

MATERIALS AND METHODS

The experiments were carried out on 69 mature female SD rats of the first category (172–176 g), which were obtained from the Department of Experimental Biomodeling of Goldberg Research Institute of Pharmacology and Regenerative Medicine of Tomsk NRMC. Animal housing and the experimental design were approved by the Bioethics Committee of Goldberg Research Institute of Pharmacology and Regenerative Medicine of Tomsk NRMC (IACUC Minutes No. 19212021) and complied with Directive 2010/63/EU of the European Parliament and the European Union Council and GOST R no. 33044-2014 “Guidelines for Good Laboratory Practice” dated August 01, 2015.

The experimental design, sample size, experimental protocol, and choice of statistical analysis methods were determined optimally for this type of study and allowed for the acquisition of reliable data for result interpretation. The animals were divided into groups randomly using body weight (±10%) as a criterion. The animals were euthanized in a CO_2 chamber.

The substrate for the study was 4,10-di(ethoxyacetyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,$0^{3,11}$,$0^{5,9}$]dodecane (hereinafter referred to as "the ethowurtzine") from a new class of hexaazaizowurtzitanes, with an acceptable safety profile (LD_{50} > 2,000 mg/kg with no animal lethality). This molecule is a colorless crystalline product with an assay of 99.6% (as per the HPLC method) and a melting point of 230.5–231.5 ºC. The ethowurtzine is water-insoluble.

Ethowurtzine was administered *per os* through an atraumatic probe in the dose range of 12.5–100 mg/kg [11]. The reference drug diclofenac (Ozon LLC, Russia) was administered *per os* at a dose of 5 mg/kg, equivalent to the average daily dose for humans; purified ampoule water was used as the solvent [5]. Purified water was used as the solvent for the ethowurtzine, with an addition of 20 µl Twin-80 (Polysorbate LAUROPAN T/80, Italy) per 1 mL water. The substances were administered at 0.7 mL solvent / 200 g rat body mass. The animals in the control group had a water – Twin-80 solution received in a similar fashion.

Chronic inflammation [11] was modeled in female SD rats. A sterile cotton swab (13 mg) was implanted under the spinal skin using a needle (A1-20 x 40-117I25). The ethowurtzine and diclofenac were administered intragastrically an hour before placing the cotton swab for 7 days. On day 8, the rats were euthanized. The cotton swabs with granulation tissue around them were excised, weighed on an Adventurer electronic balance (USA), and dried in a thermostat at 60 ºC to the constant weight. The proliferative response (%) was evaluated by the difference between the mass of the dry granuloma tissue and the initial mass of the cotton swab. The exudative response (%) was evaluated by the difference between the wet and dry weights of the granuloma tissue (exudate).

ULCEROGENIC EFFECT OF ETHOWURTZINE

The ulcerogenic effect of the ethowurtzinewas examined upon completion of the chronic inflammation modeling in female SD rats by the standard method [11]. At day 8, during autopsy, the rat stomachs were excised using tweezers and sharp-ended scissors, dissected along the lesser curvature, rinsed with cold normal saline to remove the contents, spread flat on a white substrate, and examined macroscopically using a special backlighting lens to assess the ulcerogenic effect by a 4-grade scale: 0 – no damage, 0.5 – hyperemia, 1 – single minor damage (1 or 2 hemorrhages), 2 – multiple hemorrhages (erosions, single hemorrhages); 3 – significant and multiple damage (erosions, single hemorrhages), 4 – extensive damage encompassing the whole mucosa (massive hemorrhages, erosions, and perforations). The average number of ulcerations per animal in the group and the percentage of animals with ulcers were estimated. The criterion for the ulcerogenic effect

was considered to be manifestations corresponding to grade 2 and higher.

In an *in vitro* inflammation model, the nitric oxide (NO) production by macrophages isolated from the peritoneal cavity of 25 intact C57Bl/6 mice was evaluated by the concentration of nitrites in the cell supernatant following 48-h cell incubation in the presence of various concentrations of the ethowurtzine and/or lipopolysaccharide (LPS) by adding the Grace's insect medium (Sigma-Aldrich, USA) in a 1:1 ratio and measuring the optical density of the solution on the Titertek Multiskan® MCC multichannel spectrophotometer (Labsystems, Finland) at a wavelength of 540 nm [12]. Macrophages ($2.5–3 \times 10^6$) were cultured for 48 h (37 °C, 5% CO_2, 100% humidity) in a complete growth medium (CGM) RPMI-1640 (Sigma, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, UK), 20 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES, Sigma, USA), 0.5 mM 2-mercaptoethanol (Sigma, USA), 50 μg/ml gentamicin (Sigma, USA), and 2 mM L-glutamine (Sigma, USA) in 96-well plates in the presence of various concentrations of ethowurtzine and/or 0.1 μg/ml LPS (Sigma, USA).

The effect of the ethowurtzine on the macrophage proliferation was determined by the classical MTT test (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA)) in the macrophage lysate by adding it 4 h before the end of the incubation at a final concentration of 200 μg/ml. The supernatant was then dissolved with dimethyl sulfoxide (Sigma, USA), and the optical density was measured on the Titertek Multiskan® MCC multichannel spectrophotometer (Labsystems, Finland) at a wavelength of 540 nm.

The statistical analysis of the obtained data was carried out using Statistica 8.0 software. The Shapiro – Wilk test was used to check for normality of distribution of random variables. The distribution in the *in vivo* experiment was non-normal; therefore, the Kruskal–Wallis test was used for multiple comparisons, the Wilcox–Mann–Whitney *U*-test was applied for intergroup comparisons, and the Fisher's angular transformation (φ) was used to find statistical significance of qualitative variables. The distribution of random variables in the *in vitro* experiment was normal; therefore, the one-way ANOVA and the Dunnett's test for multiple comparisons were employed to compare group means of several experimental samples with a control. For each sample, the mean and the standard error of the mean ($X \pm m$) were estimated, which are presented in the summary tables together with the value n (the number of variants). In all cases, the null hypothesis was rejected at $p < 0.05$ [13].

RESULTS

The potential analgesic agents may have an inhibitory effect on the development of inflammatory reactions, regardless of the nature of the damaging factor, phase, and stage of the process [1, 6], which explains the relevance of studying their action in modeling chronic inflammation in comparison with NSAIDs of varying selectivity [1, 6, 11].

As can be seen from Table 1, the administration of the ethowurtzine at all doses in the chronic inflammation model resulted in a significant decrease in the exudate burden: by 1.7 times at a dose of 12.5 mg/kg ($p < 0.01$, 40%), by 1.5 times at 25 mg/kg ($p < 0.01$, 35%), by 1.5 times at 50 mg/kg ($p < 0.01$, 32%), and by 1.5 times at 100 mg/kg ($p < 0.01$, 34%) compared to the controls. It should be noted that the anti-exudative effect of the ethowurtzine was significantly superior (44.5%) to that of diclofenac (8.7%).

Table 1

Parameters of Anti-Inflammatory and Anti-Ulcer Effects of Ethowurtzine (in the Dose Range of 12.5–100 Mg / Kg, *Per Os*) Compared to Diclofenac (5 Mg / Kg, *Per Os*) in the Modified Proliferative Inflammation Model in Female SD Rats

Study group, dose	Exudate weight $X \pm m$	Exudation suppresion, %	Weight of dry granuloma, $X \pm m$	Weight of granuloma tissue $X \pm m$	Proliferation suppression, %	Number of rats with ulcers per group, %
1.Control, $n = 19$	144.1 ± 10.9	0	97.2 ± 9.7	84.2 ± 9.7	0	0
2. Diclofenac, 5 mg/kg, n = 10	127.0 ± 6.8	12	68.1 ± 9.4 1–2*	55.1 ± 9.4 1–2*	35	30% 1–2**
3. Ethowurtzine, 12.5 mg/kg, $n = 10$	86.7 ± 5.0 1–3** 2–3**	40	54.9 ± 6.2 1–3**	41.9 ± 6.2 1–3**	50	0
4. Ethowurtzine, 25 mg/kg, $n = 10$	93.3 ± 6.4 1–4** 2–4**	35	56.6 ± 5.5 1–4**	43.6 ± 5.5 1–4**	48	0

End of table 1

Study group, dose	Exudate weight $X \pm m$	Exudation suppresion, %	Weight of dry granuloma, $X \pm m$	Weight of granuloma tissue $X \pm m$	Proliferation suppression, %	Number of rats with ulcers per group, %
5. Ethowurtzine, 50 mg/kg, $n = 10$	98.6 ± 6.7 1–5** 2–5**	32	58.6 ± 5.5 1–5**	44.5 ± 6.1 1–5**	47	0
6. Ethowurtzine, 100 mg/kg, $n = 10$	95.7 ± 4.5 1–6** 2–6**	34	63.5 ± 6.9 1–6**	50.5 ± 7.0 1–6**	40	0

Note: the numbers of comparison groups are given in front of the significance level, n – the number of animals. * $p < 0.05$, ** $p < 0.01$.

The statistically significant decrease in the weight of fibrotic granuloma tissue showed that the ethowurtzine in the dose range of 12.5–100 mg/kg effectively suppressed proliferation of crude granuloma burden by 50 and 40%, respectively, versus 35% for diclofenac (Table 1). However, the detected activity of the compound did not differ significantly from that of diclofenac. In previous studies, the anti-inflammatory activity of the compound in the chronic inflammation model was comparable to that of the selective COX inhibitor meloxicam [11], however, the current summary dataset indicated that the compound exhibited a better effect than the non-selective COX2 inhibitor diclofenac. It should be noted that the chemical and physical properties make it impossible to calculate the ED_{50} due to the dose-independent effect of the compound [8, 10].

The gastric mucosa of the rats that received the ethowurtzine had no ulcers. The dataset clearly indicated no gastrotoxicity of the compound and supposedly no COX2-dependent mechanism of its anti-inflammatory effect (Table 1). In contrast, in 3 out of 10 animals (30%, $p < 0.01$) who received the non-selective COX inhibitor diclofenac in the same administration regimen, grade 2 ulcers were revealed.

The LPS added to the macrophage culture caused a significant increase in NO production in the *in vitro* inflammation model (Table 2). The added ethowurtzine at various concentrations together with LPS reduced the NO production in a dose-dependent manner, and the NO production decreased significantly compared to the group of LPS alone when the test compound (750 and 1,000 μg/ml) was administered at high doses (control 2).

The proliferative macrophage activity decreased both when LPS alone and the ethowurtzine + LPS were added compared to macrophage parameters with no drugs used (control 1) (Table 2). However, the use of the compound at different concentrations together with LPS did not induce any significant changes in the macrophage proliferation relative to control 2.

Table 2

The Effect of Ethowurtzine at Different Concentrations on the Nitric Oxide Synthesis by Peritoneal Macrophages of the Intact C57BL/6 Mice, $X \pm M$

Test compound	Concentration, μg/ml	Nitrite concentration, μM	Proliferation, optical density units
Control 1 (macrophages + medium)	–	5.58±0.21	0.514±0.005
Control 2 (macrophages + LPS)	0.1	87.13±0.53*	0.446±0.008*
Ethowurtzine (+LPS)	50	81.52±0.28*	0.449±0.005*
	100	75.52±0.40*	0.466±0.007*
	200	75.86±0.23*	0.462±0.002*
	300	75.68±0.36*	0.455±0.003*
	500	75.13±0.26*	0.480±0.002*
	750	67.09±0.40*#	0.477±0.002*
	1,000	63.70±0.27*#	0.449±0.002*

Note: number of wells $n = 5$. * $p < 0.05$ compared to control 1; # $p < 0.05$ compared to control 2.

DISCUSSION

The overproduction of pain and inflammation mediators, such as interferon gamma, tumor necrosis factor, prostaglandins, interleukins-1,6, and bacterial lipopolysaccharides that induce peripheral and central pain sensitization, has a decisive role in the development of a subclinical inflammatory reaction [1, 4, 14]. It has now been proven that these substances are activators of the inducible calcium-independent NO synthase isoenzyme, thereby enhancing the NO production at the inflammation site [14], which in turn stimulates the synthesis of proinflammatory cytokines

and increases exudation, leukocyte migration, and connective tissue proliferation [15].

Evidence behind the effect of ethowurtzine on the production of NO, which is a pro-inflammatory mediator and a key player in the pathogenesis of inflammation [14], was obtained in the *in vitro* inflammation model. The addition of the ethowurtzine with LPS to the culture of peritoneal macrophages of the experimental animals suppressed the NO production in a dose-dependent manner, which may be one of the mechanisms of the identified anti-inflammatory effect of the substance.

CONCLUSION

The newly synthesized analgesic ethowurtzine outperformed the non-selective COX inhibitor diclofenac by its anti-exudative effect in the chronic proliferative inflammation model while inhibiting the granulation tissue proliferation to the same extent as NSAIDs. A lack of ulcerogenic effect of the ethowurtzine, as confirmed in this study, may indicate an alternative mechanism of its anti-inflammatory action not related to the blockade of COX-2. The results obtained in the *in vitro* inflammation model backed this assumption. The addition of the ethowurtzine together with LPS to the culture of peritoneal macrophages of the test animals inhibited the production of nitric oxide which is a pro-inflammator mediator and a key player in the pathogenesis of inflammation. The revealed activity of the test substance may be one of its anti-inflammatory action mechanisms.

The analgesic and anti-inflammatory effects of the ethowurtzine combined with its authentic action mechanism and the lack of gastrotoxicity substantiate the relevance of its further preclinical trials, while the design of a drug on its basis for the treatment of chronic inflammatory diseases associated with pain may become a safe alternative to NSAIDs.

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Author contribution

Krylova S.G. – conception and design of in vivo experiments, participation in experiments, drafting of the manuscript. Kiseleva E.A. – carrying out of experiments, statistical analysis. Sherstoboev E. Yu. – conception and design of in vitro experiments, participation in experiments, statistical analysis of results. Ligacheva A.A. – carrying out of in vitro experiments, description of results in the article. Rybalkina O.Yu. – participation in experiments. Zueva E.P. – critical revision of the manuscript for important intellectual content. Sysolyatin S.V. – design of synthesis of the study object. Kulagina D.A. – synthesis and supply of the study object. Eremina V.V. – synthesis of the study object. Zhdanov V.V. – critical revision of the manuscript for important intellectual content, final approval of the manuscript.

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Features of the Course of Pregnancy Complicated by Gestational Diabetes and a Novel Coronavirus Infection

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ABSTRACT

Aim. To study the role of metabolic disorders in the development of perinatal complications of the novel coronavirus infection.

Materials and methods. The analysis of the course of pregnancy and childbirth in pregnant women who had a novel coronavirus infection (170) and without it (100), and their newborns (270).

Results. A novel coronavirus infection (NCI) during pregnancy leads to the development of complications: preeclampsia (p = 0.012), premature birth (p = 0.038), premature detachment of the normally located placenta (p = 0.05), fetal growth retardation (p = 0.028), gestational diabetes mellitus (GDM) (p = 0.023), intrauterine infection (p = 0.048) and asphyxia of the newborn (p = 0.04). Gestational diabetes mellitus is 2 times more likely to accompany a moderate form of NCI, as opposed to a mild one (p = 0.001). Infection with the SARS-CoV2 virus on the background of previous GDM contributes to the development of moderate NCI (p = 0.005). Hyperglycemia in GDM after moderate NCI more often than after mild requires the appointment of insulin (p = 0.03). The combination of NCI and GDM is characterized by the development of polyhydramnios (p = 0.02), the risk of which increases in the presence of hereditary thrombophilia. The neonatal period is more often complicated by intrauterine pneumonia if the mother has a combination of NCI and GDM.

Conclusion. The risk of developing metabolic disorders and perinatal complications in pregnant women who had a novel coronavirus infection is significantly higher than in pregnant women without a novel coronavirus infection.

Keywords: COVID-19, pregnancy, gestational diabetes mellitus, polyhydramnios

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Особенности течения беременности, осложненной гестационным диабетом и перенесенной новой коронавирусной инфекцией

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РЕЗЮМЕ

Цель. Изучение влияния новой коронавирусной инфекции на развитие метаболических нарушений и перинатальных осложнений.

Материалы и методы. Проведен анализ течения беременности и родов у беременных, перенесших новую коронавирусную инфекцию (170) и без таковой (100), и анализ историй их новорожденных (270).

Результаты. Новая коронавирусная инфекция (НКИ), перенесенная во время беременности, приводит к развитию осложнений: преэклампсии (p = 0,012), преждевременным родам (p = 0,038), преждевременной отслойке нормально расположенной плаценты (p = 0,05), недостаточному росту плода (p = 0,028), гестационному сахарному диабету (ГСД) (p = 0,023), внутриутробной инфекции (p = 0,048) и асфиксии новорожденного (p = 0,04). Гестационный сахарный диабет в 2 раза чаще сопровождает среднетяжелую форму НКИ в отличие от легкой (p = 0,001). Инфицирование вирусом SARS-CoV2 на фоне предшествующего ГСД способствует развитию среднетяжелой степени НКИ (p = 0,005). Гипергликемия при ГСД после перенесенной среднетяжелой НКИ чаще, чем после легкой, требует назначения инсулина (p = 0,03). Для сочетания НКИ и ГСД характерно развитие многоводия (p = 0,02), риск которого возрастает при наличии наследственной тромбофилии. Период новорожденности чаще осложняется внутриутробной пневмонией при наличии у матери сочетания НКИ и ГСД.

Заключение. Риск развития метаболических нарушений и перинатальных осложнений у беременных, перенесших новую коронавирусную инфекцию, значимо выше по сравнению с беременными без новой коронавирусной инфекции.

Ключевые слова: COVID-19, беременность, гестационный сахарный диабет, многоводие

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INTRODUCTION

Analyses of the consequences of the novel coronavirus infection (NCI) pandemic have demonstrated the multisystem nature of vital organ damage. As early as 2003, during the predominance of atypical viral pneumonia caused by SARS-CoV coronavirus, studies demonstrated the expression of angiotensin-converting enzyme receptor type 2 (ACE2) in bronchi, adipose tissue, skeletal muscle, lung parenchyma, ileum, cardiovascular tissues, kidneys, liver, gastrointestinal tract, and pancreas [1, 2]. The cases of type 1 diabetes

mellitus identified at that time demonstrated the direct impact of the coronavirus family on endocrine organs [3]. The significant increase in gestational diabetes mellitus (GDM) incidence during the NCI pandemic is likely due to a similar pathogenesis. The problem of increased metabolic disorders in pregnant women with novel coronavirus infection is relevant due to GDM's significant contribution to maternal and neonatal complications.

The aim of this study was to investigate the impact of novel coronavirus infection on the development of metabolic disorders and perinatal complications.

MATERIALS AND METHODS

A comparative analysis of 270 pregnancy, delivery, and neonatal histories was performed from January to December 2021. Based on novel coronavirus infection status during pregnancy, patients were divided into two groups: the study group consisted of 170 women with NCI, and the control group included 100 patients without NCI.

Comparison of anthropometric, demographic, and anamnestic data between the clinical groups showed statistical comparability for the main parameters (Tables 1.1–1.3).

Table 1.1

Age of Subjects, , *Me* (Q_1; Q_3) [25-75 %]

Parameter	Clinical groups		*p*
	study (*n* = 170)	control (*n* = 100)	
Age, years, *Me* (Q_1; Q_3)	31 (28; 36)	31 (26; 36)	0.90

Note. The Mann–Whitney test was used to calculate the significance level of *p*.

Table 1.2

Obstetric and Gynaecological History, *n* (%)

Parameter	Clinical groups		*p*
	study (*n* = 170)	control (*n* = 100)	
First-time pregnant women, *n* (%)	28 (16.5)	26 (26)	0.059
Recurrent pregnancies, *n* (%)	142 (83.5)	74 (74)	0.059
First-born women, *n* (%)	62 (36.4)	32 (32)	0.45
Repeat births, *n* (%)	108 (63.6)	68 (68)	0.45
Abortion, *n* (%)	54 (31.7)	29 (29)	0.63
Miscarriage, *n* (%)	50 (29.4)	25 (25)	0.43
Ectopic pregnancy, *n* (%)	24 (14.1)	12 (12)	0.62

Note. The x^2criterion with Yates correction was used to calculate the significance level of *p*.

The inclusion criteria for the study were as follows: absence of severe extragenital pathology, including severe forms of type 1 and type 2 diabetes mellitus (DM); absence of HIV infection; absence of fetal malformations; singleton pregnancy; and the patient's informed consent to participate in the study.

Table 1.3

Structure of Extragenital Diseases, *n* (%)

Parameter	Clinical groups		*p*
	study (*n* = 170)	control (*n* = 100)	
Arterial hypertension, *n* (%)	17 (10)	10 (10)	1.0
Type 2 diabetes mellitus, *n* (%)	4 (2.3)	2 (2)	0.85
Anemia, *n* (%)	95 (55.8)	57 (57)	0.86
Obesity, *n* (%)	31 (18.2)	23 (23)	0.345

Note. The x 2criterion with Yates correction was used to calculate the significance level of *p*.

Treatment of novel coronavirus infection (NCI) during pregnancy was conducted in respiratory hospitals at the Tomsk Medical Center No. 2 and the Tomsk Maternity Hospital No. 4. Delivery and subsequent examination of the patients were performed at the Tomsk Regional Perinatal Center named after I.D. Yevtushenko.

Upon admission to the regional perinatal center, patients underwent a standard examination as outlined in clinical protocols. Diagnostic procedures included ultrasound and Doppler ultrasonography using Nemio XG equipment (Toshiba, Japan). During labor, cardiotocography (CTG) monitoring was conducted with Sonicaid Team devices (Sonicaid Ltd/Huntleigh Healthcare, UK) and FC 1400 systems (Bionet, South Korea).

Fibrinogen is a significant modulator of coagulation and inflammatory processes [4], so patients from both groups were examined for hereditary thrombophilia, including the fibrinogen (FGB) gene polymorphism (examination was conducted using kits manufactured by DNA Technology LLC, Russia).

The results of the study were processed via the variational statistics method using the Statistica 12 application software package. Absolute and relative frequencies were used to describe qualitative data. The x^2criterion with Yates correction, n (%) was used to compare qualitative data. The Mann–Whitney test was used to compare nonparametric quantitative data from the two groups. The results are presented in the form of the median and the interquartile range *Me* (Q_1; Q_3). The significance level is $p < 0.05$.

RESULTS

The course of pregnancy and delivery in women who experienced novel coronavirus infection (NCI), in contrast to uninfected women, was complicated

in 16.5% of cases (28/170) versus 5% (5/100) in the control group by preterm labor (p = 0.038). In 7.7% (13/170) of patients (versus 2% (2/100) in the control group), pregnancy was complicated by premature detachment of the normally located placenta (p = 0.05). Pre-eclampsia (PE) occurred in 16.5% (28/170) versus 5% (5/100) in the control group (p = 0.012). Gestational diabetes mellitus (GDM) developed in 39% (64/170) of pregnant women versus 24% (24/100) in the control group (p = 0.023).

The frequency of insulin prescriptions had no significant differences between the groups, regardless of NCI status (p = 0.7). In the study group, GDM was diagnosed twice as often after NCI infection rather than preceding it. There is a dependence of insulin prescription in GDM on the time of NCI infection — developing before NCI, GDM in the study group more often required insulin therapy (Table 2).

Table 2

Dependence of Insulin Prescription in GDM on the Time of NCI Infection, *n* (%)

Parameter	GDM (64 in total)		*p*
	GDM before NCI (21)	GDM after NCI (43)	
Insulin prescription for correction (%)	8 (38)	5 (12)	0.014
Diet correction (%)	13 (62)	38 (88)	

Note. The x^2 criterion with Yates correction was used to calculate the significance level of p.

The correlation between the severity of NCI and the probability of complications in our study was found only for the moderately severe form of NCI and GDM (Table 3).

Table 3

Gestational Complications Depending on the Severity of Novel Coronavirus Infection, *n* (%)

Parameter (number of cases)	Mild NCI n = 131	Moderately severe NCI n = 39	*p*
Preeclampsia (28)	19 (14,5)	9 (23)	0.2
Premature detachment of the normally located placenta (13)	10 (7.6)	3 (7.7)	0.8
Fetal growth retardation (35)	28 (21.3)	7 (17.9)	0.6
Premature birth (25)	19 (14.5)	6 (15.3)	0.89
Gestational DM (64)	41 (31.3)	24 (61.5)	0.001

Note. The x^2 criterion with Yates correction was used to calculate the significance level of p.

Additionally, a moderately severe degree of NCI developed significantly more often if the patient already had GDM at the time of SARS-CoV 2 infection (Table 4).

Table 4

Dependence of the Severity of Novel Coronavirus Infection on the Presence of Gestational DM Before Infection, *n* (%)

Parameter	Study group (n = 170)		*p*
	Mild, 131 (77)	Moderately severe, 39 (23)	
GDM before NCI (n = 21)	11 (8.4)	10 (25.6)	0.005
No GDM before NCI (n = 149)	120 (91.6)	29 (74.4)	

Note. The x^2 criterion with Yates correction was used to calculate the significance level of p.

Insulin administration for the correction of hyperglycemia in GDM in our study was required more frequently after moderate NCI than after mild NCI (p = 0.03).

Changes in the amount of amniotic fluid are pathognomonic for both intrauterine infection (IUI) and GDM. In the group of pregnant women who developed polyhydramnios, the formation of this complication was found to be dependent on NCI. In the control group, there was no correlation between the development of polyhydramnios and the presence of GDM (Table 5).

Table 5

Dependence of the Presence of Polyhydramnios in Gestational Diabetes Mellitus on the Novel Coronavirus Infection, *n* (%)

Parameter	Study group (n = 170)		*p*
	Gestational DM (n = 64)	Normoglycaemia (n = 106)	
Polyhydramnios, (n = 24)	14 (21.8)	10 (0.9)	0.02
Parameter	Control group (n = 100)		*p*
	Gestational DM (n = 38)	Normoglycaemia (n = 62)	
Polyhydramnios, (n = 11)	4 (10.5)	7 (11.2)	0.9

Note. The x^2 criterion with Yates' correction was used to calculate the significance level of p.

When analysing the possible factors for the development of polyhydramnios (fetal abnormalities, multiple pregnancies, GDM), we found a dependence of the increased amount of amniotic fluid on the presence of fibrinogen gene polymorphism. Polyhydramnios is significantly more likely to develop after an NCI in the presence of a homozygous carrier of the A allele in the FGB-455 fibrinogen gene (Table 6).

We can assume that there is a negative synergy of infectious and dysmetabolic processes in the formation of polyhydramnios. The outcome of the latter is usually premature birth, fetal distress, and asphyxia during labour.

Table 6

Dependence of the Presence of Polyhydramnios in Novel Coronavirus Infection on the Presence of a Polymorphic Variant of the Fibrinogen Gene, *n* (%)

FGB 455 genotype	Study group (*n* = 170)		*p*
	Polyhydramnios (n = 24)	Normohydramnios (n = 146)	0.0034
FGB 455 G/A, G/G (*n* = 160)	19 (79)	141 (96)	
FGB 455 A/A (*n* = 10)	5 (21)	5 (4)	

Note. The x^2 criterion with Yates' correction was used to calculate the significance level of *p*.

Analysis of perinatal complications in both groups showed that 20.6% of patients in the study group (35/170) (compared to 9% (9/100) in the control group) were diagnosed with fetal growth retardation ($p = 0.028$). Additionally, antenatal fetal death was recorded in 3% of patients in the study group (5/170) (compared to 1% (1/100) in the control group) ($p = 0.56$).

The Apgar score at 1 minute in newborns from mothers who had suffered from NCI was significantly lower than in the control group. No statistically significant difference was found when comparing anthropometric indicators of newborns in both groups (Table 7).

Table 7

Newborns' Records, *Me*, Q_1-Q_3 [25-75%]

Parameter	Clinical groups		*p*
	Study group (*n* = 170)	Control group (*n* = 100)	0.90
Height, cm	53 (50; 55)	53 (51; 55)	
Weight, kg	3,320 (2,997.5; 3,702.5)	3,340 (3,025; 3,635)	0.65
Apgar score at 1 min, points	8 (8.5; 9)	9 (9; 9)	0.004
Apgar score at 5 min, points	8 (8; 9)	9 (9; 9)	0.6

Note. The Mann-Whitney test was used to calculate the significance level of *p*.

When analysing the condition of newborns, changes characteristic of both the entire study group and the combination of NCI and GDM were revealed. Asphyxia during labour was diagnosed in 10% of newborns from mothers with NCI (OR 3.59, $p = 0.04$). 14% (24/170) (compared to 5% (5/100) in the control group) of newborns from the study group required transfer to the Intensive Care Unit of the Neonatal Pathology Department ($p = 0.02$). However, there were no significant differences between the groups in the incidence of complications of neonatal adaptation, such as hypoglycaemia, wet lung syndrome, supraphysiological weight loss, and early jaundice up to 2 days.

In the study of neonatal complications in the main group, the negative contribution of maternal GDM to the development of intrauterine infection was confirmed (Table 8).

Table 8

Comparative Characteristics of Neonatal outcomes After NCI During Pregnancy Depending on the Presence of GDM, *n* (%)

Parameter	Study group (*n* = 170)		*p*
	GDM (*n* = 64)	No GDM (*n* = 106)	0.5
- fetal growth retardation, *n* (%)	6 (9.3)	13 (12.2)	
- fetal macrosomia, *n* (%)	4 (6.2)	6 (5.6)	0.5
- birth asphyxia, *n* (%)	2 (3.1)	6 (5.6)	0.45
- unspecified IUI, *n* (%)	4 (6.2)	1 (0.9)	0.048

Note. The x^2 criterion with Yates correction was used to calculate the significance level of p

The "unspecified intrauterine infection: pneumonia of unspecified aetiology" diagnosis was slightly more common in women in the study group with concomitant GDM ($p = 0.048$). SARS-CoV-2 virus was not detected during the examination of newborns, and blood and sputum cultures revealed no growth of pathogenic flora. Subsequently, with standard therapy, the symptoms were quickly relieved, laboratory inflammatory markers returned to normal, and the children were discharged in satisfactory condition.

DISCUSSION

This study highlights the role of NCI in the development of gestational complications and the formation of metabolic disorders. The combination of NCI and GDM in pregnant women is associated with an increased risk of polyhydramnios. GDM was twice as frequent in moderate NCI compared to mild NCI.

The significant correlation between moderate NCI and the presence of GDM during pregnancy, as identified in this study, has a logical explanation. It is well established that normal pregnancy is characterised by a balance between insulin resistance, driven by counterregulatory hormones, and increased insulin production due to hyperactivity of pancreatic β-cells [5]. The SARS-CoV-2 coronavirus binds to angiotensin-converting enzyme type 2 receptors in critical metabolic organs and tissues, including the pancreas and adipose tissue. This interaction results in the development of insulin resistance [6]. A reduction in insulin synthesis and impaired signal transmission

are attributed to the secretion of inflammatory cytokines and the activation of pro-inflammatory signaling pathways, such as TNF-α, IL-6, IL-1β, C-reactive protein, and NF-κB [6, 7]. Consequently, the mechanism underlying GDM development following NCI involves direct effect of the virus on cells, insulin resistance development, and systemic inflammation.

According to existing literature, GDM preceding the onset of NCI undoubtedly influences its progression and outcomes. Firstly, hyperglycaemia makes patients with GDM more susceptible to infections, including NCI, by causing immune dysfunction that adversely affects neutrophil chemotaxis, macrophage function, and phagocytic response [8, 9]. Secondly, systemic inflammation during NCI in addition to carbohydrate metabolism disorders can induce metabolic dysregulation and exacerbate the course of viral infections. Furthermore, NCI is more severe in patients who develop GDM concurrently with or prior to SARS-CoV-2 infection compared to those who develop GDM later [10]. Several studies corroborate that gestational DM is a hypercoagulable state [11]. Hyperglycaemia in GDM leads to endothelial dysfunction and platelet activation; when compounded by NCI, this creates a vicious cycle that exacerbates endothelial damage in addition to infection while further aggravating it [12]. This information is supported by evidence linking the development of polyhydramnios in NCI and GDM to the presence of a mutation in the fibrinogen gene.

Polyhydramnios often accompanies the pathological course of pregnancy [13]. The increase in the incidence of polyhydramnios recorded in the study group may have a mixed mechanism. In cases of maternal hyperglycaemia, the increase in fetal urine excretion is, on the one hand, associated with an increase in osmotic diuresis, which contributes to an increase in amniotic fluid production [14]. On the other hand, the increased proinflammatory response characteristic of GDM leads to the acceleration of "physiological ageing" and the terminal form of epithelial-mesenchymal transition in the amniotic membrane, resulting in destabilisation of the matrix and membrane condition, increased production and impaired fluid absorption [15].

The inflammatory response serves as the link between the pathogenesis of viral infection and metabolic disorders. It is logical to assume that any factors modulating inflammation will influence the course of the pathological process. The association of fibrinogen gene polymorphism with increased amniotic fluid production found in this study aligns with this concept. According to epidemiological and biochemical studies, the *FGB* 455 G-A polymorphism is one of the strongest genetic variations associated with increased plasma fibrinogen [16]. The role of fibrinogen as a modulator not only of coagulation but also of inflammatory processes has been experimentally proven. In addition to local activation of inflammatory reactions at sites of fibrin deposition, a dependence of chronic inflammation in patients with obesity on fibrinogen concentration and the degree of fibrin polymerisation has been documented [4]. Many researchers [17] have also reported the significant role of fibrinogen as a mediator of inflammation in SARS-CoV-2 infection and its close relationship with key proinflammatory cytokines, particularly IL-6. In pregnancy, the proinflammatory contributions of NCI and GDM may be enhanced by fibrinogen hyperproduction due to gene polymorphism present in the patient. "Cytokine storm" products, binding to ACE2 receptors in syncytiotrophoblasts, cytotrophoblasts, and villous endothelium, affect the placenta. The resulting parenchymatous placentitis, intervillous thrombangiitis, perivillitis, and villitis with inflammatory infiltration lead to decreased resorption and increased production of amniotic fluid.

Pathological changes in the mother and the placenta results in alterations to the intrauterine environment. It is known that NCI frequently causes the development of infectious and inflammatory conditions in the fetus. Respiratory disorders, congenital pneumonia, infections during the perinatal period, intraventricular hemorrhage, and hyperbilirubinemia occur significantly more often. The association with NCI demonstrates a clear correlation with the time interval between the infection and the onset of labour [18]. Maternal GDM can lead to the development of diabetic fetopathy, characterised by liver and myocardial dysfunction, as well as a high risk of ante- and intrapartum death if left untreated.

The correlation between the incidence of intrauterine infection (IUI) and associated birth asphyxia in cases where gestational diabetes coexists with maternal NCI during pregnancy aligns more closely with a model of chronic inflammation rather than infectious disease. In accordance with the study design, mothers during the early post-COVID period were excluded from participation. The mean time interval between disease and delivery was 130 days.

The possibility of vertical transmission of the SARS-CoV-2 virus from mother to fetus remains a subject of debate; however, documented cases of viral detection in newborns are rare.

In this study, no instances of NCI births were recorded. An increase in the incidence of intrauterine infection caused by non-specific pathogens in patients with both GDM and NCI is likely. This phenomenon is attributed to the dual negative effects of SARS-CoV-2 virus and hyperglycaemia, manifesting as immune and endothelial dysfunction. These effects lead to increased expression of pro-inflammatory cytokines, heightened vascular permeability, and induction of syncytiotrophoblast apoptosis, resulting in ischaemic damage to the placenta and facilitating the penetration of non-specific pathogens to the fetus [19].

In cases where maternal infectious disease coincides with gestational diabetes, respiratory disorders may arise through several additional pathogenesis pathways, including disruption of surfactant structure, blockade of epithelial sodium channels, and inhibition of nitric oxide synthesis. Clinically, these changes may present as transient tachypnoea or manifest as pneumonia or respiratory distress syndrome.

Studies on respiratory abnormalities in uninfected newborns born to mothers who had contracted coronavirus infection let Man et. al. to describe an immunological pathway for lung damage in the absence of a pathogen. The authors demonstrated that prenatal exposure to SARS-CoV-2 can activate an inflammatory cascade in the newborn's airways, leading to dysregulation of ciliary rhythm in airway epithelium. Proteins such as IL-18, IL-1B, and CASP1 were found to be associated with NLRP3 inflammasome activation in preterm infants with respiratory distress syndrome. Biological processes related to inflammation, chemotactic reactions, and cellular responses–including IL8 production–were significantly elevated in preterm infants. These findings suggest that such changes may underlie the development of fibrosis and allergic diseases during later stages of childhood development [20].

CONCLUSION

The negative impact of factors such as NCI, carbohydrate metabolism disorders, and the development of pre-eclampsia on the course of pregnancy and the condition of the newborn, when considered in isolation, has been well established. This study confirmed that pregnancies and labours in women with NCI, compared to uninfected women, were more frequently complicated by GDM, pre-eclampsia, preterm labour, and premature detachment of normally situated placenta. The leading role of the systemic inflammatory response, characterised by hyperproduction of pro-inflammatory and anti-angiogenic molecules, is recognised as a universal mechanism underlying all these complications.

Intrauterine fetal hypoxia, which occurs more often in pregnancies complicated by NCI, has been found to increase the risk of antenatal fetal death. Clinically, newborns typically present under the "mask" of an unspecified intrauterine infection, which obscures the vertical transmission of the maternal systemic inflammatory response. The factors determining the likelihood of complications for both mother and fetus are not limited to the characteristics of the pathogen itself — such as the virus strain, its aggressiveness towards cell receptors, and cytokine production — but also include maternal comorbidities, particularly changes in immune and hemostasis systems. This is evidenced by the correlation between the severity of NCI and the probability of developing gestational diabetes, as well as by descriptions of one trigger for polyhydramnios: fibrinogen gene polymorphism, which increases the risk of this complication.

Practical applications of these study findings lie in providing earlier diagnosis of GDM in pregnancies complicated by NCI (and other infectious diseases with similar pathogenesis). Early initiation of specific insulin therapy can help prevent negative outcomes. The timeliness of preventing complications from infectious diseases in pregnant women with underlying metabolic disorders depends on the precision and speed with which pathological and compensatory reactions are assessed. These issues necessitate further detailed investigation. It is likely that omics technologies will hold particular scientific and practical value in advancing this area of research.

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Glycemic-dependent Changes of Skin Autofluorescence Level in Children and Adolescents with Type 1 Diabetes Mellitus

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ABSTRACT

Aim. To study the effect of glycated hemoglobin level, average daily glycemia and its variability on UV-induced skin autofluorescence in children and adolescents with type 1 diabetes.

Materials and methods. The study included 47 children and adolescents with type 1 diabetes living in a restricted-access administrative and territorial unit. The autofluorescence spectra of the skin from the inner surface of the shoulder and nails of patients were recorded using an original compact spectrofluorometer based on STS-VIS OCEAN OPTICS © USA microspectrometer with UVA excitation. The statistical analysis was performed using Statsoft Statistica 12.0 software. The fluorescence spectra were normalized to the average value of the UV LED signal and the moving average smoothed using a 10 nm window. Then, the renormalization of spectra was carried out, minimizing their spread from the average sample spectrum.

Results. The study revealed the most changeable regions of UV-induced skin autofluorescence spectrum with variations in the level of glycated hemoglobin, average daily glycemia, and glycemic variability.

Conclusion. The study confirms the prospects of using skin autofluorescence measurements as a non-invasive tool for assessing the state of carbohydrate metabolism.

Keywords: skin autofluorescence, type 1 diabetes mellitus, children, adolescents, glycated hemoglobin, average daily glycemia, glycemic variability

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee of Prof. V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (Minutes No. 114 dated October 5, 2022).

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Изменение уровня аутофлуоресценции кожи детей и подростков с сахарным диабетом 1-го типа в зависимости от гликемических показателей

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РЕЗЮМЕ

Цель: исследовать влияние уровня гликированного гемоглобина, среднесуточной гликемии и ее вариабельности на УФ-индуцированную аутофлуоресценцию кожи у детей и подростков, страдающих сахарным диабетом 1-го типа.

Материалы и методы. В исследование включены 47 детей и подростков с сахарным диабетом 1-го типа, проживающих на территории закрытого административно-территориального образования. Проведена регистрация спектров аутофлуоресценции кожи с внутренней поверхности плеча и ногтя пациентов с помощью оригинального компактного спектрофлуориметра на базе микроспектрометра STS-VIS OCEAN OPTICS © USA с UVA-возбуждением. Статистический анализ проводился с помощью программного обеспечения Statsoft Statistica 12.0. При выполнении анализа выполнялась нормировка спектров флуоресценции на среднее значение сигнала УФ светодиода и сглаживание методом скользящего среднего с окном 10 нм. Затем проводилась перенормировка спектров, минимизирующая разброс спектров от среднего спектра по выборке.

