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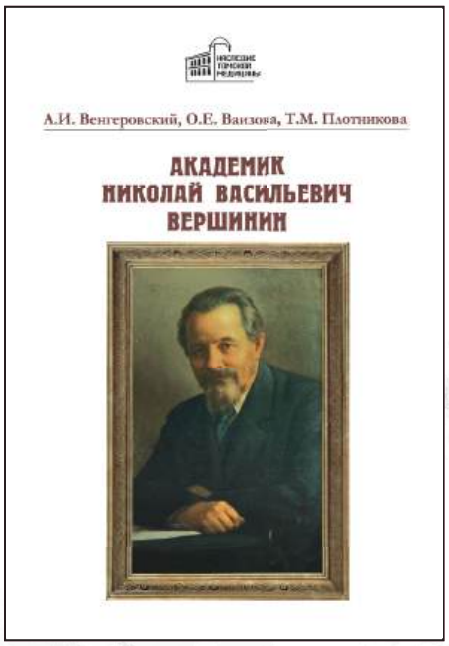

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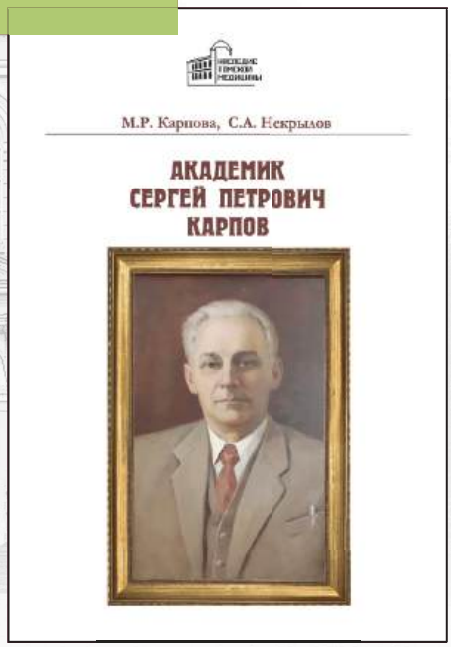

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Course of arterial hypertension during breast cancer chemotherapy with anthracyclines

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ABSTRACT

Aim. To study the characteristics of the course of arterial hypertension (AH) and subclinical cardiac damage during breast cancer chemotherapy with doxorubicin.

Materials and methods. The study included a total of 27 women with breast cancer (BC) and a history of controlled hypertension who were to receive chemotherapy with anthracyclines. Twelve women had stage 1 hypertension; 15 women had stage 2 hypertension. The patients received dual antihypertensive therapy according to clinical guidelines. All patients underwent echocardiography and 24-hour blood pressure monitoring at baseline, after the last course of chemotherapy, and 12 months after the end of chemotherapy. The control group included 35 women with BC without a history of AH, who also were to receive anthracycline chemotherapy.

Results. A significant relationship between pre-existing AH and the development of left ventricular systolic dysfunction 12 months after the completion of chemotherapy ($p = 0.01$) was found. According to 24-hour blood pressure monitoring, 15 women (55.6%) showed deterioration of blood pressure control after the completion of chemotherapy, which required modification of antihypertensive therapy by adding one more drug to the treatment regimen. At 12 months after the end of chemotherapy, in 13 women, hypertension control was reached with triple antihypertensive therapy. In two women, hypertension became resistant, which required prescription of a four-component antihypertensive regimen.

Conclusion. Pre-existing AH plays an essential role in the development of anthracycline-induced cardiotoxicity, despite the quality of blood pressure control. Polychemotherapy with anthracyclines may deteriorate blood pressure control in patients with AH, which requires addition of antihypertensive drugs to the treatment regimen.

Keywords: arterial hypertension, chemotherapy, anthracyclines, heart failure, cardio-oncology

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

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Течение артериальной гипертензии на фоне химиотерапии рака молочной железы антрациклинами

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РЕЗЮМЕ

Цель. Изучение особенностей течения артериальной гипертензии (АГ) и развития субклинического поражения сердца на фоне химиотерапии доксорубицином рака молочной железы (РМЖ).

Материалы и методы. В исследование включены 27 женщин с РМЖ, имеющих в анамнезе контролируемую АГ, которым планировалась полихимиотерапия (ПХТ) с использованием антрациклиновых антибиотиков. У 12 женщин зарегистрирована гипертоническая болезнь 1-й стадии, у 15 женщин – 2-й стадии. Пациентки получали двойную антигипертензивную терапию согласно клиническим рекомендациям. Всем пациенткам проводились эхокардиография и суточное мониторирование артериального давления (АД) исходно, после последнего курса и через 12 мес после окончания ПХТ. В группу контроля включены 35 женщин с РМЖ без АГ анамнезе, которым также планировалась терапия антрациклинами.

Результаты. Наблюдалась значимая взаимосвязь между ранее существовавшей АГ и развитием систолической дисфункцией левого желудочка через 12 мес после завершения химиотерапии ($p = 0,01$). По данным суточного мониторирования АД, у 15 женщин (55,6%) зарегистрировано ухудшение контроля АД после окончания ПХТ, что потребовало модификации антигипертензивной терапии путем добавления в схему лечения дополнительного препарата. Через 12 мес после окончания ПХТ у 13 женщин АГ имела контролируемый характер течения, что было достигнуто тройной антигипертензивной терапией; у двух женщин АГ приобрела резистентный характер течения, что потребовало назначения четырехкомпонентной схемы гипотензивной терапии.

Заключение. Ранее существовавшая АГ играет очень важную роль в развитии кардиотоксичности, вызванной химиотерапией на основе антрациклинов, несмотря на качество контроля АД. Полихимиотерапия антрациклинами может ухудшать контроль АД у больных с АГ, что сопровождается необходимостью добавления дополнительных антигипертензивных препаратов.

Ключевые слова: артериальная гипертензия, химиотерапия, антрациклины, сердечная недостаточность, кардиоонкология

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 ГБУЗ «Городская клиническая поликлиника № 16» (протокол № 245 от 25.11.2020).

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INTRODUCTION

Arterial hypertension (AH) is one of the most common diseases in the general population. Its prevalence reaches up to 30-45% and increases with age [1]. Cancer is also a common pathology, which is the second leading cause of death worldwide [2]. The average age of population is increasing, and cancer patients have longer overall survival due to improved anticancer therapy [2]. In addition, some risk factors associated with the development of AH, such as obesity, type 2 diabetes mellitus, smoking, and sedentary lifestyle, are also associated with the development of malignant neoplasms [3]. Therefore, it is not surprising that an increasing number of cancer patients seek medical care with a history of AH. In a review by M. Jain et al., the incidence of AH in cancer patients reached 37%, making it the most common concomitant cardiovascular disease reported in patients with malignant neoplasms [4]. On the other hand, AH is also a common complication in cancer patients which develops during certain types of chemotherapy [5, 6].

Childhood Cancer Survivor Study, which compared more than 10,000 adult survivors of childhood cancer with 3,000 siblings, showed that patients with a history of cancer were more likely to have AH than the general population. The researchers found that the prevalence of AH was 40% versus 25% at the age of 45 years, respectively [7]. AH may increase the risk of chemotherapy-induced cardiotoxicity and, if poorly controlled, can lead to discontinuation of treatment for the malignancy [5, 6]. AH can develop in patients receiving various types of chemotherapy following direct effects or an indirect effect through mechanisms associated with nephrotoxicity [8].

The main anticancer drugs which effect may be complicated by AH are vascular endothelial growth factor (VEGF) inhibitors and tyrosine kinase inhibitors (TKIs). A recent meta-analysis assessed the risk of cardiovascular disease during TKI therapy compared to standard chemotherapy. Seventy-one randomized controlled trials involving more than 29,000 patients were included in the review. The results showed that the relative risk of developing AH during TKI therapy was 3.78 (95% confidence interval (CI) 3.15–4.54) compared to the age- and comorbidity-matched controls [9]. AH was reported in 50% of patients receiving anti-VEGF therapy [10]. In the meantime, there are practically no studies on the effect of anthracyclines on the course of AH which was manifested before the initiation of cancer chemotherapy.

The aim of the study was to investigate the characteristics of the course of AH during breast cancer chemotherapy with doxorubicin.

MATERIALS AND METHODS

The protocol for the 12-month prospective study included patients who signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at City Clinical Hospital No. 16 (Protocol No. 245 of 25.11.2020).

Inclusion criteria: 1) women aged 40–60 years with BC; 2) use of AC (doxorubicin + cyclophosphamide) or TAC (doxorubicin + cyclophosphamide + docetaxel) chemotherapy regimens; 3) complete remission for BC within 12 months after the end of polychemotherapy (PCT); 4) controlled stage 1–2 hypertension.

Exclusion criteria: 1) confirmed cardiovascular pathology before the initiation of chemotherapy, except for hypertension (heart valve disease, cardiomyopathy, chronic heart failure, history of primary pulmonary hypertension); 2) the use of targeted therapy drugs and aromatase inhibitors for

the treatment of BC; 3) relapse of BC or emergence of new cancer during the follow-up; 4) type 1 and 2 diabetes mellitus; 5) concomitant severe renal, hepatic or multiple organ failure; 6) indications of poor drug tolerance; 7) anemia; 8) chronic alcoholism, mental disorders.

The patients were followed up at three stages: before the initiation of anthracycline therapy, after the last course of PCT, and 12 months after the completion of BC therapy. At the indicated observation points, echocardiography (echo) and 24-hour BP monitoring were performed. During echo, linear and volumetric heart chamber dimensions were assessed; left ventricular ejection fraction (LVEF) was calculated by the Simpson method, and the thickness of the walls of the left and right ventricles was measured. Left ventricular myocardial mass (LVMM) and left ventricular mass index (LVMI) were determined from M-mode measurements in accordance with the recommendations of the American Society of Echocardiography (ASE). Given prevalence among the examined individuals with excess body weight, LVMI was calculated using the formula LVMM / height$^{2.7}$. Global longitudinal strain (GLS) of the LV was studied by two-dimensional speckle tracking echocardiography.

The characteristics of the course of AH were assessed in 27 women with BC during chemotherapy and for 12 months after its completion. We also assessed initial signs of asymptomatic myocardial dysfunction according to cardiotoxicity criteria after BC chemotherapy with anthracyclines proposed by the European Society of Cardiology in 2022 [6]: LVEF ≥ 50% and a decrease in left ventricular GLS by more than 15% from the baseline value.

The control group included 35 women with BC without a history of AH, who also received BC therapy using AC or TAC regimens. In this group, the development of AH and other cardiovascular diseases, including anthracycline-induced cardiac dysfunction, was assessed within 12 months after the end of PCT.

Statistical processing of the results was carried out using the STATISTICA 13.3 software package (StatSoft, Inc., USA). Quantitative variables were presented as the median and the interquartile range Me (Q_{25}; Q_{75}). To compare two independent samples, the Mann – Whitney test was used. The critical significance level p for all statistical procedures was taken equal to 0.05.

RESULTS

Prior to PCT, the studied groups of women with BC were comparable in age, body mass index, and LVEF (Table 1).

Table 1

Baseline characteristics of patients depending on the presence of AH

Parameter	Patients with AH, n = 27	Patients without AH, n = 35	p
Age, years, *Me* (Q_{25}; Q_{75})	51 (48; 56)	50 (46; 53)	0.318
Body mass index, kg / m^2, *Me* (Q_{25}; Q_{75})	25.2 (23.6; 26.9)	24.8 (23.1; 26.3)	0.183
Cumulative dose of doxorubicin, mg / m^2, *Me* (Q_{25}; Q_{75})	360 (300; 360)	360 (300; 360)	0.893
Chemotherapy regimen, *n* (%): – AC; – TAC	 15 (55.6) 12 (44.4)	 20 (57.1) 15 (42.9)	 0.729 0.773
Left-sided radiation therapy, *n* (%)	9 (33.3)	11 (31.4)	0.851
Stage of hypertension, *n* (%): – stage 1; – stage 2	 12 (44.4) 15 (55.6)	 – –	 – –
Heart rate, *Me* (Q_{25}; Q_{75})	72 (66; 77)	76 (68; 82)	0.095
Systolic BP, mm Hg., *Me* (Q_{25}; Q_{75})	125 (120; 130)	115 (110; 120)	0.031
Diastolic BP, mm Hg., *Me* (Q_{25}; Q_{75})	75 (70; 80)	70 (70; 80)	0.062
LVEF, %, *Me* (Q_{25}; Q_{75})	63 (59; 66)	61.0 (58; 64)	0.261
GLS, %, *Me* (Q_{25}; Q_{75})	–19.3 (–17.8; 20.5)	–19.6 (–18.0; 20.7)	0.692
LVMI, g / m^2, *Me* (Q_{25}; Q_{75})	109.4 (89.3; 126.4)	85.2 (75.1; 92.8)	<0.001

Table 1 (continued)

Parameter	Patients with AH, *n* = 27	Patients without AH, *n* = 35	*p*
Antihypertensive therapy, *n* (%):			
– ACE inhibitors;	16 (59.3)	–	–
– ARBs;	11 (40.7)	–	–
– DCCBs;	17 (63.0)	–	–
– diuretics;	7 (25.9)	–	–
– β-adrenergic blockers	3 (11.1)	–	–

Note. Here and in Table 2: AH – arterial hypertension, AC – polychemotherapy regimen (doxorubicin + cyclophosphamide), TAC – polychemotherapy regimen (doxorubicin + cyclophosphamide + docetaxel), LVEF – left ventricular ejection fraction, BP – blood pressure, LVMI – left ventricular mass index, ACE – angiotensin-converting enzyme, ARB – angiotensin receptor blocker, DCCB – dihydropyridine calcium channel blocker, GLS – global longitudinal strain. *p* – probability of type I error.

In patients with AH, a significantly increased LVMI was registered ($p < 0.001$) compared to the control group. The cumulative dose of doxorubicin and the applied PCT regimens were comparable in the groups. Before the initiation of PCT, stage 1 hypertension was registered in 12 women, and stage 2 hypertension – in 15 women with AH.

A significant correlation was revealed between pre-existing AH and the development of left ventricular systolic dysfunction 12 months after the completion of chemotherapy ($p = 0.01$) (Table 2): according to the criteria of the European Society of Cardiology [6], signs of anthracycline-induced cardiotoxicity were recorded in 6 (22.2 %) women with AH and in 2 (5.7%) patients in the control group.

In 15 women (55.6%) with AH, deterioration of blood pressure control was recorded after completion of PCT, which required modification of antihypertensive therapy by adding one more drug to the treatment regimen. In the control group, two people developed stage 1 hypertension within 12 months after the completion of PCT, the control of which was not achieved by lifestyle modification and required prescription of two antihypertensive drugs. In women with AH after the completion of PCT, a non-significant increase in LVMI was noted.

At 12 months after the end of chemotherapy, in 13 women, hypertension control was reached with triple antihypertensive therapy. In two women, hypertension became resistant, which required prescription of a four-component antihypertensive regimen with the addition of mineralocorticoid receptor antagonists (Figure).

Table 2

Changes in the main clinical and echocardiography parameters in the study groups

Parameter	At baseline	After the end of PCT	Twelve months after the end of PCT	*p*
Patients with AH, n = 27				
Stage of hypertension, *n* (%):				
– stage 1;	12 (44.4)	12 (44.4)	10 (37.0)	0.416
– stage 2	15 (55.6)	15 (55.6)	17 (63.0)	0.416
Heart rate, *Me* (Q_{25}; Q_{75})	72 (66; 77)	76 (71; 80)	70 (65; 74)	0.272
Systolic BP, mm Hg., *Me* (Q_{25}; Q_{75})	125 (120; 130)	130 (120; 135)	125 (120; 130)	0.612
Diastolic BP, mm Hg., *Me* (Q_{25}; Q_{75})	75 (70; 80)	80 (75; 85)	75 (70; 80)	0.749
LVEF, %, *Me* (Q_{25}; Q_{75})	63 (59; 66)	62 (58; 66)	59 (55; 62)	0.083
GLS, %, *Me* (Q_{25}; Q_{75})	–19.3 (–17.8; 20.5)	–18.2 (–16.9; 19.8)	–18.0 (–16.8; 19.4)	0.174
Development of anthracycline-induced cardiac dysfunction, *n* (%)	–	–	6 (22.2)	–
LVMI, g / m^2, *Me* (Q_{25}; Q_{75})	109.4 (89.3; 166.4)	107.8 (91.2; 127.5)	114.3 (90.1; 132.6)	0.153
Antihypertensive therapy, *n* (%):				
– ACE inhibitors;	16 (59.3)	16 (59.3)	16 (59.3)	1.0
– ARBs;	11 (40.7)	11 (40.7)	11 (40.7)	1.0
– DCCBs;	17 (63.0)	27 (100.0)	27 (100.0)	0.023
– diuretics;	7 (25.9)	9 (33.3)	7 (25.9)	1.0
– β-adrenergic blockers;	3 (11.1)	6 (22.2)	8 (29.6)	0.041
– MCRAs	–	–	2 (7.4)	0.932

Table 2 (continued)

Parameter	At baseline	After the end of PC	Twelve months after the end of PCT	*p*
Patients without AH, n = 35				
Stage of hypertension, *n* (%): – stage 1; – stage 2	 – –	 – –	 2 (5.7) –	 – –
Heart rate, *Me* (Q_{25}; Q_{75})	76 (68; 82)	81 (75; 88)		
Systolic BP, mm Hg., *Me* (Q_{25}; Q_{75})	115 (110; 120)	115 (110; 120)	115 (110; 120)	0.621
Diastolic BP, mm Hg., *Me* (Q_{25}; Q_{75})	70 (70; 80)	70 (70; 80)	70 (70; 80)	0.811
LVEF, %, *Me* (Q_{25}; Q_{75})	61.0 (58; 64)	60,0 (57; 64)	59 (57; 62)	0.354
GLS, %, *Me* (Q_{25}; Q_{75})	–19.6 (–18.0; 20.7)	–18.7 (–17.5; 20.1)	–19.2 (–17.9; 20.5)	0.452
Development of anthracycline-induced cardiac dysfunction, *n* (%)	–	–	2 (5.7)*	–
LVMI, g / m^2, *Me* (Q_{25}; Q_{75})	85.2 (75.1; 92.8)	86.4 (74.2; 93.8)	87.1 (75.4; 93.1)	0.632
Parameter	At baseline	Afte the end of PCT	Twelve months after the end of PCT	*p*
Antihypertensive therapy, *n* (%): – ACE inhibitors; – ARBs; – DCCBs; – diuretics; – β-adrenergic blockers; – MCRAs	 – – – – – –	 – – – – – –	 2 (5.7) - - - 2 (5.7) –	 – – – – – –

*p = 0.01 compared to the AH group.

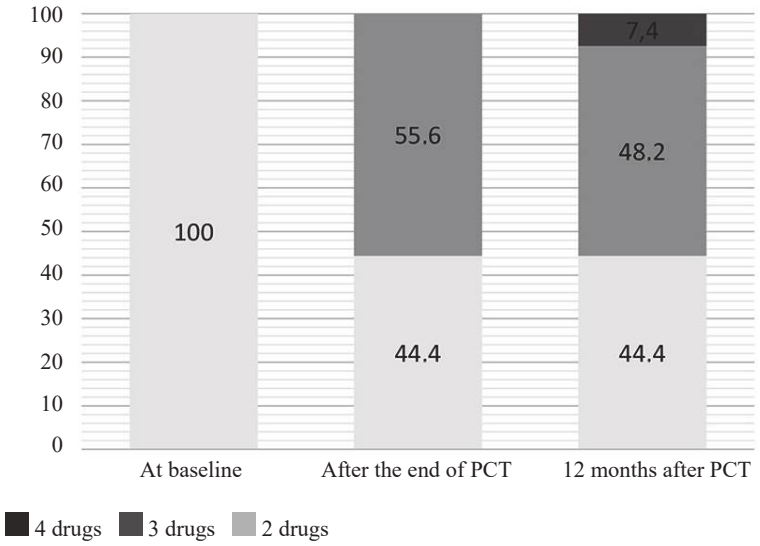

Figure. Number of antihypertensive drugs in the groups of patients with AH, %

DISCUSSION

Anthracyclines have broad antitumor activity. They are highly effective and are among the most frequently prescribed drugs for the treatment of malignant neoplasms. However, their clinical use is limited due to excessive generation of reactive oxygen species (ROS) and the development of cardiotoxicity with progression to heart failure [5, 6]. It was proven that the presence of cardiovascular diseases, including AH, is a risk factor for the development of myocardial dysfunction [5, 6].

This phenomenon was confirmed in our study: the development of anthracycline-induced cardiotoxicity 12 months after the end of PCT was more often (p = 0.01) registered in women with AH than in the control group. In the meantime, there are practically no studies on the effect of anthracyclines on the course of AH manifested before the initiation of antitumor chemotherapy. In our work, 55.6% of patients with

AH showed deterioration of blood pressure control after completion of PCT. The effect of anthracyclines on BP may be due to the development of endothelial dysfunction and activation of the renin – angiotensin – aldosterone system (RAAS) and sympathoadrenal system during and after the use of anthracyclines, which is confirmed in a number of experimental studies and clinical trials.

Recently increasing attention has been paid to anthracycline-induced endotheliotoxicity [11]. Many studies have confirmed that anthracycline-induced endotheliotoxicity, as well as cardiotoxicity and nephrotoxicity, drastically limits their clinical use [5, 6, 11]. Therefore, doxorubicin can cause severe damage to the vascular endothelium. Excessive generation of ROS is known to cause subsequent development of anthracycline-induced cytotoxicity [12], which can lead to endothelial dysfunction, affecting the course of AH.

According to M.M. Said-Ahmed et al., administration of cumulative doses of doxorubicin at a dose of 10, 15, and 20 mg / kg for two weeks resulted in a dose-dependent increase in plasma endothelin-1 (ET-1) levels by 85, 76, and 97%, respectively. In the meantime, the level of nitric oxide (NO) in the blood plasma did not change, while the production of NO in the myocardium significantly increased [13]. A recent study demonstrated a decrease in the effectiveness of NO-dependent mechanisms regulating vascular tone during a single dose of chemotherapy at 4 mg / kg [14]. On the other hand, a number of studies have demonstrated an increase in NO production, which is associated with upregulation of the inducible NO synthase (*iNOS*) and endothelial NO synthase (*eNOS*) genes [15, 16]. High concentrations of NO produced by the iNOS or eNOS enzymes stimulate generation of peroxynitrite following a reaction with superoxide anion. The resulting peroxynitrite leads to lipid peroxidation [17], which can result in damage to endothelial cells.

Studies examining the ET-1 / NO axis found low eNOS activity in the presence of severe endothelial damage, which may lead to increased ET-1 levels [18]. J. Yamashita et al. showed that monitoring plasma ET-1 levels can help detect subclinical cardiotoxicity of doxorubicin [19]. Excessive levels of intracellular calcium, which are caused by anthracyclines, are known to contribute to mitochondrial dysfunction, high-energy phosphate depletion, increased muscle stiffness, impaired contractile function, and cell death. ET-1 can induce the production of inositol phosphates, which increase intracellular Ca^{2+} levels by releasing it from intracellular stores. This process results in calcium overload of endothelial cells [20].

To date, the influence of anthracyclines on the activity of the RAAS, which is a complex hormone system crucial for both normal functioning of the cardiovascular system and regulation of the fluid and electrolyte balance of the body, has been proven. An imbalance in the RAAS leads to diseases, such as AH, heart failure, and even cancer [21]. Available data make it clear that increased angiotensin II (ATII) activity is one of the key events in anthracycline-induced endothelial dysfunction. The significant effect of ATII may be due to its increased synthesis or enhanced signaling from receptors.

M. Zheng et al. noted a three-fold increase in the level of ATII in the blood plasma of animals treated with doxorubicin compared to the controls [22]. High levels of ATII were also found in the myocardium and paraventricular nucleus of the hypothalamus (one of the centers regulating the cardiovascular system) [23]. These results indicate that under the influence of anthracyclines, ATII affects not only the heart and blood vessels, but also central control of the cardiovascular system. The ability of anthracyclines to induce excitation of the sympathetic nervous system at the central level has been proven [24].

In addition, it has been shown that under the influence of anthracyclines, renin activity increases, stimulating greater conversion of angiotensinogen to angiotensin I (ATI) [25]. A significant increase in ATI levels after doxorubicin administration may be an indirect sign of increased renin activity. At the same time, aliskiren, which is a renin inhibitor, caused a decrease in the concentration of ATI [25].

Another mechanism is associated with increased activity of angiotensin-converting enzyme (ACE). Long-term treatment with doxorubicin led to a significant, almost 2.3-fold increase in ACE activity in the heart of hamsters compared to control animals [26].

ATII exerts its effects through angiotensin type 1 (AT1R) and angiotensin type 2 receptors (AT2R). However, AT1R appears to play a key role in the development of anthracycline-induced endothelial dysfunction. It was shown that doxorubicin stimulated AT1R mRNA expression with an increasing drug

dose in cardiomyocytes [27]. In contrast to AT1R, AT2R, which mediates the protective effects of ATII, is inhibited by doxorubicin [28].

Therefore, we can conclude that the use of anthracyclines results in an imbalance of the main RAAS axes, namely hyperactivation of the angiotensinogen / ATII / AT1R axis. Consequently, the interference of doxorubicin in the secretion of essential endothelial factors and the RAAS, which play a crucial role in the functioning of the cardiovascular system, leads to a decrease in its adaptability. There is now growing evidence that anthracyclines may directly increase the risk of developing AH. Potential mechanisms include decreased capillary density, impaired neovascularization, and histologic changes in the vasculature, including intimal hyperplasia, luminal stenosis, and loss of smooth muscle cells [29]. In addition, vasomotor dysfunction resulting from decreased eNOS activity leads to decreased NO generation by endothelial cells, potentially contributing to the development of AH [30–32].

Antihypertensive therapy for AH in patients receiving chemotherapy has some distinctive features. In our study, 12 months after the end of PCT in 13 women, hypertension control was reached with triple antihypertensive therapy. In two women, AH became resistant, which required the prescription of a four-component antihypertensive regimen.

Changes in lifestyle and reduced sodium intake are recommended, regardless of anticancer therapy used, as they have a BP-lowering effect in many patients. However, strict adherence to recommendations for non-pharmacological BP control is challenging for many patients [33].

The choice of antihypertensive drugs in cancer patients is influenced by several factors. For example, the use of hydrochlorothiazide (HCTZ) was associated with the development of nonmelanoma skin cancer in two large studies performed in Denmark [34] and in the UK [35]. Researchers of the latter found an association between the use of HCTZ and the risk of developing basal and squamous cell skin cancer. On the other hand, studies conducted in Taiwan [36] and Korea [37] showed no association of HCTZ application with the development of skin cancer. However, the use of thiazide-like diuretics, such as chlorthalidone and indapamide, was not associated with an increased risk of skin cancer; therefore, some experts recommend the use of these drugs rather than HCTZ for the treatment of hypertension [38].

Several studies suggested that use of ACE inhibitors (ACEIs) may be associated with an increased risk of lung cancer. However, these studies were heavily criticized due to various limitations in data collection and interpretation, leading to the conclusion that there is currently insufficient evidence for altering clinical practice [39]. The European Society of Cardiology recommends angiotensin receptor blockers (ARBs) and dihydropyridine calcium channel blockers (DCCBs) as first-line therapy for AH. With the development of resistant hypertension, it is recommended to add β-adrenergic blockers, spironolactone, and NO donors, such as isosorbide mono/dinitrate or hydralazine, to therapy [6].

Regardless of the type of a drug used, more than one drug is required to treat AH caused by anticancer therapy. J.B. Cohen et al. suggested that patients with BP above the target values should start receiving DCCB in the absence of proteinuria and ACEI / ARB in the presence of proteinuria, titrated to an effective dose. The next step in BP management should be adding a drug from the class that was not initially prescribed (i.e. ACEI / ARB if CCB was received first, and vice versa). The third step in treatment intensification should be the use of a diuretic, unless contraindicated, followed by a MCRA or a β-adrenergic blocker [3].

Thus, AH is a common comorbidity as well as an adverse event in cancer patients and should, therefore, be closely monitored. The effect of antitumor therapy on the development of AH and treatment effectiveness should be thoroughly studied, since the prevention of cardiovascular morbidity and mortality is of paramount importance both in active cancer patients and in people with a history of cancer.

CONCLUSION

Pre-existing AH plays a crucial role in the development of anthracycline-induced cardiotoxicity, despite the quality of BP control. PCT with anthracyclines may deteriorate BP control in patients with AH, which requires addition of new antihypertensive drugs to the treatment regimen.

Despite efforts made to understand the role of various antineoplastic agents in increasing the risk of AH and influencing its course, many mechanisms

have not been fully defined, and clinical studies assessing the effectiveness and safety of specific antihypertensive drugs in cancer survivors are limited. Survivor populations are heterogeneous and have rich treatment history; therefore, the diagnosis and treatment of AH may need to be tailored to different subgroups. Understanding the mechanisms responsible for changes in BP in cancer patients will facilitate identification of new therapeutic targets in patients with AH.

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Authors' contribution

Berezikova E.N., Shilov S.N. – conception and design, analysis and interpretation of the data, drafting of the manuscript. Popova A.A., Grakova E.V. – conception and design, justification of the manuscript. Neupokoeva M.N., Kopeva K.V., Yushin A.Ju. – analysis and interpretation of the data. Teplyakov A.T., Kalyuzhin V.V. – final approval of the manuscript for publication.

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Assessing functional suitability of a lyophilized formulation containing designed ankyrin repeat proteins for radionuclide imaging of HER2/neu overexpression in malignant tumors

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ABSTRACT

Aim. To study *in vitro* and *in vivo* the functional suitability of ^{99m}Tc-labeled lyophilized formulation containing designed ankyrin repeat protein (DARPin) G3-$(GGGS)_3$Cys for radionuclide imaging of HER2/neu overexpression in malignant tumors.

Materials and methods. To create a targeted protein, a modified genetic construct with the sequence encoding DARPin G3-$(GGGS)_3$Cys was used. To generate the experimental probe, we used a lyophilized formulation containing DARPin G3-$(GGGS)_3$Cys with auxiliary substances and ^{99m}Tc sodium pertechnetate (500 MBq) incubated at 60 °C for 30 min. Radiochemical purity of ^{99m}Tc-G3-$(GGGS)_3$Cys was analyzed by thin-layer radiochromatography. SKOV-3, BT-474, and DU-145 cell lines were used to test binding specificity *in vitro*. The dissociation constant was determined via a saturation binding assay on SKOV-3 cells with a range of protein concentrations from 0.2 to 40 nM. Nu/j mice bearing HER2-positive SKOV-3 xenografts and HER2-negative Ramos xenografts were used to evaluate the targeting properties and biodistribution.

Results. A radiocomplex based on ^{99m}Tc and a lyophilized formulation with DARPin G3-$(GGGS)_3$Cys was obtained with the radiochemical purity of more than 96%. Binding of ^{99m}Tc-G3-$(GGGS)_3$Cys to the cells was specific (K_D 3.9 ± 0.5 nM) and proportional to the level of HER2/neu expression in the cells. The uptake of ^{99m}Tc-G3-$(GGGS)_3$Cys in SKOV-3 xenografts was significantly higher than in Ramos xenografts. ^{99m}Tc-G3-$(GGGS)_3$Cys demonstrated rapid blood and renal clearance and had low activity in the salivary glands and stomach. Liver uptake was about 5–7%ID/g. In addition, ^{99m}Tc-G3-$(GGGS_{)3}$Cys exhibited very low uptakes in the lungs, muscles, small intestine, and bones.

Conclusion. The ^{99m}Tc-labeled lyophilized formulation with DARPin G3-$(GGGS)_3$Cys is functionally suitable for imaging HER2/neu overexpression in tumors, as it binds specifically to the receptor, is stable *in vivo*, and has favorable biodistribution in organs and tissues. The radiocomplex based on ^{99m}Tc-G3-$(GGGS)_3$Cys was obtained by a simple method with high radiochemical purity.

Keywords: malignant tumors, Her2/neu, radionuclide diagnosis, DARPin G3, oxotechnetium, lyophilized formulation

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Оценка функциональной пригодности лиофилизата таргетных каркасных белков с анкириновыми повторами для радионуклидной визуализации гиперэкспрессии HER2/neu в злокачественных опухолях

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РЕЗЮМЕ

Цель – изучить *in vitro* и *in vivo* функциональную пригодность лиофилизата таргетных каркасных белков с анкириновыми повторами DARPin G3-$(GGGS)_3$Cys, меченых ^{99m}Tc, для радионуклидной визуализации гиперэкспрессии HER2/neu в злокачественных опухолях.

Материалы и методы. Для наработки таргетного белка использовали модифицированную генетическую конструкцию с последовательностью, кодирующей белок DARPin G3-$(GGGS)_3$Cys. Для получения экспериментального препарата использовали лиофилизат, содержащий DARPin G3-$(GGGS)_3$Cys со вспомогательными веществами, и раствор натрия пертехнетата, ^{99m}Tc (500 МБк) при инкубации 60 °C, 30 мин. Анализ радиохимической чистоты (РХЧ) ^{99m}Tc-G3-$(GGGS)_3$Cys проводили тонкослойной радиохроматографией. Для оценки специфичности *in vitro* использовали клеточные линии: SKOV-3 > BT-474 > DU-145. Константу диссоциации определяли с помощью анализа насыщения на SKOV-3 в диапазоне концентраций белка от 0,2 до 40 нМ. Для оценки таргетных свойств и биораспределения использовали мышей линии Nu/j, несущих ксенотрансплантаты SKOV-3 (HER2/neu позитивные) и ксенотрансплантаты Ramos (HER2/neu негативные).