Результаты. В ходе исследования выявлены наиболее изменчивые области спектра УФ- индуцированной аутофлуоресценции кожи при вариации уровня гликированного гемоглобина, среднесуточной гликемии и вариабельности гликемии.

Заключение. Исследование подтверждает перспективность использования измерения аутофлуоресценции кожи в качестве неинвазивного инструмента оценки состояния углеводного обмена.

Ключевые слова: аутофлуоресценция кожи, сахарный диабет 1-го типа, дети, подростки, гликированный гемоглобин, среднесуточная гликемия, вариабельность гликемии

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом ФГБОУ ВО КрасГМУ им. проф. В.Ф. Войно-Ясенецкого Минздрава России (протокол № 114 от 05.10.2022).

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INTRODUCTION

According to the definition of clinical guidelines, type 1 diabetes mellitus (T1DM) is a disease that occurs as a result of autoimmune destruction of insulin-producing β-cells of the pancreas, with subsequent development of absolute insulin deficiency. Studying this disease is relevant due to the early development of irreversible vascular complications and disability. Considering the severity of manifestation and the lability of the disease course in children and adolescents, early diagnosis and dynamic monitoring of pathology development are of no less importance.

The glycated hemoglobin (HbA1c) indicator has been recommended by the World Health Organization (WHO) since 2011 [1] and has been used for more than 25 years as a diagnostic criterion for carbohydrate metabolism disorders [2].

HbA1c shows the average blood sugar level over the past 90 days, so new methods are required to understand changes in the glycemic profile better.

With the introduction of continuous glucose monitoring (CGM) methods into clinical practice by diabetologists, the term glycemic variability appeared, which shows fluctuations in the average blood sugar level and is considered an independent predictor of diabetes complications due to the impact on target organs through oxidative stress, glycation, low-grade chronic inflammation, endothelial dysfunction, platelet activation, impaired angiogenesis, and renal fibrosis [3].

Modern CGM systems include a sensor that measures glucose levels in the interstitial fluid at intervals of 1 to 5 min, collects, and transmits them to the third component (receiver) in real time. The use of this technology provides information on glycemia at the time of the study, dynamics in glucose levels, its current direction, and the rate of change, which facilitates timely decision-making on glycemic correction [4]. To simplify the interpretation of the large amount of glycemic data obtained through CGM, the following percentage values were identified:

1. Average glucose level.

2. Glucose Management Index (GMI) is a calculated score that was developed based on the observed differences between average CGM glucose levels and laboratory-measured HbA1c. GMI is calculated using a formula that was developed and validated based on a regression line of a graph with glucose concentration on the x-axis and simultaneous HbA1c measurement on the y-axis: GMI (%) = 3.31 + 0.02392 × (average glucose, mg/dL).

3. TIR is time in range which in this case is 70–180 mg/dL (within normal limits): target > 70%. TBR is time below range < 70 mg/dL (level 1 hypoglycemia): target < 4%. Time below range < 54 mg/dL (level 2 hypoglycemia): target < 1%.

4. TAR is the time above range > 180 mg/dL: target < 25%.

5. CV is coefficient of variation for glucose levels calculated as (standard deviation of glucose / average glucose value) × 100 and includes duration, frequency, and amplitude of shifts in blood glucose levels between low and high levels, target ≤ 36% [5, 6].

Despite the undeniable advantages of existing methods of glycemic control, they are still invasive, which leads to low compliance and insufficient glycemic control in patients.

In this regard, the search for non-invasive diagnostic methods of the listed parameters of carbohydrate metabolism is undoubtedly an urgent task. Therefore, methods based on optical spectroscopy of the patient's skin are of great interest. In recent years, many studies have been published on this topic, confirming the feasibility of the method [7, 8]. However, in each case this approach solves only one diagnostic problem. There are currently no ideas for developing a multi-task method for non-invasive diagnostics of the biochemical parameters of carbohydrate metabolism in patients with diabetes mellitus.

The aim of our work was to study UV-induced skin autofluorescence spectra in children and adolescents with type 1 diabetes mellitus and to assess the correlations of these spectra with glycated hemoglobin, average daily glycemia, and variability.

MATERIALS AND METHODS

The study was conducted at Clinical Hospital No. 51, a branch of the Federal Siberian Research Clinical Center Clinical Hospital No. 42. The skin autofluorescence test was conducted in 47 patients with T1DM. The group of children included 29 individuals (61.7%), and the group of adolescents included 18 individuals (38.3%). The majority of the study group were boys – 57.4%. The average duration of the disease in patients at the time of the examination was 4.47 years, minimum and maximum levels of HbA1c were 6.0 and18.7%, respectively.

All children were on constant insulin replacement therapy from the moment when the disease was detected: 10 patients (21.2%) used continuous

subcutaneous insulin infusion (CSII) and 37 individuals (78.7%) used a syringe pen. All patients observed during the study underwent continuous glucose monitoring (CGM), with a predominance of Libre flash monitoring. Patients with T1DM and diseases affecting the accuracy of HBA1c were excluded from the study. During the study, the patients were divided into groups that are traditionally accepted in pediatric practice. Depending on the level of glycated hemoglobin: group 1 with glycated hemoglobin ≤ 7.0% (n = 2), group 2 with glycated hemoglobin of 7.1–10% (n = 18), and group 3 with its level ≥ 10.1% (n = 27). Considering the small number, group 1 was combined with group 2. According to the average daily glycemia, we identified the following groups of patients: 1. ≤ 10 mmol/l; (n = 23); 2. ≥ 10 mmol/l;(n = 24). Based the coefficient of variability, two subgroups of patients were identified:1. ≤36% (n = 16); 2. ≥36% (n = 41).

Autofluorescence spectra were collected from the inner surface of the shoulder for 30 seconds using an original compact spectrofluorometer based on the STS-VIS OCEAN OPTICS © USA microspectrometer with UVA excitation generated by a light-emitting diode (375 nm) [9].

The skin fluorescence spectra obtained using the device comprise two wide contours. The first contour in the range of 400–700 nm represents, in fact, skin autofluorescence, and the second contour in the range of 700–820 nm shows the spectrum of the UV light-emitting diode excitation at 375 nm, in the second diffraction order of the diffraction grating (Fig. 1).

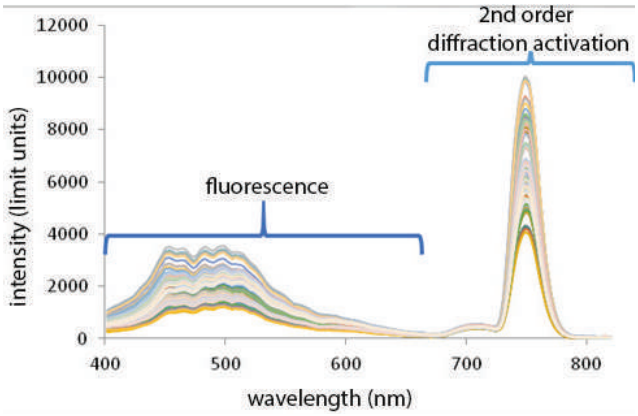

Fig. 1. Skin fluorescence spectra of patients with diabetes mellitus obtained directly using a spectrofluorometer with LED UV excitation (375 nm).

For further analysis, the fluorescence spectra were normalized to the average value of the UV LED signal and leveled using the moving average method with a 10 nm window. This spectra normalization method is further referred to as D-normalization (Fig. 2, *a*).

Additional normalization was used to compare the shape of the spectra. For this purpose, the average spectrum was calculated for the entire group of patients $F(\lambda)$ and for each spectrum $F_i(\lambda)$, the linear regression coefficients a_i, b_i were calculated using the least squares method so that after subsequent renormalization, these spectra were as close as possible to the average. Then, the spectra were renormalized by taking into account the coefficients obtained:

$$f_i(\lambda) = \frac{F_i(\lambda) - b_i}{a_i}$$

The result of applying additional normalization is a decrease in the standard deviation (Fig. 2, *b*). Such normalization is called I-normalization.

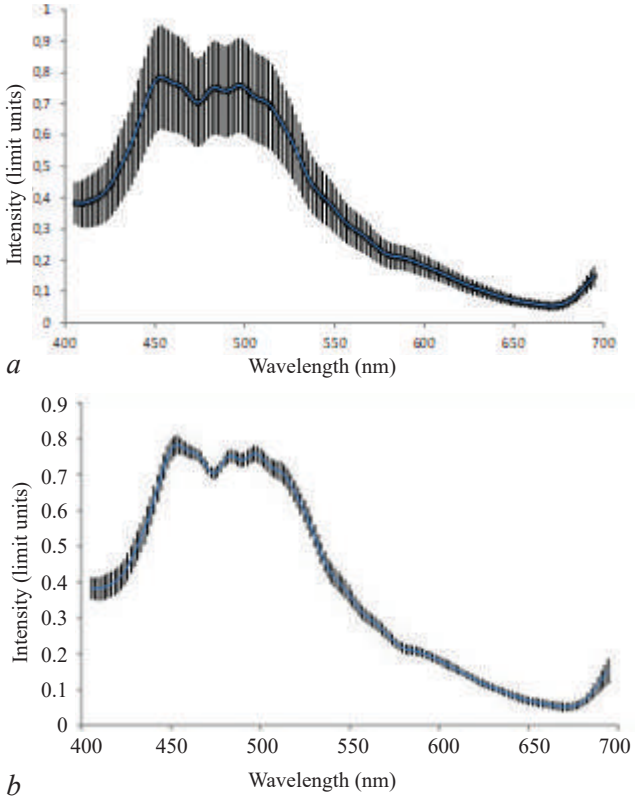

Fig. 2. Skin autofluorescence spectra of a group of patients with T1DM using different normalization methods. *a* – D; *b* – I

Statistical analysis was performed using the Statsoft Statistica 12.0 software package and Microsoft Excel. The spectra were processed using normalization and leveling algorithms. Data analysis was performed using descriptive and nonparametric statistics. The Mann–Whitney test (data were presented as a spectrum of Z-evaluation) was used for the paired comparison

of the fluorescence spectra. The main differences in the points of the spectrum are presented by the median value of fluorescence intensity and interquartile range *Me* [Q_1; Q_3].

RESULTS

The results of using D- and I-normalization with subsequent comparison of autofluorescence spectra in groups of patients with T1DM depending on the level of glycated hemoglobin are presented in the graph (Fig. 3). Significant differences are observed in both methods of spectrum normalization. However, I-normalization led to more significant differences in groups 1 and 2. The greatest difference in the spectrum was observed in the region of the Soret band of hemoglobin at 433 nm ($p < 10^{-4}$) and the region of the NADH peak at 487 ($p < 0.005$) isosbestic point of the alpha band of oxy and deoxyhemoglobin at 592 nm ($p < 0.001$).

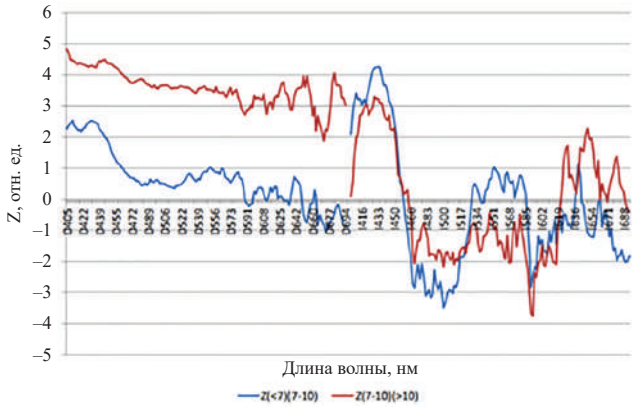

Fig. 3. Spectra of pairwise Z-scores based on the level of glycated hemoglobin

Table 1

Significant Differences in Fluorescence Intensity at Specified Wavelengths According to Mann-Whitney Test for Different Levels of Glycated Hemoglobin

Wave-length	Comparison group, *Me* [Q_1; Q_3]		*p* (based on Mann–Whitney test)
	HbA1c < 7,0	HbA1c = 7,1–10,0	
I433	0.540 [0.512; 0.551]	0.514 [0.498; 0.531]	$<10^{-4}$
I487	0.740 [0.730; 0.750]	0.748 [0.739; 0.760]	0.002
I592	0.197 [0.191; 0.204]	0. 202 [0.194; 0.206]	0.014
	HbA1c = 7.1–10.0	HbA1c > 10.1	
I433	0.514 [0.498; 0.531]	0.534 [0.518; 0.557]	0.001
I592	0. 202 [0.194; 0.206]	0.194 [0.186; 0.199]	$<10^{-3}$

Note. Differences were considered statistically significant at $p < 0.05$ (here and in Tables 2, 3).

The graph in Fig. 4 shows D-normalization, indicating significant differences in autofluorescence spectra at different values of average daily glycemia.

Fig. 4. Spectra of Z-evaluation when comparing the average daily glycemia with different methods of normalization

Table 2

Significant Differences in Fluorescence Intensity at Specified Wavelengths According to Mann-Whitney Test for Average Daily Glycemia

Wave-length	Average daily glycemia, *Me* [Q_1; Q_3]		*p* (based on Mann-Whitney test)
	<10 mmol/L	=> 10 mmol/L	
D470	0.771 [0.656; 0.826]	0.701 [0.618; 0.764]	0.004
D652	0.073 [0.064; 0.081]	0.066 [0.058; 0.074]	0.002

Significant regions of the spectrum that distinguish groups of glycemic variability are present in both normalization methods. The graph of the spectrum of Z-scores for comparing glycemic variability is shown in Fig. 5. As shown in the graph, a finer structure of the spectrum when using I-normalization allows analysis of changes in individual metabolites at different levels of glycemic variability. Thus, the most significant sections of the spectrum are represented by the Soret band of hemoglobin at 427 nm ($p < 0.005$), the peak of NADH fluorescence at 485 nm ($p < 0.005$), the peaks of β and α bands of oxyhemoglobin at 539 nm ($p < 0.01$) and 581 nm ($p < 0.001$), respectively, as well as the region of porphyrin fluorescence at 660 nm ($p < 0.01$).

Fig. 5. Spectra of Z-scores for comparing the glycemic variability

Table 3

Significant Differences in Fluorescence Intensity at Specified Wavelengths According to Mann-Whitney Test for Glycemic Variability

Wavelength	Glycemic variability, *Me* [Q_1; Q_3]		*p* (based on Mann-Whitney test)
	<36%	=> 36%	
I427	0.481 [0.468; 0.494]	0.460 [0.441; 0.481]	0.001
I485	0.748 [0.730; 0.750]	0.750 [0.742; 0.760]	0.004
I539	0.417 [0.397; 0.431]	0.430 [0.415; 0.440]	0.006
I581	0.203 [0.196; 0.217]	0.216 [0.210; 0.223]	$<10^{-3}$
I661	0.063 [0.061; 0.069]	0.060 [0.053; 0.066]	0.01

DISCUSSION

Although glycated hemoglobin is spectrally indistinguishable from other hemoglobin derivatives, its presence in the systemic bloodstream obviously causes hypoxic changes in peripheral tissues, which can be recorded using the spectrofluorometric method. The majority of studies devoted to the relationship between autofluorescence level and HbA1c in patients with diabetes mellitus indicate a correlation between these indicators, both in children and adults [10–12].

According to the results of the study, the overall reflection level (scattering) of UV radiation from the skin was the most significant parameter, depending on average daily glycemia.

As the study shows, an increase in average daily glycation decreases fluorescence in the entire spectral range in relation to the reflected excitation radiation. This may be due to an increase in the reflection coefficient and not a simultaneous change in all metabolites. An increase in the reflection coefficient, in turn, is associated with a change in the refractive index of blood plasma with an increase in the concentration of glucose in it. This finding is consistent to a certain extent with data on changes in the refractive index of the skin with an increase in glycemia [13, 14].

Moreover, when assessing the relationship between autofluorescence spectra and glycemia variability indices, it was found that a finer spectrum structure at I-normalization apparently allows analysis of changes in individual metabolites at different levels of glycemia variability. The results showed a statistical relationship between skin autofluorescence spectra and glycemia variability. Furthermore, a comparison of autofluorescence spectra revealed not only hypoxic shifts in the case of high variability, which is expected in severe diabetes mellitus, but also an increase in the pool of porphyrins.

In 1949, Sterling et al. first reported an association between the development of diabetes mellitus and porphyria. In the studies conducted, marked increase in serum glucose and insulin levels were observed in patients with porphyria.

However, despite numerous studies, the exact mechanism by which patients with porphyria, especially asymptomatic patients, experience increased insulinemia remains unknown [15]. In the present study, the cause of the increase in porphyrins can be presumably associated with a decrease in the insulin response during adolescence, when hormonal changes are observed, mainly due to the level of growth hormone and sex hormones [16].

CONCLUSION

When changing laboratory parameters of glycated hemoglobin level, average daily glycemia, and glycemic variability, there appear significant changes in the spectrum of UV-induced fluorescence of the skin in children with type 1 diabetes mellitus.

It was revealed that significant differences in the skin autofluorescence spectra under UV excitation were detected both in the overall signal level and at wavelengths coinciding with the absorption peaks of hemoglobin in the Soret band region, alpha and beta bands, isosbestic points of oxy and deoxy hemoglobin, the fluorescence peak of NADH, and porphyrins. In this regard, the discovered relationship of skin autofluorescence in the region of the porphyrin peak at 660 nm with the degree of glycemic variability in children with T1DM is poorly understood.

The results obtained in the study enable to conclude that it is possible to create a non-invasive method for monitoring various metabolic changes in diabetes mellitus based on UV-induced autofluorescence spectroscopy of the skin, which simultaneously solves the problems of such diagnostic methods as determining the level of glycated hemoglobin, average daily glycemia, and glycemia variability. This possibility can be realized through metabolic connections of the indicated clinical indicators with endogenous chromophores and fluorophores of the skin.

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Proskurina M.V. – conception and design (collection of clinical and laboratory data, measurement of skin autofluorescence level, database creation), justification of the manuscript or critical revision for important intellectual content. Kiseleva N.G. – conception and design. Taranushenko T.E. – conception and design, final approval of the manuscript for publication. Salmin V.V. – conception and design, analysis and interpretation of data, final approval of the manuscript for publication.

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Response of Systemic Hemodynamic Parameters to Changes in Blood Viscosity in Spontaneously Hypertensive Rats

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ABSTRACT

Aim. To study the response of systemic hemodynamic parameters to a decrease in blood viscosity in spontaneously hypertensive rats (SHR) compared to normotensive Wistar rats.

Materials and methods. Systemic hemodynamic parameters were recorded using the MP150 system (Biopac Systems, Inc., USA). Blood viscosity was measured using a Brookfield DV–II+Pro rotational viscometer (Brookfield Engineering Labs Inc., USA) at 36°C and a shear rate of 450 s^{-1}. Blood viscosity was reduced using isovolemic hemodilution.

Results. The decrease in the blood viscosity in Wistar rats was not accompanied by significant changes in the parameters of systemic hemodynamics. Only a slight decrease in the mean blood pressire was revealed, probably associated with the experimental conditions and the effect of isoflurane anesthesia. Unlike normotensive animals, in SHR isovolemic hemodilution led to a marked decrease in total peripheral vascular resistance, heart rate, blood pressure, and an increase in stroke volume. At the same time, in SHR rats, the hypotensive reaction of blood pressure in response to a decrease in blood viscosity was 3 times greater than in Wistar rats, which indicates impaired vascular tone regulation in response to a change in shear stress.

Conclusion. Thus, in normotensive animals, a decrease in blood viscosity as a result of isovolemic hemodilution does not cause changes in the main parameters of systemic hemodynamics. In contrast, in spontaneously hypertensive animals, total peripheral vascular resistance and blood pressure decrease alongside with blood viscosity, indicating impaired endothelium-dependent vascular tone regulation in response to changes in shear stress. The results obtained substantiate the use of drugs that reduce blood viscosity as a promising direction in the complex pharmacotherapy of hypertension and its complications.

Keywords: arterial hypertension, blood pressure, blood viscosity, total peripheral resistance, minute blood volume, stroke volume, normotensive Wistar rats, spontaneously hypertensive rats

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Реакция параметров системной гемодинамики на изменение вязкости крови у спонтанно гипертензивных крыс

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РЕЗЮМЕ

Цель: исследование реакции параметров системной гемодинамики в ответ на снижение вязкости крови у спонтанно гипертензивных крыс линии SHR по сравнению с нормотензивными крысами стока Вистар.

Материалы и методы. Параметры системной гемодинамики регистрировали с помощью системы MP150 (Biopac Systems, Inc., США). Вязкость крови измеряли на ротационном вискозиметре Brookfield DV–II+Pro (Brookfield Engineering Labs Inc., США) на скорости сдвига 450 $с^{-1}$. Снижение вязкости крови проводили с помощью изоволемической гемодилюции.

Результаты. Снижение вязкости крови у крыс стока Вистар не сопровождалось значимыми изменениями параметров системной гемодинамики, выявлено лишь небольшое снижение артериального давления, вероятно, связанное с условиями эксперимента и действием изофлуранового наркоза. В отличие от нормотензивных животных у крыс SHR изоволемическая гемодилюция приводила к выраженному снижению общего периферического сопротивления сосудов, частоты сердечных сокращений, артериального давления и увеличению ударного объема. При этом у крыс SHR гипотензивная реакция артериального давления в ответ на снижение вязкости крови была в 3 раза больше, чем у крыс стока Вистар, что свидетельствует о нарушении регуляции тонуса сосудов в ответ на изменение напряжения сдвига.

Заключение. Таким образом, у нормотензивных животных снижение вязкости крови в результате изоволемической гемодилюции не вызывает изменений основных параметров системной гемодинамики. Напротив, у спонтанно гипертензивных животных общее периферическое сопротивление сосудов и артериальное давление пассивно следуют за снижением вязкости крови, что свидетельствует о нарушении эндотелийзависимой регуляции тонуса сосудов в ответ на изменение сдвигового напряжения. Полученные результаты обосновывают применение препаратов, снижающих вязкость крови, в качестве перспективного направления комплексной фармакотерапии артериальной гипертензии и ее осложнений.

Ключевые слова: артериальная гипертензия, артериальное давление, вязкость крови, общее периферическое сопротивление, минутный объем крови, ударный объем, нормотензивные крысы стока Вистар, спонтанно гипертензивные крысы линии SHR

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Arterial hypertension (AH) is a major risk factor for cardiovascular diseases [1]. Despite the availability of a number of highly effective and well-tolerated drug therapies for AH, blood pressure control parameters leave much to be desired [2]. This justifies the need to search for new therapeutic strategies for the treatment of patients with AH.

The hyperviscosity syndrome that develops in AH is one of the links in the pathogenesis of this disease [3, 4, 5]. Increased blood viscosity (BV) in AH significantly contributes to an increase in total peripheral resistance, impaired systemic hemodynamics, and microcirculation disorder [3, 4]. However, there are complex relationships between BV and hemodynamic parameters [4, 6]. BV has two opposite effects on total peripheral vascular resistance, which includes effects on hydrodynamic resistance and on vascular tone through mechanotransduction involving the vascular endothelium. On the one hand, an increase in BV in AH leads to an increase in total peripheral resistance [3]. On the other hand, BV determines the shear stress acting on the endothelium of the vascular wall, and with an increase in BV, the shear stress on the vascular endothelium increases, which can lead to a decrease in vascular tone [7].

Experimental studies have been conducted to investigate the relationship between BV and blood pressure [8, 9]. However, these studies contain contradictory data on changes in systemic hemodynamic parameters in response to changes in BV in normotensive animals. Similar studies have not been conducted on spontaneously hypertensive rats (SHR). At the same time, SHR are the most adequate model of essential arterial hypertension.

The aim was to study the reaction of systemic hemodynamic parameters in response to a decrease in blood pressure in SHR compared to normotensive Wistar rats.

MATERIALS AND METHODS

The experiments were conducted on 8 outbred male Wistar rats aged 13–14 weeks, obtained from the Department of Experimental Biological Models of Goldberg Research Institute of Pharmacology and Regenerative Medicine of Tomsk NRMC and 9 spontaneously hypertensive male rats aged 13–14 weeks, obtained from the vivarium of the Institute of Bioorganic Chemistry, Russian Academy of Sciences, Pushchino. In the vivarium of Goldberg Research Institute of Pharmacology and Regenerative Medicine, the animals were kept in a partial barrier system with the following environmental parameters: temperature of 20–24 ºC, relative humidity of 50±20%, air exchange rate of 12–15 room volumes per hour, and light conditions of 12:12 h. The animals were kept and cared for in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Directive 2010/63/EU). The research protocol (No. 207012023) was approved by the Committee for the Control of the Care and Use of Laboratory Animals of Goldberg Research Institute of Pharmacology and Regenerative Medicine. The experiments were conducted under isoflurane anesthesia. For isoflurane inhalation, an Ugo Basile 21050 anesthesia apparatus (Ugo Basile, Italy) was used. A decrease in BV was achieved using isovolemic hemodilution, which was carried out by equivolume replacement of 10% of the circulating blood volume with plasma obtained from a donor rat. The circulating blood volume was determined for each rat based on 7.5% of the body weight [10]. The isovolemic hemodilution procedure was performed in recipient rats by withdrawing blood from the jugular vein and simultaneously transfusing plasma from a donor rat into the femoral vein using an SN-50C6 infusion syringe pump at a rate of 0.17 ml/min. Blood was collected from donor rats through a catheter from the common carotid artery; heparin was used as an anticoagulant. The blood was centrifuged at 1600 g for 15 min to obtain plasma. The mean arterial pressure (MAP) was recorded in the common carotid artery of the animal. The stroke volume (SV) of the heart was determined using the tetrapolar rheography method. The SV of blood was calculated using the formula:

$$SV = \rho \cdot (L/Z_0)^2 \cdot dz/dt_{max} \cdot LVET$$

where ρ is the specific resistance of the blood; L is the distance between the outer measuring electrodes; Z_0 is the total impedance (resistance); dz/dt_{max} is the amplitude of the first derivative of the rheogram; LVET is left ventricular ejection time. Based on the SV, heart rate (HR) and animal weight, the values of the cardiac index (CI) and total peripheral resistance (TPR) were calculated. The parameters of systemic hemodynamics were recorded using a high-speed data acquisition and analysis system MP150 (Biopac Systems, Inc, USA) with a DA100C MAP recording unit with a TSD104A sensor and an EBI100C impedance recording unit

with the AcqKnowledge 4.2 for MP150 software. BV was assessed on a Brookfield DV–II+Pro rotational viscometer (Brookfield Engineering Labs Inc., USA) at a shear rate of 450 s^{-1} at a temperature of 36 °C.

Registration of systemic hemodynamic parameters and BV was performed before isovolemic hemodilution and 30 minutes after.

Statistical processing of the obtained results was performed using the Statistica 8.0 statistical software package. The data are presented as M±SE, where M is the mean value, SE is the standard error of the mean. The nonparametric Mann–Whitney *U*-test was used to assess the statistical significance of intergroup differences. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The baseline of the BV in Wistar rats was 3.64±0.07 mPa·s (Fig. 1a). After isovolemic hemodilution, the BV in Wistar rats significantly decreased by 16% compared to the baseline. The HR, SV, CI, and TPR indices in Wistar rats after isovolemic hemodilution did not differ from the baseline (Fig. 1c, Fig. 1d, Fig. 1e, Fig. 1f). A slight decrease in MAP was revealed after isovolemic hemodilution in Wistar rats (by 7±2%) (Fig. 1b).

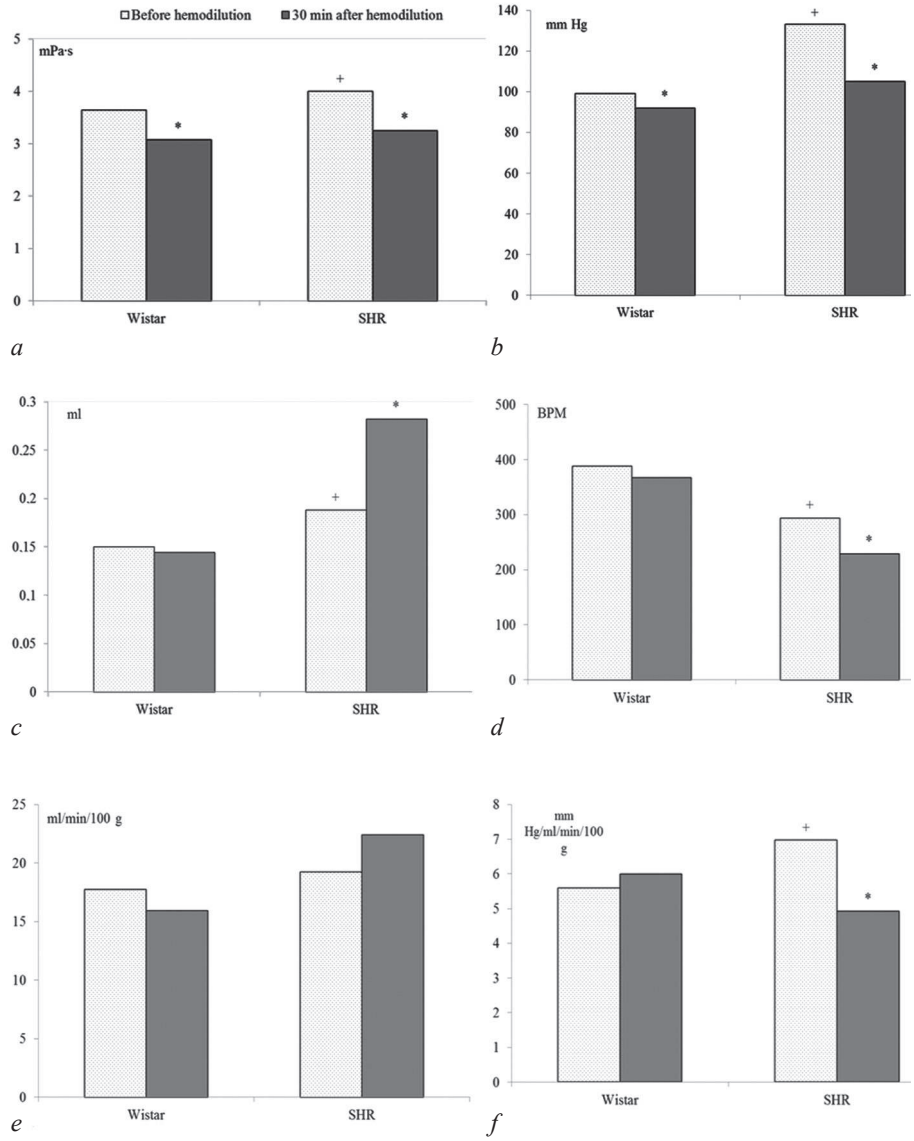

Fig. 1 Changes in blood viscosity (*a*), mean arterial pressure (*b*), stroke volume (*c*), heart rate (*d*), cardiac index (*e*) and total peripheral resistance (*f*) in normotensive Wistar rats and SHR after isovolemic hemodilution. $^{+}$ – statistically significant difference ($p < 0.05$) compared to values in Wistar rats; * – statistically significant difference ($p < 0.05$) compared to values before hemodilution.

The baseline of BV MAP, SV, and TPR in SHR was significantly higher, and HR was lower than in Wistar rats (Fig. 1a, Fig. 1b, Fig. 1c, Fig. 1d, Fig. 1f). Isovolemic hemodilution in SHR resulted in a 19% decrease in BV compared to the baseline. In SHR, isovolemic hemodilution resulted in a statistically significant decrease in HR (by 22%) and TPR (by 29%), while SV increased (by 50%). After isovolemic hemodilution, a statistically significant decrease in MAP by 21±2% was observed in SHR. Moreover, the hypotensive reaction of arterial pressure in response to a decrease in BV in SHR was 3 times greater than in Wistar rats.

DISCUSSION

To correct the syndrome of increased BV and microcirculation disorders in hypertension, the use of pharmacological agents that reduce BV seems to be a theoretically sound approach. However, the ambiguous effect of BV on TPR requires further study of this phenomenon.

Data on changes in hemodynamic parameters after a decrease in hematocrit and, consequently, in BV were obtained in normotensive animals. The experiments of Bonnin et al. demonstrated that in Wistar rats, with a change in hematocrit in the range of 35–46%, arterial pressure, CI, and blood flow in the renal artery remain stable [8]. A decrease in hematocrit in awake Syrian hamsters by 8.4% caused an increase in arterial pressure and CI, while vascular resistance remained stable [9]. In our study, a decrease in BV in normotensive animals did not lead to significant changes in the parameters of central hemodynamics. A slight decrease in arterial pressure after isovolemic hemodilution in Wistar rats was probably associated with the experimental conditions, in particular, the effect of isoflurane anesthesia [11]. The absence of changes in central hemodynamic parameters after a decrease in BV in Wistar rats is associated with the involvement of endothelium-dependent mechanotransduction in the regulation of vascular tone.

In SHR, unlike normotensive animals, isovolemic hemodilution resulted in significant changes in central hemodynamic parameters. A decrease in BV in SHR resulted in a decrease in TPR, indicating a disturbance in the regulation of vascular tone in response to a change in shear stress. A decrease in TPR and HR with a decrease in SV in SHR after isovolemic hemodilution could lead to an increase in SV. A decrease in MAP in SHR after hemodilution was more pronounced than in Wistar rats and is probably associated, in addition to the effect of anesthesia, with a decrease in TPR due to a decrease in BV. A previous study showed that in normotensive animals, before and after hemodilution, there are no correlations between BV and arterial pressure. Whereas in SHR, there is a positive correlation of medium strength between BV and arterial pressure, which persists after isovolemic hemodilution [12].

The involvement of endothelial cells in the regulation of vascular tone with changes in shear stress has been proven in a number of studies. Thus, inhibition of eNOS and NO production in normotensive animals resulted in a direct relationship between changes in BV and peripheral resistance [8, 13]. Violation of the relationship between blood flow velocity and tail artery diameter was demonstrated in normotensive Wistar rats with endothelium damaged by the CHAPS detergent, as well as in SHR [14]. Endothelial cells play a key role in the regulation of vascular tone due to their ability to respond to increased shear stress with increased NO production. In hypertension, elevated blood pressure adversely affects the vascular endothelium [15], which leads to a decrease in its vasodilating activity in response to humoral stimuli [15, 16, 17] with the development of endothelial dysfunction [18, 19]. In addition, patients with hypertension develop a syndrome of increased BV [3, 4, 5], and increased viscosity negatively affects endothelial function [20].

CONCLUSION

Thus, in normotensive animals, a decrease in BV as a result of isovolemic hemodilution does not cause significant changes in the parameters of central hemodynamics. Conversely, in spontaneously hypertensive animals, TPR and arterial pressure passively follow a decrease in BV, which indicates a violation of the regulation of vascular tone in response to a change in shear stress. The obtained results substantiate the use of drugs that reduce BV as a promising direction in the pharmacotherapy of AH and its complications.

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Polymorphic Variant of *NQO1* rs1800566 and Antipsychotic-induced Metabolic Disorders in Patients with Schizophrenia

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ABSTRACT

Aim. To conduct an associative analysis between antipsychotic-induced metabolic disorders and the polymorphic variant *NQO1* rs1800566.

Materials and methods. The study included 603 patients with schizophrenia, who underwent a comprehensive clinical, anthropometric and laboratory examination. Metabolic syndrome (MetS) was established based on the 2005 International Diabetes Federation criteria. Genotyping of the polymorphic variant *NQO1* rs1800566 was performed in the studied sample of patients. Statistical processing of the results was performed using Statistica 12.0 software package (StatSoft, Russia).

Results. Among patients receiving basic therapy with atypical antipsychotics, the T allele had an effect predisposing to the development of MetS (odds ratio: 1.63, 95% confidence interval: 1.01–2.62), while the *C* allele was statistically significantly more common among patients without metabolic syndrome (odds ratio: 0.61, 95% confidence interval: 0.38–0.99). In carriers of the *TT* genotype, serum triglyceride levels are statistically significantly higher than in carriers of the *CC* or *CT* genotypes ($p = 0.049$).

Conclusion. The results of the study for the first time revealed associations of the polymorphic variant *NQO1* rs1800566 with MetS and hypertriglyceridemia in patients with schizophrenia receiving pharmacotherapy with second-generation antipsychotics. The results of this study confirm the contribution of the genetic component to the development of metabolic disorders in patients with schizophrenia and open up prospects for further search for genetic markers for the prevention and correction of this undesirable phenomenon.

Keywords: molecular genetics, *NQO1*, single nucleotide polymorphism, metabolic disorders, schizophrenia, antipsychotics, adverse effects of therapy

Conflict of interest. The authors declare no obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients included in the study signed a voluntary informed consent. The study was approved by the Ethics Committee of Mental Health Research Institute of Tomsk NRMC (Minutes No. 165 dated September 18, 2023). .

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Полиморфный вариант *NQO1* rs1800566 и антипсихотик-индуцированные метаболические нарушения у пациентов с шизофренией

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РЕЗЮМЕ

Цель. Провести ассоциативный анализ между антипсихотик-индуцированными метаболическими нарушениями и полиморфным вариантом гена НАД(Ф)Н-хиноноксидоредуктазы-1 (*NQO1*) rs1800566

Материалы и методы. В исследование включены 603 пациента с шизофренией, у которых было проведено комплексное клиническое, антропометрическое и лабораторное обследование. Метаболический синдром (МС) устанавливался на основании критериев Международной федерации диабета (IDF), 2005. Проведено генотипирование полиморфного варианта *NQO1* rs1800566 в исследуемой выборке пациентов. Статистическая обработка результатов осуществлена с использованием программного обеспечения Statistica for Windows V.12.0 (StatSoft, Россия).

Результаты. Среди пациентов, принимающих базовую терапию атипичными антипсихотиками, аллель *T* обладал эффектом, предрасполагающим к развитию МС (отношение шансов (ОШ) 1,63; 95%-й доверительный интервал (ДИ): 1,01–2,62), в то время как аллель *C* статистически значимо чаще встречался среди пациентов без метаболического синдрома (ОШ 0,61; 95%-й ДИ: 0,38–0,99). У носителей генотипа *TT* уровни триглицеридов в сыворотке крови статистически значимо выше, чем у носителей генотипов *CC* или *CT* (p = 0,049).

Заключение. В результаты проведенного исследования впервые были обнаружены ассоциации полиморфного варианта *NQO1* rs1800566 с МС и гипертриглицеридемией у больных шизофренией, принимающих фармакотерапию антипсихотиками второго поколения. Результаты данного исследования подтверждают вклад генетической компоненты в развитие метаболических нарушений у больных шизофренией и открывают перспективы для дальнейшего поиска генетических маркеров с целью превенции и коррекции этого нежелательного явления.

Ключевые слова: молекулярная генетика, *NQO1*, однонуклеотидный полиморфизм, метаболические нарушения, шизофрения, антипсихотики, нежелательные эффекты терапии

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Соответствие принципам этики. Все пациенты, включенные в исследование, подписали добровольное информированное согласие. Исследование одобрено этическим комитетом НИИ психического здоровья Томского НИМЦ (протокол № 165 от 18.09.2023).

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INTRODUCTION

The ambiguity of the use of long-term antipsychotic pharmacotherapy lies in its dual effect on the organism of patients with mental disorders. On the one hand, these drugs successfully reduce the psychopathological symptoms of schizophrenia, which has been proven by numerous studies [1, 2]. On the other hand, antipsychotics, especially with long-term use, cause serious somatic, neurological, and other complications [2–4].

Both conventional and atypical antipsychotics can lead to weight gain, although the pathogenesis of this adverse effect is somewhat different. Conventional antipsychotics more often lead to hyperprolactinemia by blocking D2 dopamine receptors in the tuberoinfundibular pathway [5], while atypical antipsychotics cause dyslipidemia, increase insulin resistance [6], and thereby contribute to the development of obesity or metabolic syndrome (MetS). Increased body weight significantly reduces the quality of patients life, often leading to a decrease in their compliance and even to a complete treatment refusal [5].

Oxidative stress is characterized by increased synthesis of free radicals, in particular active forms of oxygen: singlet oxygen, superoxide radical, hydrogen peroxide, etc., as well as disruption of the prooxidant and antioxidant systems. Typically, free radical oxidation plays an important role in the bactericidal action of neutrophils, regulation of blood pressure, polymerization of proteins, and lipid peroxidation. In pathology, oxidative stress leads to the formation of atherosclerotic plaques, the development of coronary heart disease, hypercholesterolemia, carcinogenesis, and a predisposition to thrombosis [7].

One of the aspects of schizophrenia pathogenesis is considered to be the development of oxidative stress [8], which leads to impaired neuroplasticity and increased neurodegeneration and is detected regardless of whether patients have taken antipsychotics [9] or not [10]. There is evidence that oxidative stress is a probable mechanism for the development of tardive dyskinesia, one of the most severe adverse effects of conventional and some atypical antipsychotics [11, 12].