Результаты. Получен радиокомплекс на основе ^{99m}Tc и лиофилизата таргетных белков DARPin G3-$(GGGS)_3$Cys с РХЧ более 96%. Связывание ^{99m}Tc-G3-$(GGGS)_3$Cys с клетками является специфичным с K_D 3,9 ± 0,5 нМ и пропорционально уровню экспрессии HER2/neu в клетках. Поглощение ^{99m}Tc-G3-$(GGGS)_3$Cys в ксенотрансплантатах SKOV-3 было значимо выше, чем в ксенотрансплантатах Ramos. ^{99m}Tc-G3-$(GGGS)_3$Cys продемонстрировал быстрое выведение из крови, почечный клиренс, низкие уровни актив-

ности в слюнных железах и желудке. Уровень накопления активности в печени составил около 5–7 %ВД/г. Кроме того, ^{99m}Tc-G3-$(GGGS)_3$Cys имел очень низкое поглощение в легких, мышцах, тонком кишечнике и костях.

Заключение. Лиофилизат таргетных каркасных белков DARPin G3-$(GGGS)_3$Cys, меченый ^{99m}Tc, функционально пригоден для визуализации гиперэкспрессии HER2/neu в опухолях, поскольку специфически связывается с рецептором, стабилен *in vivo* и имеет благоприятное биораспределение в органах и тканях. Радиокомплекс 99mTc-G3-(GGGS)3Cys получен по простой процедуре с высокой радиохимической чистотой.

Ключевые слова: злокачественные опухоли, Her2/neu, радионуклидная диагностика, DARPin G3, оксотехнеций, лиофилизат

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

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INTRODUCTION

Overexpression of transmembrane tyrosine kinase receptors, which are normally expressed on the surface of all epithelial cells in the body, often correlates with progression of malignant tumors. Human epidermal growth factor receptor type 2 (HER2/neu), which plays an essential role as an oncoprotein in malignant tumors [1] of the breast, gastrointestinal tract, ovaries, etc., is of particular interest in this process. This receptor is overexpressed in ovarian, breast, esophageal, gastrointestinal, lung, and other cancers [2].

Breast cancer (BC) is characterized by a severe disease course, low overall and recurrence-free survival, and *HER2/neu* gene amplification in 15–20% of cases. Therefore, this tumor marker is used as a target in the diagnosis and targeted therapy in patients with HER2/neu overexpression [3].

Monoclonal antibodies, antibody – drug conjugates, and tyrosine kinase inhibitors are used as targeted therapeutic agents that depend on specific recognition of HER2/neu [4]. The first step is to determine the presence and / or absence of HER2/neu overexpression on the tumor cell surface. Only after this, therapy is initiated. As a drug, trastuzumab (herceptin) is used, which is the gold standard for the treatment of patients with HER-2/neu-positive BC, significantly increasing overall and recurrence-free survival [5]. In addition, HER2/neu-targeted drugs undergo clinical evaluation for the treatment of ovarian cancer [6], non-small cell lung cancer [7], and endometrial cancer [8].

For routine use of registered drugs and further development of such therapies, it is essential to accurately determine HER2/neu expression in tumors. Expression of the targeted protein is directly related to the antitumor effect; with low expression, patients may be at risk of severe side effects following the use of targeted drugs and cytotoxins [9]. The main problem with the use of HER2/neu-targeted drugs is variability of receptor expression in malignant tumors [10].

Biopsy (immunohistochemistry and fluorescence in situ hybridization) is a routine procedure for determining HER2/neu expression [11]. However, biopsy is challenging in the context of multiple metastases due to the invasive nature of the procedure. It is virtually impossible to assess the extent of tumor progression and to detect changes in HER2/neu expression levels after neoadjuvant therapy [11]. To address the limitations of conventional biopsy, radionuclide molecular imaging of HER2/neu expression *in vivo* has been proposed as a potential solution [12].

Following the results of preclinical [13] and some clinical [12–14] trials on various types of contrast agents for molecular imaging of HER2/neu (antibodies, scaffold proteins, antibody fragments, aptamers, peptides), it can be concluded that the most promising HER2/neu-targeting molecules are scaffold proteins. They provide higher imaging contrast in a shorter time (2–4 hours after the injection) compared to other contrast agents [12].

Designed ankyrin repeat proteins (DARPins), which are engineered, high-affinity, stable proteins of small size (14–18 kDa), have the potential to be integrated into development of radiopharmaceuticals for oncoprotein imaging. DARPins with high affinity for HER2/neu were selected using ribosome display, demonstrating a clear potential for tumor targeting [15]. R. Goldstein et al. demonstrated the feasibility of radionuclide imaging of HER2/neu expression in human tumor xenografts in mice using DARPins labeled with ^{111}In and ^{125}I [16]. Further studies revealed that DARPin G3 was the optimal candidate for the development of molecular imaging agents [17].

The radionuclide most commonly employed for imaging in nuclear medicine is ^{99m}Tc (half-life 6 hours), which provides excellent spatial resolution and a low absorbed dose for patients. ^{99m}Tc is produced from ^{99}Mo generators (half-life 65.9 h), which can be delivered to remote hospitals and supply ^{99m}Tc for up to two weeks [18]. Consequently, ^{99m}Tc is an appealing candidate for SPECT imaging.

DARPin G3 labeled with [^{99m}Tc]Tc(CO)3 via a histidine-containing tag ($(HE)_3$ - tri(histidyl-glutamate)) was studied in phase I clinical trials [13]. Clinical data have demonstrated that the use of DARPin $(HE)_3$-G3 for radionuclide diagnosis is safe when patients are exposed to low doses. The diagnostic imaging of HER2-positive BC using ^{99m}Tc-labeled DARPin $(HE)_3$-G3 provided clear imaging results four hours after injection. This imaging method reliably distinguished between HER2-positive and HER2-negative tumors.

Disadvantages of this experimental radiopharmaceutical for clinical use include a complex and time-consuming two-step labeling procedure and complex purification from radiochemical impurities requiring specialized equipment. This prompted further studies to optimize and improve radioactive labeling of DARPin G3 with ^{99m}Tc for a simpler and faster one-step procedure. Previous studies utilizing DARPin G3 variants demonstrated that the use of cysteine-containing peptide-based chelators at the C-terminus to form an oxotechnetium complex resulted in low uptake in normal tissues and high uptake by tumors [19]. New DARPin G3 variants labeled with the oxotechnetium complex also provided image contrast comparable to that of clinically validated DARPin $(HE)_3$-G3 [19]. One improved variant of DARPin G3, designated ^{99m}Tc-G3-$(GGGS)_3$Cys, contains a Gly-Gly-Gly-Ser-Cys chelator conjugated via the - $(Gly-Gly-Gly-Ser)_2$ linker at the C-terminus. This variant has been proposed for pilot clinical trials (NCT05923268).

Prior to commencing clinical trials, it is essential to ascertain the functional suitability of DARPin G3-$(GGGS)_3$Cys with its complete sequence in the form of a lyophilized formulation. A number of factors influence the functional suitability of a targeted protein molecule, including the choice of a chelating agent for ^{99m}Tc binding, labeling conditions, and composition (combination of chemical precursors). The type of a dosage form plays an essential role in affinity, targeting properties, and *in vivo* biodistribution of such molecules. Any alterations to the composition of the dosage form, including transition from a solution to a lyophilized formulation, may result in a reduction or complete loss of its functional suitability for HER2/neu imaging. Consequently, it is necessary to conduct *in vitro* and *in vivo* experiments to evaluate these properties.

It is worth noting that cysteine-containing proteins are susceptible to oxidation to non-affinity homodimers, which makes them highly sensitive to technological processes employed in the production of lyophilized formulations. These are undoubtedly the most convenient and storage-stable dosage forms for routine production of radiopharmaceuticals in a medical organization [18].

The aim of this research was to assess *in vitro* and *in vivo* the functional suitability of a ^{99m}Tc-labeled lyophilized formulation containing DARPin G3-$(GGGS)_3$Cys for radionuclide imaging of HER2/neu overexpression in malignant tumors.

MATERIALS AND METHODS

The nucleotide sequence of the *DARPin G3* gene was deduced from the amino acid sequence for DARPin G3 in the PDB (PDB access number:

2JAB) taking into account codons most common in highly expressed *Escherichia coli* genes using the freely available DNA builder software (http://www.innovationsinmedicine.org/software/DNABuilder/). The genome was assembled by the polymerase chain reaction (PCR) from chemically synthesized 50-bp long oligonucleotides with partially complementary sequences. Expression, isolation, and purification of DARPin G3-(GGGS)3Cys were performed according to the previously described method [19].

The method for producing the experimental formulation. ^{99m}Tc pertechnetate [^{99m}Tc]TcO4 was obtained from the commercial ^{99}Mo / ^{99m}Tc GT-4K generator (Karpov Institute of Physical Chemistry, Obninsk, Russia). Samples were obtained using chemically pure reagents from various suppliers, including Fluka, Acros Organics (UK), Panreac, Sigma Aldrich (USA), and others. In a lyophilized mixture containing 3.3 mg DARPin G3-$(GGGS)_3$Cys, 0.66 mg D-mannose, 0.33 mg PEG-4000, 0.075 mg tin (II) chloride dihydrate, 5 mg sodium gluconate, 0.1 mg ethylenediaminetetraacetic acid (EDTA) tetrasodium salt, and phosphate buffer, 500 µl sodium ^{99m}Tc pertechnetate solution with the activity of 500 MBq was added and mixed. The contents of the vial were incubated at 60 °C for 30 minutes. Then the contents of the vial were diluted with a sterile 0.9% sodium chloride solution to 10 ml. Subsequently, the solution was purified using a sterile syringe filter with a pore size of 0.2 µm into a depyrogenated sterile vial. The radiochemical purity of the experimental ^{99m}Tc-G3-$(GGGS)_3$Cys samples was analyzed by thin-layer chromatography using ITLC SG strips (Aglient Technologies, Inc., Folsom, USA) in the phosphate buffered saline (pH = 7.4). Radioactivity on ITLC strips was recorded in counts per minute (CPM) using the miniGITA Single radio-TLC system (Elysia Raytest, Germany). In order to obtain a sterile solution for experiments on laboratory animals, the radioactivity was measured on the radiometer (Amplituda, Russia) equipped with an ionization chamber.

Radioactivity in both *in vitro* and *in vivo* samples was quantified using the automated Wizard 2480 Gamma Counter (Pelkin Elmer, USA). HER2/neu-expressing human cancer cell lines SKOV-3 (human ovarian carcinoma) and BT-474 (human breast carcinoma), as well as DU-145 cells (human prostate adenocarcinoma) with low HER2/neu expression were purchased from PrimeBioMed LLC (Moscow, Russia). The cells were cultured in the Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 IU / ml penicillin, and 100 µg / ml streptomycin in the humidified incubator with 5% CO_2 at 37 °C.

In order to assess the specificity of the *in vitro* assay, the cells were seeded into 6-well plates at a seeding density of 7×10^5 cells per well 24 hours prior to the experiment. One plate was utilized for each cell line. A 100-fold excess of unlabeled DARPin G3-$(GGGS)_3$Cys was used as a control group. The same volume of cell culture medium was added to three petri dishes of the experimental group. The petri dishes were incubated at 37 °C for 30 minutes to saturate the HER2/neu receptors. Subsequently, the [^{99m}Tc]Tc-G3-$(GGGS)_3$Cys solution was added to each petri dish to a final concentration of 1 nM, and the samples were incubated at 37 °C for 1 h. Following this incubation period, the medium was collected, the cells were washed with the phosphate buffer, and the solutions were pooled. The cells were then detached with trypsin and collected. Radioactivity in every fraction was measured by the gamma counter, and the percentage of cell-associated activity per 1 million cells was calculated. The experiment was conducted in triplicate.

The method for determining the equilibrium dissociation constant (C_D) was described previously [19]. The experiment was performed on SKOV-3 cells. Radioactivity was measured using the gamma counter. C_D parameters and the maximum number of binding sites per cell (Bmax) were calculated by nonlinear regression using the Prism software (GraphPad Software, USA).

To assess the targeting properties and biodistribution of the ^{99m}Tc-labeled lyophilized formulation containing G3-(GGGS)3Cys, immunodeficient Nu/j mice were used bearing HER2-positive SKOV-3 xenografts and HER2-negative Ramos xenografts. Subcutaneous implantation of 10 million SKOV-3 cells or the same number of Ramos cells was performed in female Nu/j mice. The experiments were conducted three weeks after the implantation. The mean weight of the animals at the time of the experiment was (25.4 ± 1.8) g. The mean tumor weight was (0.4 ± 0.2) and (0.2 ± 0.05) g for SKOV-3 and Ramos xenografts, respectively.

The mice were administered 3 μg of ^{99m}Tc-G3-(GGGS)$_3$Cys (40 kBq, 100 μl in sterile phosphate buffered saline) via the tail vein. Following euthanasia of the animals, their blood, organs, and tissues of interest were collected and weighed. The radioactivity was then measured using the gamma counter. The uptake in organs was calculated as a percentage of the injected dose per gram of the sample (%ID/g).

When planning and conducting animal experiments, we adhered to all applicable international and national guidelines for the care and use of animals for scientific purposes. The animal research protocol was approved by the Ethics Committee at Siberian State Medical University (Protocol code 7715, 20190826).

The Mann – Whitney *U*-test was used to determine significant differences ($p < 0.05$). Statistical analysis was performed using the Prism software (version 9.0.0 for Windows; GraphPad Software, USA).

RESULTS AND DISCUSSION

The composition of the lyophilized formulation with G3-(GGGS)$_3$Cys for the production of the experimental probe was multicomponent due to the necessity of using two groups of excipients [19, 21]. The first group of excipients was designed to safeguard the functionality of the protein during lyophilization and to maintain its capacity of transition into a solution upon dissolution of the lyophilized material. D-mannose and PEG-4000 were employed as such excipients. The second group of excipients was essential for the radioactive labeling of the protein. This process is based on the reduction of heptavalent ^{99m}Tc sodium pertechnetate to a pentavalent substance, followed by the formation of an oxotechnetium complex with a chelating group (-GGSC). The reducing agents employed were tin (II) chloride, sodium gluconate, and EDTA. After adding the pertechnetate eluant from the generator to the lyophilized formulation, it was then incubated in accordance with the procedure outlined in [19]. The radiochemical purity of the ^{99m}Tc-G3-(GGGS)$_3$Cys radiocomplex was 98 ± 1 %. The method for labeling the G3-(GGGS)$_3$Cys solution, previously described in [19], was replicated for the lyophilized formulation in order to obtain the ^{99m}Tc-G3-(GGGS)$_3$Cys radiocomplex of high purity, which does not require purification.

In order to study the binding specificity of the ^{99m}Tc-labeled G3-(GGGS)$_3$Cys to HER2/neu, cell lines with varying levels of receptor expression were employed: SKOV-3, BT-474, and DU-145. The experiment was conducted by blocking the receptor with unlabeled G3-(GGGS)$_3$Cys. The study of binding specificity *in vitro* demonstrated high specific binding to cells, which was proportional to the level of HER2/neu expression in the cells. Blocking the receptors with excess unlabeled protein showed a significant decrease in ^{99m}Tc-G3-(GGGS)$_3$Cys binding in all groups of cells ($p < 0.0005$) (Fig. 1).

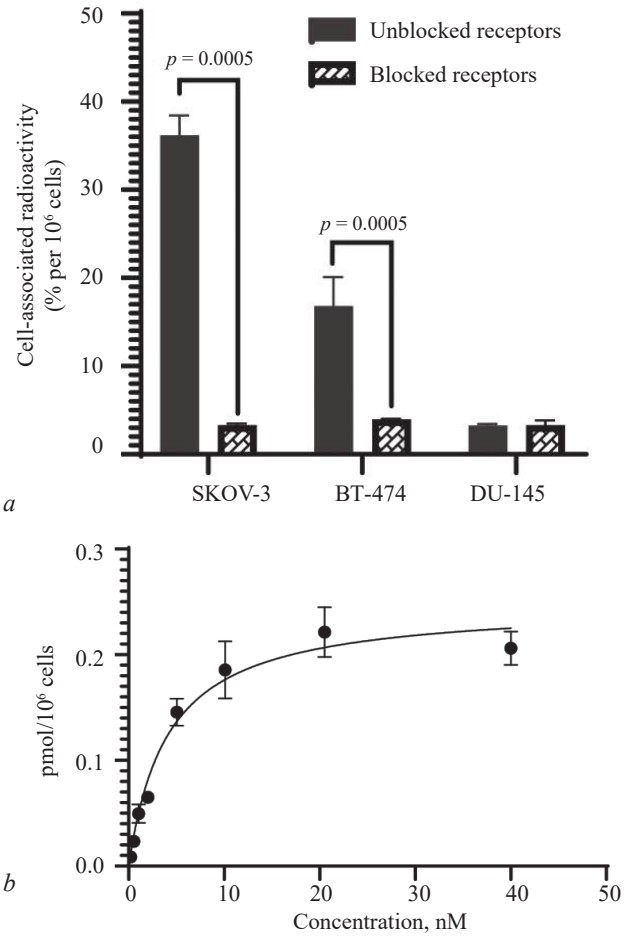

Fig. 1. Results of the *in vitro* assessment of the ^{99m}Tc-labeled lyophilized formulation with G3-(GGGS)3Cys: *a* – determination of binding specificity to HER2/neu; *b* – saturation curve on HER2/neu-expressing SKOV-3 cells. The results are presented as the mean % per 10^6 cells ± standard deviation from three samples.

Affinity assessment by the binding saturation assay demonstrated that ^{99m}Tc-G3-(GGGS)$_3$Cys binds to HER2/neu receptors on the surface of SKOV-3 cells with a nanomolar C_D (3.9 ± 0.5 nM) (Fig. 1), thereby confirming high affinity of the ^{99m}Tc-labeled protein obtained from the lyophilized formulation for its target receptors.

A comparison of the biodistribution and accumulation of ^{99m}Tc-G3-(GGGS)$_3$Cys in SKOV-3 xenografts with high HER2/neu expression and Ramos xenografts with no HER2/neu expression is presented in Figure 2. A parallel comparison of the biodistribution of ^{99m}Tc-G3-(GGGS)$_3$Cys in the SKOV-3 and Ramos xenografts revealed that the pattern of biodistribution was similar ($p > 0.05$), with the exception of radioactivity accumulation in the tumor. The accumulation of ^{99m}Tc-G3-(GGGS)$_3$Cys in the SKOV-3 xenografts was found to be significantly higher ($p < 0.005$) than in the Ramos xenografts. This indicates that the level of accumulation correlates with HER2/neu expression.

The radiopharmaceutical ^{99m}Tc-G3-(GGGS)$_3$Cys exhibited rapid blood clearance, predominantly via the kidneys. Additionally, low retention of radioactivity in the kidneys was observed (12–20% ID / g). This may be attributed to the rapid internalization of the protein following reabsorption in the kidneys and subsequent excretion of radiocatabolites containing glycine amino acid residues from the cell [17].

Low radioactivity accumulation was further observed in salivary glands and stomach, indicating the stability of the radiocomplex *in vivo*, since no hydrolysis of the complex was noted with the release of free ^{99m}Tc capable of accumulating in these organs. Furthermore, ^{99m}Tc-G3-(GGGS)$_3$Cys exhibited minimal uptake in the lungs, muscles, small intestine, and bones, which is favorable for imaging metastases in these areas [22].

Uptake in the liver was approximately 5–7% ID / g, with the tumor-to-liver ratio of less than one (approximately 0.7 times), which is suboptimal for imaging of liver metastases. Nevertheless, it should be noted that the biodistribution in mice is not entirely comparable to that observed in humans. Consequently, the uptake in the liver is unlikely to interfere with imaging of liver metastases in clinical trials [23]. It appears that biliary excretion of radiometabolites played a minor role, as radioactivity in the gastrointestinal tract with its contents was low (approximately 2%).

It can be supposed that the mechanism of radioactivity retention in the liver is not solely due to hepatobiliary excretion, but may also be influenced by ligand – receptor interactions or other mechanisms. HER2/neu expression takes place in the liver, which may contribute to the accumulation of G3-(GGGS)$_3$Cys radioactivity in this organ. The selection of the optimal protein dose in clinical trials of radiolabeled scaffold proteins allows to control this process and attain the desired contrast for visualization of liver metastases [14, 24].

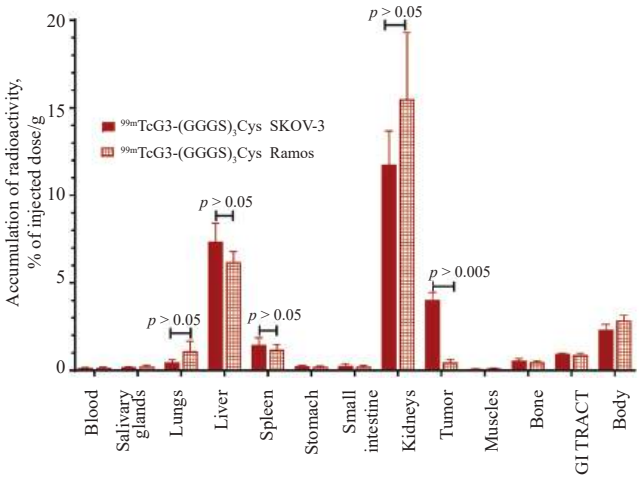

Fig.2. Comparative biodistribution of ^{99m}Tc-G3-(GGGS)$_3$Cys 4 hours after injection in HER2/neu-positive SKOV-3 and HER2/neu-negative Ramos xenografts in Nu/j mice. The data for five mice are presented as the mean % ID / g ± standard deviation. The data for the gastrointestinal tract with its contents and the rest of the body are presented as % ID for the whole sample

Since there was no direct comparison in the same batch of mice of ^{99m}Tc-G3-(GGGS)$_3$Cys obtained from the lyophilized formulation and ^{99m}Tc-G3-(GGGS)$_3$Cys obtained from the protein solution without technological interventions, it is not possible to state with any degree of reliability whether there are any changes in the functional suitability of the protein obtained from the lyophilized formulation. However, a comparison with the previously published data [19] on biodistribution and targeting properties in Nu/J mice bearing SKOV-3 xenografts revealed that the protein obtained from the lyophilized formulation in the selected composition exhibits properties identical to the native protein.

CONCLUSION

The studies *in vitro* and *in vivo* confirmed the functional suitability of the ^{99m}Tc-labeled lyophilized formulation with DARPin G3-$(GGGS)_3$Cys for radionuclide imaging of HER2/neu overexpression in malignant tumors. The radiocomplex based on ^{99m}Tc-G3-$(GGGS)_3$Cys is obtained by a simple method with high radiochemical purity, binds specifically to HER2/neu in tumor cells, is stable *in vivo*, and has favorable biodistribution in organs and tissues for subsequent transfer into clinical trials.

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Authors' contribution

Varvashenya R.N. – carrying out of radiochemical studies in vivo, in vitro, drafting of the article. Prach A.A. – carrying out of the experiments in vivo, in vitro. Plotnikov E.V., Larkina M.S. – conception and design, interpretation of the data, critical revision of the manuscript for important intellectual content, drafting of the article. Deev S.M. – conception and design. Belousov M.V. – interpretation of the data, critical revision of the manuscript for important intellectual content. Chernov V.I. – justification of the manuscript, conception and design, interpretation of the data, critical revision of the manuscript for important intellectual content, drafting of the article.

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Features of subset composition and functional activity of blood lymphocytes in tick-borne infections of different etiologies

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ABSTRACT

Aim. To perform a comparative assessment of subset composition and functional activity of peripheral blood lymphocytes in patients with tick-borne encephalitis (TBE) and ixodid tick-borne borreliosis (ITBB) in the acute phase of the disease.

Materials and methods. The study involved 22 patients with febrile and meningeal TBE, 15 patients with ITBB with and without erythema, and 11 healthy controls. Subset composition of blood lymphocytes was determined by flow cytometry. The blast transformation assay was applied to assess lymphocyte proliferation. Cytokine-producing activity of cells was studied in 24-hour incubated mononuclear cell cultures. Cytokine concentrations (interleukin (IL)-2, IL-4, IL-10, interferon (IFN)γ) were determined in the supernatants by the enzyme-linked immunosorbent assay (ELISA).

Results. Patients with TBE demonstrated an increase in the proportion of helper – inducer T-cells, a pronounced decrease in the proportion and absolute count of cytotoxic T cells, and low T lymphocyte count compared to the control values. The study in ITBB patients revealed an increase in the helper – inducer T-cell count and the proportion of NK-cells, a decrease in the cytotoxic T cell count, and the T lymphocyte count comparable to normal values. The most significant decrease in the levels of phytohemagglutinin-induced lymphocyte proliferation was found in patients with TBE. Patients of both groups showed a decrease in IL-2 secretion in the mononuclear cell culture, a rise in IL-4 and IL-10 production, and IFNγ production levels comparable to control values.

Conclusion. The study of TBE patients revealed relative lymphocytopenia with changes in the subset composition of lymphocytes characterized by an increase in the proportion of helper – inducer T-cells and a decrease in the absolute cytotoxic T lymphocyte count. Patients with ITBB demonstrated an increase in the proportion of NK-cells and a more pronounced imbalance in the T-helper / cytotoxic T lymphocyte ratio. Changes in the functional phenotype of lymphocytes, regardless of the etiology of tick-borne infection, were characterized by reduced proliferative reserve, low IL-2 secretion, increased IL-4 and IL-10 production, and depressed reactivity of lymphocytes with respect to IFNγ secretion.

Keywords: tick-borne encephalitis, ixodid tick-borne borreliosis, lymphocytes, cytokines

Conflict of interest. The authors declare the absence of obvious or potential conflict 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 Ethics Committee at Siberian State Medical University (Protocol No. 9119/1 of 30.05.2022).

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Особенности субпопуляционного состава и функциональной активности лимфоцитов крови при клещевых инфекциях разной этиологии

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РЕЗЮМЕ

Цель – провести сравнительную оценку субпопуляционного состава и функциональной активности лимфоцитов периферической крови у больных клещевым энцефалитом (КЭ) и иксодовым клещевым боррелиозом (ИКБ) в остром периоде заболевания.

Материалы и методы. В исследовании приняли участие 22 больных с лихорадочной и менингеальной формами КЭ, 15 пациентов с безэритемной и эритемной формами ИКБ и 11 здоровых лиц. Определение субпопуляционного состава лимфоцитов в крови проводили методом проточной цитофлуориметрии. Пролиферативную активность лимфоцитов исследовали в реакции бластной трансформации. Цитокинпродуцирующую активность клеток исследовали в 24-часовых культурах мононуклеарных лейкоцитов; концентрацию цитокинов (интерлейкина (IL) 2, IL-4, IL-10, фактора интерферона гамма (IFNγ)) определяли в культуральной жидкости методом иммуноферментного анализа.

Результаты. У пациентов с КЭ на фоне низкого по сравнению с контрольными значениями числа Т-лимфоцитов зарегистрировано повышение доли Т-хелперов/индукторов и выраженное снижение относительного и абсолютного количества Т-цитотоксических лимфоцитов. У больных ИКБ выявлено повышение числа Т-хелперов/индукторов и доли NK-клеток, а также сопоставимое с нормой количество Т-лимфоцитов и низкое содержание Т-цитотоксических клеток. Наиболее значимое снижение уровня ФГА-индуцированной лимфопролиферации зарегистрировано у пациентов с КЭ. У пациентов обеих групп выявлено снижение секреции IL-2 в культуре мононуклеарных лейкоцитов, повышение наработки IL-4 и IL-10 и сопоставимый с нормой уровень продукции IFNγ.

Заключение. У больных КЭ на фоне относительной лимфоцитопении регистрируются изменения субпопуляционного состава лимфоцитов, характеризующиеся повышением доли Т-хелперов/индукторов при абсолютной недостаточности Т-цитотоксических лимфоцитов. При ИКБ наблюдается повышение доли NK-клеток, а дисбаланс соотношения Т-хелперы/Т-цитотоксические лимфоциты выражен сильнее, чем при КЭ. Изменения функционального фенотипа лимфоцитов вне зависимости от этиологического варианта инфекции характеризуются снижением резерва пролиферативной активности на фоне низкой секреции IL-2, повышением наработки IL-4 и IL-10 и недостаточной реактивностью лимфоцитов в отношении секреции IFNγ.

Ключевые слова: клещевой энцефалит, иксодовый клещевой боррелиоз, лимфоциты, цитокины

Конфликт интересов. Авторы гарантируют отсутствие потенциальных и явных 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 этическим комитетом СибГМУ (протокол № 9119/1 от 30.05.2022).

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INTRODUCTION

Despite advances in the fields of prevention, diagnosis, and treatment of most infectious diseases, the problem of tick-borne infections is far from being completely resolved. High incidence of chronic ixodid tick-borne borreliosis (ITBB), pronounced polymorphism of clinical manifestations and features of the course of tick-borne encephalitis (TBE) (from suppressed forms to severe and chronic ones) with the development of long-term complications indicate a lack of scientific knowledge about the relationship between the pathogen and the host body [1, 2].

It is known that the pathogenesis of any infectious disease is a complex dynamic process during which the pathogenic potential of the microbe is realized through interaction with factors of innate and adaptive immunity of the host. At the same time, the functional viability and productive cooperative interactions of antigen-presenting, regulatory and effector immune cells during the immune response largely determine not only features of clinical manifestations, but also options for the outcome of the infectious process [3, 4]. In some cases, the absence of specific clinical and laboratory manifestations in some forms of vector-borne infections transmitted by ixodid ticks causes difficulties in differential diagnosis at the early stage of the disease [5]. In this regard, the study of the characteristics and mechanisms of development of various etiological variants of tick-borne infections has both theoretical and practical significance, in particular, to identify new biomarkers of disorders of the structural and functional phenotype of immune cells which are significant for the diagnosis and prognosis of diseases.

The aim of the study was to perform a comparative assessment of subset composition and functional activity of peripheral blood lymphocytes in patients with TBE and ITBB in the acute phase of the disease.

MATERIALS AND METHODS

The study involved 37 patients with acute tick-borne infections, of which 22 patients had acute febrile and neuroinvasive forms of TBE (mean age of the patients was 49.88 ± 2.81 years), and 15 individuals were ITBB patients with and without erythema migrans (mean age of the patients was 46.00 ± 2.79 years). The diagnosis was verified based on the medical history and the results of an objective examination, which included laboratory tests and the enzyme-linked immunosorbent assay (ELISA) with the determination of IgM and IgG to *Borrelia burgdorferi* s.l. and to TBE in the blood, as well as the TBE virus antigen. Additionally, relapsing tick-borne fever caused by *Borrelia miyamotoi*, ehrlichiosis, and human granulocytic anaplasmosis were excluded in all patients by polymerase chain reaction (PCR) (RealBest kits, Vector-Best, Russia). The control group involved 11 healthy individuals (mean age was 48.13 ± 2.76 years). The material for the study was venous peripheral blood collected from the patients upon admission to the Infectious Disease Clinic of Siberian State Medical University.

Absolute and relative lymphocyte counts, including T lymphocytes (CD3+CD19–), helper – inducer T-cells (CD3+CD4+CD45+), cytotoxic T lymphocytes (CD3+CD8+CD45+), natural killer cells (NK-cells, CD3–CD56+CD45+), and B lymphocytes (CD19+CD3–), were assessed by immunophenotyping using fluorescently labeled monoclonal antibodies (Elabscience, China) and subsequent multicolor flow cytometry on the Accuri C6 flow cytometer (BD Biosciences, USA). To correctly exclude all particles from the assay regions that did not correspond in size and granularity to living lymphocytes, some logical constraints were included into the particle distribution histograms according to small-angle and side-scatter characteristics (Fig.). The proportion of positive cells in the total cell count was measured by applying logical constraints to individual markers. At least 10^4 lymphocytes were analyzed in each sample. Absolute subset counts were determined based on their proportion (percentage) and absolute lymphocyte count in the peripheral blood using the Sysmex XN1000 hematology analyzer (Sysmex, Japan).

Spontaneous and mitogen-stimulated proliferative activity of peripheral blood mononuclear cells (PBMC) was studied in the blast transformation assay (BTA). Suspension culture was prepared by mixing heparinized venous blood with the RPMI-1640 medium in a 1:4 ratio supplemented with L-glutamine and fetal bovine serum (BioloT, Russia). Phytohemagglutinin (PHA) (Sigma, USA) (0.01 mg per 2.0×10^6 / ml) was added to one of the two samples and incubated at 37 °C and 5% CO_2 for 72 hours. After incubation, the contents of the vials were resuspended and centrifuged. Smears were prepared from the sediment, fixed and stained with Azur II Eosin. BTA intensity was determined

by standard cell morphology using light microscopy to analyze the count of non-blast and blast forms (in %). The stimulation index was calculated as the ratio of the PHA-induced blast transformation to spontaneous blast transformation.

Cytokine-producing activity of cells was studied in 24-hour incubated mononuclear cell cultures isolated from venous blood by the Ficoll density gradient centrifugation at a density of 1.077 g / cm^3 (BioloT, Russia). Cells were incubated at a concentration of 2×10^6 / ml in the complete growth medium with or without 50 μg / ml PHA. The concentrations of cytokines, including interleukins (IL)-2, IL -4, IL-10, and interferon (IFN)-γ, were assessed in the culture supernatants by ELISA using Vector-Best kits (Russia). The stimulation index was calculated as the ratio of PHA-induced cytokine secretion to spontaneous secretion.

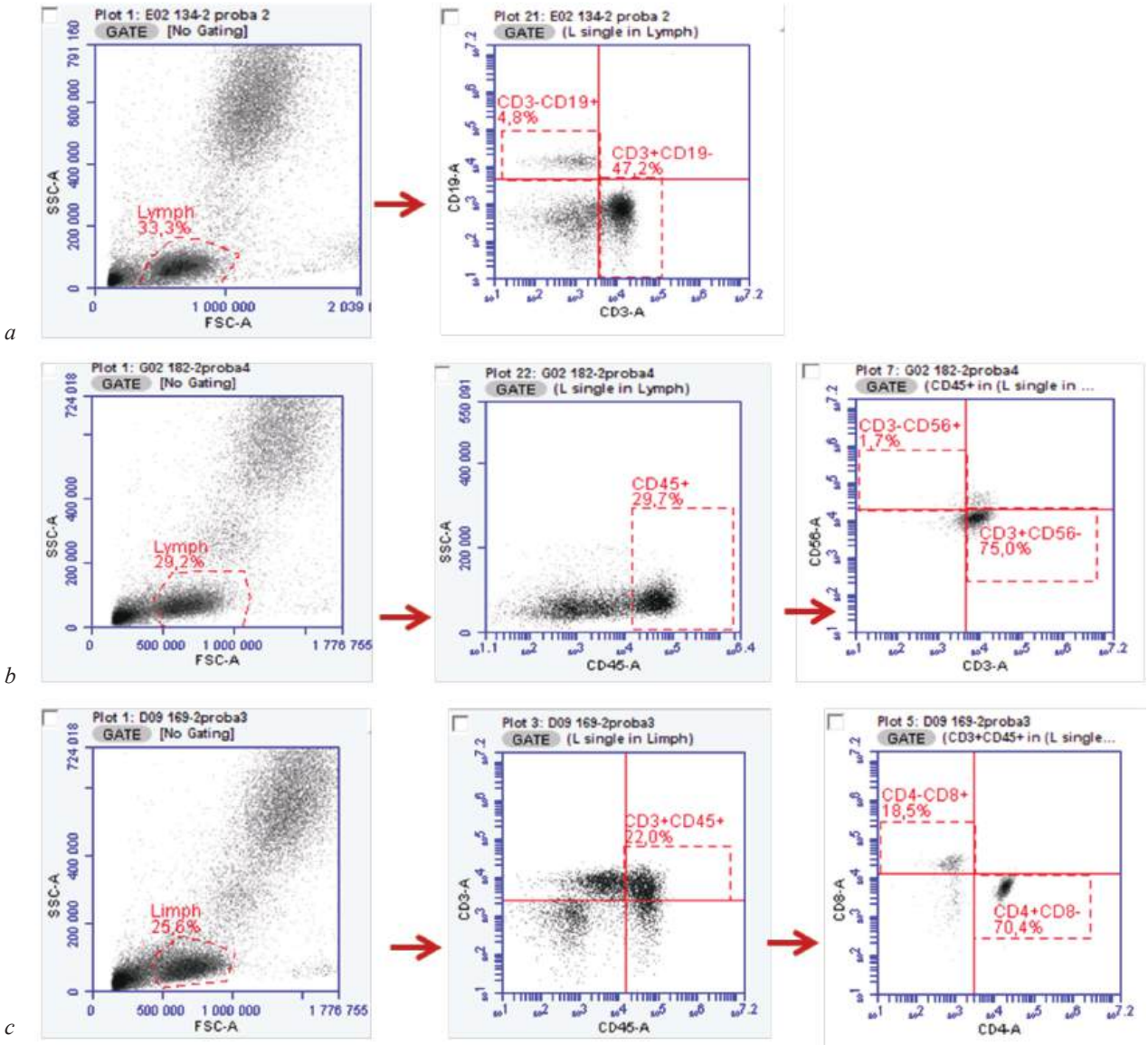

Figure. Distribution of lymphocyte subsets depending on the expression of surface CD-markers: *a* – distribution histogram of T lymphocytes (CD3+) and B lymphocytes (CD19+); *b* – isolation of NK-cells (CD3–CD56+) from the general subset of CD45+ lymphocytes; *c* – distribution histogram of helper – inducer T-cells (CD4+) and cytotoxic T lymphocytes (CD8+) after applying the logical constraint to CD45+ and CD3+

The results were processed using the Statistica 12.0 software (StatSoft, USA). Normally distributed data (Shapiro – Wilk test) were presented as the mean and the standard deviation ($M \pm SD$). Non-normally distributed data were presented as the median and the interquartile range Me (Q_1; Q_3). The Student's t-test or the Mann – Whitney U-test with the Bonferroni correction were used to compare differences between the groups. The differences between the two compared variables were considered statistically significant at $p < 0.05$.

RESULTS

When analyzing the hemogram parameters in the group of patients with TBE compared to the controls, we revealed a statistically significant decrease in the proportion of lymphocytes along with an increase in the total leukocyte count in the peripheral blood (Table 1). Nevertheless, the absolute lymphocyte counts in the peripheral blood in both groups of patients with TBE and ITBB did not differ significantly from the control values. According to the results of immunophenotyping of peripheral blood lymphocytes, patients with TBE with a decrease in the total T lymphocyte count showed an increase in the proportion of helper – inducer T-cells as well as a significant decrease in the relative and absolute cytotoxic T lymphocyte count (on average by 1.8 and 2.0 times, respectively) compared to the control values (Table 1).

In patients with ITBB, the total T lymphocyte count did not differ significantly from the values in the control group. However, they had a decrease in the cytotoxic T lymphocyte count and an increase in the absolute helper – inducer T-cell count.

Quantitative changes in these lymphocyte subsets affected the helper T cell / cytotoxic T cell ratio, which was on average 2 times higher than the control values in both groups (Table 1).

Table 1

Total leukocyte count and lymphocyte subset composition in the peripheral blood of patients with tick-borne infections, *Me* (Q_1; Q_3)

Group of the examined individuals	Total leukocyte count, $\times 10^9$ / l	Total lymphocyte count in the hemogram (in % in the numerator, $\times 10^9$ / l in the denominator)	Lymphocyte subset counts (in % in the numerator, $\times 10^9$/l in the denominator)					Helper T cell / cytotoxic T cell ratio
			NK-cells	Helper – inducer T-cells	Cytotoxic T lymphocytes	T lymphocytes	B lymphocytes	
Control group, $n = 11$	5.76 (4.32; 5.90)	36.00 (32.00; 37.01) 2.08 (1.45; 2.18)	11.02 (8.98; 12.97) 0.21 (0.19; 0.26)	49.32 (47.30; 51.12) 1.01 (0.66; 1.08)	31.00 (29.9; 32.10) 0.62 (0.47; 0.70)	80.32 (78.2; 82.03) 1.70 (1.09; 1.71)	9.07 (8.97; 9.8) 0.16 (0.14; 0.19)	1.59 (1.54; 1.70)
Patients with TBE, $n = 22$	8.36 (5.82; 10.99) $p_1 = 0.01$	20.65 (13.2; 28.9) $p_1 = 0.04$ 1.43 (1.00; 2.38)	11.80 (8.01; 13.52) 0.21 (0.12; 0.28)	58.22 (53.12; 69.1) $p_1 = 0.04$ 0.76 (0.56; 1.10)	17.37 (11.48; 22.45) $p_1 = 0.01$ 0.29 (0.17; 0.41) $p_1 = 0.01$	73.76 (66.81; 86.12) $p_1 = 0.04$ 1.12 (0.77; 1.84) $p_1 = 0.04$	9.31 (4.52; 12.66) 0.17 (0.06; 0.29)	2.85 (2.10; 5.68) $p_1 = 0.03$
Patients with ITBB, $n = 15$	7.23 (5.49; 8.57) $p_1 = 0.03$	34.40 (19.85; 43.7) $p_2 = 0.04$ 2.09 (1.72; 2.79)	14.92 (11.13; 21.53) $p_1 = 0.04$ $p_2 = 0.04$ 0.26 (0.13; 0.46)	60.99 (52.93; 64.26) $p_1 = 0.04$ 1.19 (1.14; 1.72) $p_1 = 0.04$ $p_2 = 0.03$	16.81 (14.84; 18.29) $p_1 = 0.01$ 0.34 (0.27; 0.40) $p_1 = 0.01$	77.14 (71.22; 81.87) 1.54 (1.46; 2.08)	7.83 (4.92; 10.27) 0.16 (0.09; 0.27)	3.69 (3.40; 4.20) $p_1 < 0.001$

Here and in Table 2 and 3: p_1 is the level of significance of differences when compared with parameters in the control group; p_2 is the level of significance of differences when compared with parameters in TBE patients.

It should be noted that the number of B lymphocytes in both groups of patients was comparable with the control values. At the same time, a statistically significant increase in the relative number of NK-cells was recorded in patients with ITBB compared to the control group.

Assessing the results of lymphocyte proliferative activity in the PBMC cultures *in vitro*, we found an increase in the level of spontaneous blast transformation only in the patients with ITBB compared to the healthy controls (Table 2).

The levels of PHA-induced blast transformation were significantly lower in both groups of patients than in the control group, while the most significant decrease in this parameter was found in patients with TBE, which was reflected in similar changes in the calculated PHA stimulation index (Table 2).

Table 2

The results of the blast transformation assay of the peripheral blood lymphocytes in patients with tick-borne infections, *M ± SD*

Groups of the examined individuals	Blast transformation assay, %		Stimulation index
	without PHA stimulation	with PHA stimulation	
Control group, $n = 11$	6.09 ± 0.97	61.82 ± 7.87	10.41 ± 2.41
Patients with TBE, $n = 22$	7.55 ± 3.31	26.78 ± 11.52 $p_1 < 0.001$	3.79 ± 1.19 $p_1 < 0.001$
Patients with ITBB, $n = 15$	10.40 ± 2.48 $p_1 < 0.001$ $p_2 = 0.003$	49.63 ± 7.12 $p_1 < 0.001$ $p_2 = 0.002$	4.98 ± 1.18 $p_1 < 0.001$ $p_2 = 0.03$

The capability of secreting immunoregulatory cytokines is one of the parameters determining the functional phenotype along with the proliferative activity and expression profile of peripheral blood lymphocytes. In this study, we focused on IL-2, IL-4, IL-10, and IFNγ that have para- and autocrine effects on immune cells by controlling all stages of antigen-specific proliferation, differentiation, and functional activity of T and B lymphocytes [6–8]. When analyzing the cytokine-producing activity of PBMC cultures *in vitro* obtained from both groups of patients with tick-borne infections, regardless of their etiology, we detected a decrease in the spontaneous and mitogen-induced production of IL-2, which was most pronounced in patients with ITBB (Table 3).

Basal IL-4 secretion and the levels of spontaneous and PHA-induced IL-10 secretion in the cell culture supernatants in both groups of patients were significantly higher than those in the controls. We detected that on average the levels of PHA-stimulated IL-4 production in the patients with ITBB were two times higher than those in the control group (Table 3). It should be mentioned that the concentration of spontaneous and mitogen-induced IFNγ secretion in the primary PBMC cultures in both groups of patients with tick-borne infections did not differ from that in the healthy donors (Table 3). The analysis demonstrated a statistically significant decrease in the stimulation indices of IL-2, IL-4, and IL-10 in both groups of patients compared to the control values, which indicates that the PBMC secretory activity in relation to these cytokines decreases.

Table 3

Cytokine concentrations in the supernatants of 24-hour cultures of peripheral blood mononuclear cells in the patients with tick-borne infections, *Me* (Q_1; Q_3)

Parameter		Control group, $n = 11$	Patients with TBE, $n = 22$	Patients with ITBB, $n = 15$
Concentration of IL-2, pg /ml	without PHA stimulation	44.52 (32.74; 112.13)	32.11 (11.56; 48.06) $p_1 = 0.01$	10.78 (9.78; 13.19) $p_1 < 0.001$; $p_2 = 0.03$
	with PHA stimulation	93.50 (66.96; 275.88)	27.21 (24.28; 68.74) $p_1 = 0.005$	24.27 (21.01; 27.57) $p_1 < 0.001$; $p_2 = 0.03$
	Stimulation index	2.85 (1.57; 5.52)	2.20 (1.64; 2.60); $p_1 = 0.04$	2.11 (1.68; 2.62); $p_1 = 0.04$
Concentration of IL-4, pg / ml	without PHA stimulation	2.92 (2.51; 4.93)	10.51 (6.50; 23.88) $p_1 = 0.01$	26.18 (22.13; 30.35) $p_1 < 0.001$; $p_2 < 0.001$
	with PHA stimulation	19.72 (10.20; 22.32)	19.90 (10.38; 46.97)	44.43 (41.42; 66.06) $p_1 < 0.001$; $p_2 = 0.01$
	Stimulation index	4.00 (3.49; 8.83)	2.11 (1.89; 2.19); $p_1 < 0.001$	1.99 (1.41; 2.48); $p_1 = 0.001$
Concentration of IL-10, pg / ml	without PHA stimulation	9.36 (2.45; 12.65)	37.28 (12.89; 53.71) $p_1 = 0.02$	28.77 (20.01; 90.87) $p_1 = 0.001$
	with PHA stimulation	27.39 (12.87; 59.75)	88.66 (57.09; 141.46) $p_1 = 0.04$	112.39 (48.07; 129.70) $p_1 = 0.004$
	Stimulation index	4.72 (1.38; 11.09)	2.22 (2.13; 2.73); $p_1 = 0.02$	2.20 (1.02; 5.20); $p_1 = 0.01$
IFNy concentration, pg/ml	without PHA stimulation	26.96 (9.47; 43.61)	21.27 (14.82; 25.01)	16.50 (8.81; 41.81)
	with PHA stimulation	25.59 (22.45; 49.30)	24.39 (11.49; 41.25)	21.77 (19.11; 44.34)
	Stimulation index	0.98 (0.51; 4.85)	1.02 (0.50; 2.79)	1.81 (0.43; 3.95)

DISCUSSION

It is known that the subset composition of lymphocytes in the peripheral blood in the acute phase of an infectious disease is the result of their dynamic redistribution during active migration of naive lymphocytes to peripheral lymphoid organs (where they interact with antigen-presenting cells), their further proliferation and release as mature clones into the blood, migration into tissues with return to the peripheral lymphatic organs, and apoptosis [9, 10]. The quantitative and qualitative characteristics of the immune response to infections depend on many factors, such as the antigen type, its dose, and a route of entry into the body.

According to some of the earlier studies, changes in the distribution of the peripheral blood lymphocyte subsets included a decrease in the CD3+, CD4+, and CD8+ T lymphocyte counts as well as an increase in the B lymphocyte count, typical of the acute phase of TBE [11–13]. In patients with acute febrile and neuroinvasive forms of TBE and relative lymphocytopenia, we revealed a deficit in the T lymphocyte count (CD8+ lymphocyte count), while the B lymphocyte count and NK-cell count did not differ from the control values. Moreover, we detected significant imbalances in the helper T cell / cytotoxic T cell ratio as well as a significant decrease in the PHA-induced proliferative activity of peripheral blood lymphocytes in TBE patients (Table 2).

It seems that the decrease in the CD8+-subset in TBE patients resulted from the insufficient proliferative response of lymphocytes to viral antigens. It should also be assumed that there was a direct cytotoxic effect of the TBE virus and selective damage to the T cell immune response due to its replication in the thymus, which can control maturation, differentiation, and functional activity of T lymphocytes.

Thus, some researchers point to the development of clonal exhaustion of committed T lymphocytes in viral persistence with the immune imbalance polarized toward Th2 [14, 15]. These results were consistent with our data on assessing the cytokine-producing activity of mononuclear cells in the acute phase of TBE and revealed elevated levels of IL-10 and IL-4 secretion in PBMC cultures with decreased IL-2 secretion and insufficient leukocyte cellular activity to release IFNγ (Table 3). When IL-2 secretion was reduced, lymphocytes did not have sufficient reserve to increase proliferation in response to PHA stimulation, which was probably not compensated by the effects of other proinflammatory cytokines, such as TNFα, IL-1β, IL-12, etc.

Unlike cytotoxic T lymphocytes, NK-cells as components of innate immunity do not have pathogen specific recognition function as well as in the mechanisms of antibody-dependent cellular cytotoxicity [16]. NK-cells play a pivotal role of in the immunopathogenesis of viral infections and are also involved in cytolytic effects in bacterial diseases. In bacterial infections, NK-cells can be activated both via cross-interaction with other leukocytes and via direct recognition of pathogen-associated molecular patterns [17].

To date, there is evidence that NK-cells express pattern recognition molecules, such as Toll- and NOD-like receptors [17, 18]. The capability of Borrelia spp. to induce an increase in the NK-cell count at the onset of the disease was a very important finding [19, 20]. At the same time, the cytolytic activity of NK-cells cannot be an effective mechanism for eliminating extracellular pathogens, including Borrelia spp. However, when there is cytotoxic lymphocyte deficiency and ineffective antibody synthesis, NK-cells are likely to play a pivotal role in limiting pathogen dissemination, which was confirmed by the increase in their count that we detected in the patients with ITBB. Moreover, the role of NK-cells and Th1 lymphocytes in the immunopathogenesis of Lyme disease was predominantly determined by the immune cell capability of stimulating phagocytosis via release of IFNγ and IL-12 that induce macrophage activation [21, 22].

Despite the fact that we detected elevated absolute helper T cell count in the patients with ITBB, the levels of basal and PHA-stimulated IFNγ secretion in the PBMC cultures did not differ from those in the control group. Additionally, low IL-2 production and increased secretion of IL-4 and IL-10 were revealed in both groups of patients with ITBB and TBE. The changes in the cytokine secretion profile of PBMC that we revealed in the patients with ITBB seemed quite logical and reflect the effector phase of the immune response to borrelia antigens. It was a result of cooperative interactions between macrophages and lymphocytes mediated by cytokines that induce the accumulation of mature T helper 2 cells, which

can stimulate formation of sufficient subsets of plasma cells releasing specific antibodies to borrelia antigens in the lymphoid organs.

In general, when analyzing the results obtained and comparing them with the literature data, we noted significant variability in changes in immune status parameters in different groups of patients with tick-borne infections, regarding both quantitative changes in these parameters and data interpretation in the context of complex regulatory mechanisms of the immune response [4, 11–13, 22]. Additionally, many authors note the correlation of suppression or activation levels of specific innate and adaptive immunity components with the severity of the clinical course of the disease, which, in our opinion, emphasizes the relevance of further research on immune response mechanisms in different clinical forms of tick-borne infections, including the study of dynamics of the infectious process.

CONCLUSION

The study revealed changes in the lymphocyte subset composition in the blood of patients with TBE and relative lymphocytopenia, characterized by an increase in the proportion of helper – inducer T cells and a decrease in the absolute cytotoxic T lymphocyte count. Patients with ITBB demonstrated an increase in the proportion of NK-cells and a more pronounced imbalance in the T-helper / cytotoxic T lymphocyte ratio. Changes in the functional phenotype of lymphocytes, regardless of the etiology of tick-borne infection, were characterized by reduced proliferative reserve, low IL-2 secretion, increased IL-4 and IL-10 production, and depressed reactivity of lymphocytes with respect to IFNγ secretion.

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Authors' contribution

Voronkova O.V., Ilyinskikh E.N. – conception and design, drafting of the manuscript. Hasanova R.R., Esimova I.E., Nevskaya K.V., Karpova M.R. – analysis and interpretation of the data. Chernyshov N.A., Yampolskaya A.V., Yampolskaya O.V. – implementation of laboratory research methods.

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Adipocytokine levels in patients with atherosclerosis and high triglyceride – glucose index

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ABSTRACT

Aim. To study the levels of adipocytokines and their associations with stable and unstable atherosclerotic plaques in patients with a high triglyceride – glucose (TyG) index.

Materials and methods. The study included 109 men aged 38–79 years (mean age 62.28 ± 8.19 years) with atherosclerosis hospitalized for coronary artery bypass grafting (CABG). After microscopy of the intima – media layer, the type of atherosclerotic plaque was determined: stable / unstable. The TyG index ≥ 4.49 was considered as high. Fifty-eight (60%) men had stable plaques in the CA (28 (56%) of them had TyG ≥ 4.49); 39 (40%) men had unstable plaques in the CA (15 (39%) had TyG ≥ 4.49). Blood adipocytokine level was studied using the multiplex assay and the Human Metabolic Hormone Panel V3.

Results. The final analysis included 97 patients. The level of glucose-dependent insulinotropic polypeptide (GIP) was 1.53 times greater in patients with TyG ≥ 4.49 (34.16 [18.71; 54.98] vs. 22.34 [15.02; 34.77], $p = 0.004$). In patients with TyG < 4.49, the adipsin level was 1.2 times higher in patients with unstable plaques than in patients with stable ones. In patients with stable plaques and TyG ≥ 4.49, the GIP level was 1.88 times higher than in patients with TyG < 4.49 (42.13 [25.34; 68.95] vs. 22.39 [17.00; 28.60], $p = 0.003$). In patients with unstable plaques and TyG ≥ 4.49, the level of peptide tyrosine – tyrosine (PYY) was 1.46 times greater than in patients with TyG < 4.49 (46.14 [30.49; 70.66] vs. 31.53 [24.71; 43.01], $p = 0.048$).

Conclusion. Men with atherosclerosis and TyG ≥ 4.49 had higher blood levels of GIP and PYY. Blood adipsin levels were higher in patients with unstable plaques without insulin resistance.

Keywords: TyG index, atherosclerosis, unstable atherosclerotic plaque, glucose-dependent insulinotropic polypeptide, peptide tyrosine – tyrosine, adipsin

Conflict of interest. The authors declare the absence of obvious or potential conflict 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 Committees at Research Institute of Internal and Preventive Medicine and E.N. Meshalkin National Medical Research Center (Protocol No. 2 of 05.06.2011).

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Уровни молекул, секретируемых жировой тканью, у пациентов с коронарным атеросклерозом и высоким триглицерид-глюкозным индексом

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РЕЗЮМЕ

Цель – изучение уровней адипоцитокинов, а также их ассоциаций со стабильными и нестабильными атеросклеротическими бляшками у пациентов с высоким триглицерид-глюкозным индексом (TYG).

Материалы и методы. Исследование включало 109 мужчин 38–79 лет (средний возраст 62,28 ± 8,19 лет) с атеросклерозом коронарных артерий (КА), госпитализированных на операцию коронарного шунтирования (КШ). После микроскопического исследования фрагментов интима-медиа определялся тип атеросклеротической бляшки: стабильная/нестабильная. Высоким считался TYG ≥ 4,49. Имели стабильные бляшки в КА 58 (60%) мужчин (у 28 из них (56%) TYG ≥ 4,49), 39 (40%) имели нестабильные бляшки в КА (у 15 (39%) TYG ≥ 4,49). Адипоцитокины в крови изучались при помощи мультиплексного анализа и панели Human Metabolic Hormone V3.

Результаты. В итоговый анализ вошли 97 пациентов. Уровень глюкозозависимого инсулинотропного полипептида (GIP) был в 1,53 раза выше у пациентов с TYG ≥ 4,49 (34,16 [18,71; 54,98] против 22,34 [15,02; 34,77], p = 0,004). У пациентов с TYG менее 4,49 уровень адипсина был выше у пациентов с нестабильными бляшками, чем у пациентов со стабильными, в 1,2 раза. У пациентов со стабильными бляшками и с TYG ≥ 4,49 уровень GIP был в 1,88 раза выше, чем у пациентов с TYG менее 4,49 (42,13 [25,34; 68,95] против 22,39 [17,00; 28,60], p = 0,003). У пациентов с нестабильными бляшками и TYG ≥ 4,49 уровень пептида тирозин-тирозин (PYY) был в 1,46 раза выше, чем у пациентов с TYG менее 4,49 (46,14 [30,49; 70,66] против 31,53 [24,71; 43,01], p = 0,048).

Заключение. У мужчин с коронарным атеросклерозом и TYG ≥ 4,49 в крови более высокие уровни GIP и PYY. Уровень в крови адипсина более высокий с нестабильными АСБ у пациентов без ИР.

Ключевые слова: индекс TYG, коронарный атеросклероз, нестабильная атеросклеротическая бляшка, глюкозозависимый инсулинотропный полипептид, пептид тирозин-тирозин, адипсин

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

Источник финансирования. Исследование проведено при финансовой поддержке Российского научного фонда № 24-25-00079.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальными комитетами по этике НИИТПМ – филиал ИЦиГ СО РАН и НМИЦ им. ак. Е.Н. Мешалкина (протокол № 2 от 05.06.2011).

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INTRODUCTION

The study of atherosclerosis and unstable atherosclerotic plaques is of great importance because of their key role in the pathogenesis of cardiovascular events. Atherosclerosis is a chronic inflammatory disease characterized by the emergence of atherosclerotic plaques in the walls of arteries, leading to stenosis and impaired blood flow. In particular, unstable atherosclerotic plaques are of clinical importance because they are prone to rupture, blood clots, and subsequent acute cardiovascular events. Recent studies have focused on the role of inflammation, lipid metabolism, and plaque destabilization mechanisms in the progression of atherosclerosis and the development of unstable plaques, emphasizing the importance of further research in this area to address the global burden of cardiovascular diseases [1–3].

Abdominal obesity is a well-established risk factor for the development and progression of atherosclerosis, largely due to the release of proinflammatory adipokines and cytokines from visceral adipose tissue, resulting in chronic low-grade inflammation and endothelial dysfunction, which play a key role in the pathogenesis of atherosclerosis [4]. Moreover, insulin resistance, a hallmark of the metabolic syndrome that often accompanies abdominal obesity, can further exacerbate atherosclerosis, contributing to dyslipidemia, oxidative stress, and inflammation, which together contribute to the formation and progression of atherosclerotic plaques [5].

It was found that the TyG index, a new marker of insulin resistance, is closely associated with atherosclerosis and cardiovascular risk, mainly due to its close relationship with dyslipidemia and impaired glucose metabolism, which play a key role in the pathophysiology of atherosclerosis [6]. Understanding the complex relationship between abdominal obesity, insulin resistance, and the TyG index is important for developing targeted strategies for the prevention of atherosclerosis and related cardiovascular complications.

The aim of our research was to study the levels of adipocytokines (C-peptide, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), interleukin-6 (IL-6), leptin, monocytic chemoattractant protein-1 (MCP-1), pancreatic polypeptide (PP), peptide tyrosine – tyrosine (PYY), tumor necrosis factor alpha (TNFa), plasminogen activator inhibitor-1 (PAI-1), lipocalin, ghrelin, glucagon, adiponectin, adipsin, resistin, omentin, visfatin) and their associations with stable and unstable atherosclerotic plaques in patients with insulin resistance (IR) using the triglyceride – glucose (TyG) index.

MATERIALS AND METHODS

The study was conducted as part of joint research of the Research Institute of Internal and Preventive Medicine – branch of the Institute of Cytology and Genetics of SB RAS and E. Meshalkin National Research Medical Center of the Ministry of Health of the Russian Federation. After patients signing a written consent to participate in the study, we collected their data and blood samples. The study was approved by the local Ethics Committees at both institutions (Protocol No.2 of 5.06.2011). The study was carried out with the financial support of the state assignment No. FWNR-2024-0004 and the RSF grant No. 24-25-00079.

The study included 109 men aged 38–79 years (mean age 62.28 ± 8.19 years) who were diagnosed with stable FC II–III angina pectoris and atherosclerosis of the coronary arteries (CA) following coronary angiography findings, did not have acute coronary syndrome (ACS), and were hospitalized at the clinic of E. Meshalkin NRMC from 2011 to 2023 for coronary artery bypass grafting (CABG).

The inclusion and exclusion criteria and the stages of data collection, examination, and histologic examination of samples were described in detail in previous articles [7].

After microscopy of the intima – media layer (sampled during CABG), we determined whether atherosclerotic plaques were stable or unstable [8].

After CABG, 6 patients dropped out of the study because they developed complications, signed a voluntary waiver or it was impossible to contact them. It was not possible to determine the type of a plaque in 6 patients. The final analysis included 97 patients. Insulin resistance in patients was determined using the TyG index (ln [triglycerides (mg / dl) x glucose (mg/dl)] / 2.). The optimal cut-off point was 4.49, with the sensitivity of 82.6% and specificity of 82.1% (AUC = 0.889, 95% CI: 0.854–0.924) [9].

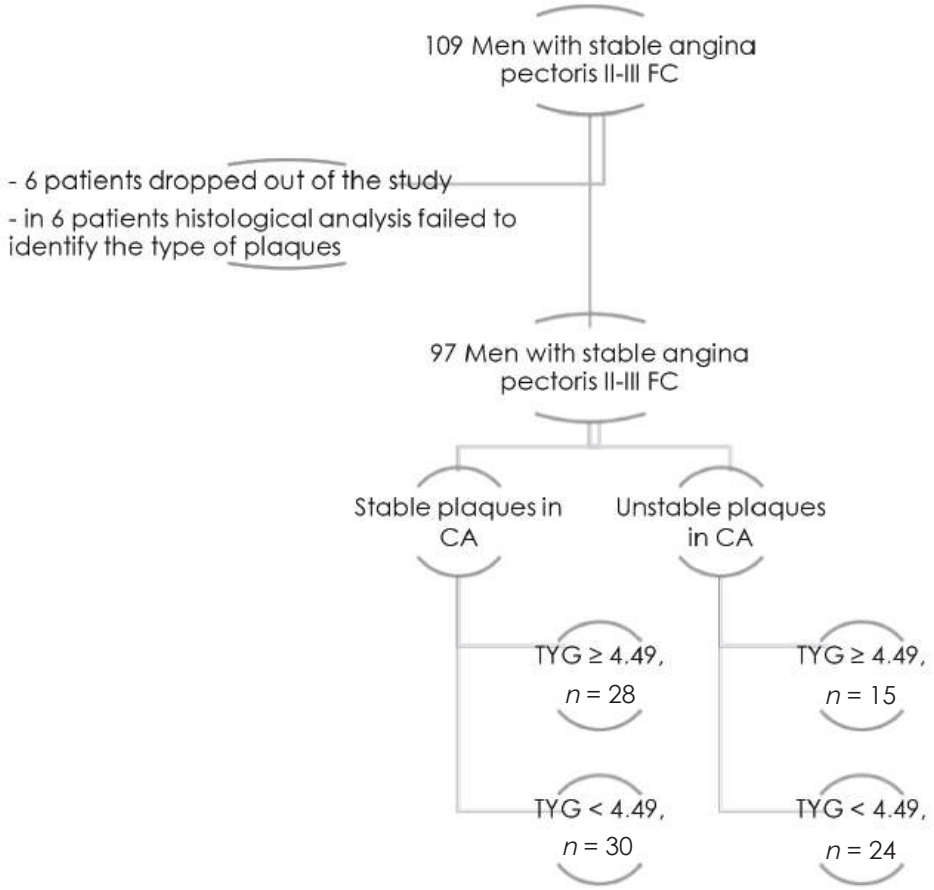

Fig. 1. Research design. CA – coronary arteries, FC – functional class, TyG – triglyceride – glucose index

In the study group, 58 (60%) men had stable plaques in CA (28 (56%) of them had TyG index ≥ 4.49, which, according to studies, is associated with insulin resistance [9]), and 39 (40%) men had unstable plaques in CA (15 (39%) had TyG index ≥ 4.49).

Biochemical studies were carried out using the enzymatic method on the Konelab 30i analyzer (Thermo, Finland). We applied the Luminex MAGPIX multiplexing system and the multiplex assay using the Human Metabolic Hormone Panel V3 (MILLIPLEX, Germany) to determine the levels of the following parameters: C-peptide, GIP, GLP-1, IL-6, leptin, MCP-1, PP, PYY, TNFa, PAI-1, lipocalin, ghrelin, glucagon, adiponectin, adipsin, resistin, omentin, and visfatin.

The statistical analysis was carried out using the SPSS 13.0 software package. The Kolmogorov – Smirnov test was used to estimate the distribution of variables. Since the distribution of quantitative variables was nonparametric, we used the median of the interquartile range *Me* (Q_{25}; Q_{75}). The Mann – Whitney *U*-test (for two independent samples) and the Kruskal – Wallis test were used to compare the samples. The Spearman's rank correlation coefficient (r_s) was applied to analyze the dependence of quantitative features of individual data from data aggregates. Qualitative variables were presented as absolute values *n* and fractions in %. The Pearson's χ^2 test was used to assess the differences between qualitative variables. The results were considered statistically significant at $p < 0.05$.

RESULTS

Table 1 presents data from patients with high and low TyG index and stable or unstable plaques. Type 2 diabetes mellitus was more common in patients with unstable plaques and TyG ≥ 4.49 than in patients with stable plaques and TyG ≥ 4.49 (53% vs. 11%, $p = 0.006$) (Table 1). All patients were diagnosed with essential hypertension and received antihypertensive therapy to achieve the target blood pressure values.

Dyslipidemia in patients of both groups was determined by an increase in the level of lipoproteins and lipids above the optimal value [1].

The patients included in the study had a very high cardiovascular risk, so dyslipidemia was determined at LDL-C levels > 55 mg / dl and at TG levels > 150 mg / dl. All patients with coronary artery disease received statin therapy at maximum tolerated doses, regardless of the presence of dyslipidemia.