It has also been shown that lipid peroxidation processes occur rapidly in obesity [13], which in itself can cause oxidative stress. Progressive growth of adipose tissue leads to increased production of reactive oxygen species and pro-inflammatory mediators, disruption of the balance of prooxidant and antioxidant systems and, consequently, to the development of oxidative stress [14, 15], which forms a vicious circle [7].

The *NQO1* gene encodes the flavoenzyme NAD(P)H quinine oxidoreductase-1. This enzyme catalyzes the reduction of quinones to less toxic hydroquinones and is also involved in the detoxification of superoxide radicals to hydrogen peroxide. *NQO1* expression increases in oxidative stress [16]. There is also evidence that *NQO1* is found in the brain, where it is involved in dopaminergic neurotransmission [17] and neuroprotection [18].

Genetic factors have been proven to play significant role in the development of schizophrenia, the formation of its clinical picture and the severity of drug-induced side effects of antipsychotic therapy [19, 20]. The polymorphic variant *NQO1* rs1800566 is functional: it leads to the replacement of proline with serine and a significant decrease in enzyme activity [21]. In this regard, we hypothesized that polymorphic variants of the *NQO1* gene may participate in the formation of metabolic disorders in patients with schizophrenia when they are receiving antipsychotic therapy.

Thus, the aim of the study was to analyze the associations between antipsychotic-induced metabolic disorders and the *NQO1* rs1800566 polymorphic variant.

MATERIALS AND METHODS

Patients. The study was conducted at the Department of Endogenous Disorders of the Mental Health Research Institute of Tomsk National Research Medical Center. A total of 603 patients with schizophrenia (302 men and 301 women) were examined. All patients included in the study underwent in-patient treatment, signed a voluntary informed consent, and received antipsychotic therapy in the average therapeutic doses recommended by the manufacturer.

Inclusion criteria were as follows: age 18–55 years; follow-up history of at least 1 year; Slavic ethnicity; verified diagnosis of schizophrenia according to ICD-10 criteria; consent to participate in the study.

Exclusion criteria were as follows: the presence of organic, neurological, severe somatic diseases leading to organ failure; the presence of concomitant addictive or other mental disorders; refusal to participate in the study.

All subjects completed the “Basic card of socio-demographic and clinical-dynamic features for patients with schizophrenia” [22], which we had

previously tested in clinical trials.

*Mental status assessment***.** The severity of the mental state was verified using the Positive and Negative Syndrome Scale (PANSS) [23] in the adapted Russian version (SCI-PANSS) [24].

*Anthropometric study***.** All subjects underwent anthropometric measurement, which included the measurement of height, weight, and waist circumference, and the calculation of body mass index (BMI). Waist circumference was measured midway between the lower rib and the iliac crest.

*Laboratory parameters***.** Blood for biochemical tests was taken on an empty stomach between 8.00 and 9.00 a.m. Glucose, triglycerides (TG), and high-density lipoproteins (HDL) were measured in blood serum samples using standard biochemical methods.

*Metabolic syndrome***.** MetS was defined according to the criteria of the International Diabetes Federation (IDF) (2005) [25]:

– abdominal obesity: waist circumference ≥ 94 cm in men or ≥ 80 cm in women;

– dyslipidemia: elevated triglyceride (TG) levels ≥ 1.7 mmol/l;

– dyslipidemia: low HDL levels < 1.03 mmol/l in men or < 1.29 mmol/l in women;

– blood pressure ≥ 130/85 mm Hg;

– fasting plasma glucose levels ≥ 5.6 mmol/l.

*Molecular genetic analysis***.** Genotyping of the polymorphic variant *NQO1* rs1800566 was performed by real-time PCR using SNP Genotyping Assay kits (ThermoFisher Scientific, USA) on a StepOnePlus device (Applied Biosystems, USA).

Based on the genotyping results, patients were divided into 3 groups according to the identified genotypes for further comparison of quantitative indicators.

Statistical analysis was performed using Statistica for Windows V.12.0 software (StatSoft, Russia). The Shapiro–Wilk test was used to test whether data set was normally distributed. The obtained data did not obey the normal distribution law. Therefore, they are presented as the median with the interquartile range *Me* [Q_1; Q_3]. Qualitative data are presented as frequency indicators in absolute and relative units *n* (%). When comparing qualitative data, Pearson's $\chi 2$ was used, including taking into account the Yates correction, and Fisher's exact test (if one or more of the study groups had less than 5 people). Quantitative data were compared using the Kruskal–Wallis test (H). The odds ratio (OR) with the calculation of the 95% confidence interval (CI) was used as a quantitative measure of the degree of association of a genetic marker with MetS. The threshold level of statistical significance is $p = 0.05$.

This study did not use animals in the experiments. All the procedures performed comply with the ethical standards of the research institute and/or national Ethics Committee and the Helsinki Declaration of 1964 and its subsequent amendments or comparable standards of ethics.

RESULTS

Socio-demographic and clinical characteristics of patients are presented in Table 1.

Table 1

Socio-Demographic and Clinical Characteristics of the Examined Patients

Characteristic	Parameter
Sample size, *n*	603
Gender, *n* (%)	Men: 302 (50.1%)
	Women: 301 (49.9%)
Age, years, *Me* [Q_1; Q_3]	39 [31; 49]
Age of manifestation, years, *Me* [Q_1; Q_3]	24 [20; 30]
Duration of disease, years, *Me* [Q_1; Q_3]	13 [7; 21]
PANSS, points, *Me* [Q_1; Q_3]	Total score: 102 [92; 102]
	Positive symptoms: 23 [19; 27]
	Negative symptoms: 25 [22; 29]
	General psychological symptoms: 52 [46; 58]
Duration of basic therapy, years, *Me* [Q_1; Q_3]	8 [3; 17]
Chlorpromazine equivalent, mg, *Me* [Q_1; Q_3]	434.8 [225; 758.7]
Pharmacological profile of antipsychotics, *n* (%)	Conventional: 370 (61.4)
	Atypical: 234 (39.3)
Metabolic syndrome , *n* (%)	Yes: 156 (25.9%)
	No: 447 (74.1%)

At the first stage of the study, an association analysis was performed between the frequency of occurrence of genotypes and alleles of the selected single-nucleotide polymorphic variant *NQO1* rs1800566 and MetS. No statistically significant associations were found in the total sample of patients. At the second stage, patients were divided into groups depending on the pharmacological profile of the basic antipsychotic. In the case of using conventional antipsychotics, no relationship was found between the selected polymorphic variant and MetS. Among patients receiving basic therapy with atypical antipsychotics, the T allele had an effect predisposing to the development of MetS, while the C allele was statistically significantly more common among

patients without MetS (Table 2).

Anthropometric and laboratory parameters were compared among patients receiving basic atypical antipsychotic therapy, for which they were divided into subgroups with different genotypes of the studied polymorphic variant *NQO1* rs1800566. It was shown that in carriers of the TT genotype, serum triglyceride levels were statistically significantly higher than in carriers of other genotypes (Table 3).

Table 2

Comparison of Genotype and Allele Frequencies of the *NQO1* Rs1800566 Polymorphic Variant in the Group of Patients Receiving Basic Therapy with Atypical Antipsychotics, *n* (%)

Polymorphic variant			Without MetS	With MetS	OR		χ^2/F	*p*
					Value	95% CI		
General sample								
rs1800566	genotypes	*CC*	309 (65.7)	92 (57.1)	0.69	0.48–1.00	4.71	0.09
		CT	140 (29.8)	63 (39.1)	1.51	1.0 –2.20		
		TT	21 (4.5)	6 (3.7)	0.96	0.38–2.45		
	alleles	*C*	0.806	0.767	0.79	0.58–1.07	2.29	0.13
		T	0.194	0.233	1.26	0.93–1.72		
Patients receiving conventional antipsychotics as basic therapy								
rs1800566	genotypes	*CC*	182 (65.2)	52 (59.1)	0.77	0.47–1.26	2.91	0.23
		CT	84 (30.1)	34 (38.6)	1.42	0.86–2.34		
		TT	13 (4.7)	2 (2.3)	0.54	0.12–2.46		
	alleles	*C*	0.803	0.784	0.89	0.59–1.35	0.29	0.59
		T	0.197	0.216	1.21	0.74–1.69		
Patients receiving atypical antipsychotics as basic therapy								
rs1800566	genotypes	*CC*	111 (68.1)	37 (53.6)	0.54	0.30–0.96	4.34	0.11
		CT	46 (28.2)	28 (40.6)	1.83	1.00–3.32		
		TT	6 (3.7)	4 (5.8)	2.00	0.53–7.48		
	alleles	*C*	0.822	0.739	0.61	0.38–0.99	4.13	0.04*
		T	0.178	0.261	1.63	1.01–2.62		

* statistically significant differences (here and in Table 3).

Table 3

Comparison of Anthropometric and Biochemical Parameters in a Group of Patients Receiving Basic Therapy with Atypical Antipsychotics with Different Genotypes of the *NQO1* Rs1800566 Polymorphic Variant, *Me* [Q_1; Q_3]

Parameter	Genotype			*H*	*p*
	CC	CT	TT		
Waist size, cm	87 [78; 98]	87 [78; 100]	91 [83; 96]	0.61	0.73
BMI, kg/cm^2	26.2 [22.5; 31.1]	23.2 [20.5; 26.3]	27.6 [22.5; 31.5]	0.31	0.86
Glucose, mmol/l	4.91 [4.5; 5.48]	5.1 [4.5; 5.42]	5.2 [5; 5.8]	1.78	0.41
Triglycerides, mmol/l	1.32 [1; 1.92]	1.3 [0.9; 1.84]	1.94 [1.6; 2.3]	5.65	0.049*
HDL, mmol/l	1.01 [0.82; 1.25]	1 [0.82; 1.3]	1.09 [0.6; 1.45]	0.003	0.999

DISCUSSION

Along with the choice of the most effective pharmacotherapeutic tactics in the treatment of schizophrenia, the problem of genetic predisposition to the development of certain undesirable metabolic phenomena when using antipsychotic drugs has remained no less relevant for biological psychiatry for a long time.

It is assumed that *NQO1* acting as an antioxidant enzyme prevents the overproduction of reactive oxygen species, leading to vascular dysfunction, promoting the activation of adipocyte transcription factors and disruption of the regulation, and synthesis of fatty acids and lipids [26]. The results obtained in the course of the study may mean that carriers of the mutant allele have reduced *NQO1* activity and, consequently, a shift in the balance towards the prooxidant system. Together with the increased risk of cardiometabolic disorders when taking some atypical antipsychotics and the development of oxidative stress with increased formation of adipose tissue, which could explain the differences obtained.

There is no information in the literature on studies of the association of polymorphic variants of the *NQO1* gene with antipsychotic-induced metabolic disorders. The overwhelming majority of publications regarding the sought-after polymorphic variant are devoted to its association with cancer [26–28], since oxidative stress plays an important role in carcinogenesis processes. Therefore, we consider the present study to be a pilot. Anecdotal data on the presence or absence of an association of this polymorphic variant, as well as other polymorphic variants of the *NQO1* gene with the development of metabolic disorders, obesity, or MetS are presented.

A relationship was found between the T allele and hypertriglyceridemia, and reduced HDL levels in a Mexican population of patients suffering from MetS [30]. Elevated triglyceride levels were associated with carriage of the homozygous T allele [31]. Mice homozygous for the knockout of the *NQO1* gene have been shown to have increased TG levels [32]. While administration of beta-lapachone, a natural *NQO1* substrate that activates the enzyme, to mice was accompanied by a decrease in the concentration of TG, cholesterol, free fatty acids, leptin, glucose, insulin, and body weight in mice with an experimental model of obesity and diabetes mellitus [33].

A number of studies involving patients with type 2 diabetes mellitus have not revealed associations between this disease and the *NQO1* rs1800566 polymorphic variant [34, 35]. According to the authors, the lack of associations may be due to the influence of concomitant therapy with fibrates and statins [34]. This further emphasizes the importance of assessing the effect of antipsychotic therapy in our study.

It is worth mentioning that one of the limitations of this study is the fact that the sample was recruited from patients with chronic schizophrenia. Patients received long-term antipsychotic therapy; however, it cannot be confirmed that all patients had high compliance in the long term. However, the results were obtained on a sufficient sample size and through correct statistical processing and therefore reflect objective clinical data. In the future, they can form the basis for a more detailed study of the contribution of oxidative stress to the development of antipsychotic-induced obesity in patients with schizophrenia.

CONCLUSION

For the first time, we have found found associations of the polymorphic variant *NQO1* rs1800566 with MetS and hypertriglyceridemia in patients with schizophrenia receiving pharmacotherapy with second-generation antipsychotics. The obtained data confirm the contribution of genetics to the development of metabolic disorders in patients with schizophrenia and open up prospects for further search for genetic markers for the purpose of preventing and correcting this on-treatment adverse event.

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Predictors of Positive Steps in the Five-Step Stress Echocardiography Protocol in Patients with Postinfarction Cardiosclerosis

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ABSTRACT

Aim. To study the frequency and predictors of positive steps in five-step stress echocardiography (SE) in patients with previous myocardial infarction (MI).

Materials and methods. The single-center study included 75 patients (61.6 ± 9.8 years, 84% men) with previous MI. The median duration of MI was 1,231.0 (381.5; 2,698.5) days. All patients underwent exercise SE according to the five-step protocol. At step A wall motion abnormalities (WMA) were detected, at step B – the sum of B-lines, at step C – contractile reserve (CR) of the left ventricle (LV), at step D – coronary reserve (CorR) in the left anterior descending artery, and at step E – heart rate reserve.

Results. The frequency of positive steps was 36.0% for step A, 18.7% for step B, 80.0% for step C, 53.3% for step D, and 50.7% for step E. Following the multivariate analysis, predictors of a positive step A (resting diastolic blood pressure (BP), $p = 0.030$, resting WMA index, $p = 0.007$), step B (taking β-blockers, $p = 0.035$; left ventricular (LV) mass index, $p = 0.005$), step C (increase in systolic BP (SBP), $p = 0.011$; increase in LV end-diastolic volume, $p = 0.019$; increase in LV ejection fraction, $p = 0.008$), and step D (taking angiotensin II receptor blockers, $p = 0.026$; increase in SBP, $p = 0.012$; increase in LV force, $p = 0.038$) were revealed.

Conclusion. Identification of predictors of WMA during exercise, subclinical pulmonary congestion, and a decrease in CR and CorR in patients with previous MI may be a target for therapeutic intervention in order to delay the development of adverse cardiovascular events.

Keywords: ABCDE stress echocardiography, myocardial infarction, contractile reserve, coronary reserve, chronotropic reserve

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Предикторы положительной пробы пятиступенчатого протокола стресс-эхокардиографии у пациентов с постинфарктным кардиосклерозом

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РЕЗЮМЕ

Цель. Изучить частоту и предикторы положительных шагов пятиступенчатой стресс-эхокардиографии (СЭ) у пациентов с перенесенным инфарктом миокарда (ИМ).

Материалы и методы. В одноцентровое исследование включены 75 пациентов (61,6 ± 9,8 лет, 84% – мужчины) с перенесенным ИМ. Медиана давности ИМ составила 1231,0 (381,5; 2698,5) сут. Всем пациентам была проведена СЭ с физической нагрузкой по пятишаговому протоколу. На шаге А выявляли нарушение локальной сократимости (НЛС), на шаге В – сумму В-линий, на шаге С – сократительный резерв (СР) левого желудочка (ЛЖ), на шаге D – коронарный резерв (КР) в левой передней нисходящей артерии, а также резерв частоты сердечных сокращений на шаге Е.

Результаты. Частота положительных результатов составила 36,0% для шага А, 18,7% – для шага В, 80,0% – для шага С, 53,3% – для шага D и 50,7% – для шага Е. В результате многофакторного анализа выявлены предикторы положительного шага А (диастолическое артериальное давление (АД) в покое, $p = 0{,}030$; индекс НЛС в покое, $p = 0{,}007$), шага В (прием β-блокаторов, $p = 0{,}035$; индексированная масса миокарда ЛЖ, $p = 0{,}005$), шага С (прирост систолического АД, $p = 0{,}011$; прирост конечно-диастолического объема ЛЖ, $p = 0{,}019$; прирост фракции ЛЖ, $p = 0{,}008$) и шага D (прием блокаторов рецепторов ангиотензина II, $p = 0{,}026$; прирост систолического АД, $p = 0{,}012$; прирост силы ЛЖ, $p = 0{,}038$).

Заключение. Выявление предикторов НЛС на нагрузке, субклинического легочного застоя, снижения СР и КР у пациентов с перенесенным ИМ может быть мишенью для терапевтического воздействия с целью отдаления развития неблагоприятных сердечно-сосудистых событий.

Ключевые слова: ABCDE-стресс-эхокардиография, инфаркт миокарда, сократительный резерв, коронарный резерв, хронотропный резерв

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все лица подписали информированное согласие на участие в исследовании. Исследование одобрено комитетом по этике Медицинского института РУДН.

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INTRODUCTION

Cardiovascular diseases and primarily myocardial infarction (MI) are the most urgent problems of practical public health care due to high mortality and disability rate. Given wide introduction of reperfusion therapy methods into medical practice, the number of surviving patients after acute MI (AMI) is growing, and, accordingly, the prevalence of heart failure (HF) and mortality is increasing [1, 2]. The role of imaging techniques for risk stratification of distant complications after AMI is being actively studied. The modern protocol of stress echocardiography (SE) can provide the physician with important additional information. The procedure can help reveal induced myocardial ischemia, subclinical pulmonary congestion, and decreased contractile (CR) as well as coronary (CorR), and chronotropic reserves, which is currently regarded as the pathophysiological cascade in ischemic stroke and can be of fundamental importance in determining the patient management strategy to improve their prognosis [3–6].

Cardiac function testing with ABCDE-CE provides a comprehensive view of patient's risk factors using an extremely simple, low-cost test with minimal risk and zero radiation exposure. An exercise protocol is the most physiologic of all stress testing options. The main practical applications of ABCDE-SE include identification of functional mechanisms of disease and symptoms, long-term risk stratification for therapy adjustment or objective assessment of therapy efficacy, and evaluation of long-term prognosis in a broad group of patients.

There is evidence in the literature of the impact of each ABCDE-SE component on patient's prognosis in terms of the development of various adverse cardiovascular events [3–15].

Each step in the protocol defines a specific phenotype, a risk biomarker, and a potential selective target of personalized therapy [10]. Abnormal step A indicates the need for anti-ischemic therapy with beta-blockers, calcium channel blockers, or nitrates and, possibly, revascularization. Diuretic therapy is recommended in patients with pulmonary congestion, which is identified qualitatively and quantitatively in step B. Angiotensin-converting enzyme inhibitors are recommended in patients with asymptomatic left ventricular (LV) dysfunction after MI, which is identified by a decrease in CR in step C. Selectively abnormal step D implies the presence of coronary microvascular disease, and statins are recommended. Abnormal step E implies reduced cardiac sympathetic reserve, potentially amenable to treatment with various techniques that reduce overactive sympathetic nervous system by blockade of the β-adrenergic or renin–angiotensin–aldosterone systems or neuromodulation therapy aimed at restoring the autonomic balance by a sympathomodulatory intervention, such as, for example, renal denervation. All these parameters individually and combined may be important for the selection or adjustment of therapy to prevent adverse events [10, 15, 16].

Thus, the practical and prognostic value of revealing possible predictors of positive steps in the five-step protocol is beyond doubt, as it may help potentially identify possible additional variants of therapeutic intervention on various markers of stress-induced ischemia.

The aim of the study was to investigate the predictors of positive steps in five-step SE in patients with a history of MI.

MATERIALS AND METHODS

The single-center study included 75 patients with a history of MI receiving therapy according to current guidelines. The vast majority of patients in the experimental group were men (n = 63.84%), mean age 61.6 ± 9.8 years. Median time elapsed from MI was 1,231.0 (381.5; 2,698.5) days.

All patients underwent exercise testing on a Schiller treadmill (TM) ergometer MTM-1500 Med or a Schiller Ergosana ERG 911S/LS horizontal bicycle ergometer (BE) as part of a clinical examination [17]. Criteria for discontinuation of the test included new areas of wall motion abnormality (WMA), severe chest pain, diagnostic ST segment displacement, excessive blood pressure elevation (systolic blood pressure (SBP) ≥ 240 mm Hg, diastolic blood pressure (DBP) ≥ 120 mm Hg), exercise-limiting dyspnea, maximum predicted heart rate (HR), and significant arrhythmias. Antianginal medications were usually not discontinued before testing.

Step A included an assessment of WMA. The WMA index (wall motion score index – WMSI) was calculated in each patient at baseline and at peak exercise. Step B evaluated B-lines using lung ultrasound and simplified scanning at four points: from the mid axillary to the mid clavicular line at the third intercostal space. Step C detected CR as a stress-to-rest ratio of force; LV force was calculated as the ratio of SBP to LV end-systolic volume (ESV). Coronary flow velocity reserve (step D) was assessed as the

ratio of peak-to-rest left anterior descending artery (LAD) blood flow velocities. HR reserve (step E) was calculated as the ratio of peak-to-rest HR from ECG data. Criterion A was considered to be positive when new areas of WMA appeared or viable myocardium was identified. Subclinical pulmonary congestion was diagnosed when the sum of B-lines increased by 2 or more. A load-to-rest ratio ≤ 2.0 was taken as a decrease in CR. Step D was considered to be positive when the ratio of velocities in LAD at peak and at rest was ≤ 2.0. A decrease in chronotropic reserve was recognized as a decrease in HR by less than 1.8 times [10]. We calculated an increase in LV ejection fraction (EF) at loading compared to rest (Δ LV EF), LV end-diastolic volume (Δ LV EDV), LV force (Δ LV force), double product ((DP), SBP multiplied by HR at the peak of the load).

The sample size was calculated according to the method of K. A. Otdelnova (set power of the study 80%; significance level 0.05). SPSS software (version 22.0) was used for statistical data processing. Quantitative variables were described as the arithmetic mean and the standard deviation *M* ± *SD* (for normal distribution), or as the median and the interquartile range *Me* ± *IQR* (for non-normal distribution). The significance of differences between the studied distribution and normal distribution was assessed by the Lilliefors-corrected Kolmogorov – Smirnov test. The differences were statistically significant at $p < 0.05$. The direction and strength of the correlation between the parameters were evaluated using the Spearman's rank correlation coefficient. The dependence of binary parameters on quantitative and categorical ones was revealed by binary logistic regression (single- and multivariate analysis) with the determination of the odds ratio.

RESULTS

The most common comorbidities were hypertension (*n* = 70, 93.3%), dyslipidemia (*n* = 64, 85.3%), obesity or overweight (*n* = 33, 44.0% and *n* = 30, 40.0%, respectively), and NYHA class 1–2 chronic HF (*n* = 30, 40%). The median NTproBNP level was 50.3 (27.5; 118.9) pg/ml. Smoking history and/or current smoking was reported by 19 (25.3%) patients, alcohol abuse – by 4 (5.3%) patients. Chest pain bothered 48 (64.0%) patients (nonanginal – 4 (5.3%), atypical – 8 (10.7%), typical – 36 (48%)).

Coronary angiography data were known in 71 patients. Fifty-one patients (68.0%) had multivessel coronary lesions. The most frequent lesion site was the LAD (59 (78.7%) cases). Stenting was performed in 59 (78,7%) patients, coronary artery bypass grafting – in 1 (1.3%) individual. There was a stent in the LAD in 35 (46.7%) patients, in the right coronary artery – in 26 (34.7%) patients, in the circumflex branch – in 18 (24%) patients, in the trunk of the left coronary artery – in 5 (6.7%) patients. Sinus rhythm was registered in all patients at the time of the study, left bundle branch block was registered in 2 patients, and nonspecific ST depression was registered in 5 patients. The most frequent components of therapy were β-blockers (*n* = 54, 72.0%), statins (*n* = 62, 82.1%), and antiplatelet agents (*n* = 63, 84.0%).

The exercise test was performed on a BE in 57 (76%) patients and on a TM in 18 (24%) patients. Reasons for discontinuation of the test were reaching the preset HR (*n* = 28, 37.3%), appearance of new WMA zones (*n* = 7, 9.3%), intolerable fatigue / dyspnea (*n* = 26, 34.7%), and increased arterial hypertension (*n* = 14, 18.7%). The achieved % of predicted HR was 94 (85; 100)%, and the % of predicted physical activity was 79 (68.5; 96.0)%. The mean DP was 252 ± 43. The most frequent complaints of patients were fatigue (*n* = 35, 46.7% of patients) and dyspnea (*n* = 21, 28.0%). Low exercise tolerance was demonstrated by 7 (9.3%) patients, moderate – by 17 (22.7%) patients, and high and very high – by 45 and 6 (68%) patients, respectively. A hypertensive response to exercise was registered in 33 (44%) patients.

LV contractility at rest was preserved in 55 patients (73.3%). Diastolic dysfunction was most often of grade 1 (E/e' 3.68 ± 1.08; left atrial volume index was 27.5±7.1 ml/m^2), concentric LV remodeling was registered in more than half of the patients (43 patients, 57.3%). The resting and load-dependent echocardiography parameters are presented in Table 1. The results of the SE are presented in Table 2.

Table 1

Quantitative Parameters of SE Stages

Parameter	Rest	Load
LV EF, %, *M* ± *SD*	53.9 ± 7.5	57.0 ± 8.4
Δ LV EF, %, *M* ± *SD*	3 ± 6	
LV EDV, mL, *Me* ± *IQR*	93 ± 32	86 (70; 110)
ΔLV EDV, mL, *M* ± *SD*	–1.0 (–8.0; 13.5)	
LV ESV, mL, *Me* ± *IQR*	41 (30; 53)	36 (29; 49)
GLS, %, *M* ± *SD*	–14.2 ± 3.2	–15.9 ± 3.4
WMSI, *Me* ± *IQR*	1.10 (1.00; 1.43)	1.13 (1.00; 1.50)
B-lines, *Me* ± *IQR*	0 (0;0)	0 (0;1)
SBP / DBP, mm Hg, *Me* ± *IQR*	132 (120; 144)/80 (75; 85)	189 (175; 207)/97 (88; 100)
LV force, mm Hg/ml, *Me* ± *IQR*	3.3 (2.5; 3.9)	5.1 (3.8; 6.8)

End of table 1

Parameter	Rest	Load
Δ LV force, mm Hg/ml, *Me* ± *IQR*	1.7 (0.6; 2.9)	
Contractile reserve, *Me* ± *IQR*	1.6 (1.2; 1.9)	
V_{LAD}, cm/s, *Me* ± *IQR*	23 (19; 26)	40 (31;50)
Coronary reserve, *M* ± *SD*	1.76 ± 0.40	
HR, beats/min, *M* ± *SD*	75 ± 12	132± 14
Chronotropic reserve, *Me* ± *IQR*	1.8 (1.6; 2.0)	

Note: EDV – end-diastolic volume, GLS – global longitudinal strain, V_{LAD} - velocity in the left anterior descending artery.

Table 2

Results of the Five-Stage SE

Parameter		Rest
Frequency of + step, *n* (%)	A	17 (22.7) – ischemia, 10 (13.3) –viable myocardium
	B	14 (18.7)
	C	60 (80.0)
	D	40 (53.3), not assessed in 9 (12.0)
	E	38 (50.7)
Sum of scores, n (%)	0	4 (5.3)
	1	12 (16.0)
	2	25 (33.3)
	3	22 (29.3)
	4	9 (12.0)
	5	3 (4.0)
Functional class of angina pectoris by double product, *n* (%)	1 (DP > 278)	22 (29.3)
	2 (DP 218-277)	38 (50.7)
	3 (DP 161–217)	14 (18.7)
	4 (DP < 150)	1 (1.3)

After identifying correlations of positive SE steps with clinical parameters, univariate and multivariate regression analysis was performed to determine their predictors.

The following parameters were found to have a significant effect on the probability of new WMA zones (Table 3).

Table 3

Characteristics of the Association of Predictors with the Probability of Positive Step A in the Five-Step SE Protocoll

Predictor	COR; 95% CI	*p*	AOR; 95% CI	*p*
DBP rest	0.934 (0.882; 0.989)	0.019	0.936 (0.882; 0.994)	0.030
LV EF rest	0.908 (0.845; 0.976)	0.009		
WMSI rest	10.0 (2.0; 49.4)	0.005	9.0 (1.8; 44.2)	0.007
WMSI load	33.0 (5.0; 217.7)	<0.001		
LV ESV load	1.03 (1.00; 1.05)	0.026		

End of table 3

Predictor	COR; 95% CI	*p*	AOR; 95% CI	*p*
DP	0.986 (0.974; 0.998)	0.019		
LV EF load	0.888 (0.826; 0.954)	0.001		

Note: COR – crude odds ratio (univariate analysis); AOR – adjusted odds ratio (multivariate analysis); 95% CI – 95%- confidence interval (here and in Tables 4–7). DP – double product.

Univariate and multivariate regression analysis was performed to identify predictors of subclinical pulmonary stasis as part of the SE protocol in patients with previous MI. Significant influence of the following parameters on the outcome was revealed (Table 4).

Table 4

Characteristics of the Association of Predictors with the Probability of Positive Step B in the Five-Step SE Protocol

Predictor	COR; 95% CI	*p*	AOR; 95% CI	*p*
Male gender	0.233 (0.061; 0.889)	0.034		
Taking β-blockers	0.232 (0.066; 0.811)	0.022	0.225 (0.056;0.902)	0.035
LVMI	0.941 (0.904; 0.981)	0.004	0.940 (0.900; 0.981)	0.005

Note: LVMI – left ventricular mass index.

Univariate and multivariate regression analysis was performed to identify predictors of CR reduction within the SE protocol in patients with previous MI. Significant influence of the following parameters on the outcome was revealed (Table 5).

Univariate and multivariate regression analysis was performed to identify predictors of decreased LV CorR within the SE protocol in patients with previous MI. Significant influence of the following parameters on the outcome was revealed (Table 6).

Statistically significant models have been developed for steps A, B, C, D to calculate the probability of an abnormal step given the identified predictors.

Univariate and multivariate regression analysis was performed to identify predictors of decreased chronotropic reserve as part of the SE protocol in patients with previous MI. The univariate analysis revealed significant influence of the following parameters on the outcome (Table 7).

We did not find any statistically significant effect of clinical, anamnestic, and echocardiography parameters on the scores resulting from the five-stage SE protocol in patients with previous MI.

Table 5

Characteristics of the Association of Predictors with the Probability Oo Positive Step C in the Five-Step SE Protocol

Predictor	COR; 95% CI	*p*	AOR; 95% CI	*p*
SBP load	0.965 (0.939; 0.991)	0.010		
LV ESV load	1.058 (1.007; 1.111)	0.024		
DP	0.984 (0.968; 0.999)	0.039		
LV EF load	0.918 (0.848;0.993)	0.033		
LV peak force	0.767 (0.635; 0.926)	0.006		
Chronotropic reserve	0.105 (0.013; 0.887)	0.038		
Δ SBP	0.942 (0.910; 0.974)	0.001	0.851 (0.751; 0.964)	0.011
ΔLV EDV	1.120 (1.056; 1.189)	<0.001	1.422 (1.060; 1.909)	0.019
ΔLV EF	0.877 (0.789; 0.973)	0.014	0.561 (0.365; 0.861)	0.008
Δ LV force	0.419 (0.270; 0.652)	<0.001		
+E	3.596 (1.027; 12.591)	0.045		

Table 6

Characteristics of the Association of Predictors with the Probability of Positive Step D in the Five-Step SE Protocol

Predictor	COR; 95% CI	*p*	AOR; 95% CI	*p*
Number of affected coronary vessels according to CAG data	3.0 (1.1; 7.9)	0.026	4.6 (1.2; 17.2)	0.024
% of BCA occlusion	1.045 (1.010; 1.081)	0.012		
Taking ARB	4.1 (1.2; 14.0)	0.026	23.3 (2.3; 240.7)	0.026
DP	0.987 (0.974; 0.999)	0.039		
LV peak force.	0.827 (0.689; 0.992)	0.041		
Contractile reserve	0.375 (0.145; 0.968)	0.043		
ΔSBP	0.974 (0.951; 0.998)	0.033	0.945 (0.904; 0.988)	0.012
Δ LV force	0.419 (0.270; 0.652)	<0.001	0.741 (0.558; 0.983)	0.038

Note: CAG – coronary angiography; BCA – brachiocephalic arteries; ARB – angiotensin II receptor blockers.

Table 7

Characteristics of the Association of Predictors with the Probability of Positive Step E in the Five-Step SE Protocol

Predictor	COR; 95% CI	*p*
Diabetes mellitus	1.926 (1.088; 3.411)	0.025
Glycemic index	1.668 (1.071; 2.596)	0.023
BP rest	1.051 (1.014; 1.098)	0.006
DBP rest	1.090 (1.027; 1.155)	0.004
LVMI	1.027 (1.002; 1.052)	0.030
Type of LV remodeling	2.251 (1.128; 4.495)	0.021
Left atrial volume index, load	1.215 (1.004; 1.469)	0.045
ΔSBP	0.973 (0.952; 0.996)	0.019
+C	3.596 (1.027; 12.591)	0.045

DISCUSSION

In our study, one of the predictors of a positive step A among clinical, laboratory, anamnestic, and instrumental parameters was resting WMSI (AOR 9.0, $p = 0.007$). These results are consistent with the current knowledge about the pathophysiology, diagnosis, and prognostic value of coronary structural and functional disorders [7, 8, 16–18], as well as with the recommended management strategy in the detection of asynergy zones [16]. Another predictor in the multivariate analysis was resting DBP (AOR 0.936, $p = 0.030$, that is, an increase by 1 mm Hg leads to a 1.1-fold decrease in the chance of a positive step A).

Currently, there are little data on the correlation between the level of DBP and the risk of adverse events in patients. On the one hand, observational cohort studies report an increased incidence of CAD and a risk of AMI in individuals with very low DBP because the heart is perfused during diastole [19–21], which is consistent with our results. On the other hand, it is important to evaluate not only office measurements of DBP, because in this way it is possible to overlook patients receiving antihypertensive therapy with masked diastolic hypotension. Thus, the problem of diastolic arterial hypertension and hypotension in the light of SE may acquire new directions for study.

B-lines in lung ultrasound determine stasis at rest and, more often, during exercise in various cardiovascular diseases characterized by possible occurrence of increased pulmonary artery occlusion pressure and accumulation of extravascular fluid in the lungs [22]. We identified predictors of a positive step B: taking β-blockers (AOR 0.225; $p = 0.035$, that is taking drugs of this group decreases the chance of subclinical pulmonary congestion by 4.4 times) and LVMI (AOR 0.940; $p = 0.005$, that is an increase in LVMI per 1 g/m² decreases the chance of subclinical pulmonary congestion by 1.1 times). Taking β-blockers as a factor reducing the chance of subclinical pulmonary stasis can be explained by its pharmacologic actions, contributing to a decrease in pulmonary artery occlusion pressure (reduces the activity of blood plasma renin, decreases the increased total peripheral resistance). The increase in LVMI as a negative predictor of subclinical pulmonary stasis is probably explained by the fact that patients with higher LVMI in our study received more intensive antihypertensive therapy, including diuretics. However, these data require confirmation in larger studies.

Predictors of a decrease in LV CR in the multivariate analysis were an increase in SBP at the peak of load (AOR 0.851; $p = 0.011$, that is an increase in SBP by every 1 mm Hg decreases the chance of LV CR reduction by 1.2 times), an increase in LV EDV (AOR 1.422; $p = 0.019$, i.e. an increase in LV EDV at exercise by every 1 mL increases the chance of CR reduction by 1.4 times); an increase in LV EF (AOR 0.561; $p = 0.008$, that is a rise in LV EF by 1% decreases the chance of CR reduction by 1.8 times). The obtained results on the SBP increase as a negative predictor of reduced LV CR correlate with the results of the study conducted by Bouzas-Mosquera et al. – the frequency of adverse cardiovascular outcomes studied by them was significantly higher in patients with a normotonic response to stress ($p < 0.001$ for all comparisons), which is associated with increased LV CR [23].

The correlation of LV EDV at stress and CR was also studied by Bombardini et al. They evaluated the effect of decreased LV CR, chronotropic reserve, and increased LV EDV on the decrease in cardiac index using the data of 1,344 patients. The binary logistic regression analysis revealed that reduced preload reserve (increase in LV EDV at SE) (OR 5.610), chronotropic incompetence (OR: 3.923), and abnormal LV CR (OR: 1.579) were independently associated with the lowest tercile of cardiac index reserve at peak exercise [5]. Thus, ABCDE-SE plays an important role in identifying the causes of decreased functional cardiac output reserve, which may be underlying separate but not mutually exclusive mechanisms (decreased chronotropic or contractile reserve) [24]. Finally, the increase in LV EF upon exercise as a negative predictor of reduced CR is explained by the methods of calculation of these values: at the same EDV, LV EF increases due to the decrease in ESV and LV CR, although the CR value is also affected by the increase in SBP. Thus, this result may be one of the examples of integration of LV EF (as a key factor for clinical classification, risk stratification, and therapeutic decision making) with other indices of LV function and may improve the characterization, in particular, of the hypercontractile phenotype [25].

Finally, independent predictors of LV CorR reduction in our group of patients were the number of affected coronary vessels according to CAG (AOR 4.6; $p = 0.024$, an increase in the number of affected vessels by 1 raises the chance of LV CorR reduction by 4.6 times), ARB intake (AOR 23.3; $p = 0.026$); increase in SBP (AOR 0.945; $p = 0.012$, an increase in SBP by 1 mm Hg decreases the chance of LV CorR reduction by 1.1 times), a rise in LV force (AOR 0.741; $p = 0.038$, an increase in LV force by 1 mm Hg / ml decreases the chance of LV CorR reduction by 1.3 times). The SBP increase as a negative predictor of reduced LV CorR correlates with the data in the study by Rimoldi et al. It was revealed according to positron emission tomography data that in patients with stage 1–2 hypertension and LV hypertrophy, LV CorR is reduced due to a lack of a proper stress response, which is inversely proportional to SBP ($p < 0.001$ for epicardial CorR; $p = 0.003$ for endocardial CorR). In patients, the degree of impairment of epicardial ($R = 0.52$, $p = 0.003$) and endocardial CorR ($R = 0.51$, $p = 0.004$) was inversely proportional to SBP [26].

The use of ARB as a positive predictor of reduced LV CorR is probably explained by frequent prescription of these drugs to patients with hypertension and previous MI to reduce the risk of associated cardiovascular morbidity and renal protection (in patients with type 2 diabetes mellitus), as part of the combined therapy of chronic HF.

Changes in force and, consequently, in LV CR can be caused by microvascular and/or epicardial disease of coronary arteries, as well as by myocardial scar,

necrosis and/or disease of the subepicardial layer, and reduced LV CorR [27]. The heart responds to inotropic stimuli by increasing its contractile function, which is accompanied by an increase in coronary blood flow [28]. Thus, in our study, LV force increment acted as a negative predictor of LV CorR reduction.

CONCLUSION

Identification of predictors of WMA at exercise, subclinical pulmonary congestion, and decreased CR and CorR as functional mechanisms of disease and symptoms in patients with MI may be a target for therapeutic intervention to delay the development of endpoints.

LIMITATIONS OF THE STUDY

The results relate to a limited number of patients with previous MI of different duration, with different intensity of coronary lesions, different degrees of comorbidity, initial symptomatology, and different therapy regimens. Not all patients had available CAG results within six months from the date of SE, and the diagnostic power was calculated with the actual number of studies per index hospitalization and anamnestic data in the remaining patients, and the conclusions were applied to the whole group. There is a clear need for a large randomized clinical trial to study the relationship between SE steps and the detection of significant coronary lesions, as well as their prognostic significance with respect to the functional status and prognosis in patients with previous MI.

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Associations of Visceral Adipose Tissue Adipokines with Metabolic Disorders in Abdominal Obesity

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ABSTRACT

Aim. To identify associations of visceral adipose tissue adipokines with metabolic disorders in abdominal obesity.

Materials and methods. The study included 101 individuals aged 25–65 years (51 men). For all patients, questionnaires were completed, anthropometric measurements and 3 measurements of blood pressure were performed, fasting blood was sampled, and biopsies of visceral adipose tissue were collected during elective surgery. The parameters of the lipid profile and glucose levels were determined in the blood by enzymatic methods. Homogenates from biopsies of visceral adipose tissue were prepared. The blood levels of adiponectin, adipsin, lipocalin-2, plasminogen activator inhibitor type 1 (PAI-1), and resistin were measured, and homogenates of adipose tissue were obtained by the multiplex analysis. Sex hormone levels in the blood of all patients (estradiol in women, testosterone in men) were determined by the enzyme-linked immunosorbent assay (ELISA) kits.