Table 1

Characteristics of patient groups depending on TyG index and plaque type (stable/unstable)

Parameter	Patients with stable plaques and TyG ≥ 4.49, *n* = 28	Patients with unstable plaques, TyG ≥ 4.49 *n* = 15	*p*	Patients with stable plaques, TyG < 4.49, *n* = 30	Patients with unstable plaques, TyG < 4.49, *n* = 24	*p*
Average age	63.00 [57.00; 67.50]	59.00 [54.00; 65.00]	0.338	64.00 [57.25; 70.75]	62.00 [56.25; 68.00]	0.567
BMI, kg / m^2	29.84 [26.97; 32.14]	29.72 [26.83; 31.70]	0.899	26.67 [25.35; 30.12]	29.11 [25.97; 32.74]	0.169
WC more than or equal to 94 cm	16 (57.1%)	9 (60.0%)	0.856	10 (33.3%)	8 (33.3%)	0.933
WC, cm	92.00 [88.00; 100.00]	93.50 [89.00; 100.50]	0.530	89.00 [82.75; 99.50]	88.00 [84.50; 94.00]	0.864
SBP, mmHg	130.00 [121.25; 140.00]	125.00 [120.00; 142.00]	0.691	135.00 [127.00; 148.33]	137.00 [132.00; 143.00]	0.535
DBP, mmHg	80.50 [80.00; 89.50]	80.00 [78.33; 81.67]	0.301	80.00 [75.00; 90.00]	80.00 [80.00; 89.50]	0.485
Smoking status, abs. %	17 (61%)	10 (67%)	0.784	24 (80%)	22 (92%)	0.230
T2DM, abs. %	3 (11%)	8 (53%)	0.006	4 (13%)	4 (17%)	0.732
Cholesterol, mmol / l	4.17 [3.46; 4.88]	4.50 [3.57; 5.09]	0.628	3.92 [2.51; 4.46]	3.51 [3.13; 4.42]	0.862
Triglycerides, mmol / l	1.09 [1.02; 1.32]	0.86 [0.77; 1.44]	0.177	0.69 [0.58; 0.86]	0.62 [0.49; 0.78]	0.246
HDL-C, mmol / l	0.59 [0.47; 0.71]	0.61 [0.53; 0.70]	0.655	0.61 [0.52; 0.80]	0.70 [0.53; 0.89]	0.293
LDL-C, mmol / l	2.98 [2.47; 3.74]	3.46 [2.26; 3.79]	0.760	2.85 [1.67; 3.39]	2.41 [1.98; 3.40]	0.947
Glucose, mmol / l	6.70 [5.63; 8.43]	6.80 [5.90; 8.00]	0.721	5.25 [4.83; 5.68]	5.25 [4.80; 6.08]	0.573

Note. BMI – body mass index, WC – waist circumference, SBP – systolic blood pressure, DBP – diastolic blood pressure, T2DM – type 2 diabetes mellitus, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol.

In all patients, we assessed the levels of adipocytokines (C-peptide, GIP, GLP-1, IL-6, leptin, MCP-1, PP, PYY, TNFa, PAI-1, lipocalin, ghrelin, glucagon, adiponectin, adipsin, resistin, omentin, visfatin) in the subgroups with low (< 4.49) and high (≥ 4.49) TyG index. The GIP level was 1.53 times higher in patients with TyG ≥ 4.49 (34.16 [18.71; 54.98] vs. 22.34 [15.02; 34.77], $p = 0.004$) (Fig. 2).

Next, adipocytokine levels were assessed in the subgroups with low (< 4.49) and high (≥ 4.49) TyG index in patients with unstable and stable atherosclerotic plaques in the CA (Table 2). In patients with TyG < 4.49, the adipsin level was 1.2 times higher in patients with unstable plaques than in patients with stable ones.

In patients with stable plaques and TyG ≥ 4.49, the GIP level was 1.88 times higher than in patients with stable plaques and TyG < 4.49 (42.13 [25.34; 68.95] vs. 22.39 [17.00; 28.60], $p = 0.003$).

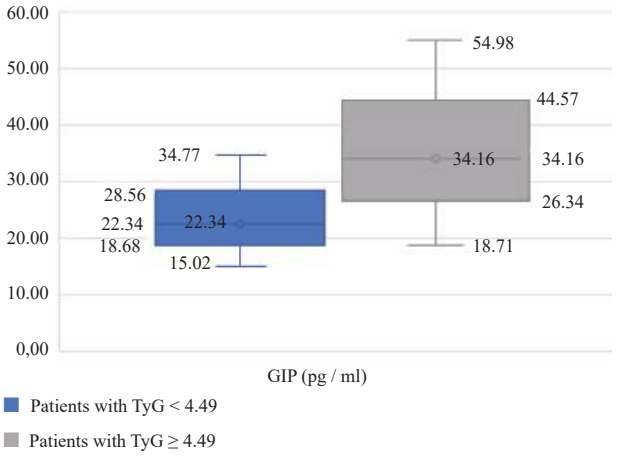

Fig. 2. GIP level (pg / ml) in patients with TyG < 4.49 and TyG ≥ 4.49

In patients with unstable plaques and TyG ≥ 4.49, the PYY level was 1.46 times higher than in patients with unstable plaques and TyG < 4.49 (46.14 [30.49; 70.66] vs. 31.53 [24.71; 43.01], $p = 0.048$).

Table 2

The content of adipocytokines depending on the TyG index and the type of atherosclerotic plaque, *Me* (Q_{25}; Q_{75})

Parameter	Patients with stable plaques and TyG ≥ 4.49, *n* = 28	Patients with unstable plaques, TyG ≥ 4.49 *n* = 15	*p*	Patients with stable plaques, TyG < 4.49 *n* = 30	Patients with unstable plaques, TyG < 4.49 *n* = 24	*p*
C-peptide, ng / ml	1.40 [0.36; 2.43]	1.57 [0.65; 1.90]	0.838	0.76 [0.14; 1.64]	0.83 [0.41; 1.65]	0.403
GIP, pg / ml	42.13 [25.34; 68.95]	26.22 [16.18; 47.27]	0.165	22.39 [17.00; 28.60]	19.55 [12.42; 38.63]	0.824
GLP-1, pg / ml	308.62 [192.49; 742.65]	403.22 [156.31; 671.80]	0.610	275.78 [185.12; 595.60]	432.65 [216.1; 680.38]	0.159
IL-6, pg / ml	5.96 [1.83; 14.48]	7.74 [3.73; 13.35]	0.462	7.14 [2.50; 13.06]	6.55 [1.67; 18.59]	0.709
Leptin, pg /ml	5,764.73 [3,516.42; 8,006.34]	5,874.95 [1,559.14; 12,266.47]	0.894	2,977.39 [869.98; 7,650.03]	4,426.24 [1,105.64; 11,135.62]	0.401
MCP-1, pg / ml	259.25 [154.81; 348.13]	215.00 [182.92; 277.42]	0.610	226.53 [142.34; 334.38]	215.81 [135.90; 325.78]	0.986
PP, pg / ml	87.44 [55.29; 165.58]	87.23 [578.68; 138.54]	0.549	65.34 [36.94; 156.61]	65.35 [39.50; 139.91]	0.914
PYY, pg / ml	46.97 [33.45; 58.99]	46.14 [30.49; 70.66]	0.908	43.01 [25.05; 73.71]	31.53 [24.71; 43.01]	0.105
TNFα, pg / ml	5.52 [3.43; 7.27]	5.66 [4.36; 7.19]	0.593	5.24 [3.11; 7.19]	5.80 [4.75; 6.70]	0.441
PAI-1, ng / ml	30.54 [13.56; 42.04]	20.55 [12.01; 46.98]	0.858	18.19 [13.34; 32.14]	21.04 [15.32; 31.66]	0.354
Lipocalin, ng / ml	551.70 [270.83; 798.91]	386.98 [196.64; 770.38]	0.537	374.40 [173.47; 597.15]	399.19 [204.80; 642.77]	0.932
Ghrelin, pg / ml	17.29 [10.19; 37.55]	13.06 [9.23; 17.29]	0.109	17.29 [9.56; 23.62]	9.23 [9.23; 23.62]	0.288
Glucagon, pg / ml	10.07 [5.89; 23.44]	7.39 [32.68; 28.38]	0.554	10.83 [3.71; 27.04]	7.87 [3.25; 15.28]	0.304
Adiponectin, mcg / ml	32.43 [15.03; 43.15]	13.85 [7.04; 30.45]	0.428	24.27 [13.80; 38.31]	27.91 [15.65; 41.89]	0.608
Adipsin, mcg / ml	9.69 [6.97; 15.96]	9.14 [7.48; 13.10]	0.067	9.39 [5.14; 12.89]	11.13 [10.08; 15.77]	0.032
Resistin, ng / ml	29.02 [13.81; 43.90]	40.76 [22.16; 62.09]	0.650	40.00 [8.95; 63.49]	29.67 [8.08; 67.54]	0.572
Omentin, ng / ml	0.84 [0.65; 1.27]	0.89 [0.73; 1.64]	0.380	1.15 [0.52; 1.38]	0.92 [0.82; 1.38]	0.827
Visfatin, ng / ml	76.84 [17.28; 135.20]	23.86 [17.84; 74.09]	0.320	101.47 [23.86; 127.87]	66.15 [22.60; 113.46]	0.581

Note. GIP – glucose-dependent insulinotropic polypeptide, GLP-1 –glucagon-like peptide-1, IL-6– interleukin-6, MCP-1 – monocyte chemoattractant protein-1, PP – pancreatic polypeptide, PYY – peptide tyrosine – tyrosine, TNFα – tumor necrosis factor alpha, PAI-1 – plasminogen activator inhibitor-1

DISCUSSION

The detection of GIP receptors on the surface of fat cells [10] led to the assumption that GIP participates in fat metabolism [11, 12]. The consumption of foods rich in fats stimulates the release of GIP more strongly than carbohydrates or proteins, and a high-fat diet leads to an increase in the expression of the *GIP* gene and an increase in its concentration in the blood. At the cellular level, activation of the GIP receptor on the adipocyte leads to anabolic effects, including increased glucose uptake into tissues, activation of lipoprotein lipases, and synthesis of free fatty acids.

These data indicate that GIP plays an important role in fat metabolism. The study by E.A. Shestakova showed significantly higher GIP levels in the group of patients with BMI ≥ 35 kg / m^2 compared to those with lower BMI both on an empty stomach and after meals. Similarly, GIP secretion was significantly higher in individuals with insulin resistance (determined by HOMA-IR) compared to individuals without it [13]. In our study, the GIP level was 1.53 times higher in patients with insulin resistance and coronary artery disease according to the TyG index, which was mostly due to its high level in the subgroup of patients with stable plaques.

The study by O.H. Ukkola et al. suggests that a high concentration of PYY in fasting blood serum is independently associated with obesity and insulin resistance [14]. The association of high PYY with high insulin levels was evident among study participants with both type 2 diabetes mellitus and normal glucose levels. Earlier data suggested that different forms of PYY may have different effects on insulin metabolism [15]. Interestingly, low serum PYY levels were associated with insulin resistance in first-degree relatives in patients with type 2 diabetes mellitus [16]. It is yet to be studied whether high insulin levels can affect PYY secretion or secretion of other intestinal peptides that affect PYY concentration. In our study, in patients with unstable plaques and insulin resistance according to the TyG index, the PYY level was 1.46 times higher than

in patients with unstable plaques without insulin resistance.

Adipsin is formed during lipolysis and stimulates appetite [17]. Adipsin levels are reported to be higher in obese people. In addition, overweight people often experience an increase in adipsin levels [18]. T. Ohtsuki et al. showed that patients with coronary artery disease without obesity have higher serum adipsin levels [19]. At the same time, the level of adipsin in the blood of patients with coronary artery disease is significantly and positively associated with the incidence of atherosclerotic plaque with a thin fibrous cap [20]. In the study conducted in patients with TyG < 4.49, the level of adipsin was higher in patients with unstable plaques than in patients with stable ones, which may indicate the association of adipsin with the progression of atherosclerotic foci.

CONCLUSION

Men with coronary artery disease and insulin resistance have higher blood levels of GIP in the general sample and PYY in patients with unstable atherosclerotic plaques. The blood level of adipsin is higher in patients with unstable atherosclerotic plaques and without insulin resistance.

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Analgesic activity of a new cannabinoid CB_1 receptor modulator

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ABSTRACT

Aim. To study the analgesic activity, the effect on motor functions, and the potential ulcerogenic effect of a new 2*H*-chromene derivative, a cannabinoid CB_1 receptor modulator (code name – CHR).

Materials and methods. The analgesic activity of the CHR compound was studied when injected intragastrically at an effective dose of 5 mg / kg in mouse models of acute chemogenic pain (formalin test), acute visceral pain (the acetic acid-induced writhing test), and thermal nociception (hot plate test and tail-flick test). It was compared to the effect of tramadol and morphine or diclofenac sodium at doses of 20.4 or 10 mg / kg, respectively. The effect of a single intragastric injection of the CHR compound at a dose of 5 mg / kg on motor activity was evaluated in the open field test. The potential ulcerogenic effect of the CHR compound at a dose of 5 mg / kg with repeated intragastric administration was compared with the effect of diclofenac sodium at a dose of 10 mg / kg.

Results. With subplantar administration of formalin to mice, the 2*H*-chromene derivative reduced the number of pain reactions by 43–63% ($p < 0.05$). With intraperitoneal administration of acetic acid to mice, it reduced the number of writhing responses by 50% and had the same analgesic effect as diclofenac sodium and tramadol. In the hot plate test, the CHR compound increased the latency time to painful stimuli by 34% ($p < 0.05$). In the tail-flick test, it increased the latency time to painful thermal sensations by 32% ($p < 0.05$). The CHR compound at an effective dose of 5 mg / kg did not change the motor activity of mice in the open field test and did not cause the formation of erosions and ulcers in the gastric mucosa when administered repeatedly to rats.

Conclusion. The 2*H*-chromene derivative CHR at an effective dose of 5 mg / kg has a pronounced analgesic effect in mouse models of chemogenic, visceral, and thermal pain, which is as strong as that of tramadol, morphine, and diclofenac sodium used at effective doses. The CHR compound at an effective dose does not inhibit motor functions and does not have an ulcerogenic effect.

Keywords: 2*H*-chromene derivative, analgesic activity, effect on motor functions, potential ulcerogenic effect, mice, rats

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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Анальгетическая активность нового модулятора каннабиноидного CB_1-рецептора

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РЕЗЮМЕ

Цель – изучить анальгетическую активность, влияние на двигательные функции и потенциальное ульцерогенное действие нового производного 2*H*-хромена – модулятора каннабиноидного CB_1-рецептора (шифр – CHR).

Материалы и методы. Анальгетическую активность соединения CHR изучали при введении в желудок мышам в эффективной дозе 5 мг/кг на моделях острой хемогенной боли (формалиновый тест), острой висцеральной боли (тест «уксусные корчи») и термической соматической боли (тесты «горячая пластина» и отдергивание хвоста от теплового излучения) в сравнении с действием трамадола, морфина или диклофенака натрия в дозах 20, 4 или 10 мг/кг соответственно. Влияние на двигательные функции соединения CHR при однократном введении в дозе 5 мг/кг в желудок мышам оценивали в тесте «открытое поле». Потенциальное ульцерогенное влияние соединения CHR в дозе 5 мг/кг при многократном введении в желудок крысам сравнивали с действием диклофенака натрия в дозе 10 мг/кг.

Результаты. В эксперименте с субплантарным введением формалина мышам производное 2H-хромена CHR уменьшало количество болевых реакций на 43–63% ($p < 0,05$). При внутрибрюшинном введении мышам уксусной кислоты оно снижало количество «корчей» на 50% и не уступало анальгетическому эффекту диклофенака натрия и трамадола. В тесте «горячая пластина» соединение CHR увеличивало латентное время до наступления болевой реакции на 34% ($p < 0,05$). В тесте отдергивания хвоста от теплового излучения увеличивало срок до появления термической боли на 32% ($p < 0,05$). Соединение CHR в эффективной дозе 5 мг/кг не изменяло двигательную активность мышей в тесте «открытое поле» и не вызывало образования эрозий и язв в слизистой оболочке желудка при многократном введении крысам.

Заключение. Производное 2*H*-хромена CHR в эффективной дозе 5 мг/кг оказывает выраженное анальгетическое действие при экспериментальной хемогенной, висцеральной и термической боли, по анальгетическому действию не уступает опиоидным анальгетикам трамадолу и морфину и нестероидному противовоспалительному средству диклофенаку натрия, использованным в эффективных дозах. Соединение CHR в эффективной дозе не тормозит двигательные функции и не обладает ульцерогенным влиянием.

Ключевые слова: производное 2*H*-хромена, анальгетическая активность, влияние на двигательные функции, потенциальное ульцерогенное действие, мыши, крысы

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

Источник финансирования. Исследование выполнено с привлечением собственных средств.

Соответствие принципам этики. Исследование одобрено локальными этическими комитетами СибГМУ (протокол № 20/23 от 08.12.2023) и ООО «Ифар» (протокол № 127/2022 от 14.02.2022).

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INTRODUCTION

Cannabinoids are a class of natural and synthetic compounds with a pronounced analgesic effect. They activate CB_1 receptors in spinal ganglia, dorsal horns of the spinal cord, nucleus raphe magnus, periaqueductal gray matter, limbic system, and cerebral cortex [1–3]. When metabotropic presynaptic G_i-protein-coupled CB_1 receptors are activated, the production of cAMP is inhibited, the permeability of voltage-gaited calcium channels is decreased, and potassium leak channels open [4]. The developing hyperpolarization prevents the release of glutamic acid and its involvement in the transmission of pain potentials [1, 5]. The use of cannabinoids as analgesics is limited due to their psychotropic properties, in particular the risk of catalepsy and drug addiction [2, 6].

Among 2*H*-chromene derivatives, the cannabinoid CB_1 receptor modulator, designated CHR, has the most pronounced analgesic activity [7, 8]. The CHR compound has a heterocyclic structure which is characteristic of cannabichromene. This cannabinoid has analgesic and anti-inflammatory effects [9].

The aim of this work was to study the analgesic activity, the effect on motor functions, and the potential ulcerogenic effect of a new 2*H*-chromene derivative, the cannabinoid CB_1 receptor modulator (codenamed CHR).

MATERIALS AND METHODS

The 2*H*-chromene derivative was (*2R,4aR,7R,8aR*)-4,7-dimethyl-2-(thiophene-2-yl)octahydro-2*H*-chromene-4-ol (Fig. 1). It was synthesized at Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences (Novosibirsk) [7].

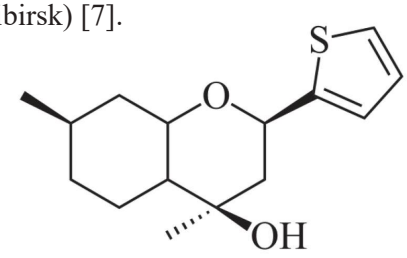

Fig. 1. Structural formula of the CHR compound

The experiments were carried out at the R&D Center (IPHAR LLC, Tomsk, Russia) on 96 specific pathogen-free male CD-1 mice and 15 male Sprague Dawley rats. The animals were kept in standard plastic cages (VELAZ, Czech Republic) at 20–23 °C and relative humidity of no more than 50%, with the exhaust – supply ratio of 8 : 10 and a 12 : 12 light / dark cycle. The animals were kept and cared for in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Directive 2010/63/EU). The study was conducted in compliance with the principles and rules of Good Laboratory Practice and approved by the Ethics Committees at Siberian State Medical University (Protocol 20/23 of 08.12.2023) and IPHAR LLC (Protocol 127/2022 of 14.02.2022).

In all experiments, the CHR compound at an effective dose of 5 mg / kg was orally administered to the animals 1 h before exposure to pain-inducing agents. The effective dose was determined in preliminary exploratory experiments. The CHR compound was dissolved in a 2% aqueous solution of Tween 80 (NeoFroxx GmbH, Germany). The control animals received this solvent according to the similar regime. Cannabinoid-based analgesics are not registered in the Russian Federation; therefore, the most frequently prescribed analgesics for pain control of different intensity were used as comparison drugs – the opioid analgesics tramadol at a dose of 20 mg / kg [10] and morphine at a dose of 4 mg / kg (both obtained from Moscow Endocrine Plant, Moscow, Russia) [11], and the non-steroidal anti-inflammatory drug diclofenac sodium at a dose of 10 mg / kg (Hemofarm, Serbia) [12, 13]. Aqueous solutions of tramadol and diclofenac sodium were orally administered to the animals, morphine was subcutaneously injected in isotonic sodium chloride solution. The mice were euthanized by cervical dislocation, the rats – by carbon dioxide.

Formalin test. Mice (4 groups, $n = 6$ animals per group) were injected with 0.02 ml of a 0.5% aqueous formalin solution (Sigma-Aldrich, USA) into the plantar aponeurosis of the hind limb. For 60 min,

we recorded the pain response by the number of licks and shakes of the injured hind paw. In the first 10 min, the acute phase (phase 1) took place due to direct activation of nociceptors by algogens. In the next 50 min, the tonic phase (phase 2) caused by inflammation developed. After 60 min, the animals were euthanized, and the weight of their hind limb amputated at the ankle joint was measured [14].

Acetic acid-induced writhing test. Mice (4 groups, n = 6 animals per group) were intraperitoneally injected with 0.75% aqueous acetic acid solution (Sigma-Aldrich, USA) in a volume of 0.1 ml per 10 g of body weight. Within 20 minutes, we evaluated the number of abdominal muscle contractions (writhings) and the latency time to the first writhing [14].

Hot plate test. Mice (3 groups, n = 6 animals per group) were placed on a metal plate preheated to 55 ± 1 °C (HWT-75 thermal table, Russia). We measured the latency to the first pain response, which was registered by paw withdrawal and licking. The mice were kept on the hot plate for no more than 1 min to avoid injury [14].

Tail-flick test. The tails of mice (3 groups, n = 6 animals per group) were placed under the heat source of the laboratory analgesia meter (Hugo Sachs Elektronik, Germany). We measured the time until the tail was removed from the source [14].

The effect of the CHR compound on the motor function of mice was studied in the *open field test* (2 groups, n = 6 animals per group). The CHR compound was administered to mice at a dose of 5 mg / kg. Their motor activity was evaluated 1 h after the administration using the LE 8811 infrared actimeter and the ActiTrack software (Panlab, Spain) [14].

To study the potential ulcerogenic effect, intact rats (3 groups, n = 15 animals per group) were orally administered the CHR compound at a dose of 5 mg / kg or diclofenac sodium at a dose of 10 mg / kg four times (with an interval of 24 h). Three hours after the last administration, the animals were euthanized, and the condition of their gastric mucosa was assessed using the dissecting microscope (Observational Instruments, Saint Petersburg, Russia) at ×10. The degree of mucosal damage was graded in points: 0 – no damage, 0.5 – localized mucosal hyperemia, 1 – generalized mucosal hyperemia covering most (> 50%) or all of the gastric mucosa, 2 – the presence of up to three hemorrhages without erosion, 3 – the presence of more than three hemorrhages or erosions, 4 – the presence of more than three erosions or no more than two ulcers, 5 – the presence of more than two ulcers, total thinning of the gastric mucosa [14].

The results were statistically processed using the Statistica v. 8.0 software (StatSoft, USA). The Shapiro – Wilk test showed that the data were not normally distributed and were presented as the median and the interquartile range *Me* (Q_1; Q_3). The sample sizes were minimally sufficient for statistical analysis and complied with 3Rs [15]. The statistical significance of the differences between the groups was assessed using the Kruskal – Wallis test followed by the Mann – Whitney test for post-hoc pairwise comparisons. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In the formalin test, the CHR compound at a dose of 5 mg / kg reduced the number of licks and shakes of the damaged hind limb in the mice in the acute phase (response to algogen) by 43% and in the tonic phase – by 63% ($p < 0.05$, Table 1). The effects of the CHR compound were no weaker than those of morphine at a dose of 4 mg / kg and tramadol at a dose of 20 mg / kg ($p > 0.05$). Both opioid analgesics reduced the number of pain responses in both phases by 47–83%. The limb weight in the mice that received the CHR compound was 45 (36; 55) mg and did not differ from that in the mice that received Tween 80 (47 (38; 49) mg). This means that the analgesic effect of the 2*H*-chromene derivative CHR is not associated with an antiinflammatory effect.

Table 1

The number of pain responses in the formalin test at oral administration of the CHR compound (5 mg/kg), tramadol (20 mg/kg), and morphine (4 mg/kg) to the mice, *Me* (Q_1; Q_3), n = 6

Number of pain responses	2% aqueous solution of Tween 80 (control)	CHR compound	Tramadol	Morphine
Acute phase	54 (52; 56)	30 (26; 34)*	32 (24; 38)*	26 (24; 28)*
Tonic phase	24 (20; 25)	9 (5; 13)*	4 (3; 5)*	2 (1; 7)*

*here and in Table 2 $p < 0.05$ compared to the control values.

In the acute visceral pain model, the CHR compound exhibited an analgesic effect that was comparable to that of diclofenac sodium and tramadol ($p < 0.05$, Table 2). The CHR compound reduced the

number of writhings by 50%, diclofenac sodium and tramadol — by 52 and 56%, respectively. At the same time, the CHR compound did not affect the latency time to the first writhing and did not increase the pain threshold.

Table 2

Analgesic activity of the CHR compound (5 mg / kg), diclofenac sodium (10 mg / kg), and tramadol (20 mg / kg) when administered orally to the mice in the acetic acid-induced writhing test, *Me* (Q_1; Q_3), *n* = 6

Parameter	2% aqueous solution of Tween 80 (control)	CHR compound	Diclofenac sodium	Tramadol
Latency to the first writhing, sec	330 (315; 332)	507 (505; 508)	665 (660; 669)*	326 (316; 331)
Number of writhings	22 (21; 23)	12 (10; 13)*	11 (8; 15)*	11 (8; 14)*

In the hot plate test, the CHR compound at a dose of 5 mg / kg increased the latency time to the pain response by 34% ($p < 0.05$), whereas tramadol at a dose of 20 mg / kg increased this parameter by 49% ($p < 0.05$). The analgesic effect of the CHR compound at a dose of 5 mg / kg was comparable to that of tramadol ($p > 0.05$, Fig. 2).

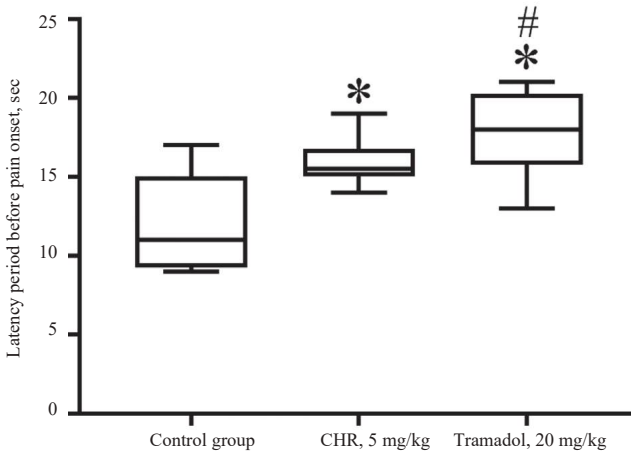

Fig. 2. Response latency in the hot plate test after oral administration of the CHR compound and tramadol to the mice.
* differences with the control group (administration of Tween 80), $p < 0.05$, # differences with the CHR group, $p > 0.05$

The CHR compound at a dose of 5 mg / kg increased the tail-flick latency by 1.3 times, and tramadol at a dose of 20 mg / kg doubled this parameter. These values differed from the control group (administration of Tween 80) ($p < 0.05$). The analgesic effect of the CHR compound in this model was weaker than that of tramadol ($p < 0.05$).

The motor activity parameters of the mice that were administered the CHR compound at a dose of 5 mg / kg were comparable to those of the animals in the control group (administration of Tween 80) ($p > 0.05$, Table 3). The CHR compound at an effective dose did not affect the behavior of the animals in the open field test.

Table 3

Effect of the CHR compound (5 mg / kg) on the motor activity of the mice in the open field test, *Me* (Q_1; Q_3), *n* = 6

Parameter	2% aqueous solution of Tween 80 (control)	CHR compound
Number of movements	146 (119; 147)	131 (110; 153)
Average speed of movements, cm / sec	1 (0; 1)	1 (1; 2)
Total distance traveled, cm	54 (34; 59)	66 (35; 77)
Number of rears	2 (0; 4)	5 (5; 6)

NSAIDs and analgesics, especially non-selective cyclooxygenase inhibitors, have an ulcerogenic effect. It was interesting to study the effect of a new 2*H*-chromene derivative with non-steroidal analgesic properties on the gastric mucosa. Multiple administration of the CHR compound did not cause hyperemia, erosion or gastric ulcers. The degree of damage was 1 (1; 1) point and did not differ from that in the control group ($p > 0.05$). After multiple administration of diclofenac sodium at a dose of 10 mg / kg, the gastric mucosa became thinner; total hyperemia, multiple erosions and ulcers were detected. The degree of damage was 5 (5; 5) points ($p < 0.05$). Multiple oral administration of the CHR compound at an effective dose had no ulcerogenic effect and probably did not inhibit cyclooxygenase-1 [16].

The CHR derivative exhibited high analgesic activity in models of chemogenic pain induced by formalin and acetic acid. In the formalin test, the analgesic equally reduced the number of pain responses in the acute and tonic phases without affecting inflammation. It can be assumed that the CHR compound activated CB_1 receptors and caused hyperpolarization of afferent pain pathways in spinal and supraspinal structures [9].

In the thermal nociception models, the CHR compound showed a greater analgesic effect in

the hot plate test, when the pain response involved predominantly supraspinal structures, such as nucleus raphe magnus, periaqueductal gray matter, limbic system, and cerebral cortex [14]. In the tail-flick test, the pain response was associated with the activation of predominantly the spinal ganglia and dorsal horns of the spinal cord. Therefore, the effect of the CHR compound was weaker [14]. Activation of CB_1 receptors in supraspinal structures makes the greatest contribution to the mechanisms of analgesic effects of the CHR compound.

According to *in vitro* studies, the CHR compound does not directly bind to cannabinoid receptors and does not affect the activity of enzymes involved in the endogenous cannabinoid metabolic pathway. However, its analgesic effects are inhibited by the CB_1 receptor antagonist rimonabant [17]. This indicates the ability of the CHR compound to activate CB_1 receptors indirectly. One of the probable mechanisms is binding to an allosteric site of the CB_1 receptor, which changes their conformation and increases their affinity for endogenous cannabinoids, such as anandamide and 2-arachidonoylglycerol [18, 19]. Unlike diclofenac sodium, the CHR compound does not have the ulcerogenic effect that is characteristic of NSAIDs. It also does not affect the motor activity of the animals.

CONCLUSION

The study demonstrated the analgesic activity of the 2*H*-chromene derivative CHR in mouse models of chemogenic, visceral, and thermal pain. The analgesic effect of the CHR compound in chemogenic pain models and in the hot plate test is the same as the effects of tramadol, morphine, and diclofenac sodium. The proposed mechanism of action of the CHR compound consists in modulation of the allosteric site of CB_1 receptors, which leads to an increase in their affinity for endocannabinoids. The studied analgesic does not damage the gastric mucosa and does not inhibit the motor activity of animals. The 2*H*-chromene derivative CHR is a promising drug for the pharmacotherapy of pain.

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Authors' contribution

Bykov V.V., Larchenko V.V. – conception and design. Gurkin N.V., Bykova A.V., Motov V.S. – carrying out of the experiments, analysis and interpretation of the data. Il'ina I.V., Volcho K.P., Salakhutdinov N.F. – chemical synthesis of the CHR compound. Khazanov V.A. – justification of the manuscript, critical revision of the manuscript for important intellectual content. Vengerovskii A.I. – final editing of the article and final approval of the article for publication.

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The role of markers of endothelial dysfunction in the pathogenesis of coronary microvascular dysfunction in patients with non-obstructive coronary artery disease

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ABSTRACT

Aim. To study the potential of non-invasive biomarkers in the diagnosis of coronary microvascular dysfunction (CMD) and prediction of the course of heart failure with preserved ejection fraction (HFpEF) in non-obstructive coronary artery disease.

Materials and methods. The 12-month observational study included 118 consecutive patients (6 patients dropped out of the study due to contact loss) with non-obstructive coronary artery disease (CAD) and HFpEF (62 [59; 64]%). At the beginning of the study, serum levels of several biomarkers were assessed using the enzyme immunoassay: N-terminal pro-B-type natriuretic peptide (NT-proBNP), vascular endothelial growth factor (VEGF), and endothelin-1. Coronary flow reserve (CFR) was examined using dynamic single photon emission computed tomography. In the absence of obstructive CAD, CMD was defined as a global decrease in CFR ≤ 2. Echocardiography was used to determine parameters of hemodynamics, LV diastolic dysfunction, and myocardial stress. LV global longitudinal strain (GLS) was assessed using 2D speckle tracking.

Results. The patients were divided into groups depending on the presence of CMD: group 1 included patients with CMD (n = 43), group 2 included those without it (n = 75). In patients in group 1, serum levels of endothelin-1 were 1.9 times higher (p = 0.012), levels of VEGF were 2.16 times higher (p = 0.008), and the concentration of NT-proBNP was 2.6 times higher (p = 0.004) than in patients in group 2. According to the ROC analysis, the concentrations of endothelin-1 ≥ 6.9 pg / ml (AUC = 0.711; p = 0.040) and VEGF ≥ 346.7 pg / ml (AUC = 0.756; p = 0.002) were considered as markers associated with the presence of CMD in patients with non-obstructive CAD. The multivariate regression analysis showed that only the presence of CMD (odds ratio (OR) 2.42; 95% confidence interval (95% CI) 1.26–5.85; p < 0.001) and an increase in NT-proBNP ≥ 760.5 pg / ml (OR 1.33; 95% CI 1.08–3.19; p = 0.023) were factors associated with adverse events, and their combination increased the risk of HFpEF progression by more than 3 times (OR 3.18; 95% CI 2.76–7.98; p < 0.001), whereas markers of endothelial dysfunction were not independent predictors.

Conclusion. Endothelin-1 ≥ 6.9 pg / ml and VEGF ≥ 346.7 pg / ml can be used as non-invasive markers for the diagnosis of CMD. However, markers of endothelial dysfunction were not independent predictors of HFpEF progression in patients with non-obstructive CAD during 12-month follow-up.