Results. We identified correlations between serum levels of adipsin and adipose tissue and between adipsin from adipose tissue and PAI-1 in the blood serum. A weak negative relationship was found between the level of adiponectin and waist circumference, body mass index, and insulin resistance indices: triglyceride glucose index (TyG), lipid accumulation product (LAP), and visceral adiposity index (VAI). The level of adiponectin in visceral adipose tissue was inversely correlated with overweight in males and in the 45–65 age group. The level of resistin in visceral adipose tissue showed an inverse correlation with diastolic blood pressure, which persisted in the age group of 25–44 years.

Conclusion. Of the studied adipokines, a relationship with cardiometabolic parameters was shown for adiponectin and resistin. At the same time, adiponectin was inversely correlated with overweight in the group of men and in the age group of 45–65 years, while resistin was inversely correlated with diastolic blood pressure in the age group of 25–44 years.

Keywords: adipokine, visceral adipose tissue, adiponectin, adipsin, lipocalin-2, PAI-1, resistin

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All study participants signed an informed consent. The study was approved by the Ethics Committee at IIPM – Branch of IC&G SB RAS (Minutes No. 66 dated October 24, 2023).

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Ассоциации адипокинов висцеральной жировой ткани с метаболическими нарушениями при абдоминальном ожирении

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РЕЗЮМЕ

Цель: выявить ассоциации между адипокинами висцеральной жировой ткани с метаболическими нарушениями при абдоминальном ожирении.

Материалы и методы. В исследовании приняли участие 101 человек в возрасте 25–65 лет. Проводилось анкетирование, антропометрия, измерение артериального давления, а также забор крови натощак и биоптатов висцеральной жировой ткани во время плановой операции. Энзиматическими методами в крови были определены показатели липидного профиля и глюкозы. Из биоптатов висцеральной жировой ткани были приготовлены гомогенаты, в которых методом мультиплексного анализа определялся уровень адипонектина, адипсина, липокалина-2, ингибитора активатора плазминогена 1 типа (PAI-1), резистина. У всех пациентов с помощью наборов enzyme-linked immunosorbent assay (ELISA) проведено измерение в крови уровня половых гормонов (у женщин – эстрадиола, у мужчин – тестостерона).

Результаты. Были выявлены связи между уровнями адипсина в сыворотке крови и жировой ткани, адипсина в жировой ткани и PAI-1 в сыворотке крови. Обнаружена слабая отрицательная связь между уровнем адипонектина и показателями окружности талии, индекса массы тела и индексами инсулинорезистентности (индекс триглицериды-глюкоза (TyG), индекс lipid accumulation product (LAP), visceral adiposity index (VAI)). Уровень адипонектина в висцеральной жировой ткани обратно ассоциирован с избыточной массой тела среди лиц мужского пола и в возрастной группе 45–65 лет. Уровень резистина в висцеральной жировой ткани демонстрировал обратную зависимость от диастолического артериального давления, что сохранялось для возрастной группы 25–44 лет.

Заключение. Из изученных нами адипокинов связь с кардиометаболическими параметрами была показана для адипонектина и резистина. При этом адипонектин обратно ассоциирован с избыточной массой тела в группе мужчин и возрасте 45–65 лет, а резистин – с диастолическим артериальным давлением в возрастной группе 25–44 лет.

Ключевые слова: адипокин, висцеральная жировая ткань, адипонектин, адипсин, липокалин-2, PAI-1, резистин

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование выполнено в рамках бюджетной темы по государственному заданию № FWNR-2024-0004 и при финансовой поддержке гранта Российского научного фонда № 21-15-00022.

Соответствие принципам этики. Все участники подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом НИИТПМ – филиал ИЦиГ СО РАН (протокол № 66 от 24.10.2023).

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INTRODUCTION

Obesity makes a significant contribution to the pathogenesis of cardiovascular diseases (CVD) and metabolic disorders, such as type 2 diabetes mellitus (T2DM), arterial hypertension (AH), and dyslipidemia [1]. The distribution of adipose tissue is a key factor in this process. Abdominal obesity (AO), which involves the accumulation of fat near internal organs, is associated with the highest risk of developing cardiovascular and metabolic diseases [1, 2]. Only recently it has been discovered that adipose tissue is not merely an energy storage but also an endocrine organ [3]. Despite this, the list of biomolecules synthesized by adipocytes, known as adipokines, continues to grow. Currently it includes over 700 adipokines [4]. All of them are involved in the pathogenesis of obesity and participate in the formation of other components of the metabolic syndrome. Most studies are limited to the determination of adipokines in the blood as the most accessible biomaterial for study. The study of these biomolecules in adipose tissue, in particular in the visceral depot, is associated with a number of limitations, therefore, the number of works on this topic is not large [5–8]. Meanwhile, this issue is of fundamental and clinical interest.

The aim of this study was to identify associations between adipokines of visceral adipose tissue and metabolic disorders observed in patients with AO.

MATERIALS AND METHODS

The study included 101 people aged 25–65 years who were hospitalized in the Surgical Department of the City Clinical Hospital No.2 for elective surgery (surgery for anterior abdominal wall hernia, or cholecystectomy for cholelithiasis or polyps, or diverticulosis of the colon).

The patients completed questionnaires covering their medical history and underwent anthropometric measurements (height, weight, waist circumference (WC), and hip circumference (HC)). Body mass index (BMI) was determined by the formula: BMI (kg/m²) = weight, kg/height, m². The examination included three measurements of blood pressure (BP) (with an interval of two minutes on the right arm in a sitting position after 5-minute rest using an OMRON automatic blood pressure monitor with the recording of the average value of the three measurements).

Before surgery, blood serum samples were taken from patients on an empty stomach, after a 12-hour overnight fasting period. Enzymatic methods using TermoFisher reagents on an automatic biochemical analyzer KoneLab 30i (Finland) in the blood were used to determine the parameters of the lipid profile: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and glucose. The levels of low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedwald formula [9], non-high-density lipoprotein cholesterol (non-HDL-C) level was calculated using the TC–HDL-C formula. We calculated the ratio of TG/HDL-C. The TyG index was calculated according to the formula (Ln (TG in mg / dl × glucose in mg/dl))/2. The LAP (lipid accumulation product) index was calculated according to the following formulas: for men: (WC in cm – 65) × TG in mmol/l; for women: (WC in cm –58) × TG in mmol/l. VAI (visceral obesity index) for men was calculated according to the formula: WC/(39.68 + 1.88 × BMI) × (TG/1.03) × (1.31 / HDL-C); for women: WC / (36.58 + 1.89 × BMI) × (TG / 0.81) × (1.52 / HDL-C), where the values of TG and HDL-C are given in mmol/l [10]. In addition, the level of the following adipokines was determined in blood by the multiplex analysis using the MILLIPLEX MAP Human Adipokine Panel 1 kit for the determination of human adipokines: adiponectin, adipsin, lipocalin-2, plasminogen activator inhibitor type 1 (PAI-1), and resistin.

During the surgery, visceral adipose tissue biopsies (3–5 g) were collected. Homogenates were prepared from the biopsies, in which the adiponectin, adipsin, lipocalin-2, PAI-1, and resistin levels were determined by the multiplex analysis using the MILLIPLEX MAP Human Adipokine Panel 1 kit for the determination of human adipokines.

Sex hormone levels (estradiol in women and testosterone in men) were measured in all patients using enzyme-linked immunosorbent assay (ELISA) kits for subsequent standardization of this parameter in statistical analysis.

Statistical processing of the results was carried out using the SPSS software package (version 20.0). The normality of data distribution was evaluated using the Kolmogorov–Smirnov test. When comparing the groups, the nonparametric Mann–Whitney *U*-test was used for continuous data, and $\chi 2$ was applied for discrete data. The correlation analysis was carried out using the Spearman's rank correlation coefficient. To find associations with cardiometabolic disorders, the linear regression analysis was performed with the inclusion of adipokines as dependent variables. Categorical variables were presented as absolute (*n*)

and relative (%) values, continuous variables were presented as the median and the interquartile range *Me* (Q_{25}; Q_{75}). The critical significance level of the null hypothesis was calculated at $p \leq 0.05$.

RESULTS

The patients were divided into 2 groups depending on the presence of abdominal obesity (AO) according to the criteria of the All-Russian Scientific Society of Cardiology (2009): WC > 80 cm in women and WC > 94 cm in men. The main group included 74 people with AO (44 men, 30 women), the control group encompassed 27 people (7 men, 20 women) ($p = 0.033$). The groups did not differ in age: the median age was 52.50 [41.00; 61.00] years and 51.00 [41.00; 63.00] years, respectively. Significant differences in BMI were revealed: in the main group, the BMI was 31.62 [27.66; 35.51] kg/m², in the control group – 23.63 [20.31; 29.00] kg/m² ($p < 0.001$). There were no significant differences for systolic (SBP) and diastolic blood pressure (DBP), as well as in the presence of AH, coronary heart disease (CHD), ischemic stroke, T2DM, non-alcoholic fatty liver disease (NAFLD), and smoking.

Among patients from the main group, the TG level was 1.3 times higher ($p = 0.002$), and HDL-C was 1.3 times lower ($p = 0.002$) than in the control group. In the main group, TG/HDL-C was 1.6 times ($p < 0.001$), TyG – 1.04 times ($p = 0.002$), LAP – 2.6 times ($p < 0.001$), and VAI – 1.4 times ($p = 0.001$) higher than those in the control group.

Thus, among patients with AO, compared to individuals without it, the following metabolic disorders were identified: expected higher BMI, a higher TG level, a lower HDL-C level, as well as higher insulin resistance indices.

The next step was to determine the levels of the studied adipokines in visceral adipose tissue, depending on the presence of AO, overweight, and obesity.

Adiponectin, adipokine, lipocalin-2, PAI-1, and resistin did not demonstrate significant differences in protein concentration in visceral adipose tissue between patients from the main and control groups.

Table 1

Clinical Characteristics of the Patients Included in the Study, Depending on the Presence of Abdominal Obesity, *Me* (Q_{25}; Q_{75})

Parameters	Group without AO, $n = 27$	Group with AO, $n = 74$	p
Men	7 (26%)	44 (60%)	0.033
Age, years	51.00 [41.00; 63.00]	52.50 [41.00; 61.00]	0.923
BMI, kg/m²	23.63 [20.31; 29.00]	31.62 [27.66; 35.51]	0.0001
sBP, mm Hg	126.50 [113.50; 138.00]	129.75 [120.50; 143.00]	0.171
dBP, mm Hg	82.00 [75.00; 87.00]	82.25 [77.50; 91.13]	0.473
Smoking	6 (22%)	27 (36%)	0.178
History of AH	9 (33%)	41 (55%)	0.051
History of CHD	0 (0%)	6 (8%)	0.129
History of IS	0 (0%)	3 (4%)	0.291
History of T2DM	1 (4%)	10 (14%)	0.163
History of NAFLD	1 (4%)	12 (16%)	0.098
TC, mmol/l	5.35 [4.41; 5.87]	5.01 [4.04; 5.71]	0.313
TG, mmol/l	1.18 [0.90; 1.69]	1.50 [1.20; 2.01]	0.002
HDL-C, mmol/l	1.69 [1.21; 2.00]	1.30 [0.98; 1.55]	0.002
LDL-C, mmol/l	3.18 [2.10; 3.71]	2.96 [2.07; 3.56]	0.602
non-HDL-C, mmol/l	3.67 [2.72; 4.44]	3.76 [2.78; 4.51]	0.724
Glucose, mmol/l	5.60 [5.40; 6.40]	6.05 [5.50; 6.70]	0.159
TG / HDL-C index	0.78 [0.56; 0.94]	1.23 [0.89; 1.73]	0.0001
TyG index	4.20 [4.12; 4.34]	4.38 [4.23; 4.56]	0.002
LAP index	24.32 [13.84; 45.90]	64.40 [37.00; 96.06]	0.0001
VAI index	1.32 [0.72; 1.71]	1.80 [1.25; 2.96]	0.001

Note: non-HDL-C – non-high-density lipoprotein cholesterol, VAI – visceral adiposity index, LAP – lipid accumulation product.

Table 2

Adipokines of Adipose Tissue Depending on the Presence of Overweight and Obesity, *Me* (Q_{25}; Q_{75})

Adipokines	BMI < 25.0 kg / m²	BMI 25.0–29.9 kg / m²	p_1	BMI < 30.0 kg / m²	BMI ≥ 30.0 kg / m²	p_2
Adiponectin, mcg/mg of tissue	10.15 [6.24; 11.99]	6.76 [4.95; 8.56]	0.015	7.25 [5.77; 10.78]	5.84 [4.19; 8.38]	0.030
Adipsin, mcg/mg of tissue	1.22 [0.90; 2.11]	1.92 [1.21; 2.82]	0.067	1.62 [1.05; 2.26]	1.76 [1.09; 3.17]	0.273
Lipocalin-2, mcg/mg of tissue	0.12 [0.06; 0.23]	0.08 [0.05; 0.21]	0.378	0.09 [0.05; 0.21]	0.15 [0.06; 0.39]	0.041
PAI-1, ng/mg of tissue	0.89 [0.44; 1.17]	0.84 [0.51; 1.55]	0.715	0.85 [0.50; 1.18]	1.05 [0.53; 2.65]	0.144
Resistin, ng/mg of tissue	23.86 [3.74; 61.96]	13.45 [4.72; 25.13]	0.413	17.81 [4.46; 37.34]	17.85 [7.05; 65.37]	0.277

Note: p_1 – significance of differences between groups of patients with normal weight and overweight, p_2 – significance of differences between groups of patients without obesity and with obesity.

When studying these adipokines in visceral adipose tissue, depending on the presence of overweight (BMI < 25 kg/m² versus BMI 25.0–29.9 kg/m²), the level of adiponectin in patients with normal body weight was 1.5 times higher than in overweight patients ($p < 0.05$).

Obese patients (BMI ≥ 30.0 kg/m²) had lower levels of adiponectin in visceral adipose tissue compared to non-obese patients (BMI < 30.0 kg/m²). It was reduced by 1.24 times ($p < 0.05$). Lipocalin-2, on the contrary, was 1.67 times higher ($p < 0.05$) in individuals with BMI ≥ 30.0 kg/m² (Table 2).

When performing the correlation analysis to assess the relationship between the studied biomarkers in the blood serum and visceral adipose tissue, a relationship was found between the levels of adipsin ($r = 0.316$; $p = 0.007$), as well as adipsin in the adipose tissue and PAI-1 in the blood serum ($r = 0.278$; $p = 0.019$) (Table 3).

The correlation analysis of adipokines of visceral adipose tissue and clinical characteristics of patients showed a weak negative relationship between the level of adiponectin and WC ($r = –0.210$; $p = 0.044$) and BMI ($r = –0.263$; $p = 0.011$). An inverse correlation was observed for adiponectin and insulin resistance indices: for the TyG index $r = –0.268$ ($p = 0.009$), for LAP $r = –0.284$ ($p = 0.006$), for VAI $r = –0.205$ ($p = 0.049$) (Table 4).

Table 3

Correlation Analysis of Adipokines in Blood Serum and Visceral Adipose Tissue Using the Spearman's Rank Correlation Coefficient

Adipokines in VAT / Adipokines in blood	Adiponectin, mcg/mg of tissue	Adipsin, mcg/mg of tissue	Lipocalin-2, mcg/mg of tissue	PAI-1, ng /mg of tissue	Resistin, ng/mg of tissue
Adiponectin, mcg/ml	0.125 $p = 0.340$	0.160 $p = 0.211$	–0.044 $p = 0.732$	–0.220 $p = 0.091$	–0.015 $p = 0.906$
Adipsin, mcg/ml	0.123 $p = 0.326$	0.316 $p = 0.007$	–0.063 $p = 0.605$	–0.221 $p = 0.075$	–0.188 $p = 0.122$
Lipocalin-2, mcg/ml	0.192 $p = 0.122$	0.013 $p = 0.916$	–0.067 $p = 0.579$	0.056 $p = 0.655$	–0.016 $p = 0.898$
PAI-1, ng/ml	0.125 $p = 0.317$	0.278 $p = 0.019$	0.064 $p = 0.600$	0.011 $p = 0.928$	–0.017 $p = 0.892$
Resistin, ng/ml	0.035 $p = 0.784$	–0.059 $p = 0.628$	–0.080 $p = 0.518$	0.162 $p = 0.201$	0.057 $p = 0.647$

Table 4

Correlation Analysis of Visceral Adipokines and Metabolic Parameters Using the Spearman's Rank Correlation Coefficient

Adipokines in VAT / Metabolic parameters	Adiponectin, mcg/mg of tissue	Adipsin, mcg/mg of tissue	Lipocalin-2, mcg/mg of tissue	PAI-1, ng/mg of tissue	Resistin, ng/mg of tissue
WC, cm	–0.210 $p = 0.044$	0.133 $p = 0.202$	0.143 $p = 0.161$	0.166 $p = 0.139$	0.038 $p = 0.714$
BMI, kg/m²	–0.263 $p = 0.011$	0.132 $p = 0.203$	0.162 $p = 0.113$	0.167 $p = 0.137$	0.066 $p = 0.524$

End of table 3

Adipokines in VAT Metabolic parameters	Adiponectin, mcg/mg of tissue	Adipsin, mcg/mg of tissue	Lipocalin-2, mcg/mg of tissue	PAI-1, ng/mg of tissue	Resistin, ng/mg of tissue
SBP, mm Hg	–0.082 $p = 0.433$	0.116 $p = 0.265$	0.015 $p = 0.883$	–0.069 $p = 0.539$	–0.090 $p = 0.388$
DBP, mm Hg	–0.168 $p = 0.109$	0.013 $p = 0.901$	0.055 $p = 0.594$	–0.037 $p = 0.743$	–0.077 $p = 0.456$
TG / HDL-C index	–0.164 $p = 0.117$	0.065 $p = 0.532$	0.149 $p = 0.146$	**0.231** $p = 0.038$	0.110 $p = 0.290$
TyG index	–0.268 $p = 0.009$	–0.011 $p = 0.920$	0.170 $p = 0.095$	0.161 $p = 0.150$	0.194 $p = 0.060$
LAP index	–0.284 $p = 0.006$	0.058 $p = 0.576$	0.169 $p = 0.098$	0.198 $p = 0.077$	0.124 $p = 0.233$
VAI index	–0.205 $p = 0.049$	0.023 $p = 0.823$	0.169 $p = 0.098$	0.215 $p = 0.054$	0.118 $p = 0.254$

The next stage of the study was to include the studied adipokines of visceral adipose tissue in the linear regression analysis. The independent variables included cardiometabolic parameters (WC, BMI, SBP, DBP, as well as the presence of obesity, overweight, AO, BP, blood glucose ≥ 6.1 mmol/l, HDL-C < 1 for men, 1.2 mmol/l for women, LDL-C ≥ 3 mmol/l, TG ≥ 1.7 mmol) and insulin resistance indices (TG / HDL-C, TyG, LAP, VAI) with standardization by age, sex, and sex hormone levels. As a result of this analysis, it was found that the level of adiponectin in the visceral adipose tissue was inversely associated with overweight in the general group (–3.542 [–5.318; –1.766], $p = 0.0001$). This association was also observed in men (–4.303 [–6.842; –1.764], $p = 0.0001$) and in the 45–65 age group (–4.662 [–7.105; –2.219], $p = 0.001$).

The level of resistin in visceral adipose tissue, when age, sex, AO, DBP, glucose, and TG were included in the model, was found to be dependent on DBP in the overall group (–3.891 [–6.979; –0.803], $p = 0.014$) and in the 25–44 age group (–7.496 [–13.182; –1.810], $p = 0.012$).

No significant associations were found between the levels of adiponectin, lipocalin-2, and PAI-1 in visceral adipose tissue and the studied parameters.

DISCUSSION

Adiponectin is a protein with a complex tertiary structure, synthesized by adipocytes. The impact of adiponectin on the body is facilitated by the AdipoR1 and AdipoR2 receptors. One of the most significant effects of adiponectin is its ability to overcome insulin resistance. It enhances insulin sensitivity in target organs, such as the liver and skeletal muscles, by promoting fatty acid oxidation and stimulating glucose utilization through the activation of the AMPK signaling pathway. The level of adiponectin in the blood serum is inversely proportional to BMI, triglyceride levels, and insulin resistance [11].

Furthermore, adiponectin has the potential to inhibit inflammation and possibly atherogenesis by suppressing the migration of monocytes and macrophages, as well as their transformation into foam cells [12]. Studies on adiponectin in adipose tissue are scarce and often conflicting. The study of adiponectin was conducted in the visceral adipose tissue of different localizations – the epicardial and perivascular fat depots in patients with CHD. The study by O.V. Gruzdeva et al. revealed a decrease in adiponectin mRNA concentration in patients with CHD. Moreover, the more pronounced the atherosclerotic lesion of the coronary artery, the lower the level of adiponectin gene expression [5].

In the study by A. Sirbu et al., no association was found between the level of adiponectin mRNA in visceral adipose tissue and BMI or WC, as well as serum adiponectin. However, participants with obesity and insulin resistance, as assessed by the HOMA-IR index, exhibited lower adiponectin expression levels compared to participants without insulin resistance [13]. Studies by T. Hörbelt et al. [14] and M.I. Jonas et al. [15] demonstrated a decrease in adiponectin levels in visceral and subcutaneous adipose tissue in individuals with obesity. Our data also show an inverse relationship between adiponectin in visceral adipose tissue and overweight, with this relationship persisting in men and in the older age group.

One of the first discovered functions of resistin was the formation of insulin resistance, which is the basis of its name. Subsequently, it was demonstrated that resistin exerts a wide range of effects, including influencing lipid metabolism, promoting the synthesis and secretion of proinflammatory cytokines, and

facilitating the differentiation of monocytes into macrophages. Furthermore, it influences heart contractility, smooth muscle cell activity, angiogenesis, endothelial permeability, and renal function [16]. Resistin was first discovered in rodent adipocytes and was initially thought to be a protein exclusively synthesized in adipose tissue. However, the highest levels of its expression in humans were found in bone marrow cells [17]. Elevated levels of resistin mRNA and protein, accompanied by a simultaneous decrease in adiponectin in subcutaneous adipose tissue, were observed in individuals with obesity. However, no significant differences were observed in the visceral adipose tissue [15].

Another study concluded that there is a link between adipose tissue resistin and impaired fasting plasma glucose in South Asian women [18]. Our findings suggest a link between resistin and DBP levels, first discovered for adipose tissue. Interestingly, this relationship is inverse, meaning that as DBP levels increase, resistin concentrations decrease. However, the link between BP, high normal blood pressure, and resistin levels has already been established for its circulating form [19–21]. The underlying mechanism of resitin action in the presence of hypertension remains unknown.

A study conducted on mice suggests that the activation of the renin–angiotensin–aldosterone system (RAAS) by resistin through the TLR4/P65/Agt pathway is responsible for this effect. This activation leads to an increase in the expression of angiotensinogen, the precursor of angiotensin II, the primary effector of RAAS. This theory is further supported by the absence of an increase in blood pressure following the administration of resistin to mice, even after pre-treatment with angiotensin-converting enzyme inhibitors [22]. Another potential mechanism involves reducing the expression of endothelial nitric oxide synthase (eNOS) and decreasing the bioavailability of NO, which in turn disrupts endothelium-dependent vasorelaxation [23]. Our discoveries necessitate further clarification and explanation of the inverse relationship between resistin levels and DBP.

CONCLUSION

Research on adipokines in adipose tissue, particularly their association with cardiometabolic parameters, remains limited. In our study, we successfully established the link between adiponectin and resistin of visceral fat tissue, as well as cardiometabolic parameters. Adiponectin is inversely associated with overweight in the male group and in the 45–65 age group, while resistin is inversely associated with diastolic blood pressure in the 25–45 age group.

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Atherosclerosis and Inflammation the Path from Pathogenesis to Treatment: Review of the Current State of the Issue (Part 2)

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ABSTRACT

Numerous studies addressing the fundamental aspects of atherosclerosis emphasize the importance of systematically organizing the accumulated data. The second part of this lecture provides an analysis of the critical mechanisms involved in the development of atherosclerosis. This analysis includes a discussion on the roles of inflammasomes, hemodynamic disorders within the vascular wall, vasa vasorum pathology, endothelial cell dysfunction, matrix metalloproteinases, and the Notch and Wnt signaling pathways in the process of atherogenesis. Additionally, it explores the specific characteristics of the pathogenesis of vascular calcification associated with atherosclerosis. A dedicated section thoroughly reviews contemporary pharmacotherapeutic strategies for managing atherogenic dyslipidemia. A comprehensive analysis of current concepts regarding the pathogenesis of atherosclerosis, along with promising approaches to drug therapy, will facilitate the identification of future research directions within the field of lipidology. This endeavor has the potential to elevate preventive cardiology to a new standard.

Keywords: atherosclerosis, inflammation, inflammasome, atheroma, PCSK9 inhibitors

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Атеросклероз и воспаление – путь от патогенеза к терапии: обзор современного состояния проблемы (часть 2)

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РЕЗЮМЕ

Достижения многочисленных исследований в изучении фундаментальных аспектов атеросклероза диктуют необходимость систематизации накопленных данных. Во второй части лекции представлен анализ роли ключевых механизмов реализации воспалительного процесса в развитии атеросклероза. Рассмотрена роль инфламмасомы, нарушений гемодинамики в сосудистой стенке, патологии *vasa vasorum*, дисфункции эндотелиоцитов, матриксных металлопротеиназ, сигнальных путей Notch и Wnt в атерогенезе, а также ассоциированные с атеросклерозом особенности патогенеза кальцификации сосудов.

Отдельным разделом представлен обзор современных фармакотерапевтических подходов к лечению атерогенной дислипидемии. Комплексный анализ современных представлений о патогенезе атеросклероза и перспективных методов лекарственной терапии позволит обозначить дальнейшие направления исследований в липидологии и вывести возможности профилактической кардиологии на потенциально новый уровень.

Ключевые слова: атеросклероз, воспаление, инфламмасома, атерома, ингибиторы PCSK9

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Atherosclerosis is one of the primary challenges in preventive cardiology, which has traditionally received significant attention in the development of national programs for the primary and secondary prevention of atherosclerosis-associated cardiovascular diseases (aCVD) and cardiac rehabilitation programs [1-4].

According to data from the multicenter study ESSE-RF, which included respondents aged 25-64 years from 13 regions of the Russian Federation (RF), the prevalence of hypercholesterolemia (total cholesterol (TC) in the blood ≥5.0 mmol/L) averaged 58.40±0.34%. This indicates an extremely high frequency of atherogenic dyslipidemia within the study population [5]. In the United States, data from the National Health and Nutrition Examination Survey revealed that levels of TC over 200 mg/dL and low-density lipoprotein cholesterol (LDL) ≥130 mg/dL were found in 32.8% and 36.2% of examined individuals, respectively [6]

According to the multicenter, cross-sectional, observational study EURIKA (European Study on Cardiovascular Risk Prevention and Management in Usual Daily Practice), which included data from 12 countries (Austria, Belgium, Germany, France, Greece, Turkey, and others, including Russia) with a final sample size of 7,641 patients, the proportion of individuals with atherogenic dyslipidemia was over20% [7]. The EURIKA population comprised European patients aged at least 50 years who had at least one risk factor for cardiovascular disease (CVD) but no history of CVD in their medical records. Additionally, the STEPs 2021 study reported that the proportion of individuals with atherogenic dyslipidemia (based on all lipidogram indicators) among the population of the Islamic Republic of Iran was 81.0% [8].

A cross-sectional study conducted as part of the China-PEACE project involved 2,660,666 individuals aged 35 to 75 years from all provinces of the People's Republic of China between 2014 and 2019. Among those examined, the prevalence of atherogenic dyslipidemia was found to be 33.8% [9].

These findings indicate that atherogenic dyslipidemia is a global problem, as evidenced by the prevalence rates of lipid metabolism disorders observed across diverse populations with varying national dietary habits. Consequently, studying the pathogenesis of atherosclerosis and developing new therapeutic methods aimed at normalizing lipid metabolism and stabilizing inflammatory status are critically important. The role of inflammation in the development of ASCVD is well established and underscores the urgency of this research.

Currently, atherosclerosis is perceived by the scientific community as an inflammatory disease of the arteries that triggers the mechanisms of vascular aging and damage to target organs [10, 11]. Given this fact, the study of atherogenesis problems from the standpoint of inflammatory theory is a relevant fundamental direction with direct access to real clinical practice [12-16].

In the second part of this lecture, attention will be directed towards examining the clinically relevant aspects of inflammation pathogenesis in the context of atherosclerosis development. Furthermore, a summary of therapeutic methodologies, grounded in the latest progressions in clinical lipidology, will be presented.

The Role of Inflammasome in Atherogenesis

In the context of the leading role of inflammation in the pathogenesis of atherosclerosis, it is worth emphasizing the role of the inflammasome, since this intracellular multiprotein complex is known to play an crucial role in the relationship between lipid metabolism and low-grade inflammation of the vascular wall [17]. Cholesterol crystals and oxidized lipoproteins activate monocytes and macrophages, generating an inflammatory response followed by the production of proinflammatory interleukins (IL) - IL-1β and IL-18. Oxidized LDL is recognized by CD36 receptors on recruited monocytes, which leads to activation of the NLRP3 inflammasome [18]. In lipopolysaccharide (LPS)-treated monocytes, saturated fatty acids can induce the release of IL-1β, which is not observed with unsaturated fatty acids [19]. Like monocytes, endothelial cells also demonstrate NLRP1 activation after stimulation with plasma containing high levels of triacylglycerols and VLDL [20]. In addition to lipid metabolism disorders, other mechanisms are involved in triggering atherogenesis-associated inflammation.

Hypoxia and hypoxia-associated signaling through hypoxia-inducible factor (HIF)-1α in atherosclerotic plaques enhance NLRP3 expression in macrophages and slow the degradation of proIL-1β [21].Hemodynamically induced shear stress increases the expression of sterol regulatory element-binding protein 2 (SREBP2) via mechanotransduction, triggering a new wave of atherogenesis. In this context, elevated NLRP3 expression in endothelial

cells plays an crucial role in maintaining aberrant lipid metabolism [22]. The development of dysfunctional autophagy in atherosclerotic plaques is also significant in the process of atherogenesis, as evidenced by the increased expression of autophagy markers ATG13 and LC3 in aortic endothelial cells. Notably, in mice lacking the ATG5 protein which is essential for autophagy, there is an increase in inflammatory activity and plaque size. These findings underscore the importance of autophagy in the pathogenesis of ASCVD [23]. In mice fed a high-cholesterol diet, hematopoietic deletion of NLRP3, ASC, or IL-1α/IL-1β resulted in reduced atherogenesis and lower levels of IL-18 [24]. Furthermore, pharmacological inhibition of NLRP3 with colchicine increases the number of smooth muscle cells (SMCs) and collagen within the atherosclerotic plaque, promoting its transition to a more stable phenotype [25].

Vascular Shear Stress and Atherosclerosis

Under normal conditions, uniform laminar blood flow acting on the intima of the arteries induces the secretion of nitric oxide (NO). In turn, NO released under physiological conditions regulates the tone of the vascular wall and helps maintain the anti-inflammatory and antithrombotic properties of the endothelium. It is well established that the formation and progression of atheroma occurs focally, primarily around bifurcations or at the points where lateral branches depart from the artery, that is, in areas characterized by uneven (turbulent) blood flow [26]. This nature of the blood flow creates low wall shear stress (WSS), which induces vascular inflammation and contributes to the development of atherosclerosis. WSS refers to the tangential force of mechanical friction exerted by flowing blood, acting longitudinally on the endothelium surface of the arterial wall [27].

Specific endothelial biomechanical receptors within the endothelial glycocalyx detect mechanical stimuli and differentiate between laminar and turbulent types of blood flow, converting WSS into biochemical signals [28]. Consequently, endothelial dysfunction induced by WSS is closely linked to inflammation and lipid metabolism disturbances in the vascular wall, thereby promoting the progression of atherosclerosis. It is worth noting that, although atherogenesis initially occurs in regions of the arterial wall exposed to low WSS, areas of high WSS that develop around growing atherosclerotic plaques are associated with the formation of an unstable plaque phenotype [29].As WSS increases, the functioning of the mechanoreceptor KLK10 diminishes, which mediates the transformation of the normal transcriptome signature of arteries into an emergency response profile [30]. Inflammatory changes within the plaque lead to hypoxia, initiating neovascularization from the adventitial vasa vasorum, which contributes to increased plaque vulnerability [31]. In discussing the vasa vasorum, it is important to highlight the theory that atherosclerosis may initiate specifically from these microvessels within the vascular wall of the arteries [32]. The microvascular network of the vasa vasorum (including arterial, venous, and lymphatic vessels of varying calibers) serves as a crucial anatomical and functional structure that meets the metabolic needs of the adventitia and perivascular adipose tissue, as well as the outer part of the medial layer of large arteries [33]. Dysregulation of blood flow in the vasa vasorum is implicated in the pathogenesis of atherosclerosis, as evidenced by the presence of multiple neuroimmune cardiovascular interfaces (NICIs) in the outer layers of atherosclerotic arteries. These interfaces are characterized by axon terminals located near the SMC media and macrophages in perivascular adipose tissue [34].Numerous newly formed vasa vasorum are abundant in lipid-rich plaques and express elevated levels of cell adhesion molecules, such as ICAM-1 and VCAM-1. This expression facilitates an excessive influx of immune cells and is associated with plaque instability [35].

Although the concept of initial vasa vasorum pathology in the initiation of atherogenic changes currently has several gaps, their role in atherogenesis is extremely important, both within the framework of the “outside-in” concept and in the classical approaches to study. During vascular wall inflammation, vascular endothelial (VE) cadherin is phosphorylated by Src kinase 3 at the intercellular junctions of the endothelium. Concurrently, dephosphorylation of VE cadherin by VE protein tyrosine phosphatase (VE-PTP) prevents its internalization and stabilizes the adhesive junctions between endothelial cells [36, 37].

Additionally, the dissociation of VE-PTP from VE cadherin leads to leukocyte diapedesis and increased vascular permeability in vivo, as demonstrated in a model induced by vascular endothelial growth factor (VEGF) and endotoxin [38]. It is known that lymphocyte binding to the adhesion molecule VCAM-1, along with the stimulation of endothelial cells by VEGF, triggers a common signaling cascade

that includes Ras-associated botulinum toxin substrate C3, NADPH oxidase, reactive oxygen species, and proline-rich tyrosine kinase 2 [39, 40]. However, the molecular mechanisms regulating the kinetics of the interaction between VE-PTP and VE-cadherin remain largely unexplored. Signaling protein 2 containing the CUB-EGF domain (SCUBE2) ensures the integrity of the vascular wall by recruiting VE-PTP to dephosphorylate VE-cadherin. This process promotes the stabilization of endothelial adherens junctions and preserves the barrier function of the intima [41]. Studies involving genetic overexpression and pharmacological induction of SCUBE2 further support the concept that therapeutic regulation of SCUBE2 may be beneficial for stabilizing the vascular bed [42].

Inflammation also stimulates the development of dystrophic calcification in the necrotic lesion of atherosclerotic plaques as a healing response to the inflammatory activation of macrophages [43]. The death of macrophages and SMCs releases vesicles that serve as "nucleation sites" for the deposition of hydroxyapatite crystals. Their aggregation leads to the formation of microcalcifications with diameters of less than 50 μm, which can penetrate the fibrous cap of the plaque [44, 45]. Microcalcifications significantly contribute to the instability of atherosclerotic plaques; furthermore, they induce mechanical stress within the fibrous capsule, generating new inflammatory impulses within the plaque [46]. It is also important to note that ectopic deposition of calcium hydroxyapatite salts occurs long before the onset of atherocalcinosis.

In atherosclerotic inflammation, various cell types, including vascular SMCs, resident pericytes, circulating stem cells, and adventitial cells, differentiate into osteoblastic cells, leading to vascular calcification [47]. For example, SMCs lose part of their contractile phenotype, as evidenced by downregulation of α-smooth muscle actin (α-SMA) and SM-22 expression, followed by abnormal upregulation of genes involved in osteogenesis, such as Runt-related transcription factor 2 (Runx2), osteopontin, osteocalcin, etc. [48, 49]. Vascular calcification is initiated by matrix vesicles produced by osteoblast-like cells that serve as deposition sites for hydroxyapatite crystals [50]. Meanwhile, the overexpression of matrix metalloproteinase MMP-9 leads to the degradation of elastin, which in turn promotes the transition of SMCs from a contractile to a producing phenotype [51].

The Role of Inflammation in Plaque Destabilization

Atherosclerotic plaques are primarily composed of extracellular matrix (ECM), which includes collagen, elastin, proteoglycans, and glycosaminoglycans synthesized by SMCs in the arterial wall [52]. Under conditions of atherogenic inflammation, cytokines such as IL-1β andtumor necrosis factor α (TNF-α) induce the secretion of metalloproteinases, particularly MMP-1, MMP-8, MMP-9, MMP-12, and MMP-13, by macrophages under the regulation of microRNA [53-55].

MMPs catalyze the destruction of interstitial collagen, leading to thinning and weakening of the fibrous capsule, which contributes to plaque instability [56]. In addition, the stability of the fibrous capsule is influenced by the cross-linking of collagen fibers, a process mediated by the enzyme lysyl oxidase (LOX), which is expressed by endothelial cells [57]. Endothelial dysfunction and the phenotypic transition of SMCs are associated with a decrease in LOX activity, resulting in abnormal collagen cross-linking. This weakens the fibrous capsule and increases the presence of soluble collagen forms that are subject to MMP-mediated degradation [58].

In unstable atherosclerotic plaques, the activity of MMP-7 and MMP-9 is increased, and tissue expression of MMP-2 and MMP-9 raises alongside a decrease in the expression of type IV collagen [59]. Among the three types of unstable atheromas, lipid-type plaques exhibit the highest tissue expression of MMP-9 compared to dystrophic-necrotic and inflammatory-erosive types, while type IV collagen expression is predominant in dystrophic-necrotic atherosclerotic plaques. In addition to MMPs, an 8-fold significant increase in APOE gene expression ($p<0.001$) was observed in unstable atherosclerotic plaques of the dystrophic-necrotic type. In contrast, stable atherosclerotic plaques showed an 8-fold statistically significant increase in LDLR and APOB gene expression ($p<0.001$) [60].

Interestingly, the level of adiponectin in an atherosclerotic plaque is directly proportional to serum levels of HDL-C, while secretin levels are inversely proportional. Furthermore, the glucagon levels in conditionally intact intima are 2.1 times lower than those in fragments with stable atherosclerotic plaque; it has also been established that secretin levels are directly associated with plaque stability [61].

In recent decades, more and more attention has been paid by researchers to such a phenomenon as atherosclerotic plaque erosion. Plaques that have undergone superficial erosion demonstrate less lipid accumulation, a less pronounced necrotic core, a moderate number of inflammatory cells, and an intact fibrous capsule [62]. Thrombi formed as a result of superficial erosions are white and rich in platelets, while thrombi associated with plaque rupture are red (rich in fibrin and erythrocytes) [63].

Parallels between Notch and Wnt Signaling Pathways and Atherosclerosis

Notch is a cellular signaling pathway that mediates intercellular communication and is involved in the regulation of homeostasis [64]. The Notch cascade protects against endothelial dysfunction induced by pro-inflammatory cytokines and regulates the phenotypic transition of cells [65]. Increasing evidence suggests that Notch plays a crucial role in signaling related to changes in WSS [66].

Activation of the Notch pathway creates an anti-inflammatory, anti-atherogenic environment that helps maintain endothelial integrity, including the preservation of adherens junctions between endothelial cells [67]. Additionally, Notch is a key signaling cascade for regulating the structure and function of SMCs. Expression of Notch receptors 2 and 3, as well as the primary ligand Jagged1, has been observed in SMCs [68]. Mutations in Notch 2 and 3 can lead to defects in SMC development, providing a strong evidence for the involvement of Notch signaling in regulating vascular differentiation during angiogenesis [69]. Furthermore, Jagged1-Notch3 signaling mediated through nidogen-2 is essential for maintaining the contractile phenotype of SMCs in vitro and in vivo [70].

Wnt is a multitarget signaling cascade characterized by three main intracellular signaling pathways: the canonical pathway (Wnt/β-catenin), the non-canonical Wnt/PCP pathway (which regulates cytoskeletal dynamics through the activation of JNK (C-Jun N-terminal kinase) by small G proteins), and the Wnt/Ca^{2+}-dependent pathway [71]. In addition to its roles in cell proliferation and differentiation, the Wnt pathway is also involved in regulating lipid metabolism [72]. The stabilization of β-catenin via Wnt signaling, along with the activation of fatty acid synthesis via Akt/mTOR signaling, plays a central role in lipid metabolism in steatotic liver [73]. An inverse relationship has been demonstrated between Wnt activation and the severity of atherosclerosis. Specifically, activation of the Wnt pathway following lipid depletion enhances the IL-4 response in macrophages via the PGE2/STAT3 axis. Dickkopf-2 (DKK2),a negative regulator of Wnt/β-catenin signaling, is implicated in macrophage activation during atherosclerosis [74].

Knockdown of DKK2 significantly reduces the expression of genes associated with the polarization of macrophages toward the pro-inflammatory M1 phenotype while increasing the level of polarization markers associated with the anti-inflammatory M2 phenotype. This knockdown also significantly attenuates the formation of foam cells [75].

The Role of Microrna in the Pathogenesis of Atherosclerosis

The role of microRNA in atherosclerosis is multifaceted. For example, miR-520c-3p protects endothelial cells from damage and stabilizes endothelial function by regulating key aspects of pathogenesis, such as cell proliferation, apoptosis, and endothelial cell adhesion [76]. Moreover, miR-181a-5h, miR-181a-3p, and miR-250bmodulate the severity of chronic low-grade inflammation in the vascular wall by suppressing the expression of the nuclear factor NF-κB, thereby slowing the progression of stromal-vascular dystrophic changes [77]. Conversely, miR-488 [78] and miR-183-5p [79] exhibit proatherogenic effects by stimulating functional reorganization of SMCs and exacerbating inflammatory infiltration in the vascular wall. MicroRNAs also demonstrate a dual effect on macrophages. Thus, miR-10a, miR-210, and miR-383 stabilize mitochondrial metabolism and the redox status of cells, leading to a reduction in apoptosis and necroptosis [80]. Notably, miR-181a-3p/5p and miR-155-5p have pronounced atheroma-stabilizing effects [81]. However, high levels of miR-155 correlate with NLRP3 activation via ERK1/2 kinase [82]. In addition, miR-216a exhibits proatherogenic potential by enhancing inflammation through the Smad3/NF-κB cascade [83].

A Look at Lipid-Lowering Therapy through the Prism of the Inflammatory Theory of Atherogenesis

In parallel with the active study of the molecular mechanisms of atherogenesis, the drug arsenal of lipid-lowering therapy is expanding, which increases the capabilities of modern cardiology.

The basic drugs of lipid-lowering therapy are traditionally considered to be HMG-CoA reductase

inhibitors – statins (in particular, rosuvastatin, pitavastatin and atorvastatin) both without and in combination with ezetimibe - a selective inhibitor of cholesterol absorption targeting the sterol transporter Neimann-Pick-like1 (NPC1L1) [84]. This combination is considered generally accepted and complies with the recommendations of both the Russian and European Cardiology Societies.

In the context of this lecture, it is important to focus on the anti-inflammatory potential of statins. Analyzing the mechanism of action of statins reveals that part of their pleiotropic effects can be attributed to the blockade of the mevalonate pathway of cholesterol synthesis, which reduces the levels of isoprenoid intermediates such as farnesyl pyrophosphate and geranyl-geranyl pyrophosphate. A decrease in these levels changes the prenylation of proteins, influencing the effects of statins on autophagy and inflammation [85]. Moreover, statins can suppress the adhesion and migration of inflammatory cells by reducing the expression of the integrin dimer CD11, the immunoglobulin superfamily protein VCAM-1, and leukocyte functional antigen-1 (LFA-1). They also decrease the expression of monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8) [86].

Another anti-inflammatory mechanism of statins is their ability to reduce the levels of interferon γ (INF-γ), oxidized LDL (oxLDL), and serum apoA-I [87, 88]. Several potential mechanisms through which statins exert their anti-inflammatory effects via Toll-like receptor (TLR) signaling pathways have also been identified: inhibition of the prenylation of regulatory proteins, direct or indirect inhibition of NF-kB and MyD88/NF-kB axis, and activation of antioxidant response elements (ARE) [89]. In addition, statins can reduce signaling mediated by transforming growth factor TGF-1β in T lymphocytes, suppress oxLDL-induced maturation of human dendritic cells, impair T lymphocyte activation, and stimulate the pool of regulatory T lymphocytes [90]. Further studies are needed to elucidate the complete molecular mechanisms and multifaceted anti-inflammatory potential of statins.At the same time, several issues persist regarding statin use, particularly their side effects, such as statin-induced myopathy and hyperglycemia. Other concerns include partial and complete resistance to statins, the presence of residual cardiovascular risk, and elevated levels of triglyceride-rich lipoproteins, despite achieving target levels of total cholesterol, LDL cholesterol, and triacylglycerols [91-97]. In light of these challenges, new drugs aimed at normalizing cholesterol metabolism are currently being actively developed and introduced into clinical practice. Among the extensive list of lipid-lowering agents, the most promising include

1) PCSK9-modifying agents

Proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors, particularly evolocumab and alirocumab, are innovative drugs that are actively utilized in modern clinical practice [98-100]. The pivotal studies demonstrating the lipid-lowering potential of evolocumab and alirocumab are FOURIER [101] and ODYSSEY-OUTCOMES [102] trials. According to a meta-analysis of 41 randomized clinical trials, which included a cumulative sample of 76,304 patients(49,086 received evolocumab and 27,218 received alirocumab), PCSK9 inhibitors significantly reduce the risk of myocardial infarction, coronary artery restenosis, and ischemic stroke. Furthermore, these agents are well-tolerated and considered safe drugs while effectively lowering LDL cholesterol levels [103]. In addition to their significant beneficial effects on lipid metabolism and the the reduction of major adverse cardiovascular outcomes (MACE) [104], PCSK9 inhibitors also demonstrate significant anti-inflammatory effects. A study from the European Collaborative Project on Inflammation and Remodeling of the Vascular Wall in Intravascular Ultrasound (ATHEROREMO-IVUS) demonstrated that serum PCSK9 levels are associated with increased absolute inflammatory plaque volume and necrotic core size [105]. A clear correlation was also observed between serum PCSK9 levels and the concentrations of pro-inflammatory cytokines, including IL-6, IL-1β, TNF-α, macrophage colony-stimulating factor (M-CSF), and high-sensitivity C-reactive protein (hs-CRP) [106]. It has been established that PCSK9 enhances the infiltration of inflammatory monocytes into the vessel wall due to the interaction of PCSK9-LDLR (less pronounced with LRP5) with plaques. This interaction directly contributes to plaque destabilization [107]. PCSK9 itself induces inflammation and exacerbates atherosclerosis independently of the LDL receptor. Research has shown that PCSK9 worsens atherosclerosis in mice with a knockout of the LDL receptor gene. Adenylate cyclase-associated protein 1 (CAP1) serves as the primary transducer for mediating the inflammatory actions of PCSK9, including the induction of cytokines, Toll-like receptor 4, scavenger receptors, and the lectin-type oxidized low-density lipoprotein receptor

1 (LOX-1) [108]. Key mediators of this inflammatory cascade include spleen tyrosine kinase (Syk) and protein kinase C delta (PKCδ), which are activated following the formation of the PCSK9-CAP1 complex [109]. In human peripheral blood mononuclear cells, it has been established that PCSK9 levels positively correlate with the phosphorylation of Syk, PKCδ and p65 [110]. Thus, the anti-inflammatory effect of PCSK9 inhibition is evident and holds significant clinical relevance. In discussing drug approaches targeting PCSK9, it is important to highlight inclisiran, a drug based on small interfering RNA (siRNA) [111, 112]. Inclisiran is a double-stranded modified siRNA linked to N-acetylgalactosamine (GalNAc), which acts as a ligand for the asialoglycoprotein receptor expressed by hepatocytes. The drug specifically binds to the matrix RNA transcribing the sequence of the gene encoding PCSK9 [113]. By disrupting the translation of PCSK9 through mRNA cleavage, inclisiran effectively reduces its production. The ORION study series [114] provides robust evidence regarding its hypolipidemic potential, supported by meta-analyses [115, 116] that confirm its clinical efficacy in achieving target lipidogram indicators and reducing adverse cardiovascular outcomes. It is worth noting that some studies within the ORION series are still ongoing today.

2) Lipoprotein (a) inhibitors

Lipoprotein (a) or Lp(a), is an independent factor contributing to both overall and residual risk of CVD [117, 118]. Individuals with elevated Lp(a) levels (>125 nmol/L; >50 mg/dL) exhibit increased activity of arterial inflammation, characterized by endothelial activation due to oxidized phospholipids carried by Lp(a). This process leads to the recruitment of circulating monocytes, resulting in heightened secretion of chemoattractants and pro-inflammatory cytokines, increased expression of adhesion molecules, and enhanced leukocyte migration through the vascular wall [119]. Unfortunately, lifestyle modifications have minimal impact on Lp(a) levels; therefore, extracorporeal therapies, such as namely lipoprotein apheresis may be necessary. This approach is supported by latest American Heart Association consensus on LP(a) apheresis published in 2024 [120]. Lp(a) particles can cross the endothelial barrier, persist in the arterial wall, and promote the development of atherosclerotic plaques [121]. The oxidized phospholipids carried by Lp(a) can trigger macrophage apoptosis and contribute to the "instability" of atheromas [122]. Additionally, Lp(a) promotes inflammation within the arterial wall by increasing monocyte extravasation and endothelial activation [123].

These effects are mediated through adhesion molecules such as ICAM-1 and are associated with an increase in the activity of the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB)-3 induced by Lp(a) [124]. The development of drugs targeting high Lp(a) levels represents an innovative approach to lipid-lowering therapy, as elevated Lp(a) levels are a strong and independent risk factor for ASCVD. As of 2024, several drugs have emerged in this category: pelacarsen [125], olpasiran [126], zerlasiran [127], lepodisiran [128], and muvalaplin [129]. Notably, clinical trials involving these agents have generated great interest within the scientific community, particularly studies such as OCEAN(a)-DOSE [130], KRAKEN [131], ALPACAR [132], among others.

3) Antisense oligonucleotides

Volanesorsen and olezarsen are antisense oligonucleotides targeting apolipoprotein C3 (APOC3) mRNA and are currently under active investigation for the treatment of familial chylomicronemia syndrome [133]. Volanesorsen blocks the synthesis of apolipoprotein C3 in the nucleus of hepatocytes by inhibiting APOC3 mRNA. Two main clinical trials have been conducted with volanesorsen: APPROACH [134] and its open-label extension (OLE) [135], as well as the COMPASS trial [136]. Olezarsen represents an advancement over volanesorsen, as it is conjugated to N-acetylgalactosamine, an aminosaccharide that exhibits a strong binding affinity for the asialoglycoprotein type 1 receptor, thereby enhancing its targeting to hepatocytes [137]. Evidence supporting the efficacy of olezarsen comes from a double-blind, placebo-controlled study [138], which demonstrated that olezarsen reduces levels of apolipoprotein C3, triacylglycerols, and atherogenic lipoproteins in patients with moderate hypertriacylglycerolemia who are at high risk or have established cardiovascular disease.

4) Bempedoic acid

Bempedoic acid is a long-chain tetramethyl-substituted ketodiac acid characterized by a linear molecule structure. It belongs to the family of "rogue" fatty acids [139].

As a hypolipidemic agent, bempedoic acid functions as an inhibitor of the enzyme ATP-citrate lyase, which catalyzes one of the key reactions in cholesterol synthesis [140]. It is the first drug in its

class to act by inhibiting adenosine triphosphate citrate lyase [141]. A significant aspect of bempedoic acid's mechanism of action is that its active metabolite is formed exclusively in the liver, which minimizes the risk of muscle-related adverse reactions [142]. The safety and efficacy of long-term use of bempedoic acid have been evaluated in the CLEAR (Cholesterol Lowering via BEmpedoic Acid, an ACL-inhibiting Regimen) program, which encompasses four phase 3 studies: CLEAR Tranquility [143], CLEAR Harmony [144], CLEAR Wisdom [145], and CLEAR Serenity [146].

Bempedoic acid promotes the activation of LDL receptor expression, leading to lower LDL cholesterol levels, attenuation of atherogenesis, reduction in hepatocyte lipid levels and body weight, and improvement in glycemic control [147, 148]. In this regard, both genetic inhibition of ATP-citrate lyase (ACLY) in hepatocytes and pharmacological inhibition with bempedoic acid suppress fatty acid and cholesterol synthesis while enhancing fatty acid oxidation without increasing circulating triacylglycerol levels. Moreover, studies conducted on murine and human hepatic stellate cells have demonstrated that bempedoic acid also inhibits liver fibrosis by targeting pathways involved in collagen formation [149].

5) Evinacumab

Evinacumab is a monoclonal antibody that targets angiopoietin-associated peptide 3 (ANGPTL3), a circulating protein secreted by the liver that regulates the hydrolysis of very low-density lipoprotein (VLDL) triglycerides. This drug is typically used for the treatment of refractory homozygous familial hypercholesterolemia [150].

6) Lomitapide

Lomitapide lowers cholesterol levels by inhibiting microsomal triacylglycerol transfer protein (MTP) [151]. MTP is involved in loading triacylglycerols onto apolipoprotein B100, which is essential for VLDL assembly. After being secreted by hepatocytes, VLDL is converted to LDL. By blocking VLDL assembly, lomitapide reduces both VLDL release and VLDL-mediated triacylglycerol secretion, resulting in lower plasma LDL concentrations [152]. Lomitapide has been approved by the FDA and EMA for the treatment of adult patients with homozygous familial hypercholesterolemia as an adjunct to a low-fat diet and other lipid-lowering therapies, with or without LDL apheresis [151]. Despite the impressive therapeutic potential of new drugs, their use is limited due to the lack of large-scale double-blind randomized studies, insufficient clinical experience, and high costs. Consequently, they are considered reserve therapies and are prescribed in cases where target lipid profile indicators are not achieved with the maximum tolerated dose of statins combined with ezetimibe and/or when there is complete intolerance to statins [153, 154].

7) Colchicine

In the context of trends in contemporary cardiology, it is worthwhile to highlight the role of colchicine in the treatment of atherosclerosis. Colchicine is a significant medication whose mechanism of action is linked to its effects on cellular structure and function. This drug exhibits a biphasic effect on microtubules; at low concentrations, it inhibits microtubules growth, while at high concentrations, it promotes their depolarization [155]. Colchicine inhibits tubulin polymerization, disrupting the cellular cytoskeleton and leading to impairment of various intracellular processes, including mitosis, intracellular transport, and phagocytosis [156]. In addition, colchicine inhibits chemotaxis and the adhesion of neutrophils to inflamed endothelium, including indirectly through alterations in the expression of VE-selectin on endothelial cells [157]. Colchicine also inhibits L-selectin expression, preventing neutrophil recruitment, and affects neutrophil function by limiting their extravasation. Furthermore, colchicine normalizes macrophage activity and inflammasome functioning [158].Beyond its effect on neutrophils, colchicine exhibits antithrombotic activity by reducing leukocyte-platelet aggregation (including both monocytes and neutrophils) as well as lowering levels of surface markers associated with platelet activity, such as P-selectin and PAC-1 (activated GP IIb/IIIa) [159].

Thus, the diverse effects of colchicine, including modulation of the cell cytoskeleton, anti-inflammatory properties, and antithrombotic activity, determine its high clinical significance in reducing both overall and residual cardiovascular risk in atherosclerosis [160]. There is a substantial body of evidence supporting the use of colchicine in atherosclerosis; notable studies include COLCOT (COLchicine Cardiovascular Outcomes) [161], LoDoCo (Low Dose Colchicine) [162], COVERT-MI (Colchicine for Left Ventricular Infarct Size Reduction in Acute Myocardial Infarction) [163], and CONVINCE (Colchicine for prevention of Vascular Inflammation in Non-CardioEmbolic Stroke) [164].

8) Biologically active compounds in contemporary lipidology

In parallel with conventional drug therapy, the role of various biologically active substances with hypolipidemic activity is being actively studied. Notable examples include chitosan, ursolic acid, nattokinase, spermidine, taurine, grape and pomegranate seed extracts, as well as many other naturally derived compounds that are positioned as atheroprotective and hypolipidemic substances [165, 166]. This topic is traditionally considered controversial. Unfortunately, the available data on the effectiveness and safety of these compounds are limited, difficult to compare, and sometimes even contradictory. Nonetheless, this does not exclude their potential benefits, which have been supported by large placebo-controlled, double-blind randomized studies. For example, the COSMOS (COcoa Supplements and Multivitamin Outcomes Study) study demonstrated a 27% reduction in cardiovascular mortality rates associated with cocoa flavonoids [167]. Additionally, a network meta-analysis encompassing 131 studies with a total sample size of 13,062 patients compared the effectiveness of various dietary supplements such as artichoke, berberine, bergamot, garlic, green tea extract, plant sterols/stanols, policosanols, red yeast rice, silymarin, and spirulina. This analysis found that bergamot and red yeast rice extracts exhibited the most significant atheroprotective effect [168]. It is important to note that in the vast majority of cases, while the positive effects of these compounds are statistically significant compared to placebo groups, they are not comparable to those of statins. The interpretation of data from existing studies is further complicated by the high variability in the biological properties of natural raw materials. These properties can depend on factors such as the life cycle conditions of the producing organisms and the conditions under which they are harvested, processed, and stored. Therefore, caution should be exercised when interpreting these findings. However, the significance of these results should not be underestimated; they should be considered in clinical practice, particularly, when developing personalized dietary interventions that align with clearly defined treatment goals.

CONCLUSION

Our understanding of atherosclerosis has evolved significantly beyond the concept of a mere lipid metabolism disorder. Contemporary research highlights the pivotal role of inflammation throughout the entire atherosclerotic process. Notably, both innate and adaptive immune responses are activated in atherosclerosis, initiating inflammatory reactions that occur both locally and systemically, manifesting as chronic low-grade inflammation. Consequently, circulating cytokines not only serve as indicators of heightened cardiovascular risk but also actively contribute to the progression and destabilization of atherosclerotic plaques. Understanding the role of inflammation in the pathogenesis of atherosclerosis presents significant clinical implications. The pursuit of identifying a molecular signature of the inflammatory cascade in atherosclerotic cardiovascular disease (aCVD) may facilitate the development of targeted anti-inflammatory strategies in the future. When combined with personalized medicine approaches, this advancement could significantly enhance the capabilities of preventive cardiology.

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Hyperlipidemia and Atherosclerosis: Experimental Models

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ABSTRACT

Cardiovascular diseases are the leading cause of death worldwide, and atherosclerosis is considered as the primary pathological process responsible for their development. Numerous studies have shown that high levels of low-density lipoproteins in the blood are one of the most significant risk factors for the development of atherosclerosis. Various models using both small and large animals, including genetically modified models – transgenic and knockout animals – are used to study the atherogenic process. Studies on hyperlipidemia and atherosclerosis commonly combine an atherogenic diet with genetic manipulations. However, none of the available models is ideal, as each has its own advantages and limitations in reproducing the lipoprotein profile and the extent of atherosclerosis compared to human cases.

This review presents literature data on modern models of hyperlipidemia in the most frequently studied laboratory animals: mice, rats, and rabbits.

Keywords: hyperlipidemia, atherosclerosis, experimental models

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Гиперлипидемия и атеросклероз: экспериментальные модели

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РЕЗЮМЕ

Известно, что сердечно-сосудистые заболевания являются основной причиной смертности во всем мире, а главным патологическим процессом, определяющим их развитие, считается атеросклероз. Многочисленные исследования показали, что высокие уровни липопротеинов низкой плотности в крови представляют собой один из наиболее значимых факторов риска развития атеросклеротического поражения артерий. Для изучения атерогенного процесса применяются различные модели как мелких, так и крупных животных, в том числе генетически модифицированных – трансгенных и нокаутированных.

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Как правило, в исследованиях гиперлипидемии и атеросклероза часто используют сочетанное применение атерогенной диеты и генетических манипуляций. Ни одна из предложенных к настоящему времени моделей не является идеальной, поскольку каждая имеет свои преимущества и ограничения в воспроизведении профиля липопротеинов и степени атеросклеротического поражения сосудистой стенки. В связи с этим выбор адекватной модели важен для каждого конкретного исследования.

В настоящем обзоре приведены литературные данные о современных моделях гиперлипидемии на наиболее часто используемых лабораторных животных – мышах, крысах и кроликах.

Ключевые слова: гиперлипидемия, атеросклероз, экспериментальные модели

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INTRODUCTION

Hyperlipidemia is a pathological condition characterized by a significant increase in levels of blood cholesterol and triglycerides [1]. Chronically elevated blood cholesterol levels are a major risk factor for cardiovascular diseases, resulting in the development of atherosclerosis and exerting a negative effect on the myocardium, primarily due to increased oxidative stress, mitochondrial and endothelial dysfunction, as well as induction of inflammation and apoptosis [2, 3]. Hyperlipidemia is classified either as primary, also known as familial, developing due to genetic defects and having a characteristic abnormal lipid profile, or secondary, acquired as a result of cooccurring diseases (diabetes, nephrotic syndrome, hypothyroidism, liver disease, etc.), or following increased consumption of saturated fats [1]. Clinically, hyperlipidemia is characterized by increased levels of atherogenic lipoproteins in the blood: low-density lipoprotein (LDL) cholesterol, reflected in an increase in total cholesterol in the blood, and very low-density lipoprotein (VLDL) cholesterol, reflected in an increase in triglyceride levels in the blood. Another important factor contributing to atherogenesis is a decrease of anti-atherogenic high-density lipoproteins (HDL) in the blood [1, 4].

The action of hypolipidemic drugs is aimed at eliminating such disorders. Statins, known as HMG-CoA reductase inhibitors, are first-line drugs for reducing LDL cholesterol levels [5]. However, despite adequate statin therapy, patients remain at significant risk of atherosclerosis progression and, consequently, at risk of developing cardiovascular complications [6, 7]. Therefore, there is a need for new therapeutic agents to effectively reduce the level of atherogenic cholesterol.

An important role in studying the effectiveness of new lipid-lowering drugs is attributed to experimental modeling of hyperlipidemia and atherosclerosis in laboratory animals. The following species are used for this purpose: mice, rats, hamsters, guinea pigs, rabbits, monkeys, zebrafish, minipigs, and farm pigs [1, 8, 9]. Small animals, such as mice, rats, and rabbits, are often used due to the ease of breeding, low cost of maintenance, and a relatively short period of development of hypercholesterolemia and atherosclerosis [10]. However, none of the current models accurately simulates the human lipid profile or the progression of atherosclerosis, since each has its own advantages and disadvantages [4, 10, 11].

This review summarizes current knowledge about mouse, rat, and rabbit models of hyperlipidemia and atherosclerosis. The review is based on the analysis of experimental and review articles available in the PubMed, Google Scholar, and eLIBRARY.ru databases. Key search terms present in the title or abstract were: hyperlipidemia, hypercholesterolemia, cholesterol, atherosclerosis, experimental models, atherogenic diet, mice, rats, rabbits, ApoE, Ldlr, APOE*3-Leiden, APOE*3-Leiden.CETP, PCSK9, Fbn1, SR-B1, ApoB100, CETP, WHHL rabbits, and SMHL rabbits. The search resulted in 9,767 publications: 7,915 English-language and 1,852 Russian-language articles. When studying the abstracts, 65 English-language and 3 Russian-language publications containing data from experimental and

review articles available in full-text versions were selected and included in the review.

EXPERIMENTAL MODELS

Mice, rats, and rabbits are resistant to spontaneous development of hyperlipidemia, but atherogenic diet and genetic manipulations make these animals more susceptible to the development of hypercholesterolemia [1]. The serum lipid profile of experimental animals of different species differs – in mice and rats, a significant part of the total cholesterol is contained in antiatherogenic HDL, and in rabbits, total cholesterol is more evenly distributed between the lipoprotein fractions [4]. Additionally, rabbits are characterized by high activity of the Cholesteryl Ester Transfer Protein (CETP) in the plasma, while mice and rats lack it [12]. However, the advent of technologies for the creation of transgenic and knockout animals partially solves the problem of reproducing the main features of the human disease in animal models [10, 11]. In general, animal models of hyperlipidemia and atherosclerosis are based on a combination of an atherogenic diet and genetic manipulations [13].

MOUSE MODELS WITHOUT GENETIC MANIPULATION

One of the widely used methods for inducing hyperlipidemia in mice is long-term (3 weeks for the development of hypercholesterolemia and 12 weeks for the formation of an atherosclerotic plaque) use of a diet containing cholesterol (0.5–1.25%) with additional substances, such as cholic acid (0.1–0.5%), vegetable or coconut oil, as well as corn starch and sucrose [1, 4, 14, 15]. This model of hyperlipidemia varies in the ratio of ingredients in the diet [16]. Overfeeding mice exclusively with sucrose or fructose causes hypertriglyceridemia [1]. Among inbred mouse lines, C57BL/6 mice were more susceptible to hyperlipidemia and atherosclerosis [10, 17]. Regarding the sex of mice, it is recommended to include both sexes in studies on hyperlipidemia and atherosclerosis due to the influence of sex hormones on cholesterol levels [18]. However, in practice, most studies on atherosclerosis are conducted only on male mice aged 6–8 weeks [18].

GENETICALLY MODIFIED MOUSE MODELS

The rate of atherogenesis can be significantly accelerated in genetically modified mice when fed with a high-cholesterol diet, the variants of which are presented in Table 1 [10,16–18]. Most atherogenic diets contain varying percentages of saturated fat and cholesterol, with or without cholic acid. The most frequently used diets in research are the Western-type diet and its modified analogues with a high cholesterol content [16–18]. According to the literature, these diets increase the level of total cholesterol within 2–3 weeks and lead to the formation of atherosclerotic plaques in some species within 8 weeks [18]. As for the Paigen diet and a similar modified Western diet with cholic acid, they induce atherosclerosis, and severe pulmonary hypertension and inflammatory reactions often occur [16–18].

Table 1

Most Commonly Used Atherogenic Diets in Mouse Hyperlipidemia Studies

Diet name	Diet composition
Western-type diet	21% fat, 0.2% cholesterol, 34% sucrose [16]
Modified Western-type diet with high cholesterol	4.4% fat, 1.0% cholesterol [16]
Modified Western-type diet with high cholesterol	15.75% fat, 1.25% cholesterol [16]
Modified Western-type diet with high cholesterol	21% fat, 1.25% cholesterol, 34% sucrose [18]
Modified Western-type diet with high cholesterol	21% fat, 1.25% cholesterol [18]
Modified Western-type diet with high cholesterol	40% fat, 1.25% cholesterol [10]
Modified Western-type diet with high cholesterol	21% fat, 1.25% cholesterol, 0.5% cholic acid [18]
High-sucrose diet	20% fat, 65% sucrose [18]
Palm oil diet	10% palm oil, 0.1% cholesterol [16]
Semi-synthetic diets (low- and high-fat)	2–18 % fat, 0–1.25 % cholesterol [16]
Paigen diet	15% fat, 1.25% cholesterol, 0.5% cholic acid [17]

The most commonly used mouse models for studying hyperlipidemia and atherosclerosis are apolipoprotein E (*ApoE*$^{-/-}$) and LDL receptor (*Ldlr*$^{-/-}$) gene knockout mice. [19]. These two models, however, are not universal and have both advantages and disadvantages, depending on the objectives of the study, since they differ in lipid and glucose metabolism, as well as other mechanisms involved in atherogenesis [20]. ApoE is synthesized by hepatocytes and macrophages and has a number of important antiatherogenic functions: it is a ligand for LDL receptors and LDL-associated proteins, promoting the capture of atherogenic particles from the bloodstream [21]. Therefore, homozygous deletion of the *ApoE* gene in mice results in a pronounced increase in blood LDL and VLDL cholesterol levels [20, 21].

The main disadvantage of the complete absence of the ApoE protein is that the model is dominated by high blood cholesterol levels compared to *Ldlr*$^{-/-}$ mice and, as a result, they develop severe atherosclerotic lesions of the aorta within a few weeks [19]. Significantly reduced HDL cholesterol levels and altered HDL composition are observed in *ApoE*$^{-/-}$ mice compared to *Ldlr*$^{-/-}$ mice [10]. Another disadvantage of *ApoE*$^{-/-}$ mice is that most of the cholesterol in the plasma is VLDL cholesterol, compared to LDL cholesterol in humans [21].

Thus, a limitation of the use of *ApoE*$^{-/-}$ mice is that they do not have a lipid profile similar to that in humans, unlike *Ldlr*$^{-/-}$ mice [13]. *Ldlr*$^{-/-}$ is a model that reproduces familial hypercholesterolemia (in which there is a genetic mutation in the LDL receptors), however, its main drawback is a milder degree of hyperlipidemia [1, 21]. First- and second-generation statin therapy had no hypocholesterolemic effect on *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, in contrast to third-generation statins, which, in turn, were effective only in the context of a diet containing a relatively low amount of cholesterol (0.15%) [21].

Third-generation statins were shown to suppress atheromatous plaque development in *ApoE*$^{-/-}$ mice [18]. Administration of ezetimibe, which selectively inhibits intestinal cholesterol absorption, effectively reduced cholesterol levels in the VLDL and LDL fractions and increased HDL cholesterol levels, resulting in reduction of aortic atherosclerotic lesions in *ApoE*$^{-/-}$ mice fed with a diet containing 0.15% cholesterol [20, 21]. These results were consistent with clinical observations in humans. Acyl-coenzyme A:cholesterol acyltransferases inhibitor (avasimibe) reduced blood cholesterol levels and prevented atherosclerosis in *ApoE*$^{-/-}$ mice [20].

ApoE$^{-/-}$ and *Ldlr*$^{-/-}$ models are considered to be unsuitable for evaluation of some drugs (e.g. proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors), which can be explained by the fact that human PCSK9 stimulates liver lipogenesis and aggravates the progression of atherosclerosis through mechanisms dependent on ApoE and LDL receptors [10, 18]. It is also known that monoclonal antibodies to PCSK9 did not affect the level of total cholesterol and the degree of atherosclerotic lesions in *ApoE*$^{-/-}$ mice. However, such treatment reduced the level of total cholesterol and triglycerides and weakened the severity of atherosclerosis in another mouse model – *APOE*-3Leiden.CETP* [10].

The next model was the *ApoE*$^{-/-}$/ *Ldlr*$^{-/-}$ double knockout mouse, which is a model with more severe hyperlipidemia and atherosclerosis [13]. This mouse model is considered to be suitable for studying the effect of lipid-lowering drugs without the use of an atherogenic diet [13, 21]. As for the therapeutic effect, it is known that in these mice, the acyl-coenzyme A:cholesterol acyltransferase inhibitor did not reduce the level of cholesterol in the blood, but it did reduce the degree of atherosclerotic damage to the aorta [21].

Transgenic *APOE*3-Leiden* (*E3L*) mice were generated by introducing a construct containing the human *APOE*3-Leiden* gene sequence into C57Bl/6 mice [21]. This apolipoprotein is associated with a familial form of hyperlipidemia [13]. Compared to *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, *APOE*3-Leiden* mice develop moderate hyperlipidemia (*ApoE*$^{-/-}$ mice have severe hyperlipidemia, while *Ldlr*$^{-/-}$ mice have mild hyperlipidemia) [21]. The advantage of this model over *ApoE*$^{-/-}$ mice is the absence of an inflammatory reaction [13]. Statin therapy was found to have hypolipidemic and antiatherosclerotic effects in *APOE*3-Leiden* mice [10, 21]. Avasimibe also reduced cholesterol levels and the extent of atherosclerotic lesions [23].

Transgenic *APOE*3-Leiden.CETP* mice were generated by crossing *APOE*3-Leiden* mice with mice expressing CETP [21, 23]. This model exhibits elevated basal cholesterol levels and a human-like lipoprotein profile characterized by a shift from HDL to an increased VLDL/LDL fraction [22]. Thus, *APOE*-3Leiden.CETP* mice are a preferred model for studying lipid metabolism compared to *ApoE*$^{-/-}$, *Ldlr*$^{-/-}$, *ApoE*$^{-/-}$/*Ldlr*$^{-/-}$, and *APOE*3-Leiden* mice [23]. In addition, this model has well proven itself for assessing the hypolipidemic and antiatherosclerotic effects of drugs. In addition to statins, fibrates – PPARα (peroxisome proliferator-activated receptor alpha) agonists, PCSK9 inhibitors also demonstrated their efficacy [10, 24–29]. *APOE*-3Leiden.CETP* mice were a model of choice for evaluating the efficacy of PCSK9 monoclonal antibodies (alirocumab and evinacumab) in preclinical trials [26–29].

Various mouse models have been developed to study the effects of PCSK9 on lipid metabolism and atherosclerosis. PCSK9 plays an important regulatory role in cholesterol metabolism, due to the degradation of the LDL receptor [30]. Decreased LDL receptor levels de-intensify LDL metabolism, which can lead to hypercholesterolemia [30, 31]. Liver tissue is characterized by the highest level of PCSK9 expression in mice. However, it is also highly expressed in the intestine, and lower expression is observed in the kidneys, spleen, and aorta [31]. All plasma PCSK9 is secreted by the liver [31]. PCSK9 is known to be

involved in the development of atherosclerosis, and its inhibitors are now being used as new drugs to lower cholesterol levels [26–29, 31].

Therefore, some of the most popular models are those without germline editing that overexpress the human protein PCSK9 [10]. Overexpression of PCSK9, mediated by adeno-associated virus (*PCSK9-AAV*) induced hyperlipidemia (after 3 weeks) and atherosclerosis (after 12 weeks) in mice, when combined with an atherogenic diet (21% fat and 1.25% cholesterol) [30, 32, 33]. Phenotypically, this mouse model mimics *Ldlr*$^{-/-}$ mice [34]. Also, a transgenic mouse model (*hPCSK9tg)* expressing the human *PCSK9* gene was developed [35]. A study comparing the extent of aortic atherosclerosis in *hPCSK9tg/Ldlr*$^{-/-}$ and *hPCSK9tg/ApoE*$^{-/-}$ mice found that the latter had a larger area of aortic atherosclerosis and higher levels of total cholesterol and triglycerides in the blood [36]. Studies show that *hPCSK9tg* mice are well suited for screening various PCSK9 inhibitors (PKF8-mFc and evolocumab) [37]. In *PCSK9* knockout mice (*Pcsk9*$^{-/-}$), a 2–3-fold increase in the number of LDL receptors in the liver and very low levels of LDL cholesterol in the blood were reported [38, 39]. In these mice, plasma PCSK9 levels are undetectable, but LDL cholesterol levels are reduced by only 60%, suggesting a role of extrahepatic PCSK9 in their regulation [31, 37].

Mice with a mutation in the glycoprotein fibrillin-1 gene and knockout gene for the apolipoprotein E (*ApoE*$^{-/-}$*/Fbn1*$^{C1039G+/-}$) were also created [13]. Mutations in the *Fbn1* gene lead to Marfan syndrome, a genetic disorder characterized by fragmentation of elastic fibers [40]. This model was developed primarily to study unstable atheromatous plaques, with their subsequent rupture and associated complications. [13, 40]. It turned out that the area of atherosclerotic lesions in the aorta in *ApoE*$^{-/-}$*/Fbn1*$^{C1039G+/-}$ mice was 3 times larger than in *ApoE*$^{-/-}$ mice [40]. A limitation of this model is premature mortality of mice due to aortic aneurysm rupture. [13].

Another model for studying atherosclerotic plaque rupture is scavenger receptor class B type 1 (SR-B1) and apolipoprotein E (*SR-B1*$^{-/-}$*/ApoE*$^{-/-}$) knockout mice [10]. *SR-B1*$^{-/-}$*/ApoE*$^{-/-}$ mice developed severe coronary artery disease and obliterating coronary atherosclerosis even when fed with a standard diet [41]. The main limitation of this model is early mortality at 5–8 weeks of age [10, 41].

Transgenic mice expressing ApoB100 and lacking LDL receptor (*APOB100/Ldlr*$^{-/-}$) were developed to study hyperlipidemia and atherosclerosis. ApoB-100 is a component of VLDL and LDL which affects the uptake and subsequent degradation of LDL by the liver [42]. These mice, without the use of an atherogenic diet, showed a lipid profile very similar to that in humans, but their use is limited due to accompanying locomotor disorders, which makes these mice useful as a model of Alzheimer's disease [42].

Currently, the optimal models for studying the hypolipidemic and antiatherosclerotic effects of drugs are transgenic *APOE*3-Leiden.CETP* mice. To study different PCSK9 inhibitors, the *PCSK9-AAV*, *hPCSK9tg*, *hPCSK9tg/Ldlr*$^{-/-}$, and *hPCSK9tg/ApoE*$^{-/-}$ models are also common.

LIMITATIONS OF THE USE OF MICE IN HYPERLIPIDEMIA AND ATHEROSCLEROSIS STUDIES

Despite various genetic modifications and atherogenic diets, mouse models still have a number of shortcomings, primarily related to the distribution of the atheromatous plaque and the structure of the vascular wall [10]. Thus, the main site of atherosclerotic lesions in mice is the aortic sinus and the innominate artery, while in humans, it is the coronary and carotid arteries, as well as peripheral vessels [10, 43]. In mice, unlike humans, the arterial wall consists only of endothelium, without a layer of elastic connective tissue (subendothelium). In addition, the middle layer (tunica media) is less thick, and vasa vasorum is absent [44, 45]. Moreover, thrombotic lesions in the lumen of the vessel may not persist in mice, since the fibrinolytic balance is shifted towards lysis [43].

RAT MODELS WITHOUT GENETIC MANIPULATION

Currently, a number of diets have been proposed for the development of hyperlipidemia in Wistar and Sprague Dawley rats. They are presented in Table 2 [4, 10, 46–49]. Notably, the most commonly used protocol for inducing hypercholesterolemia, as in mice, was the addition of 1.25% cholesterol, 21% fat, and 34% sucrose to the animals' diet for 2–3 weeks to develop hyperlipidemia and for 8–12 weeks to develop mild atherosclerotic lesions in the aorta [46–49]. Also, for the induction of hyperlipidemia, intraperitoneal administration of Tween-80 or poloxamer 407 is possible, which leads to a rapid increase in the level of lipids in the blood, especially triglycerides. However, after a single administration, lipid levels decrease on day 5 [4, 50]. With regard to the development of an atheromatous plaque, it has long been believed that

rats are immune to the development of atherosclerosis if they are fed with an atherogenic diet exclusively [10]. For this purpose, vitamin D2, promoting aortic lipidosis, was sometimes added to the diet [4].

A disadvantage of this model for studying the therapeutic effect is the abnormal response to some drugs, such as statins. Instead of decreasing the activity of hepatic HMG-CoA reductase, reductase, statins significantly increase it, resulting in the lack of hypolipidemic effects [51]. Overall, there is currently no compelling evidence that rat models may have advantages over mouse ones [10].

Table 2

Most Commonly Used Atherogenic Diets in Rat Hyperlipidemia Studies

Diet name	Diet composition
Western-type diet	21% fat, 0.2% cholesterol, 34% sucrose [47]
Modified Western-type diet with high cholesterol	2% cholesterol, 0.2% cholic acid [46]
Modified Western-type diet with high cholesterol	21% fat, 1.25% cholesterol, 34% sucrose [47]
Modified Western-type diet with high cholesterol	21% fat, 1.25% cholesterol [48]
Modified Western-type diet with sucrose, cholic acid, and propylthiouracil	0.5% cholesterol, 0.2% cholic acid, 5% sucrose, 0.05% propylthiouracil [46]
High-cholesterol diet	1% cholesterol [46]
Modified Western-type diet with high cholesterol	3% cholesterol, 0.5% cholic acid, 1.5% vegetable oil [49]
High-cholesterol and cholic acid diet	2.43% cholesterol, 0.49% cholic acid [46]
Modified Western-type diet with high cholesterol, cholic acid, and propylthiouracil	3% cholesterol, 0.2% cholic acid, 0.5% propylthiouracil, 10% fat [46]
High-fat diet	33.5% fat, 1.5% soybean oil [46]
Modified Western-type diet with high cholesterol	1% cholesterol, 2% coconut oil [46]
High-cholesterol and cholic acid diet	2% cholesterol, 0.25% cholic acid [46]
High-cholesterol diet with cholic acid and propylthiouracil	4% cholesterol, 1% cholic acid, 0.5% propylthiouracil [46]
Modified Western-type diet with high cholesterol	12.5% palm oil, 12.5% fat, 5% cholesterol, 2% cholic acid [46]
High-cholesterol diet	2% cholesterol [49]
High-fat diet	60% fat [46]
High-fat diet	42% fat [46]
High-fat diet	33.5% fat, 1.5% soybean oil [46]
Modified Western-type diet with fat and sucrose	10% fat, 20% sucrose, 2% cholesterol, 1% cholic acid [46]
High-cholesterol diet	6% cholesterol [46]
High-cholesterol and cholic acid diet	2% cholesterol, 0.5% cholic acid [46]
Thomas-Hartroft diet	40% oil, 5% cholesterol, and 5% sodium cholate [10]
Paigen diet	15% fat, 1.25% cholesterol, 0.5% cholic acid [46]
High-fat, vitamin D, and nicotine diet	20% fat, vitamin D3 300 000 IU/kg/day, nicotine 25 mg/kg/day [4]
High-sucrose diet	20% fat, 65% sucrose [47]

The Prague hereditary hypercholesterolemic (PHHC) rat is a rat line obtained by crossing with Wistar rats. It models hypercholesterolemia on an atherogenic diet [1]. In this line, most of the cholesterol is VLDL cholesterol [1, 52]. However, despite the presence of hypercholesterolemia, PHHC rats do not develop atherosclerosis even after receiving a 2% cholesterol diet for 6 months [51].