Keywords: coronary microvascular dysfunction, endothelial dysfunction, heart failure, preserved ejection fraction, non-obstructive coronary artery disease

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

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Роль маркеров эндотелиальной дисфункции в патогенезе коронарной микроваскулярной дисфункции у пациентов с необструктивным поражением коронарных артерий

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РЕЗЮМЕ

Цель. Изучение потенциала неинвазивной биомаркерной диагностики коронарной микроваскулярной дисфункции (КМД) и прогнозирования течения сердечной недостаточности с сохраненной фракцией выброса (СНсФВ) при необструктивном атеросклеротическом поражении коронарного русла.

Материалы и методы. В 12-месячное обсервационное исследование последовательно было включено 118 пациентов (шесть пациентов выбыло из исследования по причине утери контакта) с необструктивным поражением коронарных артерий (КА) и сохраненной фракцией выброса левого желудочка (ЛЖ) (62 [59; 64]%). В начале исследования с помощью иммуноферментного анализа в сыворотке крови оценивали уровень некоторых биомаркеров: N-концевого пропептида натрийуретического гормона B-типа (NT-proBNP), VEGF- васкулоэндотелиального фактора роста (VEGF) и эндотелина-1. Резерв коронарного кровотока (CFR) исследовали в ходе динамической однофотонной эмиссионной компьютерной томографии. В отсутствии обструктивного поражения КА, КМД определяли как глобальное снижение CFR ≤ 2. С помощью эхокардиографии определяли параметры гемодинамики, диастолической дисфункции ЛЖ и миокардиального стресса. Глобальная продольная деформация ЛЖ (GLS) оценивалась с помощью 2D-speckle tracking.

Результаты. Пациенты были разделены на группы в зависимости от наличия КМД: в группу 1 вошли пациенты с КМД (n = 43), в группу 2 – больные без нее (n = 75). У больных в группе 1 сывороточные концентрации эндотелина-1 были выше в 1,9 раза (p = 0,012), VEGF – в 2,16 (p = 0,008), а NT-proBNP – выше в 2,6 раза (p = 0,004) по сравнению с больными в группе 2. По данным ROC-анализа, концентрации эндотелина-1 ≥ 6,9 пг/мл (AUC = 0,711; p = 0,040) и VEGF ≥ 346,7 пг/мл (AUC = 0,756; p = 0,002) были идентифицированы как маркеры, связанные с наличием КМД у больных с необструктивным поражением КА. Многофакторный регрессионный анализ показал, что только наличие КМД (отношение шансов (ОШ) 2,42; 95%-й доверительный интервал (95% ДИ) 1,26–5,85; p < 0,001) и повышение уровня NT-proBNP ≥ 760,5 пг/мл (ОШ 1,33; 95% ДИ 1,08–3,19; p = 0,023) являлись факторами, связанными с неблагоприятными событиями, а их сочетание увеличивало риск прогрессирования СНсФВ более чем в 3 раза (ОШ 3,18; 95% ДИ 2,76–7,98; p < 0,001), тогда как маркеры эндотелиальной дисфункции не являлись независимыми предикторами.

Заключение. Уровни эндотелина-1 ≥ 6,9 пг/мл) и VEGF ≥ 346,7 пг/мл могут быть использованы как неинвазивные маркеры для диагностики КМД. Однако маркеры эндотелиальной дисфункции не являлись независимыми предикторами прогрессировании СНсФВ у пациентов с необструктивным поражением КА в течение 12 мес наблюдения.

Ключевые слова: коронарная микроваскулярная дисфункция, эндотелиальная дисфункция, сердечная недостаточность, сохраненная фракция выброса, необструктивное поражение коронарных артерий

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

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INTRODUCTION

Coronary microvascular dysfunction (CMD) plays an essential role in the mechanisms of obstructive and non-obstructive coronary artery disease, as well as their complications, including heart failure with preserved ejection fraction (HFpEF) [1–8]. Studies of the last few years have shown that the presence of CMD is an early marker of cardiovascular diseases and is closely associated with higher incidence of adverse clinical outcomes compared to individuals without such microcirculatory disorders [2, 3]. In the meantime, the results of studies by a number of authors indicate that the presence of CMD is often underestimated in clinical practice. Despite irrefutable evidence of the relationship between endothelial dysfunction, impaired vasodilation of the coronary and systemic microvasculature, and HFpEF, the pathogenesis of CMD and its role in the initiation and progression of HFpEF, especially in the context of non-obstructive coronary artery disease (CAD), are being actively discussed [4, 5].

Intact endothelium of the arteries produces a large number of biologically active substances that maintain normal vasomotor activity [6]. An imbalance of vasodilation and vasoconstriction factors leads to suboptimal control of vascular tone and structure, characterized by disruption or loss of homeostatic mechanisms, which leads to elevated expression of adhesion molecules, increased oxidative stress, overproduction of prothrombotic and proinflammatory markers, increased proliferation of vascular smooth muscle cells, and increased vascular smooth muscle tone [6, 7]. Evidence accumulated over the past several decades has demonstrated that endothelial dysfunction and coronary vasomotor dysfunction play a crucial role in the pathogenesis of cardiovascular diseases [7]. However, the role of markers of endothelial dysfunction in the pathogenesis of CMD in patients with non-obstructive CAD and their role in stratifying the risk of HFpEF progression in this cohort of patients remain poorly studied.

The aim of this research was to study the potential of non-invasive biomarkers in the diagnosis of CMD and prediction of the course HFpEF in non-obstructive CAD.

MATERIALS AND METHODS

The study was approved by the Bioethics Committee at the Cardiology Research Institute of Tomsk NRMC (protocol No. 204 of 18.11.2020)) and was conducted in accordance with the principles of the Declaration of Helsinki and the guidelines for good clinical practice (GCP).

A 12-month observational study included 118 patients with HFpEF (62 [59; 64]%) without a previous history of CAD, complaining of chest pain, shortness of breath, or a combination of both. They were examined at the Cardiology Research Institute of Tomsk NRMC from 2019 to 2022. A detailed

description of the inclusion and exclusion criteria is presented in one of our previous works [8].

The presence and severity of coronary lesions were assessed by multislice spiral computed tomography (MSCT). The resulting scintigraphy images were processed on the specialized Xeleris 2 workstation (GEHealthcare, Israel), and the myocardial blood flow was assessed at rest (rest-MBF, ml / min / g) and during exercise during the administration of the stress agent ATP (stress-MBF, ml / min / g). Coronary flow reserve (CFR) was calculated according to the formula: MFR = stress-MBF / rest-MBF [9], and CMD was diagnosed at CFR ≤ 2 [10, 11].

Real-time 2D transthoracic echocardiography (2D speckle tracking) was performed on the Philips Affiniti 70 ultrasound system. LV diastolic dysfunction (peak E-wave velocity, the E/A ratio, lateral e', average E/e' ratio, left atrial volume index (LAVI), and peak tricuspid regurgitation velocity) and LV global longitudinal strain (GLS) were assessed [12].

Myocardial wall stress parameters, such as LV end-systolic elasticity (Es), arterial elastance (Ea), and LV myocardial wall stress in systole (MWSs) and diastole (MWSd), were calculated by formulas characterizing LV remodeling and presented in the work by T.A. Nechesova et al. [13].

The content of serum biomarkers (NT-proBNP; VEGF, and endothelin-1) was determined by the enzyme-linked immunosorbent assay (ELISA) using the Biomedica (Austria), Vector-best (Russia), and RayBio (USA) reagent kits, respectively (Fig. 1).

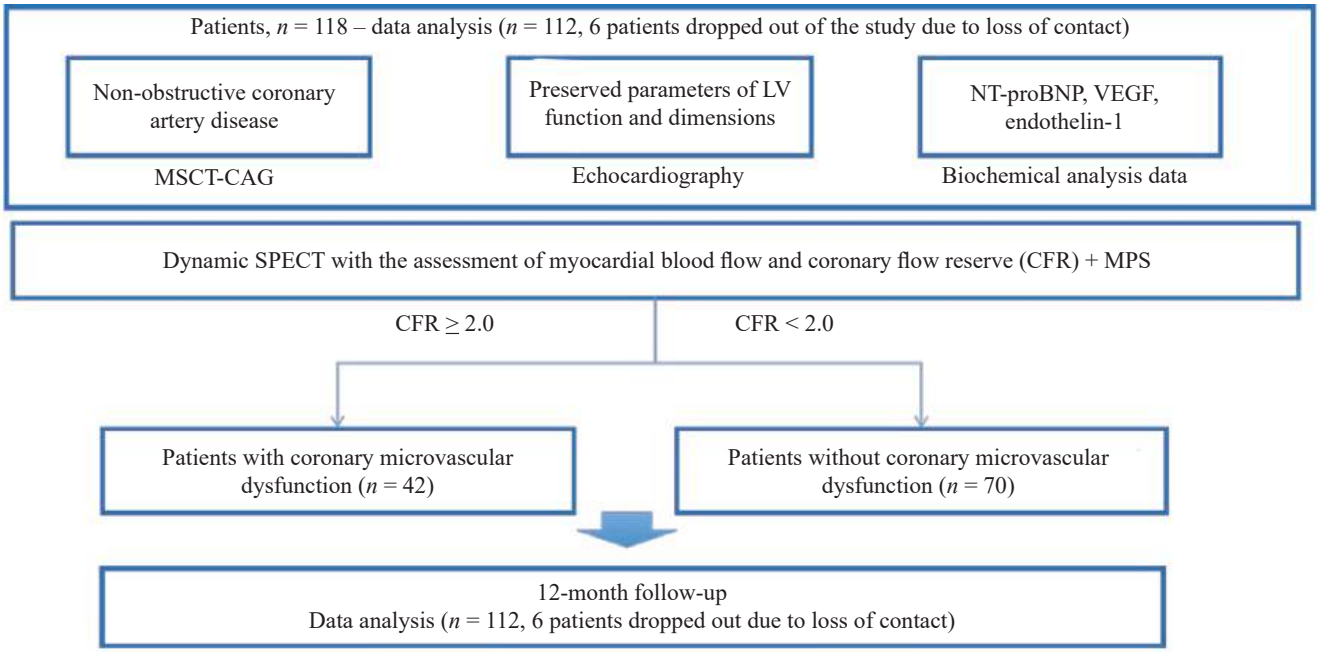


Fig. 1. Study design. NT-proBNP – NT-terminal pro-B-type natriuretic peptide, VEGF – vascular endothelial growth factor, MSCT – CAG – multislice spiral computed tomography – coronary angiography, MPS – myocardial perfusion scintigraphy

The results obtained were processed using the STATISTICA 10.0 and MedCalc 11.5.0.0 software packages. Quantitative variables were presented as the median and the interquartile range *Me* (Q_{25}; Q_{75}), qualitative variables were presented as absolute values (abs.) and percentage (%). When comparing quantitative variables in two independent groups, the Mann – Whitney test was used. When analyzing qualitative variables, contingency tables were analyzed using the Pearson's $\chi 2$ test. The univariate regression analysis with the calculation of odds ratio (OR) and 95% confidence interval (CI) was used to assess the influence of factors on the course of pathology. The multivariate regression analysis was used to identify independent predictors of adverse outcomes. To identify cut-off levels of biomarkers, the ROC analysis was used with the construction of ROC curves and calculation of area under the curve (AUC). The differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Depending on the presence or absence of CMD, the patients were divided into 2 groups: group 1 included patients with CFR ≤ 2 (CMD+, n = 42), group 2 encompassed patients with CFR > 2 (CMD-, n = 70). Myocardial blood flow parameters differed significantly between the groups (Fig.2).

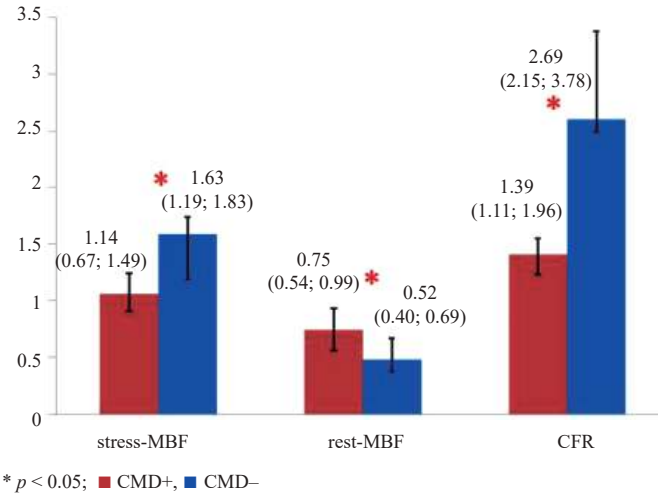


Fig. 2. Parameters of myocardial blood flow and coronary flow reserve depending on the presence of CMD. CFR – coronary flow reserve, stress-MBF – myocardial blood flow during exercise, rest-MBF – myocardial blood flow at rest.

Here and in Fig. 3, 4, p – level of statistical significance of the differences.

A history of type 2 diabetes mellitus (p = 0.003) and smoking status (p = 0.012) were significantly more often registered among patients with CMD. In this group of patients, 80.9% of those examined were diagnosed with HFpEF, while in group 2, there were significantly (p < 0.001) fewer patients with this phenotype of heart failure (34.3%). Other clinical and demographic parameters were comparable between the groups (Table 1).

The group of CMD+ patients was characterized by structural and functional changes corresponding to diastolic dysfunction in the context of non-obstructive CAD: a decrease in lateral e' values (by 35%; p = 0.009), a decrease in GLS (by 25.1%; p < 0.001) and an increase in the peak tricuspid regurgitation velocity (by 12%; p = 0.011), the E/e' ratio (by 21.4%; p = 0.041), and LAVI (by 51.2%; p = 0.038), respectively, compared to CMD- patients. Similar changes were noted in the parameters of systolic and diastolic myocardial wall stress, which were higher by 6.3 (p = 0.032) and 6.8% (p = 0.021), respectively, in the CMD+ group compared to the CMD- group. In patients of group 1, an increase in ventricular – arterial coupling (Ea/Es) by 25.9% (p = 0.032) was significantly more frequent compared to group 2, which characterizes decreased mechanical efficiency of the cardiovascular system in patients with CMD and non-obstructive CAD (Table 1).

Table 1

Characteristics of the patients at the time of inclusion in the study

Parameter	Group 1 (CMD +; n = 42)	Group 2 (CMD–; n = 70)	p
Age, years, *Me* (Q_{25}; Q_{75})	61.5 (55.0; 66.0)	62.0 (60.0; 67.0)	0.124
Men, n (%)	26 (61.9)	44 (62.8)	0.919
BMI, kg / m^2, *Me* (Q_{25}; Q_{75})	29.7 (27.6; 32.0)	30.1 (27.7; 34.1)	0.254
Type 2 diabetes mellitus, n (%)	11 (26.2)	5 (7.1)	0.003
COPD, n (%)	6 (14.3)	13 (18.6)	0.718
HFpEF, n (%)	34 (80.9)	24 (34.3)	<0.001
Smoking, n (%)	11 (26.2)	4 (5.7)	0.012
GFR (ml / min / 1.73 m^2), *Me* (Q_{25}; Q_{75})	76.8 (63.0; 81.0)	78.0 (64.0; 87.0)	0.476
Total cholesterol, mmol / l, *Me* (Q_{25}; Q_{75})	4.65 (3.67; 5.25)	4.34(3.54; 4.98)	0.932
LVEF, %, *Me* (Q_{25}; Q_{75})	62 (58.5; 65.0)	63 (61; 66)	0.183
ESD, mm, *Me* (Q_{25}; Q_{75})	40 (38; 43)	38.5 (36.5; 41.5)	0.524
EDD, mm, *Me* (Q_{25}; Q_{75})	51.0 (48.7; 53.0)	50.5 (47.5; 52.5)	0.307
Lateral e', cm / sec, *Me* (Q_{25}; Q_{75})	5.56 (4.78; 6.45)	8.56 (8.01; 9.14)	0.008
PTRV, m / sec, *Me* (Q_{25}; Q_{75})	2.98 (2.95; 3.01)	2.61 (2.3; 2.76)	0.009
E/e', *Me* (Q_{25}; Q_{75})	14 (13.5; 15.0)	11 (10; 12)	0.041
LAVI, ml / m^2, *Me* (Q_{25}; Q_{75})	38.3 (35.7; 51.1)	29.7 (27.5; 47.9)	0.038
GLS, %, *Me* (Q_{25}; Q_{75})	−14.9 (−13.1; −21.9)	−21.3 (−16.3; −22.8)	0.004

Table 1 (continued)

Parameter	Group 1 (CMD +; $n = 42$)	Group 2 (CMD–; $n = 70$)	p
MWSd, dyn / cm^2, *Me* (Q_{25}; Q_{75})	154.23 (140.11; 159.65)	140.13 (129.23; 151.54)	0.027
MWSs, dyn / cm^2, *Me* (Q_{25}; Q_{75})	172.18 (149.23; 192.34)	156.14 (134.23; 176.4)	0.022
Ea, mmHg./ ml, *Me* (Q_{25}; Q_{75})	0.61 (0.54; 0.89)	0.55 (0.52; 0.64)	0.028
Es, mmHg./ ml, *Me* (Q_{25}; Q_{75})	2.29 (1.67; 3.16)	2.78 (2.48; 3.09)	0.019
Ea / Es, *Me* (Q_{25}; Q_{75})	0.27 (0.23; 0.56)	0.20 (0.18; 0.45)	0.032

Note. BMI – body mass index, COPD – chronic obstructive pulmonary disease, HFpEF – heart failure with preserved ejection fraction, GFR – glomerular filtration rate, LVEF – left ventricular ejection fraction, ESD – left ventricular end-systolic dimension, EDD – left ventricular end-diastolic dimension, lateral e' – lateral mitral annulus velocity in early diastole, PTRV – peak tricuspid regurgitation velocity, E/e' – the ratio of transmitral E velocity to early diastolic mitral annular velocity, LAVI – left atrial volume index, GLS – global longitudinal strain, MWSd – myocardial wall stress in diastole, MWSs – myocardial wall stress in systole, Ea – arterial elastance, Es – end-systolic elastance, p – statistical significance of differences.

In patients in group 1, endothelin-1 levels were 1.9 times higher ($p = 0.012$), VEGF levels were 2.16 times higher ($p = 0.008$), and NT-proBNP levels were 2.6 times higher ($p = 0.004$) than in patients in group 2 (Fig. 3).

The ROC analysis revealed that VEGF overexpression ≥ 346.7 pg / ml (sensitivity 89.8%, specificity 72.4%; AUC = 0.756; $p = 0.002$) and endothelin-1 ≥ 6.9 pg / ml (sensitivity 84.6%, specificity 65.6%; AUC = 0.711; $p = 0.040$) had diagnostic value for identifying CMD in patients with non-obstructive CAD (Fig. 4).

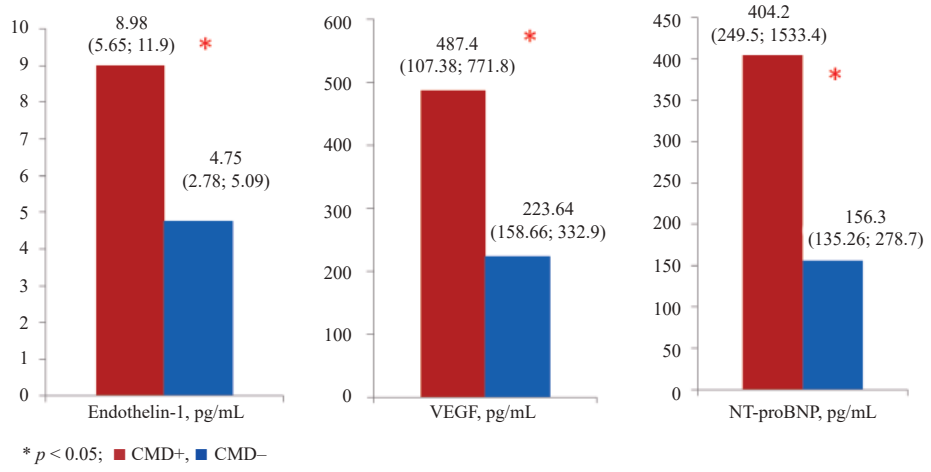
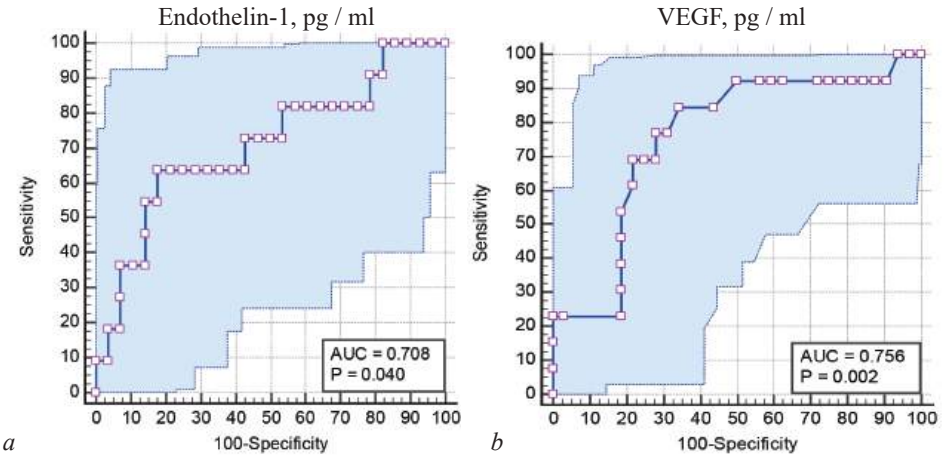


Fig. 3. Serum levels of the studied biomarkers in CMD+ or CMD- patients



a b

Fig. 4. Diagnostic value of endothelin-1 (*a*) and VEGF (*b*) levels in CMD (ROC analysis), AUC – area under the curve

During 12-month follow-up, 25 (22.3%) patients experienced adverse events (Fig. 5). The profile of adverse cardiovascular events was dominated by a complex parameter “HFpEF progression or intensification of diuretic therapy”. In one case, sudden cardiac death was registered

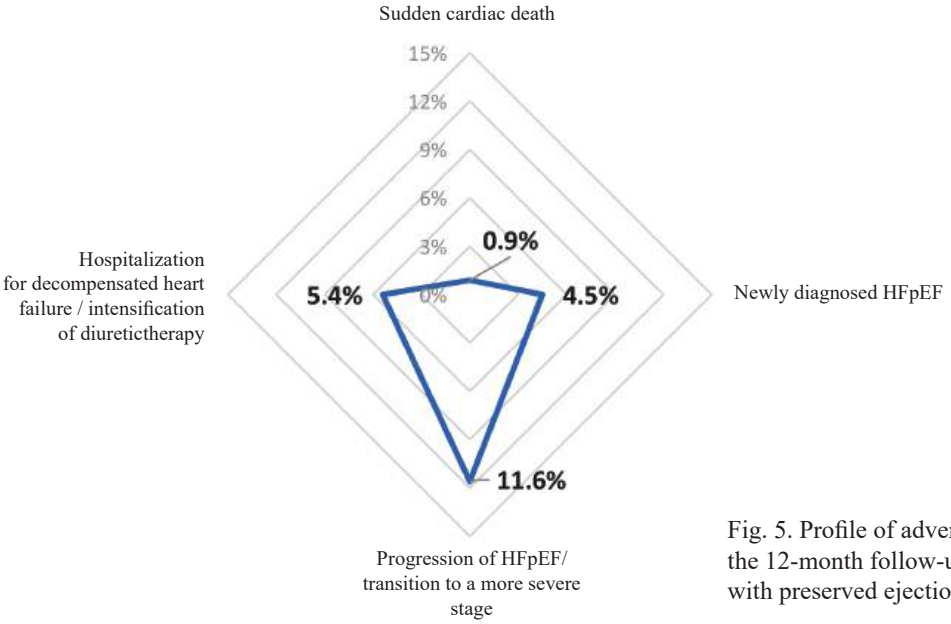


Fig. 5. Profile of adverse events registered during the 12-month follow-up. HFpEF – heart failure with preserved ejection fraction

The results of the univariate and multivariate regression models are presented in Table 2. Such factors as type 2 diabetes mellitus, CMD, smoking, overexpression of NT-proBNP ≥ 760.5 pg / ml, and endothelin-1 ≥ 4.9 pg / ml increased the risk of adverse cardiovascular events in patients with non-obstructive CAD by 1.9, 2.7, 2.3, 2, and 1.9 times, respectively. Assessing simultaneous influence of the predictors included in the analysis revealed that the factors associated with the development of adverse cardiovascular events were CMD and overexpression of NT-proBNP ≥ 760.5 pg / ml), and their combination increased the risk of HFpEF progression by more than 3 times (OR 3.18; 95% CI 2.76–7.98; $p < 0.001$). In the meantime, markers of endothelial dysfunction were not independent predictors.

Table 2

Analysis of the influence of risk factors for adverse cardiovascular events on outcomes in patients with non-obstructive coronary artery disease

Univariate regression analysis			
Factor	Odds ratio	95% CI	*p*
Type 2 diabetes mellitus	1.87	1.12–3.95	0.018
NT-proBNP (< 760.5 / ≥ 760.5 pg / ml)	1.98	1.09–3.98	0.028
GLS (>18≤/–18%)	1.98	0.99–5.98	0.002
Smoking	2.13	1.23–2.97	0.039
Coronary microvascular dysfunction	2.72	1.65–6.03	<0.001
Endothelin-1 (< 4.9 / ≥ 4.9 pg / ml)	1.95	0.98–5.87	0.002
VEGF (< 464.7 / ≥ 464.7 pg / ml)	2.65	1.76–9.12	0.001
Multivariate regression analysis			
Coronary microvascular dysfunction	2.42	1.26–5.85	<0.001
NT-proBNP (< 760.5 / ≥ 760.5 pg / ml)	1.33	1.08–3.19	0.025
CMD + NT-proBNP	3.18	2.76–7.98	<0.001

Note. NT-proBNP – N-terminal pro-B-type natriuretic peptide, GLS – global longitudinal strain, VEGF – vascular endothelial growth factor, CMD – coronary microvascular dysfunction, *p* – statistical significance of differences.

DISCUSSION

The present study found that CMD is closely associated with increased serum levels of a number of molecular biomarkers of endothelial activation and damage [14, 15]: endothelin-1 levels ≥ 6.9 pg / ml and VEGF ≥ 346.7 pg / ml can be used as non-invasive markers for diagnosing CMD. However, markers of endothelial dysfunction, according to our data, were not independent predictors of the HFpEF progression in patients with non-obstructive CAD within 12 months, which is likely associated with a more significant influence of nonspecific inflammatory factors, such as oxidative stress, profibrotic cytokines, and LV extracellular matrix remodeling, following specific changes in the structure of the sarcomeric protein titin [16, 17].

According to previously obtained data, CMD develops due to impaired endothelium-dependent and endothelium-independent vasodilation, as well as perivascular fibrosis. Moreover, CMD is one of the leading mechanisms for the development of HFpEF in patients with non-obstructive CAD in the context of increased ventricular – arterial coupling [16]. These processes lead to impaired myocardial perfusion, directing maladaptive responses of the body along the cardiovascular continuum [3–7]. Our data do not contradict the above concept. In particular, we showed that patients with CMD are diagnosed with more severe diastolic dysfunction and depressed mechanical efficiency of the cardiovascular system, manifested by an increase in Ea / Es and myocardial wall stress, associated with increased myocardial stiffness and increased LV pre- and afterload.

A recent study by S. Ohura-Kajitani et al. (2020) showed that both NO- and endothelium-dependent vasodilation were markedly impaired in patients with microvascular angina [14]. Another study found that CMD may precede epicardial dysfunction caused by oxidative stress and inflammation in early CAD [18]. The study by V. Lavin Plaza et al. (2020) showed that local inflammation in the vascular wall leads to endothelial dysfunction and accelerates the development and progression of atherosclerosis in peripheral arteries [19]. Therefore, when systemic endothelial dysfunction is detected, patients need to initiate early aggressive drug treatment aimed at restoring endothelial function and eliminating major risk factors.

Our study also found that patients with CMD had increased levels of serum biomarkers of endothelial activation and damage, which is likely associated with the influence of a number of risk factors (arterial hypertension, diabetes mellitus, hypercholesterolemia, etc.) on the development of functional and structural changes in the endothelium in the heart and the cardiovascular system as a whole. In particular, we found that increased serum levels of VEGF ≥ 346.7 pg / ml and endothelin-1 ≥ 6.9 pg / ml in patients with non-obstructive CAD can be considered as a marker of CMD presence.

Among the biomarkers of endothelial dysfunction, only asymmetric dimethylarginine (ADMA) and endothelin-1 have been studied the most in patients with CMD [20, 21]. Several investigators reported significantly higher plasma levels of ADMA and endothelin-1 in patients with CMD compared to controls and their association with adverse clinical outcomes [20, 22]. Our study also established an association of VEGF and endothelin-1 with adverse outcomes related to HFpEF progression, but these biomarkers were not independent predictors of risk stratification in the multivariate regression analysis. This is likely due to a relatively small patient sample and a small number of hard endpoints, such as readmissions and deaths.

LIMITATIONS OF THE STUDY

The main limitations of the study were a rather small sample size, heterogeneity of the sample, and short follow-up (12 months).

CONCLUSION

The results of this work, which stimulate further research in this area, can form the background for developing medical technologies for risk stratification in CMD and its early / preclinical diagnosis, which will determine the choice of personalized treatment strategy for this socially sensitive pathology and reduce the economic burden associated with its treatment costs.

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Authors' contribution

Kopeva K.V. – acquisition and interpretation of clinical data, statistical processing of the data, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Maltseva A.N. – carrying out of scintigraphy, acquisition and interpretation of the data, compilation of the database, final approval of the manuscript for publication. Mochula A.V. – carrying out of scintigraphy, assessment of blood flow parameters, acquisition and interpretation of the data, compilation of the database, final approval of the manuscript for publication. Smorgon A.V. – carrying out of echocardiography, acquisition and interpretation of the data, compilation of the database, final approval of the manuscript for publication. Grakova E.V. – interpretation of the clinical data, drafting of the article, final approval of the manuscript for publication. Gusakova A.M. – determination of serum biomarker levels, acquisition and interpretation of the data, compilation of the database, final approval of the manuscript for publication. Kalyuzhin V.V. – review of literature, interpretation of the data, drafting of the article, final approval of the manuscript for publication. Zavadovsky K.V. – coordination of the research, drafting of the article, final approval of the manuscript for publication.

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Effect of dalargin on the content of goblet cells and mucins in the colonic mucosa in experimental ulcerative colitis

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ABSTRACT

Aim. To investigate the protective effect of dalargin on the content of goblet cells and mucins in the colonic mucosa in a mouse model of ulcerative colitis.

Materials and methods. Ulcerative colitis was simulated in Balb/C mice by replacing drinking water with 5% sodium dextran sulfate in boiled water for 5 days. Dalargin was administered subcutaneously in a volume of 0.1 ml at a dose of 100 μg / kg of body weight once a day for 7 days from the beginning of ulcerative colitis simulation. Sulfasalazine as a reference-listed drug was administered intragastrically at a dose of 200 mg / kg once a day for 7 days. The mice were sacrificed on day 5, 7, and 28. The sections of the distal colon were prepared and stained with hematoxylin and eosin, alcian blue (pH = 1.0) according to Mowry or by PAS reaction. In the sections, the number of goblet cells and acid and neutral mucins was determined.

Results. In the mouse model of ulcerative colitis, the number of goblet cells (mainly at the bottom of the crypts), acid and neutral mucins decreased. Dalargin administration increased the number of goblet cells and the content of acid and neutral mucins in the colonic mucosa more effectively than sulfasalazine.

Conclusion. Dalargin has a protective effect in ulcerative colitis.

Keywords: ulcerative colitis, dalargin, sulfasalazine, goblet cells, mucins

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Conformity with the principles of ethics. The study was approved by the Ethics Committee Kursk State Medical University (Protocol No. 1 of 03.04.2023).

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Влияние даларгина на содержание бокаловидных клеток и муцинов в слизистой оболочке толстого кишечника при экспериментальном язвенном колите

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РЕЗЮМЕ

Цель – изучение протективного эффекта даларгина на содержание бокаловидных клеток и муцинов в слизистой оболочке толстого кишечника у мышей с экспериментальным язвенным колитом.

Материалы и методы. Язвенный колит моделировали у мышей линии Balb/C заменой в течение 5 сут питьевой воды 5%-м раствором декстрана сульфата натрия в кипяченой воде. Даларгин вводили подкожно в объеме 0,1 мл в дозе 100 мкг/кг массы тела 1 раз/сут в течение 7 сут с начала моделирования язвенного колита. Препарат сравнения сульфасалазин вводили в желудок в дозе 200 мг/кг 1 раз/сут в течение 7 сут. На 5, 7 и 28-е сут мышей выводили из эксперимента. На депарафинированных, окрашенных гематоксилином и эозином, альциановым синим (pH = 1,0) по Моури или реактивом Шиффа срезах дистального отдела ободочной кишки определяли количество бокаловидных клеток, содержание кислых и нейтральных муцинов.

Результаты. При модели язвенного колита в дистальном отделе ободочной кишки мышей снижается количество бокаловидных клеток (преимущественно в основании крипт), кислых и нейтральных муцинов. Даларгин эффективнее сульфасалазина увеличивает количество бокаловидных клеток, содержание кислых и нейтральных муцинов.

Заключение. Даларгин оказывает протективное влияние при экспериментальном язвенном колите.

Ключевые слова: модель язвенного колита, даларгин, сульфасалазин, бокаловидные клетки, муцины

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.

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INTRODUCTION

Goblet cells (GCs), along with absorptive colonocytes and enteroendocrine cells, are the main cell populations in the colonic mucosa [1]. GCs secrete mucus components, primarily mucins, which form the basis for the protective barrier of the mucous membrane. Mucins prevent the penetration of pathogenic and commensal microflora and toxins from the lumen into the wall of the colon and play an important role in the regulation of innate immunity [2]. There are two types of mucins: secretory and membrane-associated. Secretory mucins form an inner layer, which is impermeable to bacteria and high-molecular substances, while membrane-associated mucins form the glycocalyx [2]. Colonic barrier dysfunction and, primarily, damage to the mucin layer lead to a significant increase in permeability, penetration of bacteria into the mucous

membrane and submucosal layer, and activation of neutrophils [3], then macrophages and lymphocytes with the subsequent development of inflammation, which underlies the pathogenesis of ulcerative colitis (UC) [4].

UC is a chronic, relapsing disease of the colon. It develops in people aged 20–40 years, significantly worsens the quality of life, and often leads to disability [5]. Despite a significant number of investigations devoted to various UC aspects, its etiology and pathogenesis remain poorly understood, which results in low effectiveness of existing methods of its treatment [5]. In this regard, studying the effect of drugs exerting antioxidant, anti-inflammatory, and immunomodulatory effects on UC development is relevant.

Dalargin is one of these drugs, proposed for the treatment of peptic ulcers. Dalargin is also included in the pharmacotherapy of acute pancreatitis [6]. In this regard, it is of interest to study the effect of dalargin on the content of colonic mucins in a UC model.

The aim of the study was to investigate the protective effect of dalargin on the content of GCs and mucins in the colonic mucosa in a mouse model of UC.

MATERIALS AND METHODS

The experiments included 102 male Balb/C mice weighing 21–23 g, purchased from the Stolbovaya division of the Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency. The study was performed in compliance with the provisions of the Declaration of Helsinki proposed by the World Medical Association on Humane Treatment of Laboratory Animals (2000), the European Community Directive (86/609EC), and the Rules of Good Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 199n of 01.04.2016). Conducting experiments on the topic of the thesis research was approved by the regional Ethics Committee (Protocol No. 1 of 03.04.2023).

UC was modeled in mice by replacing drinking water with a 5% solution of dextran sulfate sodium (DSS) (Mr = 40,000, PanReac-AppliChem, Germany) in boiled water for 5 days [7]. The animals were euthanized when acute UC developed on day 5 and 7 and chronic UC developed on day 28.

Dalargin (Microgen, Russian Federation) was dissolved in normal saline and administered subcutaneously in a volume of 0.1 ml at a dose of 100 μg / kg of body weight once a day for 7 days from the beginning of UC modeling. According to the literature data, dalargin exhibits high pharmacological activity at the indicated dose [6]. Sulfasalazine (KRKA, Slovenia) was administered as a reference-listed drug into the stomach in the form of a suspension dissolved in normal saline in a volume of 0.3 ml at a dose of 200 μg / kg of body weight for 7 days from the beginning of UC modeling [8].

All animals were divided into the following experimental groups: 1) naïve mice, $n = 6$; 2) control group 1 (UC modeling + subcutaneous injection of normal saline), $n = 24$; 3) control group 2 (UC modeling + intragastric injection of normal saline), $n = 24$; 4) experimental group 1 (UC modeling + dalargin administration), $n = 24$; 5) experimental group 2 (UC modeling + sulfasalazine administration), $n = 24$.

The mice were euthanized by cervical dislocation under chloral hydrate anesthesia on days 5, 7, and 28. The distal colon was placed in neutral buffered 10% formaldehyde solution. Deparaffinized 5–6-μm slices of the colon were stained with hematoxylin and eosin, alcian blue (pH = 1.0) according to Mowry to detect highly sulfated acid mucins (AM) or by PAS reaction to detect neutral mucins (NM). Light microscopy was performed on the Nicon Eclipse Ni microscope using the NIS Elements AR software. The stained slices were scanned on the Hamamatsu NanoZoomer-SQ digital slide scanner (Japan). The resulting digital images were analyzed in the QuPath program [9] using the color deconvolution technique [10].

To assess AM and NM content in the colonic mucosa, histologic preparations after treatment with iodine acid were digitized. Areas with longitudinally oriented crypts without ulcers and erosions, but with pronounced signs of inflammation were identified in the digital images obtained. The number of GCs per 1 crypt was determined on slices stained with alcian blue. The content of AM and NM was assessed by the intensity of GC staining with alcian blue and PAS reaction after treatment with iodine acid, respectively. The coloring intensity was calculated as the average value of the decimal logarithm of the background brightness / image object point brightness ratio [11]. The intensity of histochemical

reactions varied significantly even in the control groups, which is associated with differences in slice thickness, fixation time, and staining. The intensity of GC staining was normalized by the intensity of staining of adjacent areas of connective tissue to level out these differences.

All samples were checked for normality of distribution using the Shapiro – Wilk test and for homogeneity of variance using the Levene's test. The Mann – Whitney *U*-test was used to determine the significance of differences. Nonparametric statistics methods were used due to small sample sizes, different distribution patterns, and heterogeneity of variance when comparing the samples. The results were described as the median and the interquartile range Me (Q_1; Q_3). The null hypothesis was rejected at the level of statistical significance $p \leq 0.05$. Statistical analysis of the results was carried out using the Statistica 10.0 software package.

RESULTS

The number of GCs decreased by 32.7–33.2% on day 5, by 47.1–47.6% on day 7, and by 15.4–15.9% on day 28 of the experiment ($p = 0.0024$) in both control groups of mice with experimental UC (Table). The number of GCs mainly decreased at the bottom of the crypts (Figure). GCs expanded, and their area increased. In mice with chronic UC (day 28), the number of GCs was 25.7–61.5% higher than in the animals with acute UC (day 5–7) ($p = 0.0009$). The content of AM in the colonic mucosa decreased by 3.43–3.75 times and 3.64–3.87 times and the content of NM declined by 36.3% and 38.2–39.3% on day 5 and day 7 of the experiment, respectively ($p = 0.0024$), compared to naïve animals. The content of mucins in mice with chronic UC was higher than in mice with acute UC, but was lower than in the naïve group (AM – by 42.5–43.3%, NM – by 27.5–29.4%) ($p = 0.0024$)).

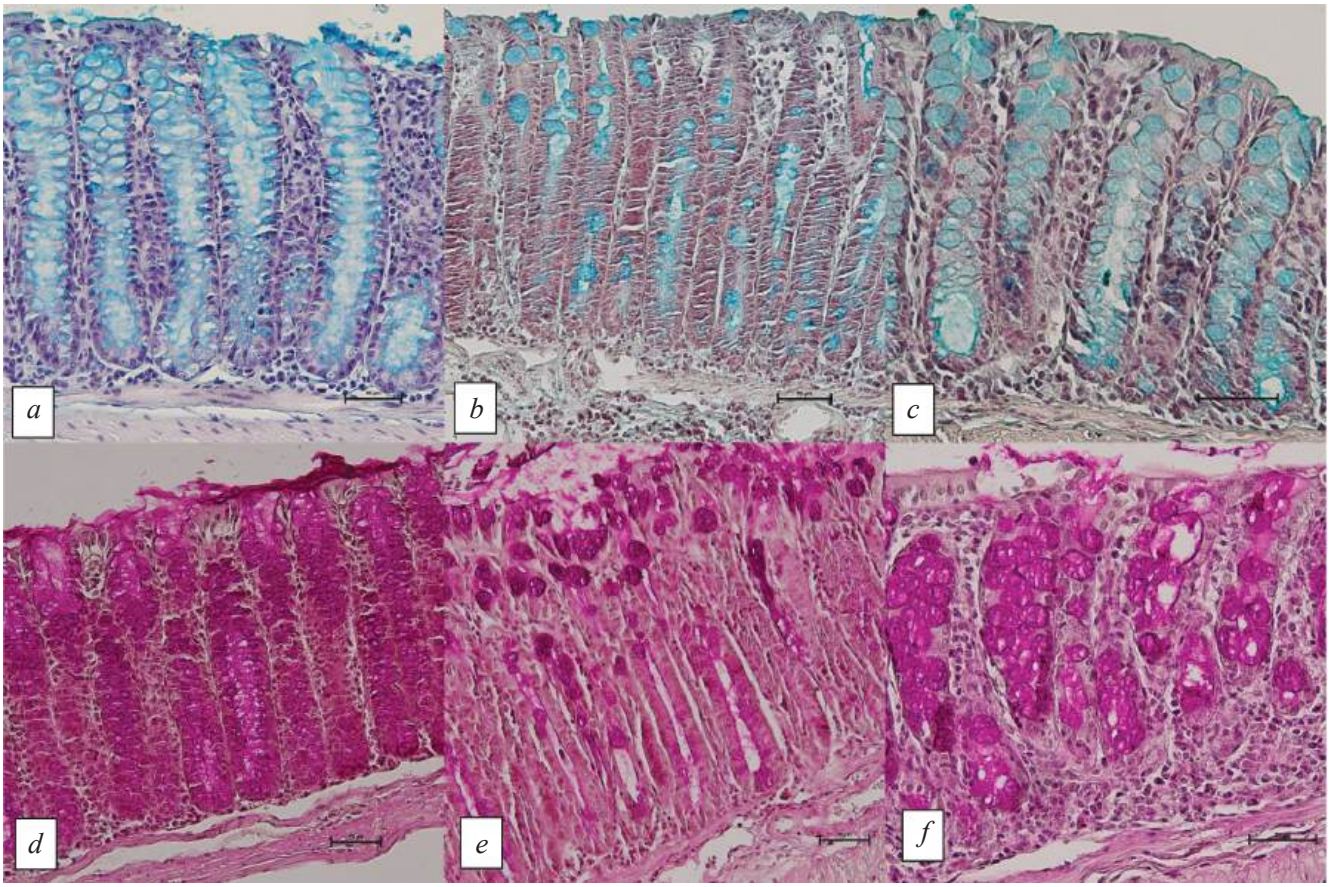

Figure. Goblet cells in the colon of male Balb/C mice: *a–c* – staining with hematoxylin and eosin + alcian blue, *d–f* – staining with hematoxylin and eosin + PAS reaction. *a*, *d* – control group, *b*, *e* – model of acute UC *c*, *f* – model of chronic UC. Bar – 50 μm

Dalargin administration was accompanied by an increase in the number of GCs in mice with the experimental UC by 3.6% (p = 0.0011) on day 5 of the experiment and by 9.3% (p = 0.0009) on day 7 compared to the control animals. Dalargin had no effect on the number of GCs in the colonic crypts of mice with chronic experimental UC. It increased the level of AM by 50.0% (p = 0.0239) on day 5 and by 54.8% (p = 0.0136) on day 7 of the experiment and increased the NM content by 6.2% (p = 0.0136) and 7.9% (p = 0.0009) on day 5 and day 7 of the experiment, respectively.

Table

The effect of dalargin and sulfasalazine on the number of goblet cells in crypts and the content of acid and neutral mucins in the colon of mice with experimental ulcerative colitis, *Me* [Q_1; Q_3]

No.	Experimental group	Duration of the experiment, day	Number of goblet cells, *n*	lg10 of the content of neutral mucins	lg10 of the content of highly sulfated acid mucins
Intact animals			20.8 [20.7; 20.9]	1.02 [1.00; 1.09]	1.20 [1.12; 1.35]
2.	Control group 1 (experimental UC + subcutaneous injection of normal saline)	5	14.0 [13.9; 14.1]^x p = 0.0024	0.65 [0.65; 0.66]^x p = 0.0024	0.32 [0.27; 0.43]^x p = 0.0024
		7	10.9 [10.7; 11.0]^x p = 0.0.0024	0.63 [0.63; 0.64]^x p = 0.0024	0.31 [0.28; 0.44]^x p = 0.0024
		28	17.6 [17.3;17.9]^x p = 0.0.0024	0.72 [0.72; 0.73]^x p = 0.0024	0.68 [0.58; 0.80]^x p = 0.0024
3.	Control group 2 (experimental UC + intragastric injection of normal saline)	5	13.9 [13.7; 14.3]^x p = 0.0024	0.65 [0.64; 0.66]^x p = 0.0024	0.35 [0.29; 0.43] ^{x} p = 0.0024; p = 0.0023
		7	11.0 [10.8; 11.2]^x p = 0.0024	0.64 [0.63; 0.64]^x p = 0.0024	0.33 [0.28; 0.37]^x p = 0.0024
		28	17.5 [17.4; 17.7]^x p = 0.0024	0.74 [0.74; 0.75]^x p = 0.0024	0.69 [0.54; 0.84]^x p = 0.0024
4.	Experimental UC + dalargin at a dose of 100 μg / kg subcutaneously	5	14.5 [14.3; 14.6]* p = 0.0011; ^1p = 0.3720	0.69 [0.68; 0.70]* p = 0.0136; ^1p = 0.2076;	0.48 [0.38; 0.56]* p = 0.0239; ^1p = 1.00
		7	13.0 [12.9; 13.2]*^{1} p = 0.0009; ^1p = 0.0009	0.68 [0.66; 0.71]*^{1} p = 0.0019; ^1p = 0.0019	0.48 [0.44; 0.60]* p = 0.0136; ^1p = 0.5635
		28	18.0 [17.7; 18.6] p = 0.0587; ^1p = 0.7527	0.78 [0.76; 0.80]^1 p = 0.0520; ^1p = 0.0023;	0.75 [0.68; 0.84] p = 0.3184; ^1p = 0.1722
5.	Experimental UC + sulfasalazine at a dose of 200 μg / kg intragastrically	5	14.3 [14.1; 14.5]* p = 0.0011	0.67 [0.66; 0.70]* p = 0.0101;	0.45 [0.38;0.55] p = 0.0831
		7	12.1 [11.9; 12.3]* p = 0.0009	0.63 [0.62; 0.64] p = 0.4623	0.45 [0.39;0.59]* p = 0.0209
		28	18.0 [17.7; 18.1] p = 0.0587	0.75 [0.74; 0.76]* p = 0.0136	0.65 [0.63; 0.75] p = 0.8337

$p < 0.05$ compared to the parameters in the: ^{x} – intact group; * – control group; ^{1} – animals treated with sulfasalazine.

The number of GCs increased by 2.9% (p = 0.0011) on day 5 and by 10.0% (p = 0.0009) on day 7 of the experiment, when sulfasalazine was administered at a dose of 200 μg / kg to mice with experimental UC, compared to the control group 2. The AM content did not change throughout the experiment, the NM content increased by 3.1% (p = 0.0101) on day 5 and by 3.1% (p = 0.0136) on day 28 of the experiment, compared to the control group 2.

Dalargin increased the number of GCs by 7.4% (p = 0.0009) on day 7 compared to the value during sulfasalazine administration. The NM content increased by 7.9% (p = 0.0019) on day 7 and by 8.3% (p = 0.0023) on day 28 only in the experiment with dalargin administration.

DISCUSSION

The results of the study confirm the literature data on a decrease in the number of GCs and the content of AM and NM in the colonic crypts both in patients with UC [12] and in a mouse model of UC [11]. The work shows a decrease in the number of GCs mainly at the bottom of the crypts. It is known that GCs located at the bottom of crypts produce the antimicrobial peptide WFDC2 in healthy people, while its secretion is impaired in patients with UC [12]. This peptide inhibits serine and cysteine proteases; therefore, it prevents premature transformation of the inner layer of the colonic mucosa into the outer layer. The outer layer is permeable to bacteria coming from the colonic lumen and serves as a growth medium for the commensal microflora; the inner layer is

impermeable to bacteria and ensures the maintenance of the colonic wall homeostasis [13]. Destruction of the inner layer under the influence of proteases promotes penetration of bacteria into the colonic wall and development of inflammation in UC [4].

Highly sulphated AM and other acid mucins are more effective than NM in preventing the destruction of the protective colonic barrier by proteases [13]. The NM content increased in the colonic mucosa of mice with experimental UC [11]. In our study, the number of NM decreased throughout the experiment. Diverse changes in the NM content are due to the formation of experimental UC of varying severity in mice of different strains. The mucin content increases in the colonic mucosa during the transition from acute to chronic UC.

Dalargin has a protective effect in mice with experimental UC and is more effective than sulfasalazine, as it increases the number of GCs and the content of AM and NM. Dalargin, as an analogue of leu-enkephalin, activates opioid δ- and μ-receptors [6]. The mechanism of the therapeutic effect of dalargin in colonic inflammation is apparently explained by the activation of opioid μ-receptors, since their activation by the selective ligand DAMGO (H-Tyr-D-Ala-Gly-N-MePhe-Gly-ol) has a protective effect on DSS-induced UC development in mice [14]. Apparently, like DAMGO, dalargin reduces disease severity, decreases myeloperoxidase activity and the concentration of proinflammatory cytokines, prostaglandins, and nuclear factor kB, and increases the production of the antiapoptotic factor Bcl-xL [10]. The authors associate this effect with the activation of peripheral μ-receptors, since DAMGO is not able to penetrate the blood – brain barrier, and the peripheral μ-receptor antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Pen-Thr-NH_2) eliminates its effect [10].

Dalargin has antioxidant and immunomodulatory effects [6]. It inhibits the activity of mononuclear cells in their pathological activation [15] and, thus, reduces the severity of inflammation. Suppression of lipid peroxidation not only weakens alterative changes at the inflammation site, but also prevents an increase in the colonic barrier permeability and penetration of pathogenic microbes into the colon.

CONCLUSION

The protective effect of dalargin on the UC development in Balb/C mice was established: an increase in the number of GCs and the content of AM and NM in the colonic mucosa was registered. The effect of dalargin is more pronounced than that of sulfasalazine. The data obtained allow to consider dalargin as a component of an effective combination drug therapy for UC.

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Cardioprotective effect of lithium ascorbate in an *in vivo* model of myocardial infarction

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ABSTRACT

The aim of the work was to study the cardioprotective effect of lithium ascorbate in an *in vivo* model of myocardial infarction. In the course of the study, we searched for compounds promising for therapy of acute myocardial infarction.

Materials and methods. Myocardial infarction was modeled in Wistar rats by ligating the left coronary artery (the duration of ischemia was 45 minutes) followed by ligature loosening and 120-minute reperfusion. All manipulations were performed under alpha-chloralose anesthesia with mechanical lung ventilation and recording heart rate, blood pressure, and ECG. Lithium ascorbate was administered intravenously at a dose of 100 mg / ml before ischemia. The area at risk (the ischemia / reperfusion zone) was detected by staining the myocardium with tightened ligature with 5% potassium permanganate. After that consecutive myocardial slices were prepared, and infarct size was determined. Differentiation of the infarct size from the area at risk was performed by staining with 1% 2,3,5-triphenyl tetrazolium chloride solution for 30 minutes at 37 ºC. The infarct size and the area at risk were determined by the planimetric method. The serum concentration of myocardial damage marker creatine kinase-MB (CK-MB) was measured using ELISA kits.

Results. Lithium ascorbate reduced the infarct size / area at risk ratio by 38% and decreased the serum CPK-MB level in the experimental animals by 42% compared to the control group. Lithium ascorbate did not affect hemodynamics parameters during coronary artery occlusion and reperfusion.

Conclusion. The cardioprotective effect of lithium ascorbate in cardiac ischemia / reperfusion *in vivo* was found.

Keywords: myocardial infarction, lithium salts, ischemia, reperfusion, arrhythmias

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

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Conformity with the principles of ethics. The study was approved by the Ethics Committee at Cardiology Research Institute of Tomsk NRMC (Protocol No. 207 of 23.12.2020).

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Кардиопротекторный эффект аскорбата лития на модели инфаркта миокарда *in vivo*

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РЕЗЮМЕ

Цель – изучение кардиопротекторного действия аскорбата лития на модели инфаркта миокарда *in vivo*. В ходе исследований проводился поиск соединений, перспективных для терапии острого инфаркта миокарда.

Материалы и методы. Моделирование инфаркта миокарда проводили на крысах линии Вистар массой 250–300 г путем наложения лигатуры на левую коронарную артерию (продолжительность ишемии 45 мин) с последующим ослаблением лигатуры и реперфузией продолжительностью 120 мин. Все манипуляции выполнялись под хлоралозным наркозом с искусственной вентиляцией легких и регистрацией частоты сердечных сокращений, артериального давления и электрокардиограммы. Аскорбат лития вводили в дозе 100 мг/мл перед ишемией внутривенно. Определяли зону риска (ЗР) – зону ишемии/реперфузии, для чего миокард с затянутой лигатурой окрашивали 5%-м перманганатом калия, после чего делали последовательные срезы миокарда и определяли зону инфаркта. Дифференцировку зоны некроза миокарда от ЗР осуществляли путем окрашивания 1%-м раствором 2,3,5-трифенил тетразолия хлорида в течение 30 мин при 37 ºС. Размер зоны инфаркта и зоны риска определяли планиметрическим методом. Концентрацию маркера повреждения миокарда креатинфосфокиназы-МВ (КФК-МВ) в сыворотке крови определяли иммуноферментным методом.

Результаты. Аскорбат лития статистически значимо уменьшал отношение зоны инфаркта к ЗР на 38% и снижал уровень КФК-МВ в сыворотке крови экспериментальных животных на 42% по сравнению с группой контроля. Аскорбат лития не повлиял на параметры гемодинамики на всех этапах развития коронарокклюзии и реперфузии.

Заключение. Установлено кардиопротекторное действие аскорбата лития при ишемии/реперфузии сердца *in vivo*.

Ключевые слова: инфаркт миокарда, соли лития, ишемия, реперфузия, аритмии

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

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INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death worldwide. Despite the development of effective emergency care and primary prevention measures, CVD prevalence remains high [1]. In Russia, more than 50,000 people die from myocardial infarction each year, which accounts for more than 2.5% of all-cause mortality [2]. Among all CVDs, myocardial infarction remains the most common acute condition with high mortality. This fact indicates the relevance of developing new and more effective cardioprotective drugs.

Currently, the main approach in complex therapy of myocardial infarction is rapid myocardial reperfusion (surgical or pharmacological), which implies restoration of blood supply to ischemic heart tissue. A related approach is cardioprotection, which involves pharmacological protection of myocardial cells in cardiac ischemia and reperfusion. Broadly speaking, cardioprotection includes all drugs and agents that can preserve the pumping function of the heart, reduce infarct size, and prevent the occurrence of life-threatening arrhythmias by reducing or preventing myocardial damage.

Such drugs include compounds from different pharmacologic classes, including, for example, beta-blockers, which have a long history of use and clinically proven cardioprotective effects. The cardioprotective effect of opioid receptor agonists has been proven in a number of studies [3, 4]. However, many drugs have not proven to be effective in clinical trials [5]. The search for new cardioprotective agents continues due to the high relevance and demand.

At the same time, some compounds are not widely used in cardiology, despite their significant cardioprotective potential. Lithium salts can refer to this group. It was previously shown that lithium chloride has a neuroprotective effect and allows to reduce neuronal tissue damage in the zone of ischemic stroke [6–8]. It should be noted that lithium salts are widely used in psychiatry as mood stabilizers for the treatment of affective disorders, so the pharmacokinetics and toxicology of lithium are well known from clinical practice. However, the potential of these drugs is not limited to the treatment of mental disorders. In particular, the protective effect of lithium in ischemic conditions has been proven. In this regard, CVDs are the most promising target for lithium therapy. The development of this area requires the study of the mechanisms of action and the search for new applications of lithium salts.

The studies published to date allow to conclude that the anti-ischemic effect of lithium may be manifested not only in relation to the brain, but also in relation to other organs [9]. It is important to note that even though lithium has been used in medicine for almost a century, the mechanism of action of lithium salts in myocardial infarction has not been studied. Previously lithium has been shown to have cardioprotective effects on isolated myocardium [10]. Most of the described studies consider inorganic lithium salts, primarily chloride and carbonate. The therapeutic potential of lithium salts can be expanded by selecting an anionic component with antioxidant activity, which increases antioxidant properties of the salt and enhances cytoprotective properties in oxidative stress.

The aim of this work was to study the cardioprotective effect of lithium ascorbate in an *in vivo* model of myocardial infarction.

MATERIALS AND METHODS

All experiments were carried out in compliance with the principles of humanity set out in the Directives of the European Community (86/609/EEC) and the Declaration of Helsinki. The equipment

of the Center for Collective Use "Medical Genomics" of Tomsk NRMC was used in the work.

Lithium ascorbate was used as a test preparation. For these experiments, lithium ascorbate was obtained *ex tempore* by reaction between ascorbic acid and lithium carbonate (ACS reagents, Sigma-Aldrich) according to the described method [11]. The white powder was obtained, which was tested for authenticity using infrared (IR) spectrometry and elemental analysis. Lithium ascorbate was dissolved in normal saline and injected intravenously during the experiment.

Wistar rats weighing 250–300 g were used as experimental animals. The animals were kept in standard conditions, with natural day and night regimen and unlimited access to food and water. Before performing the experimental procedures, the animals were randomly distributed into two groups of eight rats each (control and experimental group). In the experimental group, the drug was injected into the femoral vein at a concentration of 100 mg / ml in 1 ml of normal saline 10 minutes before ischemia. A similar volume of saline solution was injected into the femoral vein in the control group 10 minutes before ischemia.

Solution of α-chloralose (Sigma) was used for anesthesia during surgical modeling of myocardial infarction (intraperitoneal injection at a dose of 60 mg / kg). Anesthetized animals were then connected to the SAR-830 Series mechanical ventilation system (CWE Inc., USA). Heart rate (HR) and blood pressure (BP) were measured using the SS13L pressure sensor (Biopac System Inc., Goleta, USA). The sensor was connected to the MP35 data acquisition system (Biopac System Inc., Goleta, USA). Electrocardiography was also performed on this device; ECG was recorded automatically throughout the experiment. Direct surgery on the heart was carried out according to the method of J.E. Schultz et al. [12].

Ligation of the left coronary artery was performed, which resulted in controlled ischemia in its basin. After 45 minutes of ischemia, the ligature was removed and blood flow restoration was confirmed by the emergence of hypraemia. The reperfusion period lasted 120 minutes. The detection of the necrotic zone and myocardial area at risk was performed according to the method described in the study by J. Neckar et al. [13]. To do this, after completion of the reperfusion period, the heart was removed followed by retrograde flushing. The heart was flushed with normal saline through the aorta.

The area at risk, i.e. the myocardial area subject to ischemia – reperfusion injury, was identified as follows. The ligature applied for ischaemia was re-ligated, and the cardiac muscle tissue was stained through the aorta with 5% potassium permanganate solution. After flushing the heart with normal saline, it was frozen and sliced perpendicular to the longitudinal axis in 1-mm-thick slices using the HSRA001-1 precision slicer (Zivic Instruments, Pittsburgh, USA) according to standard histologic techniques.

Visual differentiation of the necrotic zone (NZ) from the area at risk (AAR) was performed by treating with a 1% solution of 2,3,5-triphenyl tetrazolium chloride for half an hour in the thermostat at 37 ºC. During this process, oxidized 2,3,5-triphenyl tetrazolium chloride is reduced under the effect of dehydrogenases, which is manifested by the emergence of permanent staining. Active dehydrogenases were absent in the zone of heart muscle necrosis, and, consequently, this zone was not stained. At the end of treatment, all slices were fixed with 10% formalin for 24 hours. The obtained myocardial tissue samples were scanned from different sides using the HP Scanjet G4050 scanner. The sizes of AAR and NZ were determined by the planimetric method. The infarct size was expressed as a percentage of the hypoperfusion zone (AAR) as the NZ / AAR ratio. The activity of CPK-MB was determined using ELISA kits (Cloud-Clone Corp, Wuhan, China). CPK-MB in blood serum was determined using the Infinite 200 PRO microplate reader (Tecan GmbH, Austria).

Statistical analysis of the data was performed using specialized GraphPad Prism 9 (GraphPad Software, CA, USA) and MS Excel (Microsoft, USA) software. The results were shown as the mean and the standard deviation ($M \pm m$). The statisctical significance of the differences between the groups were identified using the Mann – Whitney test. The differences were considered significant at $p < 0.05$.

RESULTS

The assessment of HR dynamics at various stages of infarction modeling with lithium is shown in Figure 1. The assessment of BP dynamics at various stages of infarction modeling when exposed to lithium is

presented in Figure 2. The results of the evaluation of lithium cardioprotective effect parameters on the myocardial infarction model are presented in Tables 1 and 2. The evaluation of heart rhythm disturbances during ischemia – reperfusion injury is shown in Table 3.

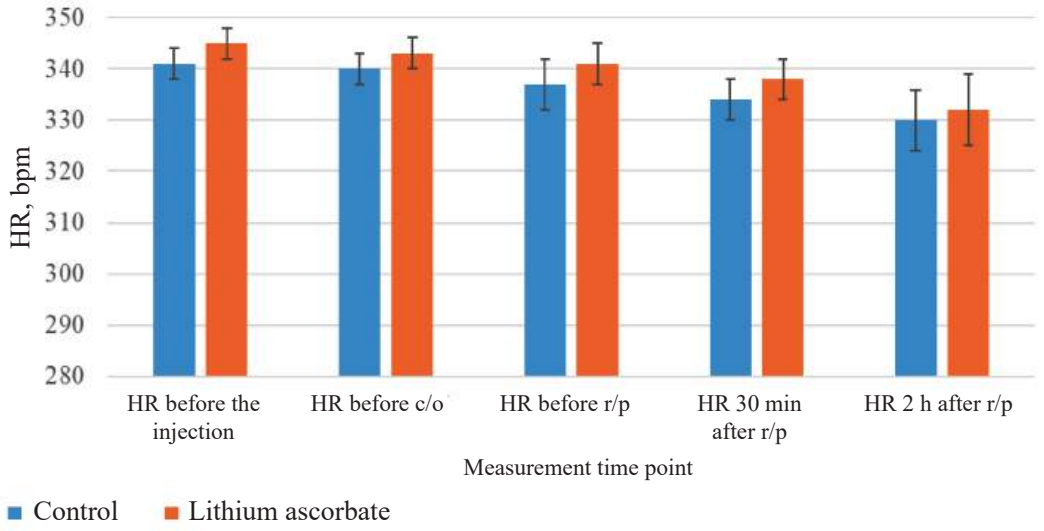

Fig. 1. Heart rate (HR) assessment in the animals at different time points during the experiment, bpm; c/o – coronary occlusion (45 minutes); r/p – reperfusion (120 minutes).

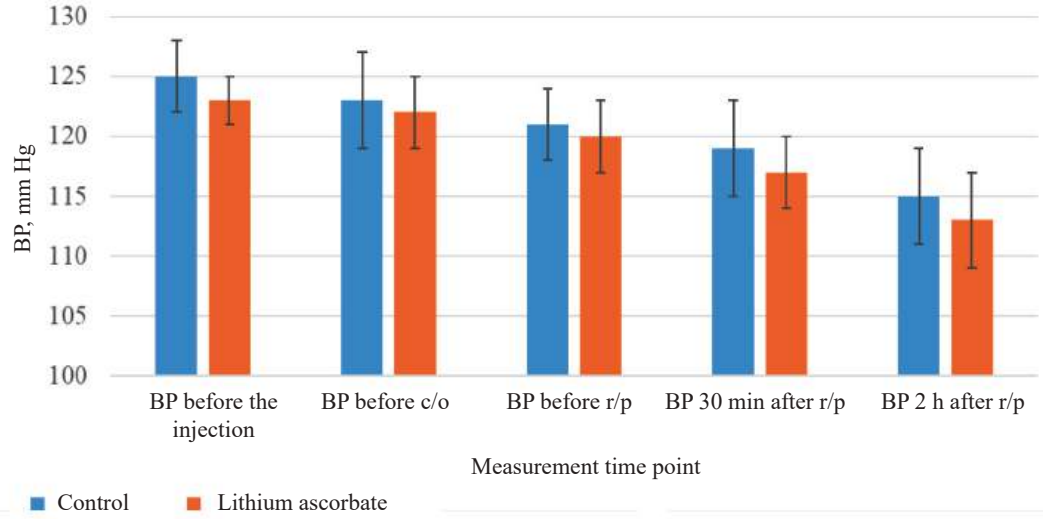

Fig. 2. Blood pressure (BP) assessment in the animals at different time points during the experiment, mm Hg; c/o – coronary occlusion (45 minutes); r/p – reperfusion (120 minutes).

Table 1

Calculated sizes and parameters of myocardial infarction area (45-minute ischemia, 120-minute reperfusion) in experimental animals, $M \pm m$

Group	Necrotic zone, mg	Area at risk, mg	NZ / AAR, %	Right ventricular mass, mg	Left ventricular mass, mg
Control, $n = 8$	271 ± 52	561 ± 101	48 ± 3	187 ± 12	988 ± 11
Lithium ascorbate, $n = 8$	119 ± 32*	394 ± 95	30 ± 2*	181 ± 10	± 11

* statistically significant difference from the control group, $p < 0.05$ (here and in Table 2)

Table 2

Creatine phosphokinase-MB concentration in the blood serum of rats with modeled myocardial infarction (45-minute coronary occlusion, 120-minute reperfusion), *M* ± *m*

Group	Serum CPK-MB concentration, U / l
Control, *n* = 8	104.3 ± 13.2
Lithium ascorbate, *n* = 8	60.5 ± 10.4*

Table 3

Frequency and types of arrhythmias in the rats with modeled myocardial infarction at the stage of ischemia (45-min coronary occlusion), %

Group	Ischemia (45 min)			
	Without arrhythmias	Multiple ventricular extrasystoles	Ventricular tachycardia	Ventricular fibrillation
Control, *n* = 8	0	100	87.5	25
Lithium ascorbate, *n* = 8	12.5	87.5	62.5	12.5

Note. Data are shown as a percentage from the corresponding animal group.