GENETICALLY MODIFIED RAT MODELS

In order to study hyperlipidemia and atherosclerosis in rats, similar methods to mouse models were employed, i.e. knockout of the apolipoprotein E gene (*ApoE*$^{-/-}$) and LDL receptors (*Ldlr*$^{-/-}$), as well as double knockout of the *ApoE*$^{-/-}$/*Ldlr*$^{-/-}$ genes [10]. For the formation of the atheromatous plaque, *ApoE*$^{-/-}$ и *Ldlr*$^{-/-}$ rats needed a diet with a high fat content (42%), but even under these conditions, aortic damage was insignificant [10, 53, 54]. *ApoE*$^{-/-}$/*Ldlr*$^{-/-}$ rats showed significant atherosclerotic lesions in the aorta only after a long time (48 weeks) [10, 54]. Additionally, a more pronounced degree of hypercholesterolemia was observed in models with double knockouts [55].

Thus, it turned out that the formation of atherosclerotic lesions in *ApoE*$^{-/-}$, *Ldlr*$^{-/-}$, and *ApoE*$^{-/-}$/*Ldlr*$^{-/-}$ rats requires a much longer period of time and a diet with a higher fat content, compared to mice [10]. A less common model of hypercholesterolemia is the rat overexpressing CETP protein (*hCETPtg*), which develops severe atherosclerotic lesions in the aorta but shows significantly high mortality [1].

LIMITATIONS OF USING RATS IN HYPERLIPIDEMIA AND ATHEROSCLEROSIS STUDIES

Rats, even genetically modified, were found to be more resistant to the development of the atheromatous plaque due to their low susceptibility to endothelial inflammation caused by hyperlipidemia [56].

RABBIT MODELS WITHOUT GENETIC MANIPULATION

Rabbits are often used as an experimental animal model to study the atherosclerotic process because their lipid metabolism is more similar to that of humans than of mouse and rat [4, 12, 57]. Also, rabbits are highly sensitive to a cholesterol diet, due to which they quickly develop severe hypercholesterolemia, leading to severe aortic atherosclerosis [12]. However, recently there has been a trend towards a decrease in the use of this animal model, probably due to the availability of genetically modified mice [13, 58].

Currently, the following types of rabbit models are used: rabbits on an atherogenic diet; rabbits with Watanabe hereditary hyperlipidemia and St. Thomas mixed hyperlipidemia; and genetically modified rabbits [59]. In rabbits fed with an atherogenic diet, more than 90% of cholesterol is VLDL and LDL cholesterol [12]. Since female rabbits have higher blood cholesterol levels than male rabbits, these characteristics make males much more commonly used for studies of hyperlipidemia and atherosclerosis [12, 51].

New Zealand White rabbits are often used to study hyperlipidemia and atherosclerosis [13, 58]. For this purpose, various variants of the atherogenic diet have been developed, presented in Table 3 [4, 13, 58]. However, when fed with a diet containing more than 1% cholesterol for a long time (more than 4 weeks), rabbits develop high hypercholesterolemia and severe atherosclerotic lesions exceeding those seen in humans, so a diet with cholesterol in the range of 0.3–0.5% is recommended [58]. Also, it is recommended to use vegetable oils (3–8% soybean, coconut, or corn oil) for 8 weeks to form hyperlipidemia and for 16 weeks to form the atheromatous plaque [58, 59]. A cholesterol-free diet enriched with casein may also cause hypercholesterolemia and atherosclerosis in rabbits [60]. It is believed that a possible mechanism of hypercholesterolemia in this case is associated with a decrease in the synthesis of bile acids and the excretion of fecal sterols, which leads to an increase in the level of total cholesterol and LDL [60]. It is worth noting that rabbits fed with casein developed less severe aortic atherosclerosis than rabbits receiving a cholesterol diet [58, 60].

Table 3

Most Commonly Used Atherogenic Diets in Hyperlipidemia Studies in Rabbits

Diet name	Diet composition
Atherogenic diet for rabbits	3–8% soybean or corn oil, 0.3–0.5% cholesterol [13]
Atherogenic diet for rabbits	3–8% soybean or corn oil, 1.0–1.5% cholesterol [58]
Cholesterol-free diet containing casein	27% casein [60]

Compared with hypercholesterolemia and atherosclerosis in humans, rabbits fed with an atherogenic diet show a number of differences. For example, the main lipoproteins are not LDL but VLDL, and there are large differences in blood lipid levels and the extent of atherosclerotic lesions due to individual differences in response to the cholesterol diet [61–63].

Watanabe heritable hyperlipidemic (WHHL) rabbits have a genetic mutation in the gene encoding the LDL receptor, leading to high blood cholesterol levels when fed with a normal diet, resembling human familial hypercholesterolemia [63]. In experimental studies of hyperlipidemia and atherosclerosis, the advantages of using WHHL rabbits compared to rabbits fed with an atherogenic diet include:

1) the lipid profile of WHHL rabbits is characterized by high LDL and low HDL levels, whereas the major lipoproteins in rabbits fed with an atherogenic diet are VLDL and LDL, while HDL levels usually do not change;

2) hypercholesterolemia is constantly present in all homozygous WHHL rabbits on a normal diet, and variations in plasma total cholesterol levels and lipoprotein ratios are small compared to rabbits fed with a special atherogenic diet;

3) in WHHL rabbits, atherosclerotic lesions have a pattern similar to the same stage of atherosclerosis in humans;

4) in WHHL rabbits, coronary atherosclerosis and myocardial infarction are often observed, which corresponds to clinical manifestations in humans [61, 62].

Thus, the WHHL rabbits are particularly suitable for studies aimed at developing lipid-lowering drugs.

St. Thomas mixed hyperlipidemic (SMHL) rabbits receiving a normal diet have elevated total

cholesterol levels, normal LDL levels, and normal or elevated blood triglyceride levels [59, 60]. When fed with a low-cholesterol diet, SMHL rabbits develop hyperlipidemia associated with excess hepatic apoB production and characterized by high levels of LDL and VLDL [60]. This rabbit model is rarely used in research [59].

When evaluating the effects of lipid-lowering drugs in New Zealand rabbits receiving an atherogenic diet and WHHL rabbits, such drugs as statins, ezetimibe, and evolocumab were effective [50, 59, 64–66]. Fibrates (PPARα agonists) significantly reduced plasma triglyceride levels in both humans and rodents, but this effect was absent or weakly pronounced in rabbits [60]. CETP inhibitors (torcetrapib, dalcetrapib, anacetrapib, and evacetrapib) in rabbits on an atherogenic diet showed a strong atheroprotective effect and significantly increased HDL levels [60].

Thus, although WHHL rabbits are advantageous for assessing the lipid profile and the extent of atherosclerotic lesions, rabbits on an atherogenic diet can also be used to assess the lipid-lowering and anti-atherosclerotic activity of drugs.

GENETICALLY MODIFIED RABBIT MODELS

Advances in genetic engineering have made it possible to create genetically modified rabbits to study the pathophysiological features of the atherosclerotic process, which may be useful in studying the effectiveness of new lipid-lowering drugs [41]. Thus, transgenic rabbits have been used to study cardiovascular diseases and lipoprotein metabolism over the past two decades [67, 68]. However, after the creation of rabbits with knockout genes, they began to be used as models of hyperlipidemia and atherosclerosis [68].

Transgenes expressed in rabbits can be broadly divided into three categories: 1) proteins that directly bind to lipoproteins, such as apo: apoAI, apoAII, apoB-100, apoCIII, apoE; 2) enzymes that participate in lipid metabolism: hepatic lipase, lipoprotein lipase, phospholipid transfer protein (PLTP), catalytic polypeptide, lecithin-cholesterol acyltransferase (LCAT); 3) proteins that may participate in the pathogenesis of atherosclerosis: matrix metalloproteinase-12 (MMP-12), 15-lipoxygenase (ALOX15), C-reactive protein, and vascular endothelial growth factor (VEGF). [58, 67].

Currently, the most widely used models for studying hyperlipidemia and atherosclerosis are rabbits with knockout of the apolipoprotein E gene (*ApoE*$^{-/-}$) and LDL receptors (*Ldlr*$^{-/-}$), as well as models with double knockout of the *ApoE*$^{-/-}$/*Ldlr*$^{-/-}$ genes [40, 68]. Thus, *ApoE*$^{-/-}$ rabbits demonstrated a mild degree of hyperlipidemia on a standard diet, and when fed with an atherogenic diet (0.3% cholesterol and 3% soybean oil), they developed severe hyperlipidemia (within 2 weeks) and atherosclerotic aortic lesions (within 10 weeks) [13, 68]. Compared to WHHL rabbits, *ApoE*$^{-/-}$ rabbits did not show changes in HDL cholesterol levels, which is a drawback of this model [69]. *Ldlr*$^{-/-}$ rabbits at 3 months of age had a 20-fold increase in total blood cholesterol and a 35-fold increase in LDL cholesterol compared to rabbits receiving an atherogenic diet [68]. They also had elevated triglyceride levels and reduced HDL cholesterol levels [68]. *ApoE*$^{-/-}$/*Ldlr*$^{-/-}$ double knockout rabbits do not require an atherogenic diet to develop severe hyperlipidemia [68, 69].

LIMITATIONS OF USING RABBITS IN HYPERLIPIDEMIA AND ATHEROSCLEROSIS STUDIES

Limitations of using rabbits are related to the anatomical and physiological features of atheromatous plaque formation. Thus, atherosclerosis develops predominantly in the arch and thoracic part of the aorta, with minimal lesions in the abdominal part, and coronary atherosclerosis is usually limited to the left coronary arteries [13]. In addition, rabbits, especially non-pedigreed ones, can respond differently to an atherogenic diet and not develop pronounced hyperlipidemia even on a high-cholesterol diet [58]. To minimize differences, rabbits can be pre-examined by feeding them with a cholesterol diet for a short period of time, and then only the rabbits that showed high levels of lipoproteins in the blood can be selected [60].

CONCLUSION

Taking into account the constant increase in life expectancy and the spread of the Western-type diet in the population, the treatment of hyperlipidemia and the prevention of atherosclerotic lesions are an urgent tasks. Currently, many models of experimental animals and variants of atherogenic diets have been proposed for the induction of hypercholesterolemia.

The most common animals for creating hyperlipidemia and atherosclerosis are mice, rats, and rabbits. Rodent models are characterized by a short life cycle, high reproduction rate, and ease of research manipulations, which makes them a convenient model for studying hypercholesterolemia. It is worth noting that various genetic manipulations with

rodents made it possible to overcome the significant difference in the lipid profiles of humans and rodents. In terms of lipoprotein metabolism, rabbits are superior to mice and rats due to their similarity in the development of pathology to humans, but rabbit hyperlipidemia and atherosclerosis models also have their limitations.

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Sensitization to Food Allergens in the Context of Atopic Comorbidity

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ABSTRACT

The lecture considers a place of food allergy in the profile of allergic and, in particular, atopic diseases and its features, distinguishing this pathology from all other allergies. Three classes of food allergens are characterized, and sensitization to them involving cells and regulatory molecules, such as neurotransmitters, neuropeptides, cytokines, and others mediators, is described in detail.

At the current level of science, the mechanisms of oral tolerance and the causes of its breakdown are considered, resulting in clinical manifestations of food allergies, characterized by high polymorphism and complexity of diagnosis. Not only is a high rate of comorbidity of food allergies emphasized, but also its exceptional risks are pinpointed in terms of the development of anaphylactic shock, which is a difficult issue to explain in nutrition and digestion. The final part of the lecture is devoted to current and future therapeutic interventions in this pathology.

Keywords: food allergens, sensitization, comorbidity, anaphylaxis, oral tolerance

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Пищевая сенсибилизация в аспекте атопической коморбидности

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РЕЗЮМЕ

В лекции рассматриваются вопросы места пищевой аллергии в структуре аллергических и, в частности, атопических болезней, ее особенностей, отличающих данную форму патологии от всех других аллергий. Дается характеристика пищевых аллергенов трех классов и детальное описание процесса сенсибилизации к ним с участием клеток и регуляторных молекул: нейротрансмиттеров, нейропептидов, цитокинов и др.

На современном уровне рассмотрены механизмы оральной толерантности и причины ее срыва, что приводит к клинической манифестации пищевой аллергии, характеризующейся высокой полиморфностью и сложностью диагностики. Подчеркивается не только высокая коморбидность пищевой аллергии, но и ее исключительные риски в аспекте развития анафилактического шока, что является трудно объяснимым вопросом в феноменологии питания и пищеварения. В заключительной части лекции рассматриваются перспективы терапевтической интервенции при данной патологии.

Ключевые слова: пищевые аллергены, сенсибилизация, коморбидность, анафилаксия, оральная толерантность

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Food intake is a complex daily physiological process, which is crucial for the existence and functioning of the human body. Therefore, any nutrition–related problems are unacceptable for good health and well-being and the work of all organs and systems, posing a threat to human life as well as an obstacle to the evolution of the species. Unfortunately, modern people often face food allergies [1], and almost half of humanity faces food intolerance – another form of pathology that is also associated with food problems, but is not related to the immune system [2].

In recent years, it has been shown that the extent to which the immune system is involved in the pathogenesis of food allergies varies in different patients. In this regard, several endotypes [3] were described: dominant immunoglobulin (Ig) E-mediated

(with high involvement of T helper 2 cells (Th2) in the pathogenesis), IgE-mediated (with low involvement of Th2 cells), and non-IgE-mediated – independent of the immune system. Special rare phenotypes are also identified, for example, alpha-gal syndrome (mammalian meat allergy).

According to various estimates, a steady increase in the incidence of food allergies and other atopies has been seen lately [4, 5]. At the same time, evolution has formed mechanisms of oral tolerance that resist the manifestations of food allergies [6]. In particular, dietary fibers should be currently added to the list of essential nutritional ingredients, which play the key role in the development of tolerogenic cells, that are crucial for maintaining oral tolerance. The sources of this component are both proper nutrition itself and the functioning of the beneficial gut microbiota [7, 8]. Although food allergies belong to the category of atopic diseases and syndromes, the development of oral tolerance mechanisms has provided this pathology with a number of features that other atopies lack: the absence of delineated subtypes of food allergies, an episodic course, a threat of severe anaphylaxis [6]. On the other hand, food allergies are characterized by pronounced atopic comorbidity [9].

The aim of the lecture was to analyze current views on food sensitization, the mechanisms of its development, and treatment approaches.

FOOD ALLERGIES AND SENSITIZATION TO THEM

Not all food proteins are allergens; therefore, to distinguish between allergen-containing and allergen-free foods, an allergenicity criterion is used (Table 1) [1, 10, 11]. Table 1 demonstrates, that allergenicity depends on many factors, including the structure and physicochemical properties of the allergen itself, as well as the influence of cofactors and the host immune system. Critical factors are the atopic constitution [6], disruption of gut barrier integrity [12, 13], and depleted tolerogenic gut microbiota [14–16].

Table 1

Allergenicity of Food Allergens [1]

Allergen-dependent factors	Biogenic cofactors	Factors of the immune system
Primary amino acid sequence in allergen epitopes	Presence of molecular patterns and adjuvants in food	Genetic predisposition to atopy
Molecular weight lower than 70 kDa		Disruption of oro-gastrointestinal epithelial barrier
Small isoelectric point (charge), low hydrophobicity, solubility in water		Route of exposure
Peculiarity of allergen molecule fold, and epitope proximity to one other		Depleted tolerogenic gut microbiota
Abundance in food		Insufficiency of sIgA
High stability and resistance to the extremes of food processing, as well as to digestive enzymes		

Among the large number of food allergens, eight stand out, which are called the “Big Eight.” The Big Eight shows the strongest allergenicity and causes up to 90% of all food allergy reactions. With age, the child outgrows allergies to cow’s milk, chicken’s egg, and wheat, acquiring oral tolerance [17, 18]. However, peanut-, fish-, shrimp-, and soy-specific memory cells tend to remain for life with a high degree of probability of anaphylactic shock. However, there is no definite answer to the question why some people develop shock when consuming a causal food allergen, while others do not [19–21]. Numerous studies have been conducted, including genetic, epigenetic, transcriptomic, and proteomic ones, which have failed to identify accurate biomarkers associated with a high risk of anaphylaxis in the target groups of patients.

There is another classification, which distinguishes three classes of food allergens [17, 22, 23]. Class 1 allergens are highly allergenic, and some of them (cow’s milk, chicken’s egg, and peanuts) are part of the Big Eight. They cause sensitization through the gastrointestinal tract and display severe clinical signs. Class 2 allergens (for example, apple, carrot, melon, and other vegetables and fruits) are cross-reactive dietary allergens with aeroallergens that trigger sensitization through the unified airway and cause an oral allergy syndrome that mimics seasonal pollen allergies. Class 3 allergens include small proteins weighing up to 10 kDa, dietary supplements, and

colorants (e.g. tartrazine), which cause sensitization through the unified airway or skin and frequently result in occupational allergies. Thus, class 2 and 3 allergens are comorbidity factors, while class 1 allergens may be life-threatening in some patients.

Food sensitization begins when a food allergen enters the body through one or more routes: 1) oral, 2) respiratory, and 3) cutaneous [17]. Another, rarer route is described – the urogenital one [6]. Following the penetration of the food allergen via any of these routes, a classic Th2-dependent IgE response develops, which involves antigen-presenting dendritic cells (DC), T helper 2 cells (Th2), and group 2 and group 3 innate lymphoid cells (ILC2 and ILC3). Subsequently, allergic inflammation develops, in which mast cells and eosinophils act as the main inflammatory cells (Fig,).

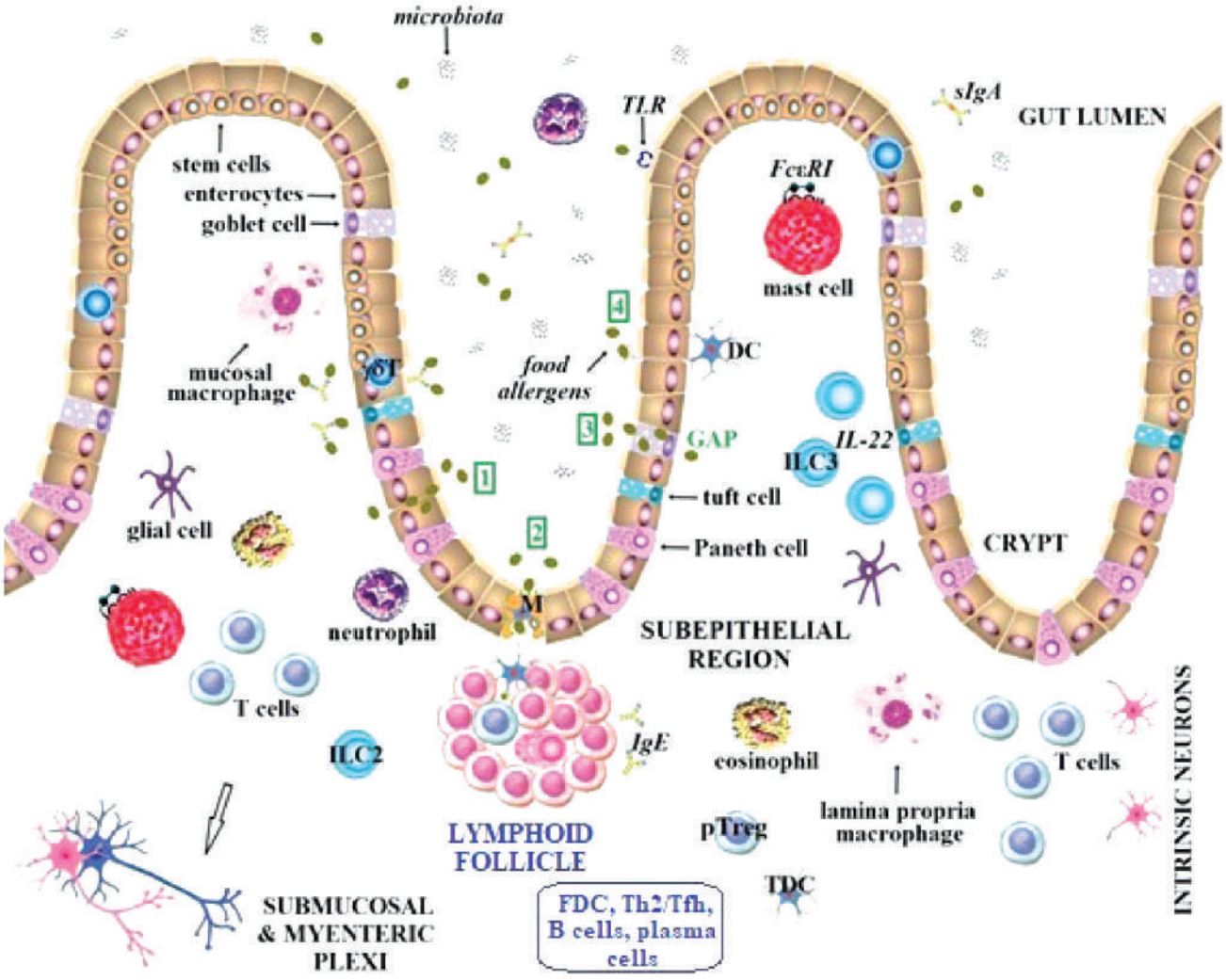

Fig. Food allergen sensitization

The gut epithelium landscape is currently revised due to a new transcriptomic technology, the single-cell RNA-sequencing. Absorptive enterocytes in the small intestine and colonocytes in the large intestine are prevalent cell lineages. In total, the gut epithelium consists of epitheliocytes and stem cells, and many interepithelial cells perform the main function to protect the subepithelial region and internal environment against invaders and allergens. However, under certain conditions, food allergens can penetrate the epithelial barrier using one or some of four routes: (1) due to impaired epithelium integrity or leak; (2) via specialized M cells; (3) by GAP; and (4) due to uptake by long dendrites of DC.

GAP – goblet cell-associated allergen passage, DC – dendritic cell, Th2 – type 2 helper T cell, Tfh – T follicular helper cell, FDC – follicular dendritic cell, ILC2 and ILC3 – group 2 and group 3 innate lymphoid cells, TDC – tolerogenic dendritic cell, pTreg – peripheral regulatory T cell, TLR – Toll-like receptors

According to the Fig., there are four possibilities for allergens to penetrate the intestinal epithelium [12]. Following the penetration, food allergens get to the submucosal layer filled with various cells of the immune system. Activated epithelium produces special alarmin cytokines [24]: interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP), stimulating ILC2, DC, and Th2 cells. Activated ILC2 secrete IL-5, IL-9, and IL-13 acting on mast cells and eosinophils. Food allergens are phagocytosed by DC, processed, and presented to Th2, which upregulate a B cell-driven Th2 response with IgE production and memory B and T cell formation. This process occurs in lymphoid follicles – Peyer's patches [25]. Th2 produce IL-4, IL-5, IL-9, IL-13, and IL-33 and stimulate allergic inflammation. IL-33 is considered as a major maturation factor for mast cells [19]. T follicular helper (Tfh) cells secrete IL-21, IL-4, and IL-13 that are important for plasma cell maturation, switching to IgE synthesis, and affinity growth [6]. Tolerogenic neurotransmitters and neuropeptides of the enteric nervous system, TDCs, and peripheral regulatory T cells (pTreg) do not allow food allergens to break allergen tolerance, however, if this occurs, especially with repeated ingestion of food allergens, food allergy manifests clinically. Its symptoms are usually very polymorphic, which makes accurate diagnosis difficult.

ORAL TOLERANCE

Oral tolerance is an essential result of evolution which mitigates threats to modern civilization associated with current changes in the nature of nutrition [1]. Table 2 shows the main mechanisms for maintaining allergen (oral) tolerance.

Table 2

Cellular and Molecular Mechanisms of Oral Tolerance

Mechanisms	References
Tolerogenic dendritic cells, including $CD103^+$	[26, 27]
Peripheral allergen-specific $FoxP3^+$ pTreg cells	[28, 29]
Subsets originated from pTreg: Tr1, Th3, and Tr1-like cells	[6, 30–32]
Regulatory B (Breg) cells and blocking antibodies	[33]
Type M2a (alternatively activated) macrophages localized close to the gut epithelium, in Peyer's patches, and lamina propria	[6, 26, 34]
Protolerogenic cytokines: IL-10, transforming growth factor (TGF) β, IL-35, and IL-27	[6, 35, 36]
Coinhibitory molecules	[37]
Protolerogenic neurotransmitters and neuropeptides	[6, 38, 39]
Tolerogenic gut microbiota	[14–16, 40, 41]

End of table 2

Note. FoxP3 is a master transcription factor of pTreg.

Oral tolerance depends on daily consumption of dietary proteins, the dynamic gut microbiota, changing influence of neurotransmitters and neuropeptides, and continuous traffic of proinflammatory cells and molecules. An important positive role is attributed to a specialized subpopulation of $CD103^+$ TDC, acting in the intestines and mesenteric lymph nodes with the involvement of heterodimeric integrin αEβ7 [27, 42].

In general, risk factors, especially for children, can be genetic predisposition to atopy, epigenetic modifications, and environmental effects [4, 43]. Among them, caesarean birth of an infant, exposure to domestic and farm animals, smoking of parents, air pollution, and insufficient care are of great importance [43]. Disrupted integrity of the epithelial barrier in the oral mucosa may predispose to food allergies, at least in profilin-mediated allergic reactions to peanuts, kiwi, celery, melon, etc. Histological signs of progressive remodeling (increased acanthosis, angiogenesis, and high-density collagen fibers) were found in the oral mucosa of patients. These histological features are comparable to those described in gingivitis and periodontal disease [44].

On the whole, oral tolerance and its loss are the result of a complex interaction between allergens in food, the microbiota that inhabits the gut, and the profile of immune and non-immune cells in gut-associated lymphoid tissue (GALT) and specialized neuromolecules found in the enteric nervous system.

FOOD ALLERGY AND ATOPIC COMORBIDITY

It has been proven that all atopic diseases and syndromes have a common basis – the atopic constitution [6], which can be characterized as a polygenic condition associated with an evolutionary dead-end – hyperproduction of IgE on proteins found in the genome of ancient mites (ectoparasites) on the skin of humans who lived about one million years

ago. This understanding resulted from genetic studies of mites of the *Dermatophagoides* genus, which are the main source of modern allergens, and their closest relatives who live a parasitic lifestyle on mammals and birds (*Psoroptidia* mites) [45, 46].

Food allergies often coexist with other atopic diseases: allergic rhinitis, allergic asthma, and atopic dermatitis. Children sensitized to food allergens are two to four times more likely to have asthma, particularly poorly controlled asthma [47, 48]. Consumption of snails by patients allergic to *Dermatophagoides* mites can exacerbate the course of severe asthma, while aeroallergens, such as wheat, fish, and seafood, can lead to so-called food-induced asthma [49, 50]. Children who are cosensitized to food and aeroallergens suffer from more severe clinical signs of allergic rhinitis [51]. In addition, exposure to airborne food particles during air travel can cause asthma attacks in predisposed patients [52].

Research has found that children with atopic dermatitis are six times more likely to develop food allergies than their healthy peers. In addition, the risk of developing IgE-mediated food allergies is about 40% in children with moderate-to-severe atopic dermatitis. It is known that allergy-free diets cannot cure atopic dermatitis, but may have adverse effects, such as nutritional deficiencies, slow body growth in childhood, and reduced quality of life [53]. In most cases, immune tolerance to causative allergens is restored due to allergen-specific immunotherapy, which results in prolonged remission of the disease. However, in some patients, tolerance is not established, and the disease may progress in the form of eczema, lichenification, and reactivation of secondary bacterial, fungal, and viral infections.

Given high comorbidity and a threat of fatal anaphylactic shock in food allergies, developing drugs for allergen-specific immunotherapy remains urgent. The first such drug for the treatment of peanut allergy was developed, passed all stages of clinical trials, and was approved [54]. This is Palforzia®. Alternative technologies are being developed for the establishment of immune tolerance in exclusively breastfed children from an early age by early introduction of a potentially dangerous product [12, 55], which is based on the fundamental theory of immune tolerance [56]. For example, after about 24 months of oral immunotherapy with hen's egg, 75% of children were able to tolerate a cumulative 5 g of an egg [5]. However, a fully effective result has not been attained [57, 58].

Currently studies on biologics in food allergies and other atopies are being carried out. In particular, phase II clinical trials showed the efficacy of lebrikizumab, a high-affinity IL-13 inhibitor based on monoclonal antibodies, in moderate-to-severe atopic dermatitis in adults [59]. However, we have to admit that new approaches to intervention in food allergies have not emerged in the last 57 years.

CONCLUSION

The steady increase in the prevalence of allergic atopic diseases and syndromes is now becoming as pressing as such problems as global warming, the threat of famine, wars, and new pandemics, because food allergies are a challenge to human civilization and our species [6].

On the one hand, the gastrointestinal tract was created by evolution as a zone of tolerance, but on the other hand, it is here that the most severe and fatal forms of allergies can originate – food anaphylactic shock [60]. Other food allergy-related problems should be mentioned [6]: significant difficulties in accurate diagnosis, high-risk diagnostic procedures, broad differential diagnosis due to the polymorphism of symptoms, and harder to achieve therapeutic efficacy compared to other atopies.

The enteric nervous system in the gastrointestinal tract produces many neutrotransmitters and neuropeptides [61, 62] and contains the largest microbial community in the body [17]. It makes it one of the control centers in the human body that interacts with the brain via the gut – brain axis [63]. It should be recognized that this system has not yet been sufficiently studied, and its value in terms of function and phenomenology is not fully understood.

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Author contribution

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Modern Ideas about the Mechanisms of Intermittent Hypoxia Hyperoxia Training and the Possibility of its Use in Cardiovascular Diseases (Literature Review)

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ABSTRACT

Intermittent hypoxia–hyperoxia therapy with individually dosed gas levels (ReOxy therapy) is a modification of the long-known method of intermittent normobaric hypoxia training. Currently, ReOxy therapy can be considered as an addition to physical training in programs of cardiological rehabilitation, primary and secondary prevention of a wide range of cardiovascular diseases, as well as an alternative to physical exercises if it is impossible to perform them.

This review examines the pathogenetic rationale, differences from traditional intermittent hypoxia training, and clinical prospects for the use of intermittent hypoxia–hyperoxia therapy in cardiovascular diseases.

Keywords: hypoxia–hyperoxia therapy, cardiovascular diseases, ReOxy therapy, rehabilitation

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Современные представления о механизмах гипоксически-гипероксических тренировок и возможности их применения при сердечно-сосудистых заболеваниях (обзор литературы)

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РЕЗЮМЕ

Интервальная гипокси-гиперокситерапия с персонализированным дозированием нагрузки (ре-окси терапия) является результатом совершенствования давно известного метода интервальных нормобарических гипоксических тренировок. В настоящее время ре-окси терапия может рассматриваться как дополнение к физическим тренировкам в программах кардиологической реабилитации, первичной и вторичной профилактики многочисленного спектра сердечно-сосудистых заболеваний, а также в качестве альтернативы физическим тренировкам при невозможности их выполнения.

В обзоре рассматриваются патогенетическое обоснование, отличия от традиционного метода гипоксически-нормоксического воздействия и клинические перспективы применения метода интервальной гипокси-гиперокситерапии при сердечно-сосудистых заболеваниях.

Ключевые слова: гипоксически-гипероксические тренировки, сердечно-сосудистые заболевания, ре-окси терапия, реабилитация

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The issues of treatment, prevention, and rehabilitation after cardiovascular diseases remain important in modern medicine. Various methods of non-drug therapies are a key component of secondary prevention and cardiac rehabilitation programs aimed at restoring physical and social wellness, improving the quality of life in the short term, and reducing the risk of recurrent cardiovascular events, while increasing life expectancy in the long term [1].

The most heavily researched and proven method of cardiac rehabilitation is physical training (PT). The indications for PT have evolved over time from increasing exercise tolerance to the main method of secondary prevention (evidence level IA) [2, 3]. The evidence base for PT as a method of cardiac rehabilitation is based on the study of the mechanisms that increase physical tolerance and improve adaptation to ischemia, as well as on the proven long-term effect of reducing the risk of adverse cardiovascular events. However, the implementation of rehabilitation programs based on PT can be complicated.

The main problem is related to low adherence of patients to PT. Recent studies have revealed that only 18% of patients are compliant with physical rehabilitation programs [4]. Moreover, current advances in the treatment of cardiovascular diseases have led to a change in the profile of a patient. The number of patients with heart failure, multifocal atherosclerosis, who underwent heart surgery, and elderly patients with severe concomitant pathology, including musculoskeletal disorders, has increased, which significantly lowers their active participation in physical rehabilitation programs [5]. These facts indicate the need to find new effective and safe methods of cardiac rehabilitation as an addition or alternative to PT with a similar mechanism of action and comparable clinical and prognostic efficacy.

As such, researchers are engaged in the study of hypoxia training which has been used to increase the endurance of healthy individuals and later became a method of prevention and rehabilitation after various diseases in which hypoxia plays a key role [6–12]. The results of experimental and clinical studies showed that enhanced exercise tolerance can be observed upon exposing a patient to short episodes of hypoxia that do not exceed the physiological threshold. This resulted in the development of intermittent hypoxia training (IHT) [9, 10]. IHT consists in alternating inspiration of hypoxic gas mixture and normal air for an average of 5 minutes for 20-40 minutes [6]. Intermittent hypoxia–hyperoxia therapy with individually dosed

gas levels (IHHT, ReOxy therapy) is the improvement of the long-known IHT method.

Aim. To substantiate the use of IHHT in the rehabilitation of patients with cardiovascular diseases based on the analysis of the best practices of using IHT in cardiology and the mechanisms of its therapeutic effect.

Methods. The authors carried out the analysis of publications in eLIBRARY.ru and PubMed databases, published from 2014 to 2024, using the following keywords: hypoxic effects, intermittent hypoxic training, hypoxia–hyperoxia therapy, ReOxy therapy, pathophysiological mechanisms of hypoxia, hypoxic preconditioning, and cardiac rehabilitation. The inclusion criteria were the results of randomized trials, systematic reviews, and original articles including a control group.

MECHANISMS OF EFFECTS OF HYPOXIA TRAINING

A number of studies have shown that hypoxia increases body adaptive capabilities to hypoxic conditions by activating numerous pathophysiological mechanisms, including modulation of sympathoadrenal reactivity, chemoreceptor sensitivity, anaerobic and aerobic energy production pathways, increased tissue resistance to hypoxia, activation of antioxidant defense systems, and many others [7, 13–17].

Essentially, IHT is one of the methods of hypoxic preconditioning that consists of short episodes of hypoxia. Such training increases the activity of the antioxidant system and trains the metabolic mitochondrial systems of cells, which subsequently prevents structural and functional damage to tissues, including the heart and brain, in severe or acute hypoxia [9, 18]. In terms of the action and end result, IHT is very similar to PT, triggering numerous hematological and non-hematological adaptation mechanisms [13–17, 19].

Currently, changes in the rate of reactive oxygen species (ROS) generation and redox signaling in the form of intracellular and intercellular electron transport chains that provide a balance between oxidative stress and antioxidant defense in the aerobic body are considered as the main mechanism through which therapeutic, modifying, and pathological effects of hypoxia are implemented [20, 21]. ROS, including free radicals and hydrogen peroxide, are generated in all major biological aerobic systems and play an important role in regulating vital processes and forming a cellular response to external stimuli. During hypoxia, a specific regulatory protein, the hypoxia-inducible factor (HIF), is activated through ROS, which plays a key role in body adaptation to hypoxia.

Currently, three types of HIF have been discovered: HIF-1 (several subtypes), HIF-2, and HIF-3. While HIF-1 and HIF-2 regulate multidirectional processes, HIF-3 is possibly associated with negative effects of hypoxia. HIF-1α increases the expression of more than 100 genes necessary for survival in conditions of oxygen deficiency, including those that activate endothelium-dependent vasodilation, angiogenesis and angioedema, energy metabolism, mitochondrial metabolism, cell division processes, erythropoiesis, iron metabolism, and many others [22, 23]. Following IHT, increased expression of HIF-1α initiates metabolic processes necessary for regeneration of damaged myocardium and improvement of cardiac functions after episodes of ischemia [23].

In 2017, the understanding of the pathophysiology of hypoxia was expanded by C.W. Pugh and P. J. Ratcliffe. They discovered that cells have a unique mechanism of direct perception of oxygen fluctuations in the blood, in which an oxygen-sensitive signal ultimately increases the level of HIF-1α through the catalytic action of a number of 2-oxoglutarate-dependent oxygenases [24]. Further in-depth studies of the pathophysiological mechanisms and the boundary between adaptive and pathological effects of hypoxia have continued to this day. Their findings will help us understand the effects of different IHT and IHHT regimens depending on the severity, duration, and intermittent regimen of hypoxia [25].

FEATURES AND MECHANISMS OF HYPOXIA – HYPEROXIA TRAINING EFFECTS

Biofeedback-controlled IHHT represents the next step in the development of IHT. ReOxy therapy is a unique treatment that has two fundamental differences compared to IHT: 1) the principle of hypoxia–hyperoxia is used instead of hypoxia–normoxia; 2) individual selection of a hypoxia regimen is based on a hypoxic test and collected biofeedback [26].

ReOxy therapy involves changing the oxygen concentration in the gas mixture (ranging from 10 to 40% O_2 max) under the control of blood oxygen saturation using pulse oximetry. The addition of hyperoxia episodes (up to 40% oxygen in the gas mixture) during the reoxygenation period makes it possible to increase the dose of oxygen free radicals without increasing hypoxia and is the main difference from the IHT method. Moreover, the hypoxia–

hyperoxia paradox serves as the physiological basis for the combination of hypoxia and hyperoxia episodes in one procedure. Cells perceive fluctuations in the concentration of free oxygen (not its absolute level) as hypoxia.

Thus, repetitive intermittent hyperoxia can induce many molecular cascades and cellular mechanisms that are normally induced by hypoxia. Hyperoxic stimuli activate angiogenesis, mitogenesis, oxygen consumption efficiency, and metabolic activity in different tissues in the same way as hypoxia, but without adverse consequences [11].

A number of studies have shown that ReOxy therapy provides more significant activation of the antioxidant enzyme system in response to a moderate increase in the concentration of free radicals compared to conventional IHT. As a result, the body tissue resistance to hypoxia is more pronounced and achieved faster [11, 27].

Personalized hypoxic load chosen after a hypoxic test and biofeedback are other important differences between ReOxy therapy and conventional IHT. The selection of an adequate hypoxic and hypoxemic load within one IHHT course is based on an individual physiological response (SpO_2 level and heart rate) to a hypoxic stimulus. It ensures high effectiveness and safety of the procedure. Biological feedback is collected by constant monitoring of the degree of oxygen saturation in the blood, which allows specialists to maintain an individually adjusted level of hypoxemia.

BEST PRACTICES AND PROSPECTS OF REOXY THERAPY IN CARDIOVASCULAR DISEASES

The safety and effectiveness of IHHT in various cardiovascular diseases has been actively studied. Studies highlighting the effects of ReOxy therapy in arterial hypertension [26], metabolic syndrome [12, 28], acute and chronic coronary artery disease, including after coronary bypass surgery [2, 29, 30], in elderly patients and patients with comorbidities [31–33], and chronic heart failure (CHF) have been already published [34]. All these studies have proven the safety of the method and demonstrated the positive effect of IHHT on exercise tolerance [29, 30], lipid and carbohydrate metabolism [12, 28], cognitive functions [34], blood pressure levels and normalization of its daily profile [26], and reduction of depression [26, 31].

After IHHT, patients with hypertension present with blood pressure stabilization and a significant systolic blood pressure decrease by 15–17% compared to baseline [12, 26, 29]. ReOxy therapy in patients with coronary heart disease improves physical tolerance and lipid profile [7, 17, 29]. Moreover, there is evidence that IHHT impacts all pathological components of the metabolic syndrome: it mediates weight loss due to a decrease in fat mass, increases physical endurance in obese patients, lowers blood pressure, glucose levels, cholesterol, triglycerides, and low-density lipoproteins [12, 28]. The findings indicate that ReOxy therapy is an effective way of reducing cardiovascular risk factors in patients with metabolic syndrome, even in the absence of physical training [28].