DISCUSSION

The experimental drug was administered to the animals intravenously at a dose of 100 mg / ml in normal saline in a volume of 1 ml 10 minutes before the onset of ischemia. The control group received intravenously normal saline in the same volume, and no significant changes in hemodynamics were observed immediately after administration (Fig. 1, 2). Lithium ascorbate did not affect HR (Fig. 1) and BP values (Fig. 2) at all stages of the ischemia development. During the development of ischemia, a slight trend toward a decrease in BP and HR was noted. However, even at the final stage of reperfusion, these parameters did not significantly differ from the baseline values in the animals.

The assessment of myocardial ischemic damage under lithium ascorbate exposure showed a decrease in cardiomyocyte death, which was expressed in a significant decrease in the NZ / AAR ratio by 38% compared to the control group (Table 1). The decrease in this parameter reflects greater myocardial preservation in AAR and a proportional decrease in the zone of ischemic heart injury during lithium exposure. Significant reduction of myocardial damage is confirmed by the biochemical analysis of serum CPK-MB level in the experimental animals (Table 2). We detected a decrease in the CPK-MB level in myocardial infarction under lithium exposure by 42% compared to the control group (blood was taken at the final stage after coronary occlusion and reperfusion).

Thus, it can be concluded that lithium administration before ischemia is accompanied by a pronounced cardioprotective effect, manifested by a decrease in the infarct size and serum concentration of CPK-MB, a myocardial cell damage marker. At the same time, lithium ascorbate administration was not accompanied by hemodynamic deterioration at the stages of coronary occlusion and reperfusion in the experimental animals. During all manipulations, HR was monitored, which is an important parameter in assessing the cardioprotector effectiveness. In this context, it is important to note that in clinical practice, life-threatening ventricular arrhythmias are a severe complication usually accompanied by a decrease in myocardial contractility [14]. The occurrence of such arrhythmias adversely affects the prognosis in patients with myocardial infarction and often leads to death [15].

In this study, various arrhythmias, including multiple ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation, were identified in the control group (Table 3). The identified arrhythmias were generally reversible and sinus rhythm was restored and, in some cases, transitioned to another cardiac rhythm disturbance, so there could be more than one type of arrhythmia in one animal. After 45-minute ischemia, no arrhythmias occurred in all experimental groups, so the antiarrhythmic effect was assessed only during ischemia. The group which received lithium ascorbate showed a decrease in arrhythmias of all types, including ventricular fibrillation that is the most critical one, but no statistically significant differences were found compared to the controls during this experiment (Table 3).

The presented results are consistent with the literature data obtained on isolated organs [16]. Several most probable mechanisms of lithium effect during ischemia explain the cardioprotective effect of lithium, including competitive antagonism to the main biological ions (Na, K), induction of nitric oxide synthesis, and influence on protein kinases and related metabolic pathways. These pathways are known to be involved in the realization of the cardioprotective effects of opioids [17]. It has been

previously shown that the effect on ATP-sensitive potassium channels (KATP channels) plays an essential role in cardioprotection and pathological process in myocardial infarction [18]. As a target of lithium, these channels are protein structures whose activity is regulated by intracellular nucleotides. They act mainly in muscles and neurons, where, under conditions of energy shortage in the form of ATP, they can reduce cell excitability, which contributes to its survival under stressful conditions. It is important to note that lithium can exert multiple effects on a number of targets simultaneously in different tissues [*9*]. The results obtained imply further study of the cardioprotective effect of lithium salts.

CONCLUSION

The results of the study revealed an infarct size-limiting effect of lithium ascorbate with no significant effect on hemodynamics. During the experiment, we detected a decrease in myocardial NZ by 38% and a fall in the serum CPK-MB level by 42% compared to the control group.

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Authors' contribution

Plotnikov E.V. – conception and design, carrying out of research, drafting of the manuscript. Chernov V.I. – analysis of the data, substantial intellectual contribution, drafting of the manuscript. Mukhomedzyanov A.V. – performing operations in vivo, drafting of the manuscript. Maslov L.N. – conception and design of the in vivo study, interpretation of the results, drafting of the manuscript. Yusubov M.S. – analysis of lithium compounds, processing of the results, substantial intellectual contribution, drafting of the manuscript. Larkina M.S. – chemical synthesis, drafting of the manuscript. Artamonov A.A. – statistical processing of the results, graphic design, drafting of the manuscript. Belousov M.V. – analysis of the research results, drafting of the manuscript. All the authors approved the final version of the article.

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The effect of a new 3-hydroxypyridine derivative LHT-2-20 on free radical oxidation in experimental periodontitis

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ABSTRACT

Aim. To study the effect of a new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) on free radical oxidation in experimental periodontitis.

Materials and methods. The experimental study was performed on 195 white mongrel mice weighing 19–23 g and 137 white mongrel rats weighing 180–220 g. The effect of a new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) on the intensity of free radical oxidation and the local state of periodontal tissues during a course of intragastric administration was studied on the experimental model of periodontitis. Statistical processing of the results was carried out using the SPSS Statistics 20.0 software package with the analysis of variance (ANOVA) and the parametric Tukey's test.

Results. The LHT-2-20 compound reduced elevated levels of primary and secondary lipoperoxidation products (conjugated dienes, malondialdehyde in plasma and in erythrocytes during spontaneous and iron-induced oxidation) already at the early stages of the experiment, bringing the studied parameters closer to the reference values by the end of the course of treatment. The use of the compound LHT-2-20 contributed to an increase in the activity of the main antioxidant enzymes (catalase and superoxide dismutase), normalizing them to baseline values by the end of the experiment. With the correction of free radical processes, the use of LHT-2-20 limited the local inflammatory response in periodontal tissues, which was confirmed by a decrease in gingival edema and hyperemia, bleeding, depth of periodontal pockets, and tooth mobility.

Conclusion. The results of this study confirm the anti-inflammatory potential of the compound and the multiplicity of its effects due to the impact on the mechanisms of oxidative stress. The expediency of further study of the drug is justified by the prospect of creating a new drug and its subsequent wide clinical application as part of the complex therapy of periodontal inflammation.

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Keywords: 3-hydroxypyridine derivatives, periodontitis, ROS, oxidative stress, bleeding, pathological mobility

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Влияние нового производного 3-гидроксипиридина ЛХТ-2-20 на процессы свободнорадикального окисления при экспериментальном пародонтите

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РЕЗЮМЕ

Цель исследования: изучение влияния нового комплексного соединения ЛХТ-2-20 (2-этил-6-метил-3-гидроксипиридиния-2-(3-бензоилфенил)-пропаноата) на процессы свободнорадикального окисления при экспериментальном пародонтите.

Материалы и методы. Экспериментальное исследование выполнено на 195 белых беспородных мышах массой 19–23 г, 137 белых беспородных крысах массой 180–220 г. На модели экспериментального пародонтита изучено влияние нового комплексного соединения ЛХТ-2-20 (2-этил-6-метил-3-гидроксипиридиния-2-(3-бензоилфенил)-пропаноата) на интенсивность свободнорадикального окисления и местное состояние тканей пародонта при курсовом внутрижелудочном его применении. Статистическая обработка результатов проводилась с использованием программного комплекса SPSS Statistics 20.0 с применением дисперсионного анализа (ANOVA) и параметрического критерия Тьюки.

Результаты. Соединение ЛХТ-2-20 снижало повышенные уровни первичных и вторичных продуктов липопероксидации (диеновых конъюгатов, малонового диальдегида в плазме и в эритроцитах при спонтанном и железоиндуцированном окислении) уже в ранние сроки эксперимента, приближая исследуемые показатели к референсным значениям к концу курсового лечения. Применение соединения ЛХТ-2-20 способствовало росту активности основных энзимов антиоксидатной системы (каталазы и супероксидисмутазы), нормализуя данные показатели к концу эксперимента до исходных значений. На фоне коррекции свободнорадикальных процессов применение ЛХТ-2-20 способствовало ограничению местной воспалительной реакции тканей пародонта, что подтверждалось уменьшением отека и гиперемии десневого края,

кровоточивости, глубины пародонтальных карманов и степени подвижности зубов.

Заключение. Результаты данного исследования подтверждают противовоспалительный потенциал соединения и множественность его эффектов за счет воздействия на механизмы оксидативного стресса. Целесообразность последующего изучения средства обоснована перспективой создания нового лекарственного препарата и его последующего широкого клинического применения в составе комплексной терапии воспалительных процессов тканей пародонта.

Ключевые слова: производные 3-гидроксипиридина, пародонтит, оксидативный стресс, кровоточивость, патологическая подвижность

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

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

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INTRODUCTION

Currently, periodontal diseases represent an important medical and social problem which is due to the widespread prevalence of this pathology. Globally, chronic periodontitis ranks 11th among all dental diseases [1]. A similar trend can be observed in the Russian Federation where the assessment of dental morbidity among the adult population revealed high prevalence of inflammatory periodontal diseases (89%), with the peak of morbidity (94.3%) occurring at the age of 40–45 years [2]. Therefore, chronic periodontitis is characterized by a marked trend toward an increase in morbidity rates [1].

Activation of free radical oxidation processes and inhibition of the antioxidant system (AOS) are of great importance in the pathogenesis of inflammatory and destructive periodontal diseases [3]. As a result, an imbalance develops between the prooxidant and antioxidant systems, leading to increased production of reactive oxygen species (ROS). These pathological changes predetermine the emergence of oxidative stress, characterized by increased production of ROS and destruction of cells [4]. Emerging disorders of oxidative homeostasis and capillary blood flow and increased vascular permeability contribute to the formation of local inflammatory changes in the periodontium, characterized by gingival hyperemia, hemorrhagic manifestations, and a pathological increase in the depth of periodontal pockets followed by pathological tooth mobility [5, 6].

The pronounced prevalence of chronic periodontitis, its continuous recurrent course, and an increase in the number of disease forms with complications demand the development of new methods for the treatment of this pathology [7]. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) used in various forms are components in the treatment of chronic periodontitis [8]. However, despite their pronounced anti-inflammatory effect, this class of drugs has limited use due to frequent complications resulting from their ulcerogenic activity [9]. In addition, NSAIDs have low antioxidant, membrane protective, and antihypoxic activity, which is manifested by their inability to correct the imbalance between two interdependent systems in order to increase antioxidant potential [8]. The above disadvantages make it necessary to include drugs with complex antioxidant and anti-inflammatory effects in periodontal therapy, which will contribute to the potentiation of therapeutic effects and leveling of adverse drug reactions.

The aim of the research was to study the effect of a new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) on free radical oxidation in experimental periodontitis.

MATERIALS AND METHODS

The experimental study was performed on 195 white mongrel mice weighing 19–23 g and 137 white mongrel rats weighing 180–220 g divided into 5 groups. All animals were obtained from the SMK STEZAR bionursery (Vladimir, Russia) and were kept under laboratory conditions at standard temperature and air humidity. The study was carried out in accordance with the requirements of the European Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes and the rules of Good Laboratory Practice (Order No. 267 of the Ministry of Health of the Russian Federation of 19.06.2003). The experimental study was approved by the Ethics Committee (Protocol No. 102 of 31.01.2021).

A new complex compound 2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate (laboratory name LHT-2-20) was synthesized at the Department of Chemistry and Technology of Synthetic Drugs and Analytical Control of the All-Union Research Center for Safety of Biologically Active Compounds (Patent of the Russian Federation for invention No. 2793537) [10]. The NSAIDs ketoprofen (2-(3-benzoylphenyl)-propanoic acid, Velpharm, Russia), which has anti-inflammatory effects, and mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate, Pharmacoft, Russia), which has antioxidant effects, were selected as reference-listed drug.

The model of experimental periodontitis was reproduced in accordance with the patented method developed by K.D. Shkolnaya et al. [11]. Prednisolone at a dose of 12 mg / kg was administered to the rats on days 1, 3, and 5. On day 5 of the experiment, under general anesthesia with 0.03 ml of zoletil 100 (Virbac Sante Animale, France) intramuscularly, the interdental papilla between the first and second maxillary molars was sutured with suture fixation and filled with Filtek Bulk Fill (3M, USA) from the vestibular side.

Group 1 included intact rats ($n = 15$) with a healthy periodontium. In group 2 (control; $n = 30$), the model of periodontitis without treatment was reproduced. Animals in groups 3 and 4 ($n = 30$ in each group) were administered ketoprofen and mexidol at doses corresponding to 2% and 5% of the LD_{50} value, respectively. Rats in group 5 ($n = 32$) received LHT-2-20 at a dose of 11.54 mg / kg corresponding to 2% of the LD_{50}. After preliminary dissolution in 1.5 ml of starch, the studied compounds were administered intragastrically (i.g.) in a volume of 1.5 ml once a day for 10 days. The animals were euthanized under isoflurane anesthesia by decapitation on day 25.

Acute toxicity of LHT-2-20 was studied in white mongrel mice of both sexes divided into groups of 5 animals each. After preliminary dissolution in starch, the compound was administered i.g. in a volume of 0.3 ml at increasing concentrations. LD_{50} was calculated using the Litchfield and Wilcoxon's method.

Free radical processes in plasma were assessed by biochemiluminescence on the Fluorate–02-ABLF-T biochemical analyzer (Promecolab, Saint Petersburg), by the level of primary and secondary lipid peroxidation products – conjugated dienes (CD), determined in blood plasma by the modified Placer method (1976), as well as by the level of malondialdehyde (MDA) in plasma and erythrocytes in spontaneous (MDA) and iron-induced oxidation (Fe-MDA), determined by the method proposed by S.G. Konyukhova (1989). Antioxidant potential was studied by the activity of catalase (CAT) in plasma and erythrocytes determined in accordance with the method developed by M.A. Korolyuk (1988) and by the activity of superoxide dismutase (SOD) in plasma determined by the method proposed by E.E. Dubinina (1983).

Local periodontal changes were identified based on the state of the oral mucosa; the severity of bleeding gums (0–3 points); grades of tooth mobility (grade 0–2); and the depth of periodontal pockets (mm).

Statistical processing of the results was carried out using the SPSS Statistics 20.0 software package. The data were presented as the mean and the standard deviation ($M \pm \sigma$), absolute and relative values (n (%)), and a 95% confidence interval (CI). The normality of data distribution was checked using the Shapiro – Wilk test. Intergroup comparisons were conducted using the two-tailed Fisher's exact test and the analysis of variance (ANOVA) with the Tukey's post hoc test. The correlation analysis was carried out using the Spearman's rank correlation coefficient. The differences were considered statistically significant at $p < 0.001$.

RESULTS

Determination of acute toxicity of LHT-2-20

LD_{50} of LHT-2-20 in mice with i.g. administration of the compound was 1,130 mg / kg. The result shows that the compound is 2.97 times less toxic

than ketoprofen, but 1.88 times more toxic than mexidol (Table 1).

Table 1

Acute toxicity of LHT-2-20 in mice

Substance (compound)	LD_{50}, mg / kg	95% CI
Ketoprofen	380	358–402
Mexidol	2,120	2,010–2,230
LHT-2-20	1,130 [A B]	1,040–1,220

[A] – the differences are significant compared to ketoprofen; [B] – the differences are significant compared to mexidol ($p = 0.001$, one-way analysis of variance (ANOVA) with the Tukey's test).

Anti-inflammatory and antioxidant effects of LHT-2-20 in experimental periodontitis

LHT-2-20 administered i.g. suppressed free radical oxidation, which was confirmed by the following changes. The plasma CD level was 50% lower ($p_1 < 0.001$) compared to the control group, thereby approaching intact values. In addition, compared to the ketoprofen and mexidol groups, it was 73.3% ($p_2 < 0.001$) and 26.7% lower ($p_3 = 0.344$), respectively. The MDA values in plasma and erythrocytes during spontaneous oxidation in the LHT-2-20 group were 6.2 ± 0.6 and 9.6 ± 0.6 μmol / l, which is 32.6% ($p_1 = 0.46$) and 28.1% ($p_1 = 0.002$) lower than the control values, respectively.

Fe-MDA levels in plasma and erythrocytes were 37.6% ($p_1 < 0.001$) and 29.5% lower ($p_1 = 0.124$) than the control values, respectively. Compared to i.g. administration of ketoprofen and mexidol, plasma MDA in the group receiving the new compound was 42.9% ($p_2 < 0.001$) and 8.8% ($p_3 = 0.417$) lower, plasma Fe-MDA was 55.6% ($p_2 < 0.001$) and 22.7% ($p_3 < 0.001$) lower, MDA in erythrocytes was 34% ($p_2 < 0.001$) and 17.5% ($p_3 = 0.328$) lower, and Fe-MDA in erythrocytes was 40.4% ($p_2 < 0.001$) and 16.8% ($p_3 < 0.001$) lower, respectively. Moreover, on day 25 of the experiment, more effective restoration of the antioxidant potential was revealed in the LHT-2-20 group: CAT activity in plasma was 0.89 ± 0.06, which is 23.9% ($p_2 < 0.001$) and 10.3% ($p_3 = 0.035$) higher than the same parameter in the ketoprofen and mexidol groups, respectively.

CAT in erythrocytes was 16.8% ($p_2 < 0.001$) and 7.9% higher ($p_3 = 0.026$), and SOD activity in plasma was 39.7% ($p_2 < 0.001$) and 22.1% higher ($p_3 < 0.001$), compared to groups 3 and 4, respectively. When conducting a biochemiluminescence study of plasma with the use of the new compound, the I_{max} and S values were 27.2% ($p_1 < 0.001$) and 37.2% lower ($p_1 < 0.001$), compared to the control values, respectively. The I_{max} value was 31.9% ($p_2 < 0.001$) and 6.0% lower ($p_3 = 0.596$), and S was 35.5% ($p_2 < 0.001$) and 10.2% lower ($p_3 = 0.001$), compared to the ketoprofen and mexidol groups, respectively (Table 2).

Table 2

Effect of LHT-2-20 on free radical processes in experimental periodontitis, $M \pm \sigma$

Parameter	Group 1 ($n = 15$)	Experimental groups			
		Group 2 ($n = 30$)	Group 3 ($n = 30$)	Group 4 ($n = 30$)	Group 5 ($n = 32$)
CD in plasma, U / ml	0.15 ± 0.02	0.34 ± 0.05*	0.29 ± 0.05	0.22 ± 0.04*^	0.18 ± 0.04*^A
Plasma MDA, mmol / l	4.89 ± 0.21	9.12 ± 0.71	8.25 ± 0.54*	6.58 ± 0.52*^	6.15 ± 0.53*^A
Fe-MDA in plasma, mmol / l	9.56 ±0.44	19.80 ± 1.52	17.68 ± 0.69*^	14.53 ± 0.63*^	12.36 ± 0.51*^AB
MDA in erythrocytes, mmol / l	8.05 ± 0.49	13.36 ± 0.71*	12.34 ± 0.88*	10.18 ± 0.59*^	9.60 ± 0.58*^A
Fe-MDA in erythrocytes, mmol / l	17.49 ± 1.42	30.26 ± 1.01	28.41 ± 0.86*^	24.28 ± 0.87*^	21.33 ± 0.82*^AB
CAT in plasma, kcat / s·l	1.17 ± 0.13	0.51 ± 0.08*	0.62 ± 0.07*	0.77 ± 0.08*^	0.89 ± 0.06*^A
CAT in erythrocytes, mkcat / s·l	2.27 ± 0.13	1.29 ± 0.11*	1.59 ± 0.13*^	1.79 ± 0.10*^	1.97 ± 0.14*^A
SOD in plasma, AU	1.31 ± 0.12	0.52 ± 0.08*	0.65 ± 0.08*	0.88 ± 0.07*^A	1.17 ± 0.06^AB
I_{max} in plasma, mV / sec	1.85 ± 0.14	3.53 ± 0.23*	3.16 ± 0.17*^	2.68 ± 0.17*^	2.57 ± 0.14*^A
S in plasma, mV / sec	26.77 ± 1.06	48.80 ± 1.76*	40.18 ± 1.73*^	33.39 ± 1.29*^	30.66 ± 1.63*^A

* – significance compared to reference values ($p < 0.001$); ^ – significance compared to control values ($p_1 < 0.001$); [A] – significance compared to the values of the ketoprofen group ($p_2 < 0.001$); [B] – significance compared to the values of the mexidol group ($p_3 < 0.001$) (one-way analysis of variance (ANOVA), Tukey's test).

When assessing the state of the oral cavity in the laboratory animals of the control group, pronounced gingival hyperemia was determined. Heavy bleeding, manifested immediately after probing (3 points), as well as an increase in tooth mobility up to grade 2 was detected in 30 rats of the control group (100%). When determining the depth of periodontal pockets, we noted their increase by 1.3 ± 0.2 mm ($p < 0.001$). These changes indicate the existing inflammatory and degenerative changes in periodontal tissues leading to the formation of pathological tooth mobility and tooth loss.

By the end of the study, the examination of the oral cavity in the ketoprofen and groups revealed a decrease in the severity of local signs of inflammation, which was manifested by a decrease in gingival edema and hyperemia. The determined depth of periodontal sulcus probing was less than the control values in the context of ketoprofen administration (by 40% ($p_1 < 0.001$)) and intragastric mexidol administration (by 32.1% ($p_1 < 0.001$)). When assessing bleeding on a 3-point scale, the following dynamics was determined in the ketoprofen group: 1 rat (3.3%) scored 1 point, 21 rats (70%) – 2 points, and 8 rats (26.7%) – 3 points. In the mexidol group, 1 rat (3.3%) scored 1 point, 18 rats (60%) – 2 points, and 11 rats (36.7%) – 3 points. We also assessed the grade of pathological tooth mobility during ketoprofen treatment and revealed that 6 rats (20%) had grade 1, and 24 rats (80%) had grade 2 mobility. During mexidol therapy, 14 rats (46.7%) and 16 rats (53.3%) had grades 1 and 2, respectively. The results obtained indicate a decrease in the percentage of animals with bleeding gums of 2–3 points and grade 1–2 pathological tooth mobility. However, despite the positive dynamics, these changes indicate insufficient effectiveness of the studied compounds in experimental periodontitis.

In the LHT-2-20 group, a pronounced decrease in the intensity of inflammatory changes was determined as evidenced by the absence of gingival hyperemia. Using a button probe, we determined a decrease in the severity of hemorrhagic manifestations. We detected 1 and 2 points in 24 rats (75%) and 8 rats (25%), respectively; animals with intense bleeding gums (3 points) were not detected. When assessing the severity of damage to the tooth-supporting apparatus by the degree of pathological toot mobility, grade 0 was determined in 12 rats (37.6%), grade 1 – in 20 rats (62.4%), and grade 2 was not diagnosed in the studied groups of rats. Assessing the depth of the periodontal pocket by probing revealed that it was 67.2% less ($p_1 < 0.001$) compared to the control group (0.4 ± 0.01 mm), which is 27.8% ($p_2 < 0.001$) and 35.7% ($p_3 < 0.001$) less compared to the ketoprofen and mexidol groups, respectively (Table 3).

Table 3

The effect of LHT-2-20 on the parameters of the local state of periodontium in experimental periodontitis

Parameter	Group 1 (n = 15)	Group 2 (n = 30)	Group 3 (n = 30)	Group 4 (n = 30)	Group 5 (n = 32)
Depth of periodontal pockets, mm, $M \pm \sigma$	0.3 ± .1	1.3 ± 0.2*	0.8 ± 0.02*^	0.9 ± 0.02*^^A	0.4 ± 0.01*^AB
Bleeding gums, points, n (%):					
1	15 (100%)	0 (0%)*	1 (3.3%)*	1 (3.3%)*	24 (75%)^ AB
2	0 (0%)	0 (0%)	21 (70%)*^	18 (60%)*^	8 (25%)*^ AB
3	0 (0%)	30 (100%) *	8 (26.7%)*^	11 (36.7%)*^	0 (0%) ^ AB
Tooth mobility, grade, n (%):					
0	15 (100%)	0 (0%)*	0 (0%)*	0 (0%)	12 (37.6%) *^ AB
1	0 (0%)	0 (0%)	6 (20%)*^	14 (46.7%)^ A	20 (62.4%)*^ AB
2	0 (0%)	30 (100%)*	24 (80%)*^	16 (53.3%)^ A	0 (0%) ^ AB

* – significance compared to the reference values ($p < 0.001$); ^ – significance compared to the control values ($p_1 < 0.001$); A – significance compared to the values in the ketoprofen group ($p_2 < 0.001$); B – significance compared to the values in the mexidol group ($p_3 < 0.001$) (one-way analysis of variance (ANOVA), Tukey's test, Fisher's exact test).

The correlation analysis revealed a positive correlation between the parameters of free radical oxidation and the data on the local status of periodontal tissues. A strong correlation was revealed between CD, MDA, and Imax biochemiluminescence values in plasma and parameters of gum bleeding, depth of periodontal pockets, and grade of tooth mobility. A very strong correlation was detected between the values of Fe-MDA and biochemiluminescence S in plasma

and the grade of tooth mobility. The assessment of the relationship between the levels of the main antioxidant enzymes and the parameters of the periodontal tissue revealed a negative correlation. Thus, a strong correlation was revealed between the levels of CAT and SOD in plasma and data on local periodontal tissue status, except for the SOD value and the grade of tooth mobility, whose correlation was very strong, thereby indicating the interrelation between the studied parameters (Table 4).

Table 4

Correlation between some parameters of free radical oxidation, the antioxidant system, and local periodontal tissue status in experimental periodontitis in rats

Parameter	Spearman's rank correlation coefficient						
	Plasma MDA, mmol / l	Fe-MDA in plasma, mmol / l	CD in plasma, U / ml	CAT in plasma, kcat / s·l	SOD in plasma, AU	I max in plasma, mV / sec	S in plasma, mV / sec
Bleeding gum, points	0.71	0.83	0.67	–0.74	–0.73	0.75	0.88
Depth of periodontal pockets, mm	0.76	0.87	0.73	–0.82	–0.88	0.77	0.89
Tooth mobility, grade	0.85	0.94	0.73	–0.84	–0.95	0.84	0.93

DISCUSSION

As a result of the study, it was found that free radical oxidation processes play an important role in the pathogenesis of experimental periodontitis, contributing to the formation of local inflammatory changes in periodontal tissues.

During the experiment, we observed enhanced free radical oxidation determined by biochemiluminescence data and the level of lipid peroxidation products in plasma and red blood cells, associated with a strong correlation with the parameters of the local status of periodontal tissues. Oxidative stress and the inflammatory response at the local and systemic levels are interconnected and involved in periodontal tissue damage during the development of periodontitis.

The course of ketoprofen therapy did not allow to inhibit free radical oxidation at the systemic level, as evidenced by the preservation of a high level of lipid peroxidation parameters compared to reference values. At the same time, the treatment course limited the inflammatory process in periodontal tissues, which was confirmed by the trend toward decreased values of the corresponding parameters by the end of the experiment, and improved the local state of periodontal tissues.

During mexidol therapy, more effective inhibition of free radical damage mechanisms was determined compared to the control group. However, no significant differences were observed compared to the ketoprofen group.

The use of a new complex compound 2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate led to the inhibition of free radical processes and activation of the antioxidant system, contributing to the normalization of the studied parameters to the values in the intact animals. Along with correction of free radical oxidation processes, we observed elimination of the local inflammatory response and a decrease in the destructive processes in periodontal tissues.

CONCLUSION

In experimental periodontitis, an increase in the activity of free radical oxidation processes was determined with a simultaneous decrease in the antioxidant enzyme potential in erythrocytes and blood plasma. The data obtained indicate the development of oxidative stress along with an increasing local and systemic inflammatory response which promotes destructive processes in the connective tissue matrix of the periodontium and contributes to the progression of the disease.

In the rats with experimental periodontitis, the new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) administered intragastrically at a dose of 11.54 mg / kg per day for 10 days led to a significant decrease in the levels of lipid peroxidation parameters and a decrease in the severity of local inflammatory changes in periodontal tissues by the end of the experiment. The results obtained confirm the presence of anti-inflammatory and antioxidant activity of the compound and also justify the relevance of its further study to create a drug for

the complex therapy of inflammatory periodontal diseases.

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Authors' contribution

Poryadin G.V. – conception and design, analysis and interpretation of the data, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Zakhvatov A.N., Skachilova S.Ya., Yasnetsov V.V. – conception and design, analysis and interpretation of the data, drafting and editing of the manuscript, final approval of the manuscript for publication. Khaydar D.A. – drafting and editing of the manuscript, final approval of the manuscript for publication. Tarasova T.V., Zakharkin I.A., Parshina A.Yu., Simakina E.A. – analysis and interpretation of the data, drafting and editing of the manuscript, final approval of the manuscript for publication.

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Beta-adrenergic reactivity of erythrocyte membranes in adolescents with supraventricular and ventricular arrhythmias before and after radiofrequency ablation

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ABSTRACT

Aim. To evaluate β-adrenergic reactivity of erythrocyte membranes (β-ARM) in adolescents with ventricular and supraventricular arrhythmias before and after radiofrequency ablation (RFA) of heart rhythm disturbances.

Materials and methods. The study included 49 adolescents aged 11–17 years, of which 15 had Wolff – Parkinson – White pattern (WPW), 13 – WPW syndrome, 10 – atrioventricular nodal reentry tachycardia (AVNRT), and 11 – ventricular arrhythmia (VA). The control group consisted of 11 adolescents without cardiovascular pathology. All patients received surgical treatment for heart rhythm disturbances (HRD) using RFA. In patients with HRD, β-ARM was determined by a set of reagents BETA-ARM AGAT (AGAT LLC, Russia) before RFA and 3 days after it. In the control group, the parameter was determined at the stage of inclusion in the study.

Results. In adolescents with supraventricular arrhythmias, median values of β-ARM did not differ significantly from the control group. RFA in adolescents in these groups did not affect the value of β-ARM on day 3 after the surgery. In adolescents with VA, the median value of β-ARM was initially higher than in the control group ($p = 0.026$). On day 3 after RFA, an increase in β-ARM was noted in this group ($p = 0.028$) compared to baseline values.

Conclusion. Activation of the sympathetic nervous system plays a significant role in the pathogenesis of VA in adolescence. The study showed the possibility of using β-ARM to assess the state of the sympathetic nervous system in patients with methodological limitations in analyzing heart rate variability.

Keywords: radiofrequency ablation, heart rhythm disturbance, adolescents, β-adrenergic reactivity of erythrocyte membranes

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

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Conformity with the principles of ethics. An informed consent was signed by parents of the patients. The study protocol was approved by the Bioethics Committee at Cardiology Research Institute of Tomsk NRMC (Protocol No. 208 of 20.01.2021).

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Адренореактивность мембран эритроцитов у подростков с суправентрикулярными и желудочковыми аритмиями до и после радиочастотной аблации

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РЕЗЮМЕ

Цель. Оценить показатель β-адренореактивности мембран эритроцитов (β-АРМ) у подростков с суправентрикулярными и желудочковыми аритмиями до и после выполнения радиочастотной коррекции нарушения ритма.

Материалы и методы. В исследование включено 49 подростков от 11 до 17 лет, из них 15 с феноменом Вольфа – Паркинсона – Уайта (ВПВ), 13 с синдромом ВПВ, 10 с атриовентрикулярной узловой реципрокной тахикардией и 11 с желудочковой аритмией (ЖА). Группу контроля составили 11 подростков, не имеющих патологии сердечно-сосудистой системы. Всем пациентам проведено оперативное лечение нарушения ритма сердца (НРС) методом радиочастотной аблации (РЧА). Пациентам с НРС определение β-АРМ эритроцитов с использованием набора реагентов БЕТА-АРМ АГАТ (ООО «АГАТ», Россия) выполняли перед проведением РЧА и через 3 сут после нее. В контрольной группе показатель определяли на этапе включения в исследование.

Результаты. У подростков в группах с суправентрикулярными аритмиями показатели β-АРМ значимо не отличались от группы контроля. Проведение РЧА у подростков этих групп не повлияло на величину показателя β-АРМ эритроцитов на 3-и сут после оперативного вмешательства. У подростков с ЖА показатель β-АРМ исходно превышал значение в группе контроля (p = 0,026). На 3-и сутки после РЧА в этой группе отмечено увеличение β-АРМ эритроцитов (p = 0,028) относительно исходных значений в группе.

Заключение. Активация симпатического отдела вегетативной нервной системы занимает существенное место в патогенезе желудочковых аритмий в подростковом возрасте. Выполненное исследование показало возможность использования показателя β-АРМ эритроцитов для оценки состояния симпатической нервной системы у категории пациентов с методическими ограничениями выполнения анализа вариабельности сердечного ритма.

Ключевые слова: радиочастотная аблация, нарушение ритма, подростки, β-адренореактивность мембран эритроцитов

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

Источник финансирования. Исследование проведено в рамках темы фундаментальных научных исследований № 122020300183-4.

Соответствие принципам этики. Информированное согласие на включение ребенка в исследование было подписано родителями. Протокол исследования одобрен комитетом по биомедицинской этике НИИ кардиологии Томского НИМЦ (протокол № 208 от 20.01.2021).

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INTRODUCTION

Heart rhythm disturbances (HRD) are some of the most common (60–70%) cardiovascular pathologies in children and adolescents. In recent years, an increase in the total number of various types of HRD in pediatric population has been noted [1–3]. It may be caused, on the one hand, by an improvement in the diagnosis and, on the other hand, by long asymptomatic development of arrhythmia in children and untimely referral to specialists. In children, several developmental periods are distinguished which are characterized by the

highest risk of developing arrhythmia: the neonatal period; 4–5 years of age; 7–8 years of age; 12–13 years of age [4, 5]. In adolescence, the most common HRD is pacemaker migration (13.5%). Other forms are much less common: bradycardia (3.5%), atrial tachycardia (2.7%), extrasystole (1.9%), Wolff – Parkinson – White (WPW) pattern and first-degree atrioventricular block (AVB) (0.5% each), and long QT interval (0.3%) [3].