In addition to the clinical and metabolic effects of IHHT, there is evidence of a positive effect of IHHT on myocardial remodeling and biochemical markers in blood plasma, which explains the mechanisms of the positive cardiological effects of ReOxy therapy [29, 30]. Thus, arranging an IHHT course for patients with myocardial infarction at the inpatient stage of rehabilitation helped increase exercise tolerance, significantly decrease the volume of the left ventricle, and increase left ventricular ejection fraction by 12% compared to the controls ($p < 0.05$) [30]. In another study, patients with stable coronary heart disease presented with an improvement in physical tolerance, an increase in hemoglobin levels, a decrease in cardiovascular reactions, and an increase in oxygen saturation during exercise [29]. Patients with hypertension exhibited normalization of blood pressure during ReOxy therapy due to increased levels of nitric oxide and HIF-1 in plasma [26]. Studies focusing on patients with CHF indicate an increase in cardiopulmonary reserve due to IHHT, which was confirmed by cardiopulmonary stress test results, and a decrease in biochemical prognostic markers, such as sodium uretic peptide, tumor necrosis factor (TNF) α, and homocysteine, thus making this method quite promising for management of patients with chronic heart failure [34].

In patients with metabolic syndrome, IHHT contributed to an improvement in the proinflammatory status by lowering the level of CRP and heat shock protein (Hsp70) [28]. Some studies show that moderate IHHT protocols enhance the innate immune system, while simultaneously exerting an overall anti-inflammatory effect by suppressing TNFα and IL-4 by more than 90% [30].

All abovementioned studies emphasize safety of

the method ensured by an individual hypoxic test and biofeedback and indicate the possibility of studying its effects in patients with severe coronary artery disease after surgical interventions on the heart and blood vessels and in the postoperative period, in patients with low left ventricular ejection fraction and acute decompensated heart failure.

In addition, ReOxy therapy has a great potential in neurology as a method of rehabilitation after ischemic stroke, transient cerebral circulation arrest, vascular dementia, Alzheimer`s disease, chronic progressive cerebral circulation insufficiency, and rehabilitation after spinal cord injuries. Presumably, this is due to mechanisms that are involved in influencing neurophysiological biomarkers and electrophysiological properties of the neural network, and not only due to the mechanisms that are involved in myocardial pathology [35–38].

CONCLUSION

According to the results of the conducted studies, IHHT proved to be a very promising method of cardiac rehabilitation, as an alternative to PT and as an addition to it, aimed at increasing exercise tolerance, reducing symptoms and improving the functional status of angina pectoris and CHF, as well as improving myocardial perfusion and metabolic status. ReOxy therapy has a clinical and pathophysiological potential to optimize long-term prognosis in cardiovascular diseases. However, to confirm this, further studies are required.

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Trends in Precision Diagnosis and Monitoring of Inflammatory Bowel Diseases: the Potential of Proteomic and Metabolomic Biomarkers

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ABSTRACT

Omics technologies, including proteomics and metabolomics approaches, provide promising opportunities to improve the accuracy of diagnosis and monitoring of the course of inflammatory bowel disease (IBD). Integration of these advanced research areas into clinical medicine not only allows for a more in-depth assessment of the pathogenesis of IBD, but also opens avenues for innovative therapeutic strategies adapted to individual patient profiles and patient cohorts.

The lecture analyzes trends in the identification of biomarkers with high sensitivity and specificity that can be used both for diagnosis and prognosis of the course of IBD subtypes, and for predicting the response to therapy, which, ultimately, will contribute not only to improved treatment outcomes, but also to an increase in the quality of life of patients.

The authors conducted a non-systematic, descriptive review of the literature with a search depth of 10 years, aimed at systematizing data on the achievements of proteomics and metabolomics approaches for the diagnosis, monitoring of the IBD course, and personalization of therapeutic strategies. The search for literary references was carried out using Scopus, Web of Science, MedLine, the Cochrane Library, EMBASE, Global Health, CyberLeninka, and RSCI databases.

The analysis of the results of experimental and clinical studies allowed to identify a number of biomarkers – candidates for testing and potential implementation in routine clinical practice. Convincing data were obtained on the potential benefits of integrating proteomics and metabolomics studies with other omics approaches. The importance of an interdisciplinary approach combining the results of clinical studies with modern approaches in bioinformatics and molecular biology for the development of more effective diagnostic tools and strategies is obvious.

Keywords: inflammatory bowel disease, Crohn's disease, ulcerative colitis, omics technologies, metabolome, proteome

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Тренды прецизионной диагностики и мониторинга воспалительных заболеваний кишечника: потенциал протеомных и метаболомных биомаркеров

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РЕЗЮМЕ

Омиксные технологии, в том числе протеомные и метаболомные подходы, предоставляют многообещающие возможности для повышения точности диагностики и мониторинга течения воспалительных заболеваний кишечника (ВЗК). Интеграция этих передовых направлений исследований в клиническую медицину не только позволяет более углубленно оценить патогенез ВЗК, но и открывает путь к инновационным терапевтическим стратегиям, адаптированным к индивидуальным профилям пациентов и когорт пациентов.

Лекция посвящена анализу трендов выявления биомаркеров с высокой чувствительностью и специфичностью, которые могут быть использованы как для диагностики и предикции течения подтипов ВЗК, так и прогнозировать ответную реакцию на терапию, что в конечном итоге будет способствовать не только улучшению результатов лечения, но и повышению качества жизни пациентов.

Авторами проведен несистематический, описательный поиск литературы с глубиной 10 лет, направленный на систематизацию данных о достижениях подходов протеомики и метаболомики для целей диагностики, мониторинга течения ВЗК и персонализации терапевтических стратегий. Поиск литературных источников проводился по базам данных Scopus, Web of Science, MedLine, The Cochrane Library, EMBASE, Global Health, CyberLeninka, РИНЦ.

Анализ результатов экспериментальных и клинических исследований позволил выделить ряд биомаркеров – кандидатов для тестирования и потенциального внедрения в рутинную клиническую практику. Получены убедительные данные о потенциальных преимуществах интеграции протеомных и метаболомных исследований с другими омиксными подходами. Очевидна значимость междисциплинарного подхода, объединяющего результаты клинических исследований, современные подходы биоинформатики и молекулярной биологии для разработки более эффективных диагностических инструментов и стратегий.

Ключевые слова: воспалительные заболевания кишечника, болезнь Крона, язвенный колит, омиксные технологии, метаболом, протеом

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Searching for disease biomarkers dates back to the formation of medicine as a science. The search for biomarkers in inflammatory bowel disease (IBD) is of no exception (Fig. 1). Precision medicine and diagnostic approaches associated with targeting interventions are becoming new hotspots and trends in modern medicine (Fig. 1). At the early stage of diagnostic research in IBD, the focus was placed on general characteristics and classical diagnostic approaches. Currently, the trend in research is increasingly shifting toward targeting IBD therapy and improving the quality of life of patients [1].

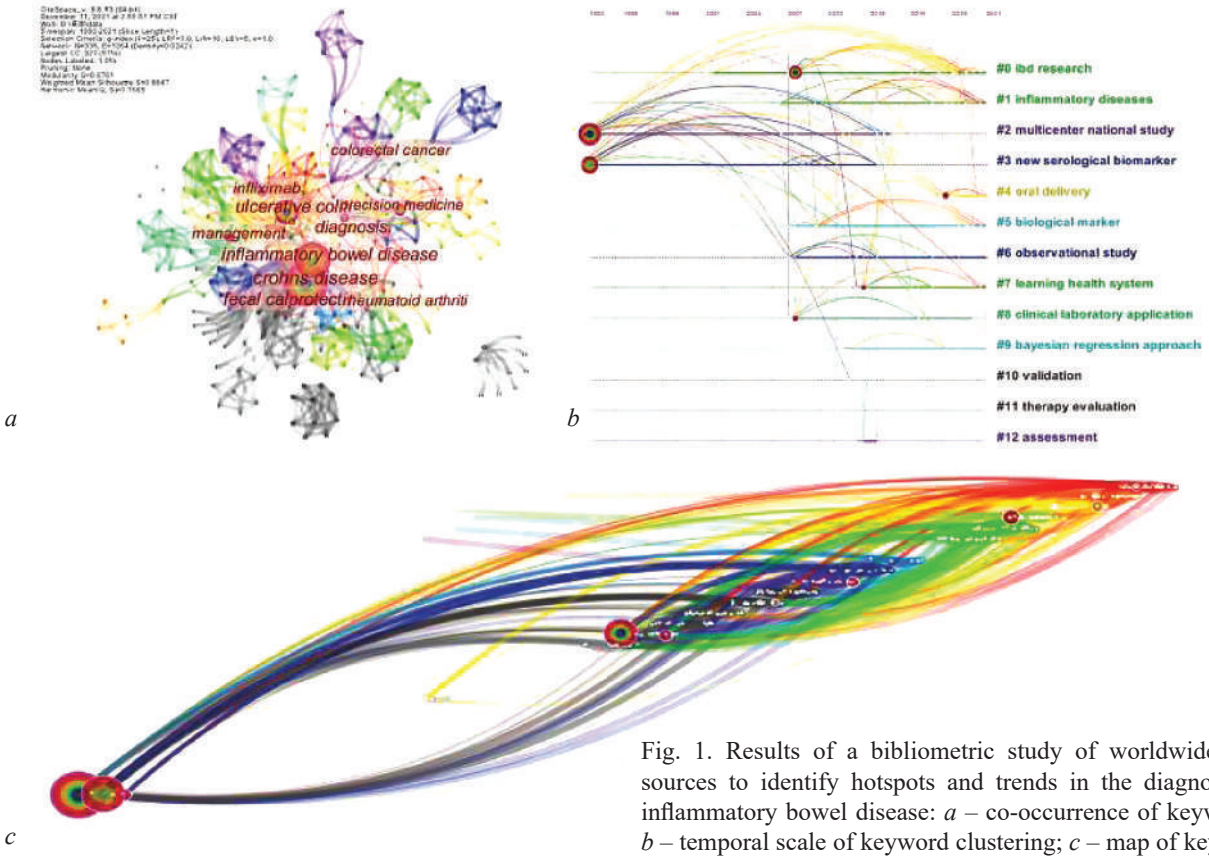

Fig. 1. Results of a bibliometric study of worldwide data sources to identify hotspots and trends in the diagnosis of inflammatory bowel disease: *a* – co-occurrence of keywords; *b* – temporal scale of keyword clustering; *c* – map of keyword time zones in the literature on accurate diagnosis and treatment of inflammatory bowel disease [1]

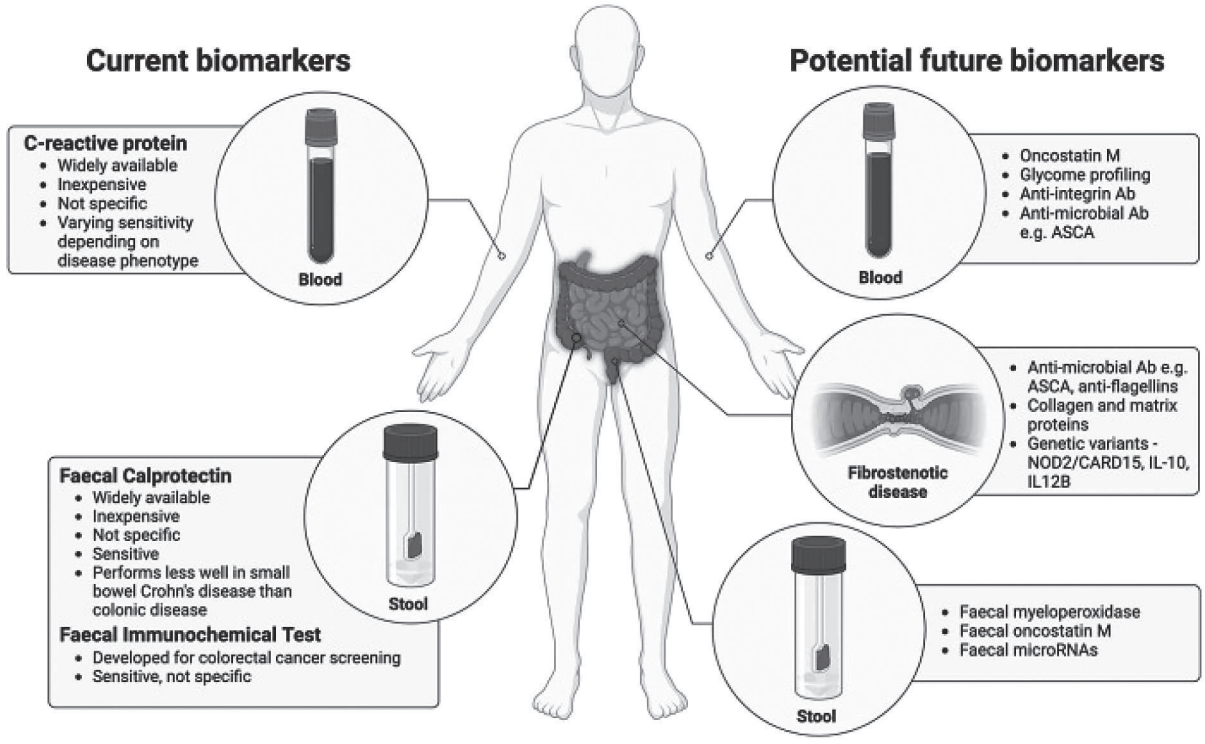

Fig. 2. Current and potential biomarkers in inflammatory bowel disease, adapted from [3]

Some of the most common keyword search markers for the diagnosis and management in IBD are C-reactive protein (CRP) and fecal calprotectin. However, they are obviously not true biomarkers of IBD, as they merely reflect the presence of inflammation and its severity, but are not specific, and changes in the values of these indicators are typical of many other conditions. They have relatively low sensitivity and specificity in patients with IBD [2]. Similarly, the evaluation of serologic biomarkers is of uncertain value in predicting disease progression or a response to treatment.

Today, omics biomarkers provide significant additional advantages for the diagnosis and management in IBD, including Crohn's disease (CD) and ulcerative colitis (UC) (Fig. 2). The introduction of omics technologies, the identification of genomic, proteomic, and metabolomic markers, and the in-depth assessment of the intestinal microbiome allow not only to assess the probability of disease development, but also to provide in-depth and comprehensive evaluation of the molecular basis and pathogenesis in IBD. Early diagnosis and understanding of the pathogenesis of CD and UC are extremely valuable for the choice of reasonable personalized pathogen-specific therapy in a variety of clinical manifestations.

Among omics biomarkers, the results of proteomics and metabolomics studies attract the attention of researchers and clinicians. Their significance as promising tools for the diagnosis, management, and control of IBD therapy in modern personalized and precision medicine is undoubted.

Proteomics and metabolomics are among the most dynamically developing areas of molecular diagnosis. The undeniable advantage of these approaches is the possibility of non-invasive assessment of a significant number of indicators.

A proteomics analysis has already identified some candidate IBD biomarkers for testing in clinical practice, such as oncostatin M and αvβ6 antibodies [4]. In addition, proteomics approaches have been actively used for identifying stool protein and peptide biomarker panels in patients at risk of IBD and in treatment strategy adjustment [5].

Metabolomic profiling also allows to differentiate IBD patients from healthy individuals and to identify CD and UC with high accuracy. Such metabolites as tryptophan and indole-3-acetic acid have been identified as potential biomarkers in IBD, with ROC curves showing high discriminatory power (AUC: 0.9738 for CD and 0.9887 for UC) [6]. Data from metabolomics studies also identify a number of biomarkers with a potential diagnostic value [7]. Simultaneuosly, , the integration of metabolomics data with other potential molecular biomarkers, such as lipidomics, can be used as an additional diagnostic advantage in IBD [8].

This work focuses on analyzing the results of current omics studies evaluating proteomic and metabolomic indices to identify potential biomarkers for the diagnosis, monitoring, and potential assessment of the response to therapy in patients with IBD.

OMICS BIOMARKER POTENTIAL

Currently, the study of omics biomarker potential and the integration of various omics data in IBD has focused on three areas of interest: identification of new diagnostic proteomic biomarkers, in-depth characteristics of disease pathogenesis, and response to treatment.

Proteomic and metabolomic biomarkers provide a holistic view of the disease, identifying molecular networks and pathways involved in the IBD pathogenesis (Fig. 3). This approach significantly helps to develop prognostic criteria for early detection of the disease and monitoring of clinical outcomes [9].

Proteome Analysis

Considering the presence of proven strong correlations between the level of protein expression and disease activity, proteomics attracts special attention as a diagnostic tool [10]. At the same time, a current trend in proteomics is the formation of diagnostic panels for the most accurate CD and UC signatures.

Diagnostic and Monitoring Capabilities

It is obvious that the diversity of clinical manifestations and insufficient sensitivity and specificity of existing biomarkers indicate the special significance of potential biomarkers for the differential diagnosis of IBD. Proteomics studies allow to differentiate IBD and other intestinal diseases with high sensitivity and specificity [11]. Proteomics approaches in IBD were first used in the works of U. Berndt et al. The studies revealed differences in protein expression by different T cell populations in CD and UC [12]. This experimental approach demonstrated high sensitivity (70%) and specificity (72.5%) in CD.

The results of the study on MMP-12 and oncostatin M are of great interest. They allowed to effectively differentiate IBD from other intestinal diseases [13].

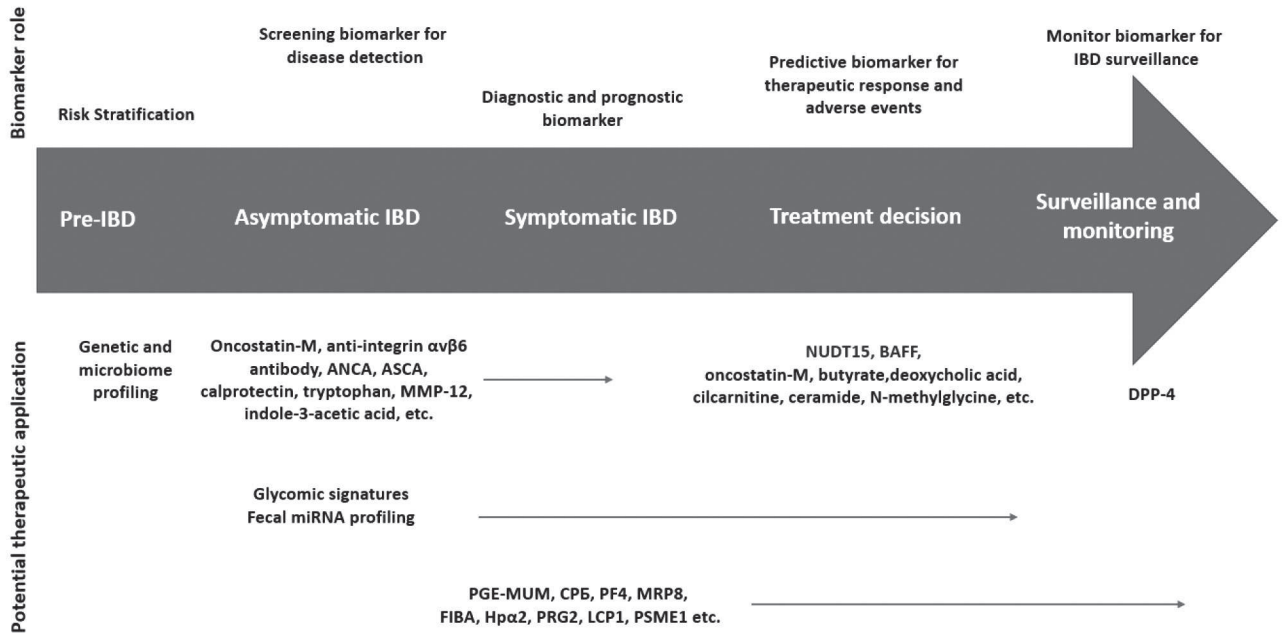

Fig. 3. Potential roles of biomarkers in IBD, examples of existing and new biomarkers that could perform these functions, adapted from [3]

Serum antibodies to αvβ6 integrin determined in the blood serum are now considered as another promising biomarker in IBD and, especially, UC [14]. The loss of intestinal epithelial barrier integrity precedes clinical manifestations of the disease, which explains the possibility of detecting antibodies to αvβ6 in the preclinical period of IBD, as well as the possible association of the level of antibodies to αvβ6 with the severity of the disease course, which is potentially prognostically significant.

Another candidate for application in clinical practice is PGE-MUM, which is determined in urine and correlates with endoscopic and histologic activity in IBD, especially in UC. PGE-MUM has a great diagnostic potential because of its better correlation with endoscopic parameters compared to CRP. In addition, threshold values for PGE-MUM were proposed to predict endoscopic and histologic activity with a reported sensitivity of 81–82% [15].

A significant trend in molecular diagnosis of IBD is the formation of diagnostic panels to evaluate protein expression in biological material of different types. Thus, four most diagnostically significant protein biomarkers were identified in serum: platelet factor 4 (PF4), calgranulin A (MRP8), fibrinogen A (α-chain) (FIBA), and haptoglobin alpha-2 (Hpα2). Hpα2 was particularly significant in differentiating UC and CD with accuracy similar to or higher than that in ANCA and ASCA serologic tests [16]. The analysis of colonic mucosal tissue samples from adult and pediatric patients allowed to form two candidate protein panels [17].

These panels were effective in the diagnosis of IBD and the differential diagnosis of CD and UC, respectively. The diagnostic panel included fatty acid binding protein 5 (FABP5), uridine diphosphate-α-d-glucose-6-dehydrogenase (UGDH), leucine-rich mitochondrial protein containing PPR motifs (LRPPRC), visfatin/NAMPT, and inorganic pyrophosphatase 1 (PPA1). Elevated levels of NAMPT and PPA1 were particularly significant in IBD. The differential diagnostic panel included mitochondrial trifunctional enzyme subunit beta (HADHB), cytosol aminopeptidase (LAP3), leukotriene-A-4 hydrolase (LTA4H), metallothionein-2 (MT2A), mitochondrial tricarboxylate transport protein (SLC25A1), heterogeneous nuclear ribonucleoprotein H3 (HNRNPH3), mitochondrial delta(3,5)-delta(2,4)-dienoyl-CoA isomerase (ECH1), transferrin receptor protein 1 (TFRC), beta-2-microglobulin (B2M), SEC61 transmembrane channel complex protein, subunit alpha 1 (SEC61A1), staphylococcal nuclease domain-containing protein 1 (SND1), , and transferrin (TF). The first nine proteins of the panel were significantly elevated in CD compared to UC patients. Thus, they can be considered as candidates for an

in-depth evaluation in the differential diagnosis of CD and UC regardless of the age of patients. In the analysis of colonic biopsy proteome, there are three newly identified biomarkers, including eosinophil major basic protein (PRG2), laminin 2 (LCP1), and proteasome activator complex subunit 1 (PSME1), that are clearly associated with active CD [18].

It is of particular interest that many biomarkers are mainly components of fatty acid metabolism [17]. This allows to consider the prospect of a possible combination of proteomics studies with the assessment of the lipid profile in IBD patients.

The proteome analysis also suggests the pathogenetic significance of mitochondrial dysfunction in the development of IBD, especially UC [19]. Decreased expression of eight mitochondrial proteins (ATP synthase subunit beta (ATP5B), mitochondrial malate dehydrogenase 2 (MDH2), heat shock protein 90 (HSPA9B), voltage-dependent anion-selective channel protein 1 (VDAC1), peroxiredoxin 1 (PRDX1), heat shock protein 60 (HSPD1), peroxiredoxin 2 (PRDX2), and prohibitin (PHB)), was particularly significant in UC. The key protein of mitochondrial complex, PHB, was decreased in biopsy specimens of colonic mucosa in UC both in remission and relapse. This allows to suggest possible early mitochondrial changes during disease formation.

B cell-activating factor (BAFF), a member of the tumor necrosis factor (TNF) superfamily, attracts special attention among proteomic biomarkers. It is produced by most cells of the innate and adaptive immunity and is of great importance for immune regulation and inflammatory changes in the intestine in IBD [20]. In IBD, BAFF levels are elevated in serum, feces, and colonic tissues and are associated with inflammation in the intestinal mucosa [21]. Pathogenetically relevant BAFF overexpression, in turn, exacerbates the proinflammatory activity of immune cells in IBD, including through the NF-κB signaling pathway and the NLRP3 inflammasome [22]. These data allow us to consider BAFF as a candidate biomarker for monitoring the course of IBD, including in the context of therapy.

Potential for Personalized Therapy and Assessment of a Treatment Response

In addition to diagnostic biomarkers, proteomics approaches can potentially be used to assess the response of IBD patients to ongoing therapy. For example, an elevated serum BAFF level, mentioned in the previous section, was initially associated with a better response to infliximab treatment in CD patients. Those with a clinical response to infliximab treatment showed a decrease in its levels after treatment, whereas those who did not respond to therapy showed an increase in the parameter [23]. In addition, specific single nucleotide polymorphisms (SNPs) in the BAFF gene, such as rs1041569, have been associated with CD susceptibility and a response to treatment [24].

The potential of BAFF blockade is now considered as one of the therapeutic strategies. It has been shown in experimental models that BAFF blockade reduces the severity of inflammation, weight loss, and histopathologic damage in colitis [25, 26]. Thus, BAFF may not only be a potential diagnostic and therapeutically predictive biomarker, but also may be considered as one of the targets for the IBD treatment.

The dynamic assessment of circulating chemokine levels and the assessment of monocyte activation have also been used as candidate biomarkers for the response to treatment with TNF inhibitors, particularly infliximab. Within 2 weeks after the initiation of therapy in patients without a clinical response, there was an obvious decrease in the level of protein from CD14+/CD86+ macrophages and the level of the chemokine CCL2 [27].

In another study, proteomics approaches were used for the response management of infliximab and prednisolone therapy in IBD children. The study proposed a candidate panel with 18 proteins and 3 microRNAs [28].

Thus, the potential of the proteomics data obtained allows to consider this approach as promising for the differential diagnosis of IBD, research on IBD pathogenesis, as well as monitoring and prediction of the treatment response. At the same time, the results of the proteome analysis in a number of cases demonstrate associative links with other areas of omics diagnosis, such as lipidomics, which allows to speak about the possibility of more in-depth studies in IBD.

Metabolomic Biomarkers

Metabolomic biomarkers have also become promising tools for diagnosing and evaluating the response to treatment of IBD, including CD and UC. Metabolomic biomarkers can be used not only to identify pathogenetic features of the disease and, as expected, diagnostic targets, but can potentially guide therapeutic decisions. Metabolomics is increasingly being used to identify biomarkers to predict a treatment response and to distinguish IBD subtypes.

Diagnostic Potential

Today, metabolomic biomarkers are used in the differential diagnosis of IBD subtypes and to identify key differences between IBD patients and healthy persons. Serum and plasma, feces, and urine are considered as the main biological samples for metabolomic biomarkers. Thus, a group of five serum metabolites – pyruvate, phenylacetylglutamine, isolithocholic acid, taurodeoxycholic acid, and glycolithocholic acid – showed high accuracy (AUC = 0.861) in the differential diagnosis between CD and UC groups. High diagnostic accuracy rates allow us to consider them as a non-invasive diagnostic alternative to the tests used in routine clinical practice [29].

Serum metabolomics studies have demonstrated an increase in tryptophan and indole-3-acetic acid levels in both CD and UC patients, while kynurenine and indole-3-propionic acid levels were elevated only in CD [6]. A study by T.Vakhitov et al. identified 14 serum metabolites, including 2-hydroxybutyric acid and creatinine, as potential biomarkers of UC [30]. Other plasma metabolites – acylcarnitine, 3-indoleacetic acid, and dehydroepiandrosterone sulfate – were associated with intestinal microbiota and immune response formation. They were highlighted as candidate markers for further in-depth analysis [31].

The analysis and identification of fecal metabolites are also being used to develop metabolic profiles of individuals with IBD. Among 78 metabolites identified by L. Ning et al. all, metabolites were classified into three major categories of nutrient metabolism, including amino acids, carbohydrates, and fatty acids [7]. According to the results, the increase in the levels of amino acids, such as tryptophan, glutamine, arginine, 5-hydroxytryptophan, and histidine, was worth noting. These data go in line with the previous results [32]. Various organic acids related to the tricarboxylic acid cycle, such as pyruvic acid, fumaric acid, malonic acid, and oxoglutaric acid, were elevated in the feces of patients with IBD, indicating abnormal energy metabolism of the intestinal microbiota.

In addition to the possibility of using fecal metabolites to identify IBD, it is also possible to perform differential diagnosis of their subtypes. For example, significant changes in fecal metabolome profiles have been described in patients with UC and CD. The metabolic signature of fecal IBD includes alterations in short-chain fatty acids, tryptophan metabolites, sphingolipids, and vitamin levels. Although there is a considerable overlap between the metabolic signatures of the two subtypes of IBD, CD is primarily characterized by enrichment of primary bile acids, whereas UC is characterized by higher levels of proteolytic fermentation products [33].

The analysis of metabolomic pathways is also of particular importance for the formation of diagnostic strategies in IBD. Studies have demonstrated that glyoxylate and dicarboxylate metabolism, alanine, aspartate and glutamate metabolism, as well as glycerolipid metabolism in patients with IBD are associated with disease activity and can be used in the differential diagnosis of IBD subtypes [6]. A decrease in the ratio of primary and secondary bile acids in IBD compared to healthy individuals is worth noting. The metabolomics analysis also associated metabolism of beta-alanine, arginine, and proline with IBD, while glycerolipid metabolism in UC and CD differed significantly [6].

Correlations between IBD activity and changes in amino acid metabolism and β-oxidation of fatty acids have been described [34]. Amino acids, such as L-glutamine, glycine, and L-arginine, have been shown to support intestinal redox balance and immune homeostasis and can potentially alleviate the severity of IBD symptoms.

In addition, the metabolomics analysis also confirms the significance of the relationships between amino acids and various signaling pathways, including mTOR and NF-kB, involved in the implementation of inflammatory responses. These pathways play an important role in regulating the balance of pro-inflammatory and anti-inflammatory cytokines in the gut [35]. Alterations in fatty acid metabolism, especially polyunsaturated fatty acids (PUFAs), are also closely associated with IBD. Changes in PUFA ω-6 and ω-3 levels correlate with inflammatory markers, suggesting their role in modulating inflammation in IBD [36]. An approach involving the evaluation of ratios between pro-inflammatory and anti-inflammatory mediators and fatty acid derivatives can also be used for diagnostic purposes in IBD. For example, an increased arachidonic acid-to-eicosadienoic acid ratio is indicative of a proinflammatory state in UC patients.

Metabolome as a Biomarker of a Treatment Response

Metabolomic profiles can be used to predict the response to a number of biological drugs. The significant role of the intestinal microbiota and its

endogenous metabolites in the IBD development is known. It is suggested that the analysis of metabolites, including endogenous metabolites, may serve as predictors of the response to biological therapy in patients with IBD. According to a systematic review that included 38 studies investigating the potential of fecal and intestinal wall microbiota and endogenous metabolomic markers as predictors of a response to biologic therapy in patients with IBD, the data on the significance of metabolomic signatures in assessing the response of patients with IBD to various biological agents were confirmed [37]. In the future, these data can be used for precision and personalized therapy. Thus, the levels of endogenous metabolites, such as butyrate and deoxycholic acid, were significantly associated with clinical remission after anti-TNF alpha drug therapy.

So, higher levels of butyrate-producing bacteria and specific metabolites, such as acetamide, have been shown to be associated with a positive response to vedolizumab [37]. In addition, lower baseline levels of acylcarnitine and ceramide and increased levels of N-methylglycine were positively associated with the response to vedolizumab [38].

CD patients with a positive clinical response to ustekinumab also showed specific bacterial signatures of the gut microbiota – the increase in *Faecalibacterium* and lower levels of *Escherichia/shigella*. This supports the suggestion that bacterial profiles can be used as predictors of a treatment response in IBD [37].

Therefore, it is clear that metabolomic profiling is of particular interest and importance in the context of precision and personalized medicine for patients with IBD.

CONCLUSION

Proteomics and metabolomics studies open significant perspectives for further study of IBD. The results of experimental and clinical studies have already identified a number of biomarkers – candidates for testing and introduction into routine clinical practice. There is compelling evidence of the potential benefits of integrating these areas with other omics approaches, such as lipidomics. The integrative biomarker analysis can be used both to assess IBD pathogenesis and to personalize patient management approaches and treatment strategy selection. The integration of multi-omics data, including those using artificial intelligence, can also be considered as a basis for tools to predict IBD development and the course of the disease [39]. Data integration and IBD datasets and biomarker atlases are of great use for predicting specific features of the disease [40].

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The Individual Contribution of Fatty Acids to the Development of Cardiovascular Diseases

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ABSTRACT

Impaired fatty acid (FA) metabolism may be an important factor that increases the development and progression of atherosclerosis and related cardiovascular diseases (CVD). However, most of the research focuses on studying the influence of classification groups of FA. Therefore, the aim of this lecture was to present both pro- and anti-atherogenic functions of each FA. This paper considers up-to-date information about the effects of saturated (myristic (C 14:0), palmitic (C 16:0), stearic (C 18:0)), monounsaturated (palmitoleic (C 16:1), oleic (C 18:1)), and polyunsaturated (linoleic (C 18:2 omega-6), alpha-linolenic (C 18:3, omega-3), dihomo-gamma-linolenic (C 20:3, omega-6), arachidonic (C 20:4, omega-6), eicosapentaenoic (C 20:5 omega-3), docosahexaenoic (C 22:6 omega-3)*)* FAs on CVD. The accumulated data expand the understanding of the role of FAs in metabolic processes, which will allow us to move from fundamental research to practical aspects of the use of these substances in the treatment of CVD. In the future, these results can be used in the interpretation and prediction of changes in lipid metabolism disorders in CVD.

Keywords: fatty acids, lipids, cardiovascular diseases, blood, risk factors

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Вклад жирных кислот в развитие сердечно-сосудистых заболеваний

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РЕЗЮМЕ

Нарушение обмена жирных кислот (ЖК) может являться значимым фактором, потенциирующим развитие и прогрессирование атеросклероза и связанных с ним сердечно-сосудистых заболеваний (ССЗ). Тем не менее большинство исследований сосредоточены на изучении влияния классификационных групп ЖК. Поэтому цель настоящей лекции – представить как про-, так и антиатерогенные функции каждой жирной кислоты.

В настоящей работе рассмотрены современные сведения о влиянии насыщенных (миристиновой (С 14:0),

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пальмитиновой (С 16:0), стеариновой (С 18:0)), мононенасыщенных (пальмитолеиновой (С 16:1), олеиновой (С 18:1)) и полиненасыщенных (линолевой (С 18:2, омега-6), альфа-линоленовой (С 18:3, омега-3), дигомо-гамма-линоленовой (С 20:3, омега-6), арахидоновой (С 20:4, омега-6), эйкозапентаеновой (С 20:5, омега-3), докозагексаеновой (С 22:6, омега-3)) жирных кислот на ССЗ. Накопленные данные расширяют представления о роли ЖК в метаболических процессах, что позволит перейти от фундаментально-поисковых работ к практическим аспектам применения данных веществ в лечении ССЗ. В перспективе эти результаты могут быть использованы при интерпретации и прогнозировании изменений метаболических нарушений липидов при ССЗ.

Ключевые слова: жирные кислоты, липиды, сердечно-сосудистые заболевания, кровь, факторы риска

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи

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INTRODUCTION

The growing prevalence of chronic non-communicable diseases, primarily cardiovascular diseases (CVDs), is a huge problem for the health care system [1]. Coronary heart disease (CHD) caused by atherosclerotic lesions of the coronary arteries is the main and most common nosology among CVDs [2]. For a long time, atherosclerosis can be asymptomatic, which is associated with a latent stage of the disease, in which morphological changes in the coronary arteries are already present [3]. However, following the growth of the atherosclerotic plaque, gradual stenosis of the coronary and other arteries occurs, leading to complications, such as myocardial infarction (MI), stroke, angina pectoris, cerebrovascular insufficiency, sudden cardiac death, etc. [4, 5]. At the same time, the rate of atherosclerosis progression is strictly individual, which necessitates preventive measures at the population and individual levels aimed at eliminating or minimizing the incidence of CVD and the associated loss of working capacity.

A growing body of evidence suggests that fatty acids (FAs) and their metabolites play an important role in atherogenesis [6]. In addition to their structural and/or energy functions, FAs are associated with the regulation of hemodynamics, inflammation, endothelial dysfunction, antioxidant defense, and other important biological processes [7, 8]. This is due to their chemical structure, showing differences for both saturated (SFA) and unsaturated FA (UNFA) [6]. Therefore, the aim of this lecture was to study the role of each FA on the risk of developing CVD. It should be noted that FAs are divided into short-chain, medium-chain, and long-chain FAs based on the number of carbon atoms in their hydrocarbon chain. In addition, according to the presence and number of double bonds in their carbon chain, they can be classified into SFAs (contain no double bonds); monounsaturated FAs (MUFA) (contain one double bond), and polyunsaturated FAs (PUFA), whose structure contains two or more double bonds [9, 10].

SATURATED FATTY ACIDS

As important energy sources, long-chain SFAs can be incorporated into lipoproteins, circulate in the blood, be stored in fat depots, and be used to synthesize other lipid compounds in the body [11]. Currently, the relationship between tissue SFA levels and the risk of atherosclerotic CVD is widely studied, mainly because SFAs can increase low-density lipoprotein cholesterol (LDL-C) concentrations [12]. Nevertheless, there is growing evidence that individual SFAs generally have different biological functions [13].

The most common SFA in the human body is palmitic acid (C16:0), which is an important component of membrane, secretory, and transport lipids, so both deficiency and excess of this SFA are harmful [14–16]. It can enter the body with food or be formed by endogenous synthesis (i.e., *de novo* lipogenesis) as a result of excess energy intake from carbohydrates and/or proteins [17]. To date, the relationship between high levels of palmitic acid in the blood and the risk of developing CVD is beyond doubt. The clinical and observational data indicate that C16:0 may be

associated with adverse cardiovascular events, as well as with overall mortality [18–20]. A population-based study by C.L. Chei et al., which was an additional study to the CIRCS (Circulatory Risk in Communities Study, Japan) [21], revealed that the average level of palmitic SFA was higher in patients with CAD than in the control group. Another population-based study, the LURIC (The Ludwigshafen Risk and Cardiovascular Health study, Germany) [19], showed a direct association with an increased risk of CVD mortality only for C16:0. Moreover, high palmitic acid intake (≈50% of total SFA intake) has been shown to elevate LDL-C [22] and interleukin-6 [18] levels and increase the risk of CHD [23, 24].

Stearic acid (C18:0) is also one of the main SFAs included in triglycerides. It can be obtained from a wide range of foods, including meat, fish, dairy products, etc. Meanwhile, under the action of palmitoyl elongase, cells can elongate C16:0 palmitic SFA to C18:0 stearic SFA [17]. Unlike palmitic FA, data on the effect of stearic SFA on lipid metabolism and, therefore, on the risk of CVD remain controversial. In the Mendelian Randomization Study [25], it was shown that a genetic predisposition to higher levels of stearic SFA in plasma was positively associated with CVD, such as stroke and venous thromboembolism. The EPIC-Norfolk study (European Prospective Investigation into Cancer, UK) [26] found that the concentration of stearic SFA in plasma was positively associated with an increased risk of CHD. At the same time, the CHS study (Cardiovascular Health Study, USA) [27] reported an inverse relationship between high C18:0 levels and all-cause mortality among elderly individuals (over 65 years of age). When studying the effect of stearic SFA, it was found that intake of C18:0 could reduce the level of total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and apolipoprotein A1 in the blood serum, compared to palmitic SFA [28].

However, no significant effect on LDL-C and HDL-C levels has been found in previous studies [22]. The Nurses' Health Study, which included data from the Health Professionals Follow-up Study [24], showed that higher intake of stearic SFA was associated with an increased risk of developing CHD over 24–28 years of follow-up. On the contrary, in the EPIC-NL study (European Prospective Investigation into Cancer and Nutrition–Netherlands Cohort, Netherlands) [29], no significant contribution of stearic acid to the development and course of CHD was found. Thus, the effect of stearic SFA on lipid metabolism, inflammation, and/or endothelial function is not uniform, and additional research in this area is certainly needed.

One of the less common SFAs is myristic acid (C14:0). At relatively low concentrations in the human body, it is also an important component of cell membranes and can systematically influence lipoprotein metabolism [30]. The amount of endogenously biosynthesized myristic FA from lauric acid (C12:0) following elongation or from palmitic SFA following peroxisomal β-oxidation is much smaller than the amount supplied by dietary sources [31]. Within the Ventimiglia di Sicilia Heart Study [32], it was found that the levels of myristic SFA in plasma were inversely correlated with HDL-C levels. The Verona Heart Study reported a strong positive relationship between myristic acid and plasma apolipoprotein CIII concentrations [30]. The study by S.O. Ebbesson et al. [33] showed positive associations between high plasma C14:0 levels and CVD risk factors: increased levels of triglycerides, LDL-C, blood pressure (BP), body mass index (BMI), plasma glucose, as well as an inverse relationship with HDL-C. In an additional study to CIRCS [21], it was noted that high serum levels of myristic SFA were associated with an increased risk of CHD.

Nevertheless, a few data suggest that morbidity and mortality from CVD depend not so much on the total amount of SFA consumed, but on their ratio to UNFA [34].

MONOUNSATURATED FATTY ACIDS

The interest in the role of MUFA is steadily growing. In addition to exogenous intake, MUFAs can be endogenously synthesized in the liver and adipose tissue using microsomal stearoyl-CoA desaturase-1 from precursors – SFA [35]. MUFAs can promote a healthy blood lipid profile, improve blood pressure, glycemic control, etc. [36]. However, the effect of MUFAs on inflammation has not been sufficiently studied. However, there is increasing evidence indicating a close relationship between MUFAs and anti-inflammatory conditions [37]. Some of the key MUFAs, from the standpoint of their functional role in the body, are considered to be omega-7 palmitoleic (C16:1) and omega-9 oleic (C18:1) acids.

Recently, palmitoleic MUFA has been considered as a lipid hormone (or lipokine) derived from adipocytes, which allows adipose tissue to regulate systemic metabolism, indicating its physiological significance [38]. It has been established that C16:1

can be detected as a cis- or trans-isomer and is also associated with cholesterol metabolism, insulin sensitivity, and hemostasis [39–41]. At the same time, its effect on the body, in particular on the cardiovascular system, is still controversial among researchers. The EPIC-Norfolk Study [26], which involved 25,639 people, found no relationship between the content of palmitoleic MUFA in plasma and CHD.

In another prospective study – CIRCS [21], involving 12,840 individuals, positive associations of serum palmitoleic MUFA levels with a higher risk of developing CHD were registered in both men and women. In a population-based study of 1,828 patients with MI and 1,828 controls [42], it was found that C16:1 in adipose tissue had an inverse relationship with acute MI. Most likely, the opposite conclusions are due to different patient samples and/or the biomaterial used. At the same time, a significant number of researchers are inclined to believe that palmitoleic MUFA can have an anti-inflammatory effect [43] and even reduce harmful effects of SFA. In particular, C16:1 promotes differentiation of primary macrophages into the anti-inflammatory M2 phenotype, protecting against the pro-inflammatory effects of palmitic acid [44]. In addition, C16:1 can reduce the levels of pro-inflammatory cytokines produced by lipopolysaccharide-stimulated macrophages (interleukin-6/-8, tumor necrosis factor α) [45].

Oleic acid accounts for approximately 80% of MUFAs in plasma phospholipids. In the PREDIMED study (PREvención con DIeta MEDiterránea, Spain) [46], researchers wanted to demonstrate that consumption of a Mediterranean diet enriched with olive oil (as a key component and source of plant oleic MUFA) was inversely correlated with CVD. However, it was shown that dietary oleic FA intake did not affect its plasma levels, since the concentrations of oleic MUFA in the blood are regulated by other factors, including *de novo* synthesis from stearic MUFA [47].

The results of the MESA (The Multi-Ethnic Study of Atherosclerosis, USA) study [47] show that elevated levels of oleic MUFA in plasma phospholipids may be a risk factor for the development of CVD and all-cause mortality. In the Aldo-DHF (Aldosterone in Diastolic Heart Failure, Germany) study [48], positive correlations were observed between the level of oleic MUFA and established cardiovascular risk factors, such as atherogenic dyslipidemia, dysglycemia, and obesity. In the population-based FINRISK study (Finland) [49], it was determined that high levels of MUFA in the blood, including oleic FA, were associated with a higher risk of CVD. Similar results were obtained with respect to arterial hypertension [50] and inflammation [51]. Despite the relevance of studying the role/influence of MUFAs in the development of CVD and their risk factors, additional studies are needed on the influence of non-dietary factors, such as genetics or younger populations.

POLYUNSATURATED FATTY ACIDS

Recently, special attention has been paid to the role and importance of nutrients, especially long-chain omega-3 and omega-6 PUFAs. It has been shown that omega-3 PUFAs may be beneficial in various diseases and conditions, such as atherosclerosis [52], obesity [53], and inflammation [54]. However, the cardioprotective properties of omega-3 PUFAs are considered to be the most studied. The biological effects of omega-6 PUFAs are still poorly understood and are the subject of active debate [55]. Although most studies report that some omega-6 PUFAs are associated with a lower risk of CVD [56], they have powerful vasodilatory, antiplatelet, and antiarrhythmic effects [57].

The alpha-linolenic acid (C18:3, omega-3) is the most common omega-3 PUFA that can be obtained only from food (mainly from plant sources: flaxseed oil, walnuts, soy, etc.) [58]. One of the large meta-analyses of the Cochrane Database [59], which included 86 randomized controlled trials lasting at least 12 months, assessed the effect of increased omega-3 FA intake on overall mortality, CVD, obesity, and lipid profile. The results showed that an increase in alpha-linolenic PUFA slightly reduced the risk of cardiovascular events. A subsequent meta-analysis [60] including the results of 47 studies confirmed that increasing alpha-linolenic PUFA intake by 1 g / day was associated with reductions in triglycerides, total cholesterol, and LDL-C, thereby preventing CVD.

In a meta-analysis of 27 observational studies [61], data on the association of alpha-linolenic PUFA and the risk of developing CVD were summarized. Observations show that total exposure to C18:3 omega-3 PUFA is associated with a moderately lower risk of CVD. Within the PREDIMED study [62], it was found that in people with high cardiovascular risk, but without previous CVD, the alpha- PUFA intake was inversely correlated with all-cause mortality. The Alpha – Omega study [63] revealed a trend toward

a reduction in the risk of CVD with alpha-linolenic PUFA consumption in patients receiving modern cardiac treatment.

In a study of the relationship between the levels of alpha-linolenic PUFA in plasma and the risk of acute coronary syndrome, T.A. Zelniker et al. [64] found significant inverse associations of C18:3 omega-3 with a lower risk of sudden cardiac death, independent of traditional risk factors and lipid levels. And in a study on mice, it was shown that a diet rich in C18:3 omega-3 can protect against endothelial dysfunction and prevent the development of atherosclerosis by suppressing the inflammatory response and the formation of foam cells [65].

Eicosapentaenoic acid (C20:5, omega-3) is considered to be an essential omega-3 PUFA. It is found primarily in fish and other seafood but can be biosynthesized in small amounts from its main precursor, alpha-linolenic PUFA [66]. There is strong evidence that eicosapentaenoic PUFA has beneficial effects on endothelial function and increases the synthesis of eicosanoids (which dilate blood vessels and reduce thrombus formation and inflammation) [67]. In addition, its potential therapeutic effects on the atherosclerotic plaque include anti-inflammatory and antioxidant activity, reduction of macrophage and foam cell accumulation in lipid spots, reduction of monocyte adhesion, and increase in the thickness of the fibrous cap of the plaque [67–70].

The JELIS (Japan Eicosapentaenoic acid Lipid Intervention Study, Japan) study [71] showed that the introduction of eicosapentaenoic PUFA at a dose of 1.8 g / day led to a decrease in CVD by 19% in patients receiving statins and a decrease in LDL-C concentration in the blood by 25% after treatment. The results of the multicenter, randomized REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial, USA) study [72] indicate that in patients with elevated triglyceride levels who received icosapent ethyl 4 g / day, the risk of major ischemic events, including sudden cardiac death, was significantly lower.

The OCEAN (Omacor Carotid Endarterectomy Intervention, UK) study [73] noted that higher levels of eicosapentaenoic PUFA in atherosclerotic plaques were associated with a decrease in the number of foam and T cells, less pronounced inflammation, and increased stability. Accordingly, the use of C20:5 omega-3 in individuals with a high risk of developing CVD as additional drug therapy helps reduce this risk [74].

Docosahexaenoic acid (C22:6, omega-3) is a very long-chain omega-3 PUFA found in high concentrations in fish, fish oil, and some algae [75]. Clinical studies using dietary supplements with high levels of docosahexaenoic PUFA have shown stable anti-inflammatory, antioxidant, antiatherogenic, and antiproliferative effects [76, 77]. In a double-blind, multigroup, placebo-controlled, randomized study [78], it was shown that C 22:6 omega-3 was more effective than C 20:5 omega-3 in reducing blood triglyceride levels, partly due to differential regulation of liver enzymes associated with lipogenesis. However, consumption of docosahexaenoic PUFA at a dose of ~3 g/day for 10 weeks may be more effective in reducing inflammatory markers, such as interleukin-18, tumor necrosis factor α, and C-reactive protein [79]. There is also evidence that consumption of docosahexaenoic PUFA increases not only C22:6 omega-3 in blood and tissues, but also C20:5 omega-3 eicosapentaenoic PUFA [80]. Moreover, the increase in the omega-3 FA index is significantly higher after supplementation with docosahexaenoic PUFA (2.7 g/day)[81]. Finally, a number of authors have found that docosahexaenoic PUFA causes a greater decrease in blood pressure, heart rate, and total peripheral resistance compared to eicosapentaenoic PUFA [82–84]. Thus, relatively high levels of free omega-3 PUFA may not always be associated with protection of the acutely damaged heart, but nevertheless have a beneficial effect on the body as a whole.

Linoleic acid (C18:2, omega-6) is the main dietary source of other omega-6 PUFAs, such as gamma-linolenic acid, dihomo-gamma-linolenic acid, and arachidonic acid. Linoleic acid is mainly obtained from vegetable oils [85]. There is increasing evidence that high linoleic acid levels are significantly associated with a reduction in the risk of development and mortality from CVD [86, 87]. According to the results of the Cochrane Database meta-analysis [88], which included 19 randomized controlled trials, higher intake of linoleic PUFA instead of SFA or carbohydrates reduced the risk of developing MI and total serum cholesterol by 6%. According to a meta-analysis of 30 prospective studies from 13 countries [56], higher levels of linoleic PUFA *in vivo* were associated with a lower risk of CVD, in particular, mortality from stroke.

In a meta-analysis of observational studies [86], high serum/dietary omega-6 C18:2 levels were inversely proportional to the risk of hypertension. In addition, the results of the International Study of

Macro-Micronutrients and Blood Pressure Study (INTERMAP) [89] show that dietary linoleic PUFA intake may contribute to the prevention and control of unfavorable blood pressure levels in the general population. In a study aimed at investigating the risks of CVD in communities (CIRCS) [21], it was found that serum levels of omega-6 linoleic PUFA were inversely associated with the risk of CHD.

In a Mendelian randomization study [90], it was shown that higher serum omega-6 C18:2 levels were inversely associated with lower levels of lipids, including LDL-C, HDL-C, and total cholesterol. In general, it can be noted that enriching the diet with a moderate amount of linoleic acid-rich oil may reduce the risk of cardiometabolic diseases [91].

Dihomo-gamma-linolenic acid (C20:3, omega-6) is considered to be one of the key omega-6 PUFAs, which has antiatherogenic effects. It inhibits the formation of foam cells, reduces the proliferation of endothelial cells, improves mitochondrial function, etc. [92]. By means of enzymatic activity, gamma-linolenic acid (C18:3, omega-6) is very quickly converted into dihomo-gamma-linolenic PUFA. The latter, in turn, can be metabolized into the anti-inflammatory eicosanoid – prostaglandin E1, via the cyclooxygenase pathway [93]. In the body, it is found in lipids (primarily phospholipids) and most cells, and C20:3 omega-6 levels are consistently increased following C18:3 omega-6 supplementation [94]. In mice, dihomo-gamma-linolenic PUFA supplementation has been shown to reduce aortic lipid content, along with macrophage and smooth muscle cell levels and ICAM-1 and VCAM-1 expression [93].

Few studies have shown an association between low levels of dihomo-gamma-linolenic PUFA and the severity of CHD [95]. In the OMEMI study [96], low serum levels of dihomo-gamma-linolenic PUFA were associated with an increased risk of all-cause mortality in elderly patients who had recently experienced MI. Similar results were obtained by S. Ouchi et al. [97], where the authors concluded that low levels of dihomo-gamma-linolenic PUFA in serum may be a predictor of permanent CVD (acute coronary syndrome, MI). In the work by T. Nagai et al. [98], lower levels of omega-6 PUFA, in particular C20:3, were associated with higher incidence of adverse events (death from all causes and observation of heart failure) after acute decompensated heart failure.

Finally, arachidonic acid (C20:4, omega-6), also known as eicosatetraenoic PUFA of the omega-6 class, is worth noting. It can enter the human body as part of various foods (meat, eggs, salmon, vegetable oils, walnuts) or be formed by endogenous synthesis due to release from phospholipids in the cell membrane by cytosolic phospholipase A2 (PLA2) [99]. It is usually esterified as triglycerides or glycerophospholipids to maintain cell membrane structure and function. It is well known that arachidonic PUFA can compete with omega-3 eicosapentaenoic PUFA for cyclooxygenase and lipoxygenase *in vivo* [100]. The arachidonic PUFA and its metabolites play an important role in the functioning of the cardiovascular system. They act as vasodilators or vasoconstrictors and modulate vasodilation in pathological and physiological conditions [101].

Nevertheless, the results of studies on the associations of circulating or tissue levels of arachidonic PUFA with CVD are rather inconclusive. A meta-analysis of 30 prospective studies [56] did not support adverse cardiovascular effects of arachidonic PUFA. Moreover, the authors suggested that higher plasma C20:4 levels may be associated with a lower risk of developing CVD. In two population-based cohort studies conducted in the Netherlands [102], no association was found between arachidonic PUFA levels and the risk of developing CHD. In the analysis of data obtained from a retrospective registry of patients with acute hypertensive stroke [103], lower serum arachidonic PUFA levels were independently associated with poor functional outcome in acute intracerebral hemorrhage. According to the results of a study using genetic variants [104], positive associations of arachidonic PUFA with atherosclerotic CVD and venous thromboembolism were found. When studying the content of arachidonic PUFA in adipose tissue, a positive association with the risk of MI in the Danish Prospective Cohort Study (DCH) was established [105].

CONCLUSION

Thus, the study of the influence of FA on the development of CVD is a promising area of research. Data on the associations of different SFA, MUFA, and PUFA with lipid and lipoprotein parameters and inflammatory markers of CVD may be of interest for obtaining new data clarifying and supplementing the mechanisms of the effect of FA on the cardiovascular system.

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To the 135th Anniversary of the Founding of the Pathological Anatomy Division of the Siberian State Medical University

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ABSTRACT

The article is devoted to the history of the Pathology Department of the Siberian State Medical University, which will celebrate its 135th anniversary on May 6, 2025, since its foundation and opening within the framework of the Siberian Imperial University established in 1878. The article presents and describes the most important historical events and achievements in scientific and pedagogical activities, as well as in practical medical and diagnostic work.

The Pathology Department has always occupied a leading and strong position among the strongest and most authoritative Departments of the University. Traditionally, from the moment the department was founded and to this day, teachers have been engaged in practical clinical activities, combine the teaching process with the full-time work of a pathologist, conduct autopsies and intravital diagnostics, examining biopsy and surgical material.

Keywords: Pathological Anatomy Division of the Siberian State Medical University, history of foundation and development

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К 135-летию со дня основания кафедры патологической анатомии Сибирского государственного медицинского университета

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РЕЗЮМЕ

Статья посвящена истории кафедры патологической анатомии Сибирского государственного медицинского университета, со дня основания и открытия которой в рамках образованного в 1878 г. Императорского Томского университета 6 мая 2025 г. исполняется 135 лет. Представлены и описаны важнейшие исторические события и достижения в научно-педагогической деятельности и практической лечебно-диагностической работе.

Кафедра патологической анатомии всегда занимала ведущие и прочные позиции в ряду сильнейших и авторитетных кафедр университета. Традиционно с момента основания кафедры и по настоящие дни преподаватели занимаются практической клинической деятельностью, совмещают педагогический процесс с полноценной работой врача-патологоанатома, проводят аутопсии и прижизненную диагностику, исследуя биопсийный и операционный материал.

Ключевые слова: кафедра патологической анатомии Сибирского государственного медицинского университета, история основания и развития

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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On May 6, 2025, the Pathological Anatomy Division of the Siberian State Medical University will celebrate its 135th anniversary since its establishment as part of the Siberian Imperial University, founded in 1878. Over more than a century of its history, the department has grown and solidified its reputation as one of the strongest and most respected departments within the university. This achievement is undoubtedly due to the people who devoted their lives to this department.

The official founding date of the Division is considered to be May 6, 1890. That year, Konstantin Nikolaevich Vinogradov was appointed professor ordinarius of the Pathological Anatomy Division. In 1870, Vinogradov K.N. graduated with honors from the Saint Petersburg Medical and Surgical Academy, where he developed an interest in histology and, under the guidance of K.F. Slavyansky, authored two works: *On the Study of Myxomas in Fetal Membranes* and *The Histological Structure of the Amniotic Membrane in Humans*. In 1873, Vinogradov K.N. defended his doctoral dissertation *Materials for the Pathological Anatomy of Glanders and Anthrax*. Despite his brief tenure as head of the department (until 1892) Vinogradov K.N. made significant contributions by creating and developing the educational process within the discipline from scratch. He also initiated the establishment of a pathology institute.

During the first academic year, Vinogradov personally delivered all lectures and practical classes. Thanks to his work, by 1891, the division was equipped with 36 Zeiss, Leitz and Reichert microscopes. Effective student training required demonstrative pathological materials, however, at that time, the university lacked both a museum of pathological anatomy and a collection of microscopic specimens. It is known that Professor Vinogradov K.N. brought 1500 histological slides with him to Tomsk from Saint Petersburg, which were used to demonstrate various pathological processes to students, along with more than 200 macroscopic specimens for the museum and practical training.

Today, the division's museum collection comprises thousands of specimens, but it was Vinogradov who laid its foundation and continued to expand it during his tenure. Vinogradov K.N. also engaged in practical work, conducting autopsies on patients with various pathologies. During one such autopsy, he discovered a previously unknown parasite in the liver, later named the Siberian fluke (Opisthorchis felineus). This discovery was the most significant in Vinogradov's career and resulted in five scientific publications during his time in Tomsk. In 1892 Professor Vinogradov left the university to accept a position as a professor ordinarius at the Military Medical Academy in Saint Petersburg [1, 2].

The second head of the division was Professor Ivan Ivanovich Sudakevich who led it for four years (1892-1896) until his death from complications of tuberculosis. Sudakevich I.I. was a representative of the Kiev school and a student of the renowned Russian pathologist and infectious disease specialist G.N. Minkh, and focused his scientific interests on infectious pathology. In 1888, he defended his doctoral dissertation *The Pathology of Leprosy*. During his time in Tomsk, he studied trichinosis (*Changes in Muscle Fibers in Trichinosis: Muscle Phagocytosis*, 1895) and explored phagocytic mechanisms in malignant tumors (*On the Phenomenon of Metachromasia in Sporozoans Parasitic in Cancer Cells*, 1892 and *Phagocytic Phenomenon in Cancer Tumors*, 1895). Most of Sudakevich's scientific work was published in international journals. During his tenure as head of the division, Sudakevich significantly expanded and enriched the collection of microscopic and macroscopic specimens. His scientifically rich and elegantly delivered lectures were consistently popular among students [1].

In 1897 the division was headed by professor ordinarius Fedor Ivanovich Romanov. Romanov graduated with honors from the Medical Faculty of Kharkov University and defended his doctoral dissertation *Changes in the Thyroid Gland with the Internal Use of Potassium and Sodium Iodides* in 1889. He moved to Tomsk in 1891, where he worked as a prosector and an acting associate professor in the Pathological Anatomy Division of the Tomsk Institute. Although Romanov officially received the title of professor ordinarius only in 1901, he was permitted to lecture during the tenure of Professor Sudakevich I.I.

Romanov's scientific work focused on parasitology, particularly echinococcosis, tumor pathology and infectious diseases. He actively participated in the activities of the Society of Naturalists and Physicians. During his time in Tomsk he published 15 scientific articles and mentored Viktor Pavlovich Mirolyubov who later became the head of the Pathological Anatomy Division [1].

The next head of the division was appointed only in 1908. Professor Mikhail Mikhailovich Pokrovsky, a 1888 graduate of the Saint Petersburg Medical and Surgical Academy, took the position. Between 1889 and 1898, Pokrovsky worked at the Moscow University and later spent six years as a prosector at the Saint Petersburg Women's Academy. His tenure as head of the Pathological Anatomy Division coincided with the division's move to the newly constructed building of the New Anatomical Complex within the university grove.

Pokrovsky completely reorganized the museum, acquiring 17 display cabinets, which have been preserved and are still in use today. Most of Pokrovsky's scientific work focused on the foundational principles of pathological anatomy and its practical applications. He authored *A Guide to Autopsies for Beginners*, *On the Techniques of Pathological Autopsy*, as well as works such as *The Beginning of Pathology: General Pathology* and *What Are Pathological Phenomena?*. Additionally he published a series of articles on infectious and oncological topics. In 1919, Professor Pokrovsky moved to Nizhny Novgorod, where he headed the Department of General Pathology and Pathological Anatomy at the Medical Faculty of Nizhny Novgorod University [1].

In 1922, the Pathological Anatomy Division was headed by Viktor Pavlovich Mirolyubov. Born into a priest's family in Saratov Province in 1910, Mirolyubov moved to Tomsk in 1890 after studying at the Balashov Theological School and Astrakhan Theological Seminary. In Tomsk, he entered the university, graduating with honors in 1896 with a degree in medicine. By May 1897, he had been appointed an acting prosector in the Pathological Anatomy Division. In 1900, Mirolyubov V.P. was called to active military medical service in China. During the Russo-Japanese War (1904–1905), he served as a field physician with the active army. After returning in 1910, Mirolyubov V.P. defended his doctoral dissertation *On the Development of Alveolar Echinococcus in Humans* in 1911 and was appointed a private docent. By 1922, he had become a full professor. His dissertation gained widespread recognition in the scientific community for its uniqueness and was the only work on this problem in Russian literature at the time. It was actively cited and translated into foreign languages. He was awarded the degree of Doctor of Medical Sciences and the title of professor in 1935.

Throughout his years at the division, Mirolyubov actively lectured on general and specific pathological anatomy, conducted practical classes with students, paid special attention to autopsy work, and, in particular, diagnosed complex biopsy and surgical material. He also consulted on challenging cases requiring differential diagnoses. Professor Mirolyubov V.P. sought to collaborate closely with Western European universities. He visited various cities, including Paris, Zurich, Freiburg and Munich, to study the nuances of teaching pathological anatomy

and the intricacies of a pathologist's work. His collaboration with the Berlin School of Pathology, led at the time by Johann Orth - a student of Rudolf Virchow - was especially notable.

As a result of this activity, Mirolyubov published the monograph *On Parenchymal Liver Cancer*. Initially featured in Virchow's Archive, it was later released as a standalone publication. The work was distinguished by its proposal of the first classification of parenchymal liver cancer. Mirolyubov was a talented, organized, and deeply dedicated teacher and a practicing physician. He also possessed exceptional humbleness and humility. He authored over 40 scientific works on various pathological issues. Among his distinguished students was academician Innokenty Vasilyevich Toroptsev [1].

Innokenty Vasilyevich Toroptsev became the head of the Pathological Anatomy Division in 1947 following the death of Professor V.P. Mirolyubov. After graduating from a Soviet labor school in 1926, Toroptsev entered the Medical Faculty of Tomsk State University. During his early years at the university, he actively participated in the scientific circle of the Department of General Pathology under the guidance of A.D. Timofeevsky. While still a student, Toroptsev received several patents for inventions. In his fourth year, he developed an interest in pathological anatomy and spent his free time attending autopsies, learning the intricacies and challenges of a pathologist's work. After graduating from the university in 1931, Toroptsev entered a postgraduate program at the Pathological Anatomy Division and became an assistant in 1933. His candidate dissertation *Scleropigment Nodules in the Spleen* was successfully defended in 1937. Ten years later, in 1947, he defended his doctoral dissertation *Materials on the Problem of Plant-Based Bactericides*, and was awarded the title of professor the same year.

Toroptsev authored 134 scientific works, including five monographs and numerous articles published in international journals. He held 16 patents for inventions, all of which were officially recognized. Toroptsev initiated research into the mechanisms of action of electromagnetic fields and their potential applications in medical practice. He established the only interdisciplinary magnetobiological laboratory in Tomsk, based on the Polytechnic and Medical Institutes. Under his scientific supervision, 30 candidate and 22 doctoral dissertations were successfully defended. Among his well-known students were prominent physicians and scientists, including E.V. Goldberg, V.P. Desyatov, A.I. Ryzhov and D.A. Gratsianov [1, 3, 4].

In 1961, for his outstanding contributions, Toroptsev received scientific recognition and was elected a corresponding member of the Academy of Medical Sciences of the USSR. In 1969, he became a full member. Practical work held a special place in Toroptsev's life. He was a meticulous autopsy technician, highly skilled in clinico-morphological analysis and an exceptionally knowledgeable, responsible, and erudite pathologist [5, 6].

Due to illness, Toroptsev stepped down from his duties in 1983, and Associate Professor Galina Viktorovna Borisova served as acting head of the division from 1983 to 1987. Borisova G.V. joined the Pathological Anatomy Division at Tomsk Medical Institute in 1953 as an assistant. In 1959, without completing a formal postgraduate program, she successfully defended her candidate dissertation *Pathological Anatomy of Listeriosis in Experimental Animals*, under the supervision of Professors I.V. Toroptsev and S.P. Karpov, a corresponding member of the Academy of Medical Sciences of the USSR.

The results of her research were summarized in a monograph and published in the *Archive of Pathology* central journal . These findings were referenced in multi-volume pathological anatomy guides and the Great Medical Encyclopedia. Borisova co-authored the monograph *Listeriosis: Microbiology, Clinic, Pathological Anatomy, Pathogenesis, Treatment, Epidemiology, Laboratory Diagnostics*, which remains a priority reference for researchers studying this disease. Over her career, Borisova published 61 scientific works. In recognition of her organizational talent and high professional and pedagogical standards, Borisova was awarded the title Honored Worker of Higher Education in 2001 [4].

In September 1987, Vladimir Mikhailovich Perelmuter was appointed the head of the Pathological Anatomy Division. Born in Daugavpils, Latvian SSR, Perelmuter graduated from Tomsk Medical Institute in 1964 after attending Secondary School 24 in Tomsk. As a student, he actively participated in the microbiology department's scientific circle, where he studied the incubation period of listerial infection under the guidance of Y.N. Odintsov.

After graduating from the institute, Perelmuter worked as a microbiologist at the Tomsk Research Institute of Vaccines and Serums while pursuing postgraduate studies in histology part-time. In 1972,

he served as a sanitary doctor for Tomsk's sanitary-epidemiological service and starting in 1973, as a pathologist at the Tomsk Regional Oncology Dispensary. In 1975, Perelmuter V.M. was appointed an assistant at the Pathological Anatomy Division. Five years later, he was appointed the head of the Inter-University Magnetic Biology Laboratory (Laboratory Number 25 at the Institute of Nuclear Physics, Tomsk Polytechnic Institute). In 1981, under the guidance of Candidate of Medical Sciences Y.N. Odintsov and Doctor of Medical Sciences Professor N.M. Tikhonova, Perelmuter V.M. successfully defended his candidate dissertation *Morphofunctional Assessment of the State of Lymphoid Organs and the Liver in Early Stages of Experimental Chronic Listerial Infection in Mice*, at the Academic Council of Novosibirsk Medical Institute. He later studied the biological effects of millimeter-range electromagnetic radiation and discovered the phenomenon of functional asymmetry in the bone marrow of mice's hind limbs and inguinal lymph nodes.

These findings led to new research into the functional asymmetry of parenchymal organs under normal and pathological conditions, becoming a primary research focus under Perelmuter's leadership. From 1987 to 2012, Perelmuter served as the head of the Pathological Anatomy Division. In 1990, the USSR State Committee for Public Education awarded him the academic title of associate professor. In 1996, he successfully defended his doctoral dissertation *Functional Asymmetry of the Thymic-Adrenal System*, supervised by Academician of the Russian Academy of Medical Sciences, Professor E.D. Goldberg. In 1998, Perelmuter was awarded the title of professor. Between 2002 and 2019, he simultaneously headed the Department of Pathology and Cytology at Tomsk Oncology Research Institute.

Perelmuter was an innovator in pedagogical and scientific activities. He introduced a rating system for evaluating students' knowledge in pathological anatomy, developed a comprehensive digital lecture course and applied an integrated approach to studying pathological processes and nosological forms, emphasizing pathogenetic and clinical parallels. Under his leadership, the department digitized its macro-specimen collection and created high-quality illustrative materials for educational purposes. A computer lab was established to enable students, interns, residents, postgraduates and physicians to study morphological changes at both macroscopic and microscopic levels.

Perelmuter's primary research interest was the mechanisms underlying the formation and progression of malignant neoplasms. He developed new scientific directions in pathology and oncology, focusing on tumor heterogeneity, the role of the tumor microenvironment and chronic inflammation in the pathogenesis of malignant tumor growth. He introduced an innovative approach to assessing the pathological manifestations of tumor heterogeneity in breast cancer. His research demonstrated that morphologically distinct carcinoma structures could be associated with varying prognoses.

Together with his student, Doctor of Medical Sciences V.N. Manskikh, Perelmuter proposed an original hypothesis explaining the selective metastasis of malignant neoplasms to specific target organs. The hypothesis included experimental predictions and potential strategies for metastasis prevention. Furthermore, they proposed a hypothesis to explain the mechanisms of lymphatic metastasis in sarcomas.

Perelmuter is a highly qualified specialist in morphological tumor diagnostics. At the Tomsk Oncology Research Institute, he established one of Russia's first immunohistochemical and molecular research laboratories for precise verification of malignant processes. These advanced methods improved diagnostic accuracy, optimized cancer treatment, and achieved better clinical outcomes. Under Perelmuter's supervision, 8 doctoral and 14 candidate dissertations were defended. His notable students include Professor M.V. Zavyalova, Professor S.V. Vtorushin, Associate Professor I.L. Purlik, Doctors of Medical Sciences V.N. Manskikh, L.A. Tashireva, and N.V. Vasiliev.

Since 2012, Pathological Anatomy Division has been headed by Professor Marina Viktorovna Zavyalova. In 2004, under the supervision of Professors V.M. Perelmuter and E.M. Slonimskaya, M.V. Zavyalova successfully defended her candidate dissertation *Features of Breast Cancer Progression Depending on the Morphological Variant of the Tumor and Background Dysplastic Processes* at the Dissertation Council of the Tomsk Oncology Research Institute. In 2009, she defended her doctoral dissertation *Relationship Between Tumor Morphological Structure, Lymphatic and Hematogenous Metastasis in Infiltrative Ductal Breast Cancer*. In 2013, she was awarded the title of professor.

Professor Zavyalova continues the legacy of V.M. Perelmuter, developing both the educational and scientific activities of the department. She restructured

the complete lecture course on pathological anatomy, adapting it to practical clinical work and current clinical guidelines. As a practicing pathologist at the Pathological Anatomy Division of Siberian State Medical University Clinics (under the Russian Ministry of Health), Zavyalova integrates her professional expertise into the educational process. This led to the creation of a digital archive of histological preparations and the development of educational materials with high-quality, original illustrations. Zavyalova M.V. introduced mentorship and gamification into the educational process to enhance student engagement.

She also places great emphasis on organizing extracurricular activities to strengthen students' knowledge and motivation. To prepare students for clinical disciplines and ensure the continuity of knowledge from junior to senior years, she organized and implemented a system of student clinical-anatomical conferences. The mentoring system allows students to acquire additional skills and competencies.

Zavyalova's scientific interests include the study of tumor progression mechanisms, particularly intratumoral heterogeneity, invasion, generalized metastasis, and primary-multiple tumor growth. As a leading researcher at the Department of General and Molecular Pathology at the Tomsk National Research Medical Center, Zavyalova has supervised the defense of 15 candidate dissertations. She actively trains personnel to strengthen the pedagogical and research activities of the division. Among her students are Associate Professor N.V. Krakhmal, N.S. Telegina, D.S. Pismenny and V.V. Alifanov.

Over the years, the division has been home to outstanding specialists, educators, and prosectors, including Professor D.A. Gratsianov, Associate Professor O.M. Ordina, T.G. Kamneva and B.V. Novitsky. Throughout its existence, the Pathological Anatomy Division has been one of the leading divisions at Siberian State Medical University, excelling in teaching, organizational, and scientific activities. It has provided students with in-depth knowledge, clinical reasoning skills, and has trained talented pedagogical and scientific personnel. The division's contributions have significantly impacted both science and practice.

Today, the division's academic staff includes 4 Doctors of Medical Sciences and 7 Candidates of Medical Sciences. Since its founding, division faculty members have traditionally combined teaching with practical work as pathologists, performing autopsies and examining biopsy and surgical material. Since 2010, the clinical base of the division has been represented by the Pathological Anatomy Division of Siberian State Medical University Clinics headed by Professor S.V. Vtorushin.

The combination of theory and practice, requiring constant professional development from instructors, makes the educational process engaging and enriching for students. For of them, the study of pathological anatomy remains one of the most memorable experiences of their medical education.

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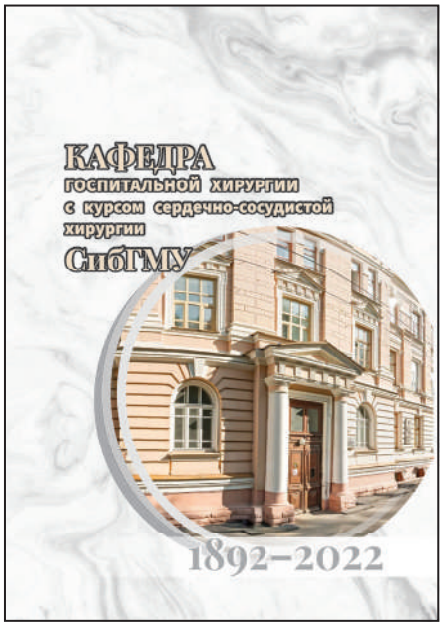
«Наследие томской медицины»



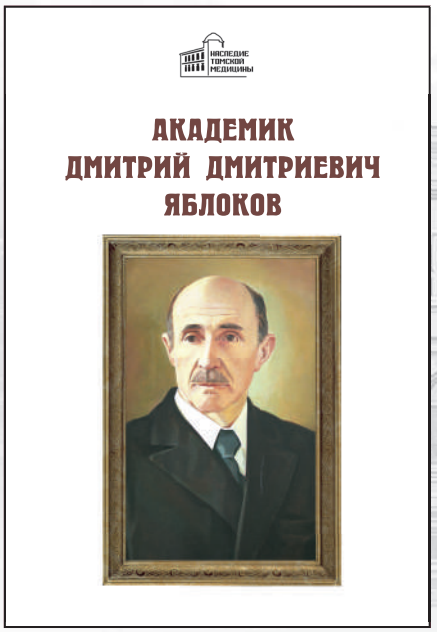
Книга посвящена 130-летнему юбилею кафедры госпитальной хирургии СибГМУ. Приведены биографические данные 79 сотрудников клиники и кафедры госпитальной хирургии в период с 1892 по 2022 г. Им предшествует подробная статья, характеризующая основные научно-практические достижения коллектива на каждом историческом отрезке. В издании упомянуты не только выдающиеся хирурги, звезды мировой величины, но и рядовые профессора, доценты, ассистенты, врачи-ординаторы, многие из которых связали с кафедрой и клиникой всю свою трудовую биографию. При изложении материала наряду с традиционными источниками информации использованы автобиографические документы, данные из семейных архивов, производственные характеристики нередко с сохранением авторского стиля.

Это позволяет полнее ощутить атмосферу в обществе и рабочем коллективе в разные годы существования клиники. Текстовая информация сопровождена богатым иллюстративным материалом, многие фотографии опубликованы впервые.

Издание предназначено для хирургов, студентов старших курсов врачебных факультетов, специалистов по истории медицины.

Трёхтомная иллюстрированная летопись одного из старейших и наиболее авторитетных медицинских вузов России – Сибирского (Томского) государственного медицинского университета является по сути первой серьёзной попыткой осветить более чем 140-летнюю историю этого прославленного университета.Особенностью издания является его богатейший иллюстративный материал, включающий более четырёх тысяч фотографий (в том числе ранее практически неизвестных), и никогда не публиковавшиеся до этого крайне любопытные и интересные факты о жизни университета, его студентов и профессоров, воспоминания и рассказы выпускников и преподавателей вуза.

Для самого широкого круга читателей, интересующихся историей российских университетов, отечественного высшего медицинского образования и науки, развитием клинических и научно-медицинских школ, здравоохранения, историей Томска, Сибири, России…



В книге представлены биография и обзор научной, педагогической и общественной деятельности выдающегося ученого, терапевта, клинициста, академика АМН СССР, Героя Социалистического труда, лауреата Сталинской премии Дмитрия Дмитриевича Яблокова (1896-1993).

Для врачей, студентов, всех интересующихся историей медицины.

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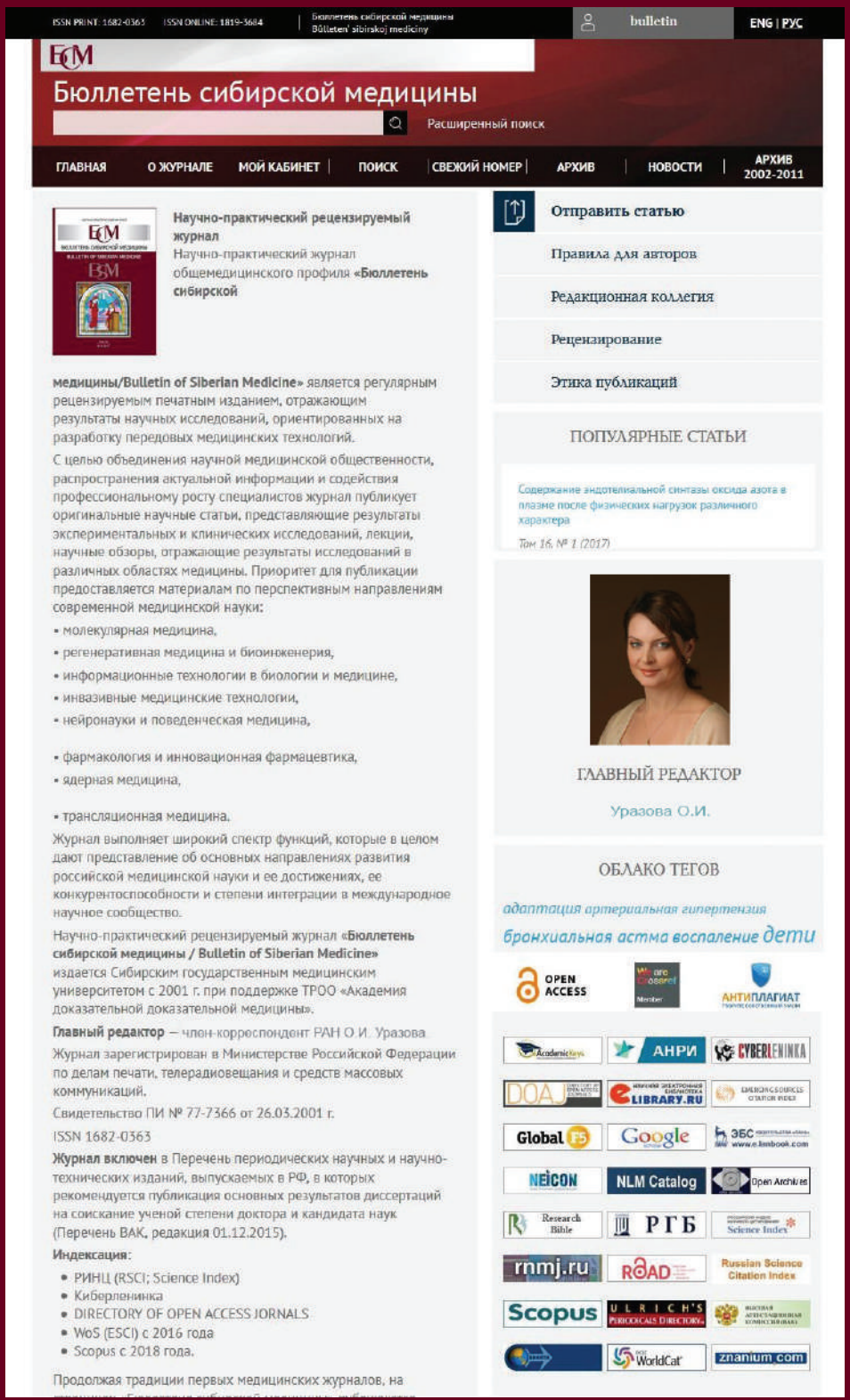

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