The limited effect of existing drug therapy for the control of supraventricular tachycardia (SVT) in children and adolescents is recognized [6]. It is noted that the impossibility of treating such HRDs as WPW syndrome, atrioventricular nodal reentry tachycardia (AVNRT), and ventricular arrhythmia (VA) with drugs in school-age children can cause the development of life-threatening arrhythmias, arrhythmogenic cardiomyopathy, and even death [7]. In these circumstances, radiofrequency ablation (RFA) is becoming the method of choice in the treatment of drug-refractory HRD in children [8].

A distinctive feature of the heart is an autonomic nervous system (ANS). At the body level, it controls interaction with the conducting system of the heart [9]. In this regulation, special importance is attributed to maintaining the balance between the sympathetic and parasympathetic divisions of ANS, as well as to the sensitivity of β -adrenergic receptors [9]. The dominance of the sympathetic division results in life-threatening HRDs and is considered as an independent risk factor for a lethal outcome [10]. This circumstance indicates the relevance of a timely assessment of the sympathetic division of the ANS in children with congenital heart disease (CHD), including after treatment of these disorders by RFA. A promising approach that allows for monitoring of the state of the sympathetic division of the ANS, including in the presence of life-threatening CHD, is β-adrenergic reactivity of erythrocyte membranes (β-ARM) [11, 12].

The aim of the study was to assess β-ARM in adolescents with ventricular and supraventricular arrhythmias before and after radiofrequency ablation (RFA) of HRD.

MATERIALS AND METHODS

The study included 49 children aged 11–17 years. VA was detected in 11 patients. WPW pattern was detected in 15 patients, WPW syndrome and AVNRT were detected in 13 and 10 patients, respectively. All patients were receiving planned treatment at the Department of Pediatric Cardiology of Cardiology Research Institute of Tomsk NRMC. The control group consisted of 11 age-matched children who did not have cardiovascular pathology.

The study was carried out in compliance with the ethical principles of the Declaration of Helsinki ("Ethical principles of research involving humans" as amended in 2000) and the Rules of Clinical Practice in the Russian Federation approved by the Order of the Healthcare Ministry of Russia No. 266 of 19.06.2003. The study was approved by the Bioethics Committee at Cardiology Research Institute of Tomsk NRMC (Protocol No. 208 of 20.01.2021).

The inclusion criteria were the following: absence of congenital heart defect, presence of persistent paroxysmal tachycardia, sustained tachycardia, permanent tachycardia, frequent supraventricular extrasystoles (SVE) accounting for more than 15% of the total number of heartbeats per day, frequent ventricular extrasystoles (VES) constituting more than 15% of the total number of heartbeats per day, absence of acute infections and exacerbations of chronic diseases, absence of laboratory signs of myocarditis, a signed informed consent.

The exclusion criteria were the presence of congenital heart defects, acute infections and exacerbations of chronic diseases, laboratory signs of myocarditis and primary electrical heart diseases. When determining the indications for RFA, national guidelines and guidelines of the American and European associations of arrhythmologists and pediatric cardiologists were used [13, 14].

Upon admission, all patients underwent clinical examinations, including taking a history and complaints, an objective examination of a child, 12 lead electrocardiography (ECG), Holter ECG monitoring (HM ECG), echocardiography (echo). In groups of adolescents with arrhythmias, ECG, HM ECG, and echo were repeated 3 days after RFA. Based on the results of HM ECG, the following parameters were assessed: maximum, minimum, and daily average heart rate (HR) during the day, the total number of SVE and VES.

Patients with HRD had their blood sampled before RFA and 3 days after it to determine β-ARM. In the control group, blood samples were taken once at the stage of inclusion in the study.

Beta-ARM in the blood samples was determined using the beta-ARM AGAT reagent kit (AGAT LLC, Russia). The method is based on increasing the osmotic stability of erythrocytes (inhibition of hemolysis) in hypo-osmotic buffer in the presence of the β-blocker 1-(1-isopropylamino)-3-(1-naphtyloxy)-2-propanol hydrochloride.

The degree of hemolysis inhibition, expressed as a percentage, was determined by the ratio of the optical density of the supernatant in the sample with the addition of the β -blocker to the incubation medium (test sample) to the optical density of the supernatant in the sample without the addition of the β-blocker to the incubation medium (control sample). Hemolysis inhibition percentage was taken as conventional units (conv. units) of β-ARM. The reference values were the β-ARM values recommended by the manufacturer within the range from 2 to 20 conv. units. At the same time, the β-ARM values over 20 conv. units indicated an increase in the degree of erythrocyte hemolysis and a decrease in β-ARM following desensitization of β-ARM in response to a persistent increase in the activity of the sympathoadrenal system.

Statistical processing of the obtained data was performed using the STATISTICA 10 software. Qualitative variables were presented as absolute and relative values *n* (%). The difference in frequencies in independent groups of patients was determined using the Pearson's $\chi 2$ test. Quantitative variables were checked for normality of distribution using the Shapiro – Wilk test. Quantitative data were presented as the median and the interquartile range (*Me* [Q_1; Q_3]). Given non-normal distribution of quantitative variables, the statistical significance of differences in three or more independent groups was assessed using the Kruskal – Wallis test. If statistically significant differences between the groups were detected, the post-hoc test was applied. Comparison of dependent data in individual groups of diseases was performed using the Wilcoxon test. The differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Table 1 presents the clinical and demographic characteristics of the sample with account of specific features of HRD. The formed groups had no significant differences in age, gender, body weight, and height. The history of HRD in all groups lasted from 1 to 2 years. Processing the HM ECG results revealed no significant differences in the daily average, minimum, and maximum HR between the studied groups of adolescents with HRD and the controls. In patients with supraventricular arrhythmia, single SVE and VES were recorded. For patients with VA, the presence of a large number of VES in the context of single SVE was noted.

Echo did not show any significant differences in cardiac parameters between the patients with HRD and healthy adolescents in left ventricular end-diastolic volume (EDV), left ventricular ejection fraction (LVEF), and left and right atrial volumes (LAV, RAV).

The results obtained in determining β-ARM are presented in Table 2. At the stage of inclusion in the study, the adolescents appeared to have similar values for the WPW pattern, WPW syndrome, and AVNRT that did not differ significantly from the values in the control group. In contrast, high baseline β-ARM values were found in the group of adolescents with VA. In this group of patients, β-ARM significantly exceeded the value in the control group ($p = 0.026$). in the meantime, no significant differences were found between β-ARM values in the groups with supraventricular arrhythmia and VA.

RFA in adolescents with WPW syndrome, WPW pattern, and AVNRT had virtually no effect on β-ARM on day 3 after the procedure (Table 2). On the contrary, in the group of children with VA, RFA resulted in an increase in β-ARM. Therefore, on day 3 after RFA, the parameter value increased significantly ($p = 0.028$).

DISCUSSION

Emergence and development of HRD in children and adolescents in most cases are not associated with organic changes in the heart, which complicates the determination of their etiology and pathogenesis. The functioning of the cardiac conducting system is regulated by ANS. The method for studying heart rate variability which allows to assess the activity of the sympathetic and parasympathetic divisions of ANS is widely used in clinical practice. At the same time, application of this method is limited in patients with ventricular ectopic beats.

Currently, the presence of α-, β1-, and β2-adrenergic receptors on erythrocyte membranes has been proven [15, 16].

Table 1

Clinical and laboratory characteristics of patients

Parameter	Groups of patients					*p*
	WPW		AVNRT	VA	Controls	
	pattern	syndrome				
Total number of patients, *n*	28		10	11	11	
	15	13				
Men, *n* (%)	11 (73.3)	8 (61.5)	2 (20.0)	6 (54.5)	6 (50.0)	0.200
Women, *n* (%)	4 (26.7)	5 (38.5)	8 (80.0)	5 (45.5)	6 (50.0)	
Age, years, *Me* [Q_1; Q_3]	13 [10; 15]	14 [10; 14]	14.5 [13; 15]	13 [11; 15]	14 [12; 16]	0.754
Body weight, kg, *Me* [Q_1; Q_3]	52 [38; 60]	51 [39; 60]	56 [40; 63]	54 [33; 61]	58 [43; 71]	0.880
Height, cm, *Me* [Q_1; Q_3]	164 [140; 172]	165 [149; 172]	160 [156; 172]	160 [144; 167]	170 [151; 179]	0.997
BMI, kg / m^2, *Me* [Q_1; Q_3]	19.7 [16.7; 22.1]	19.4 [16.6; 20.3]	19.7 [16.3; 23.0]	19.2 [15.4; 23.7]	20.6 [17.9; 21.6]	0.991
Functional class (NYHA) I–IV, *n* (%)	I, 15 (100.0)	I, 13 (100.0)	I, 10 (100.0)	I, 11 (100.0)	–	-
Age of HRD detection, years, *Me* [Q_1; Q_3]	12 [7; 13]	8[6; 12]	13[10; 15]	11 [11; 13]	–	0.119
History of HRD, years, *Me* [Q_1; Q_3]	1 [0; 2]	2 [1; 6]	1 [0; 1.75]	1 [1; 2]	–	0.875
Drugs taken, *n* (%) *	1 (6.66)	2 (15.38)	1 (10.00)	2 (18.18)	–	0.815
HM ECG findings						
Daily average HR, beats / min,*Me* [Q_1; Q_3]	77 [75; 90]	82 [69;87]	84 [75; 87]	81 [75; 93]	74 [69; 81]	0.938
Minimum HR, beats / min, *Me* [Q_1; Q_3]	50 [48; 54]	50 [44; 54]	51 [47; 54]	51 [45; 54]	42 [40; 53]	0.564
Maximum HR, beats / min, *Me* [Q_1; Q_3]	151 [147;165]	152 [140; 163]	161 [153;165]	158 [157; 163]	153 [129; 178]	0.461
Total number of SVE at baseline	single	single	single	single	–	–
Total number of VES at baseline, *Me* [Q_1; Q_3]	single	single	single	18,395.0 [12,066.0; 36,871.0]	–	–
Features of QRS morphology during HM ECG	Deformation due to permanent / intermittent preexitation	Deformation due to permanent / intermittent preexitation	–	–	–	–
ECG findings						
EDV, ml, %**, *Me* [Q_1; Q_3]	77 [57; 89], 103 [96; 105]	78 [62; 90], 101 [97; 109]	79[69; 94], 100 [94; 104]	75 [51; 96], 104 [91; 115]	94 [67; 99], 103 [96; 109]	0.816 0.847
LVEF (b), %,*Me* [Q_1; Q_3]	65 [63; 67]	65 [63; 67]	63,5 [63; 65]	63 [62; 64]	66, [66; 68]	0.375
LAV, ml, %**, *Me* [Q_1; Q_3]	33 [19; 38], 102 [96; 118]	33 [25; 42], 101 [93; 102]	37 [28; 41], 105 [99; 108]	33 [21; 380], 99,1 [94; 109]	38 [22; 39], 98 [93; 106]	0.943 0.457
RAV, ml, %**, *Me* [Q_1; Q_3]	30 [21; 34], 105 [100; 117]	31 [19; 39], 103 [102; 117]	34 [28; 40], 112 [107; 119]	31 [21; 40], 108 [95; 119]	29 [19; 41], 105 [103; 113]	0.949 0.722

* drugs taken at the time of hospitalization, including the ones to treat the underlying disease; ** percentage from the individual predicted norm.

Table 2

Beta-ARM values in adolescents with VA and supraventricular arrhythmia before and after RFA, conv. units, *Me* [Q_1; Q_3]

Parameter	Control group (healthy children), *n* = 11	WPW pattern, *n* = 15	WPW syndrome, *n* = 13	AVNRT, *n* = 10	VA, *n* = 11
β-ARM before RFA	13.1 [8.8; 16.5]	17.6 [13.3; 22.6] p_1 = 0.192	16.1 [11.5; 18.2] p_1 = 0.600	15.5 [12.9; 27.7] p_1 = 0.465	19.01 [14.3; 21.7] p_1 = 0.026
β-ARM after RFA	–	18.6 [13.2; 22.8] p_2 = 0.886	16.2 [9.7; 17.9] p_2 = 0.661	14.6 [10.1; 30.8] p_2 =1.000	29.6 [23.2; 31.7] p_2 = 0.028

Note. Statistical significance of differences in the value of the parameter between the pre-RFA group and the control group – p_1, statistical significance of differences in the value of the parameter in the group before and after RFA – p_2.

This fact allows to think that β-ARM can reflect β-adrenergic reactivity of the body as a whole. It has been convincingly shown that an increase in the content of catecholamines in the blood is accompanied by desensitization of β-adrenergic receptors and a decrease in the response from regulated organs to the stimulating effect of neurohormones [11, 17, 18]. The information value of β-ARM in the treatment of HRD in adult patients is being studied [11, 12]. At the same time, we have not found any works devoted to the study of β-ARM in HRD in adolescents, including after RFA.

At the time of inclusion in the study, the formed groups were comparable in anthropometric parameters, age of HRD detection, and duration of HRD history. ECG parameters also did not differ significantly between the groups. With the considered duration of HRD history, the presence of supraventricular arrhythmia was not accompanied by a change in β-ARM compared to the controls. In adolescents with VA, the β-ARM value was significantly higher than in the control group.

Conducting RFA in groups of adolescents with supraventricular arrhythmia did not lead to changes in β-ARM. At the same time, surgical treatment of VA was accompanied by a significant increase in β-ARM on day 3 after surgery compared to baseline values in the group. An increase in β-ARM indicates desensitization of β-adrenergic reactivity in response to an increased stimulating effect of ANS mediators. The obtained results allow to consider the role of the sympathetic nervous system in the etiology and pathogenesis of VA and an increase in its functional stress in the early postoperative period.

In children with supraventricular arrhythmia, the morphofunctional arrhythmia substrate is presented by anomalies in the embryonic development of the cardiac conducting system. Therefore, the emergence of an arrhythmogenic focus in the atria in the first 6 months after birth may be associated with its embryonic origin. At the same time, a number of researchers note that it is possible to suppose an association between rhythm disturbances and features of postnatal development of the cardiac conducting system even in the absence of obvious congenital anomalies. [19]. Studying the electrophysiological characteristics of the atrioventricular node and accessory pathways during transesophageal electrocardiography and cardiac pacing in groups of adolescents with WPW syndrome or pattern made it possible to establish a high degree of vagotonia in adolescents with WPW pattern [20]. This serves as an indirect confirmation of the results of our study on the absence of tension in the sympathetic division of ANS and desensitization of β-ARM in adolescents with supraventricular arrhythmias before and after RFA.

CONCLUSION

For adolescents with VA, a significant increase in β-ARM was revealed before RFA, which indicates tension in the sympathetic division of ANS. A significant increase in β-ARM in the early postoperative period indicates a further increase in the activity of the sympathetic division, which is accompanied by further desensitization of β-ARM. The sympathetic division of ANS plays an essential role in the pathogenesis of VA in adolescents.

Supraventricular arrhythmias (WPW syndrome, WPW pattern, AVNRT) that developed in adolescence were not accompanied by a significant increase in β-ARM compared to the healthy controls. RFA in this category of patients did not result in significant changes in β-ARM.

The study showed the possibility of using β-ARM to assess the state of the sympathetic nervous system in patients with methodological limitations in the analysis of heart rate variability.

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Rebrova T.Yu. – analysis and interpretation of the data, drafting of the article. Perevoznikova Yu.E. – collection and processing of clinical material, statistical analysis of the data. Svintsova L.I. – design of the study, critical revision of the manuscript for important intellectual content. Muslimova E.F. – processing of the biological material, analysis and interpretation of the data. Afanasiev S.A. – conception and design, final approval of the manuscript for publication. Dzhaffarova O.Yu. – selection and recruitment of patients, obtainment of informed consents, collection and processing of the clinical material.

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A decision rule for identifying patients at high risk for impaired lung diffusion capacity after COVID-19

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ABSTRACT

Aim. To elaborate a decision rule for identifying the main predictors of impaired lung diffusion capacity after COVID-19.

Materials and methods. The retrospective study included 341 patients without underlying lung diseases (median age 48 years) who experienced COVID-19 with bilateral pneumonia. The median extent of parenchymal lesion in the acute phase of COVID-19 (CT_{max}) was 50%. Spirometry, body plethysmography, and lung diffusion capacity for carbon monoxide (DLCO) test were performed. The data were analyzed by descriptive statistics, correlation analysis, one-dimensional logistic regression analysis with an assessment of odds ratios (OR), and multivariate logistic regression analysis. Receiver operating characteristic (ROC) analysis was used to assess the quality of the binary classifier model.

Results. The initial model for predicting reduced DLCO (< 80% of predicted) included the following predictors: CT_{max}, time interval from the COVID-19 onset, gender, age, body mass index. Backward stepwise regression was applied, and a binary classifier model that includes CT_{max} was obtained. The sensitivity and specificity of the model for the training sample were 80 and 67%, respectively, for the test sample – 79 and 70%, respectively. The analysis of OR showed that OR > 1 was observed at CT_{max} > 40%.

Conclusion. The decision rule was obtained for predicting impaired lung diffusion capacity after COVID-19 with virus-associated lung damage in patients without underlying bronchopulmonary diseases. Patients with CT_{max} > 40% require more thorough clinical follow-up with DLCO monitoring after the acute phase of COVID-19.

Keywords: impaired lung diffusion capacity, pulmonary function tests, binary classifier model, COVID-19

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Решающее правило для выявления пациентов с высоким риском нарушения диффузионной способности легких после перенесенного COVID-19

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РЕЗЮМЕ

Цель – построение решающего правила для определения наиболее важных предикторов нарушения диффузионной способности легких после перенесенного COVID-19 (Coronavirus disease 2019) с вирус-ассоциированным поражением легких.

Материалы и методы. В ретроспективное исследование включен 341 пациент без бронхолегочной патологии в анамнезе (медиана возраста 48 лет) после перенесенного COVID-19 с вирус-ассоциированным поражением легких. Медиана объема поражения легочной ткани в острый период COVID-19 ($КТ_{макс}$) в общей группе составила 50%. Выполнены спирометрия, бодиплетизмография, диффузионный тест (измерение трансфер-фактора монооксида углерода, DLco). Анализ данных проведен с помощью описательной статистики, корреляционного анализа, одномерного логистического регрессионного анализа с оценкой отношений шансов (ОШ) и многофакторного логистического регрессионного анализа. Для оценки качества модели бинарного классификатора использовался ROC-анализ (receiver operating characteristic analysis).

Результаты. В многофакторный логистический регрессионный анализ снижения DLco (<80% от должного значения) изначально были включены следующие предикторы: $КТ_{макс}$, временной интервал от начала COVID-19, пол, возраст, индекс массы тела. С помощью логистического регрессионного анализа с последовательным исключением наименее значимых предикторов получена модель бинарного классификатора, единственным значимым предиктором в которой стал показатель $КТ_{макс}$. Чувствительность и специфичность полученной модели на обучающей выборке составили 80 и 67% соответственно, на тестовой выборке – 79 и 70% соответственно. Анализ ОШ для полученной модели бинарного классификатора показал, что ОШ > 1 наблюдается при $КТ_{макс}$ > 40%.

Заключение. Получено решающее правило для прогнозирования снижения показателя DLco после перенесенного COVID-19 с вирус-ассоциированным поражением легких у пациентов без бронхолегочной патологии в анамнезе. Показано, что пациентам с $КТ_{макс}$ > 40% требуется более тщательное клиническое наблюдение с обязательным контролем показателя DLco после окончания острой фазы COVID-19.

Ключевые слова: нарушение диффузионной способности легких, легочные функциональные методы исследования, модель бинарного классификатора, COVID-19

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 участники исследования подписали информированное согласие. Протокол исследования одобрен независимым этическим комитетом ГВКГ им. акад. Н.Н. Бурденко (протокол № 04-22 от 20.04.2022).

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INTRODUCTION

The problem of functional disorders caused by novel coronavirus infection remains relevant in the present time. The results of previous studies showed that the most common functional disorder of the bronchopulmonary system is impaired lung diffusion capacity [1–5] following diffuse alveolar damage and pulmonary embolism [6–8]. Follow-up of COVID-19 survivors has shown that it is relevant to identify patients at high risk for imparted lung diffusion capacity in the post-COVID phase, especially with account of current restrictions on performing lung function tests during the COVID-19 pandemic [9].

The aim of the study was to elaborate a decision rule for identifying the main predictors of impaired lung diffusion capacity after COVID-19 with virus-associated lung damage.

MATERIALS AND METHODS

A retrospective study included 341 patients who experienced COVID-19 with bilateral pneumonia. We analyzed demographic data, the maximum extent of parenchymal lesion in the acute phase of COVID-19 according to high-resolution computed tomography of the chest (CT_{max}), and the time interval from the COVID-19 onset (Table 1). Lung function tests included spirometry, body plethysmography, and lung diffusion capacity for carbon monoxide (DLCO) test. The analyzed pulmonary parameters included forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), slow vital capacity (VC), the FEV_1 / VC ratio, total lung capacity (TLC), and lung diffusion capacity for carbon monoxide adjusted for the hemoglobin level (DLCO) (Table 2). Lung function tests were performed in 64.8% (221/341) of patients within 90 days, in 23.5% (80/341) of patients within 90–180 days, and in 11.7% (40/341) of patients within more than 180 days from the COVID-19 onset.

Table 1

Characteristics of the study group, *n* = 341

Parameter	Value
Gender (men), *n* (%)	262 (76.8)
Age, years, *Me* (Q_1–Q_3)	48 (41.5–57)
BMI, kg / m^2, *Me* (Q_1–Q_3)	29.9 (27–32.5)
Smoking index, pack / years, *Me* (Q_1–Q_3)	0 (0–5.13)
CT_{max}, %, *Me* (Q_1–Q_3)	50 (31–75)
Time interval from the COVID-19 onset:	
<90 days, *n* (%)	221 (64.8)
90–180 days, *n* (%)	80 (23.5)
>180 days, *n* (%)	40 (11.7)

All studies were conducted according to the national and international guidelines [10–12]. The results were expressed as the percentage of predicted values (%pred) calculated according to the European Coal and Steel Society equations (ECCS 1993) [13, 14]. The fixed values of 80%pred were taken as the lower limit of normal (LLN).

Table 2

Lung function and lung diffusion capacity parameters in COVID-19 survivors, *n* = 341

Parameter	Value
VC, %pred., *Me* (Q_1–Q_3)	102 (87–111)
VC< LLN, *n* (%)	63 (18.5)
FVC, %pred., *Me* (Q_1–Q_3)	103 (88–114.2)
FEV_1, %pred., *Me* (Q_1–Q_3)	101 (89–113)
FEV_1< LLN, *n* (%)	58 (17)
FEV_1 / VC, %, *Me* (Q_1–Q_3)	80.3 (76.4–84.3)
FEV_1 / VC < 70%, *n* (%)	25 (7.3)
TLC, %pred., *Me* (Q_1–Q_3)	98 (83.2–108)
TLC < LLN, *n* (%)	68 (19.9)
DLCO, %pred., *Me* (Q_1–Q_3)	75 (61.7–88.3)
DLCO < LLN, *n* (%)	206 (60.4)

The data analysis was performed using descriptive statistics and multivariate logistic regression analysis using the SPSS 21 and MS Excel 2016 software packages. Quantitative data with non-normal distribution were presented as the median and the

interquartile range (*Me* (Q_1–Q_3)). The Mann – Whitney test was used to compare two independent samples. Qualitative variables were presented as a percentage (%); the differences were assessed using the χ^2 test with the Yates correction or the Fisher's exact test. The relationship between the traits was studied using partial correlations. The differences were considered statistically significant at $p < 0.05$. To assess the risks of abnormal parameters, the one-dimensional logistic regression analysis with the assessment of odds ratios (OR) was applied.

To construct a binary classifier model for predicting abnormal DLCO, the multivariate logistic regression analysis was used. The decision rule for predicting abnormal DLCO was obtained in a training sample. For this purpose, the total sample was split into a training and a test (validation) sample by random selection with a 3:1 split ratio. The logistic regression coefficients were obtained in the training sample.

Z is the regression equation, which has the form of:

$$Z = \alpha_0 + \alpha_1 x_1 + \ldots + \alpha_n x_n,$$

α_0, α_1, $\ldots\alpha_n$ – model parameters (coefficients), $x_1 \ldots . x_n$ – predictors.

$P = \frac{1}{1+e^{-z}}$ is the probability of abnormal DLCO, where

The logistic regression model predicted a decrease in DLCO at $Z \geq 0$ and normal DLCO values at $Z < 0$.

To assess the quality of the binary classifier model and find the optimal cut-off value for dividing objects into subsets, the ROC analysis was performed. The criterion for choosing the cut-off value was the requirement to the maximum sum of sensitivity and specificity. The ability of the created model to recognize the presence or absence of abnormal DLCO was assessed by the area under the curve (AUC) and by the correspondence of the ROC curve to the diagonal reference line.

RESULTS

In previous studies, a moderate inverse correlation was revealed between CT_{max} and DLCO [3, 15]. Besides, the bigger the time interval between the onset of COVID-19 and the DLCO test was, the less frequently abnormal DLCO was observed [4, 5]. Considering the results obtained, we divided the general sample into two subgroups: group 1 included 39.6% (135/341) of patients with normal DLCO, group 2 encompassed 60.4% (206/341) of patients with reduced DLCO. After that, we analyzed DLCO depending on CT_{max}, the time interval from the COVID-19 onset (T), gender, age, and BMI (Table 3).

Table 3 shows that, depending on DLCO (normal or reduced), the groups differed in the time interval from the onset of COVID-19 (it was shorter in group 2) and in CT_{max} (it was greater in group 2). No differences in gender, age, and BMI were identified between the groups.

The partial correlation coefficient demonstrated a significant inverse correlation between DLCO and CT_{max} ($r = –0.601$; $p < 0.01$) at fixed values of the time interval from the COVID-19 onset, gender, age, and BMI (Fig. 1).

Table 3

Characteristics of patients depending on the DL_{CO} value, *Me* (Q_1–Q_3)

Parameter	Group 1 DLCO ≥ 80%pred. *n* = 135/341 (39.6%)	Group 2 DLCO < 80%pred. *n* = 206/341 (60.4%)	*p*
Gender (men / women), *n* (%)	107 (79.3) / 28 (20.7)	155 (75.2) / 51 (24.8)	0.467[1]
Age, years	47 (40–58)	49 (43–57)	0.539[2]
BMI, kg / m^2	29.7 (26.9–32.3)	30 (27–33)	0.579[2]
T, days	81 (42–145)	39.5 (27–93)	<0.001[2]
$CT_{max,}$ %	35 (25–52)	70 (48–80)	<0.001[2]

Note. T – time interval from the onset of COVID-19 to the DLCO test; *p* – level of significance (half bold font marks *p* values for statistically significant differences).
[1] the χ^2 test with the Yates correction; [2] the Mann – Whitney test.

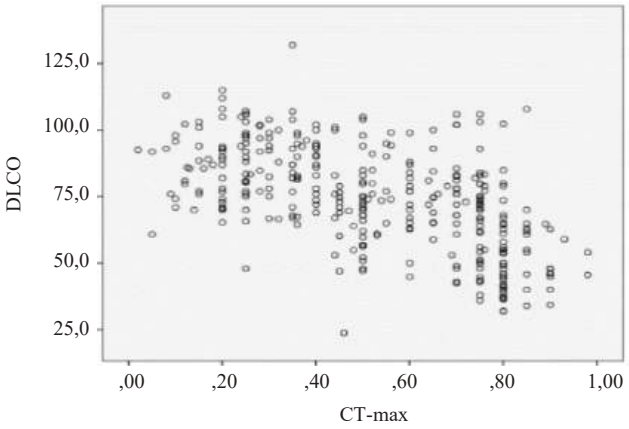

Fig. 1. Correlation analysis of the relationship between DLCO and CT_{max} (partial correlation, $r = –0.601$; $p < 0.01$)

Then we constructed a decision rule to identify patients at high risk for impaired lung diffusion capacity after COVID-19. For this purpose, the general sample was split in a 3:1 ratio into a training (n = 252) and a test (n = 89) subset. Age, gender, BMI, and parameters with a statistically significant association with reduced DLCO (CT_{max} and T) were selected as predictors. Following the conducted analysis, the following logistic regression equation was obtained:

$$Z = –1.279 + 0.05 \times x_1 – 0.004 \times x_2 – 0.046 \times x_3 + \\ + 0.017 \times x_4 + 0.653 \times x_5 \quad (1),$$

where x_1, x_2, x_3, x_4, x_5 are input parameters of the model (predictors): x_1 – CT_{max} (%), x_2 – time interval between the COVID-19 onset and the DLCO test (days); x_3 – BMI (kg/m^2), x_4 – age (years), x_5 – logistic regression coefficient encoding gender: 1 – male, 0 – female. The results of the logistic regression analysis are presented in Table 4.

Table 4 shows that the sensitivity, specificity, and accuracy for the training sample using equation 1 were 82.5, 61.2, and 74.2%, respectively.

Further, the logistic regression analysis was performed with stepwise exclusion of the least significant variables. As a result, predictors, such as gender, age, and BMI, were excluded from the model, and the following logistic regression equation was obtained:

$$Z = –1.564 + 0.046 \times x_1 – 0.003 \times x_2 \quad (2),$$

where x_1 is CT_{max} (%), x_2 is the time interval from the onset of COVID-19 to the DLCO test (days).

The results of the classification obtained at this stage are shown in Table 5.

Table 5 shows that the sensitivity, specificity, and accuracy for the training sample using equation 2 were 82.5, 61.2, and 74.2%, respectively.

The quality of the model described by equation 2 was verified using the ROC analysis (Fig.2): in the training sample, the AUC value was 0.789 (95% confidence interval (CI) 0.733–0.844), the sensitivity and specificity (at the cut–off point of 0.258) were 80 and 67%, respectively.

Table 4

Results of the classification in the training sample (predictors: CT_{max}, time interval between the COVID-19 onset and the DLCO test, age, gender, BMI)

Parameter	DLCO ≥ 80%pred., *n* (predicted)	DLCO < 80%pred., *n* (predicted)	Classified correctly, %
DLCO ≥ 80%pred., *n*	60	38	61.2
DLCO < 80%pred., *n*	27	127	82.5
Total			74.2

Table 5

The results of the classification in the training sample (predictors: CT_{max}, time interval from the onset of COVID-19 to the DLCO test)

Parameter	DLCO ≥ 80%pred., *n* (predicted)	DLCO < 80%pred., *n* (predicted)	Classified correctly, %
DLCO ≥ 80%pred., *n*	60	38	61.2
DLCO < 80%pred., *n*	27	127	82.5
Total			74.2

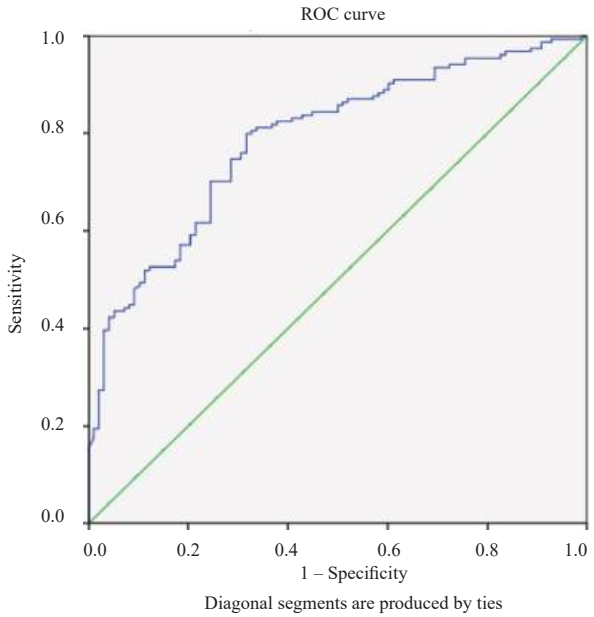

Fig. 2. The ROC analysis for the training sample (predictors: CTmax, time interval from the onset of COVID-19) to predict reduced DL_{CO}

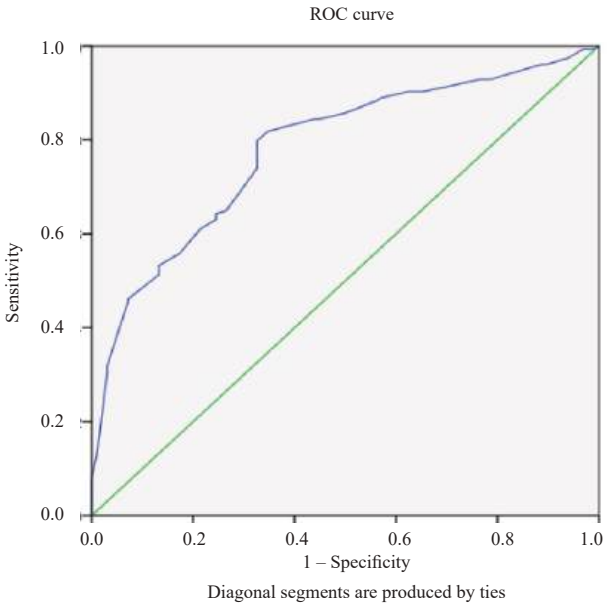

Fig. 3. ROC analysis for the training sample (predictor: CTmax) to predict reduced DL_{CO}

At $Z \geq 0.258$, reduced DLCO was predicted; at $Z < 0.258$, DLCO was within the normal range. When testing the resulting model in the test sample, sensitivity and specificity were 77 and 70%, respectively. The classifier model with a single predictor CT_{max} was also studied and the following logistic regression equation was obtained:

$$Z = -1.564 + 0.046 \times x_1 \quad (3),$$

where x_1 is CT_{max} (%).

The results of the classification obtained at this stage are shown in Table 6.

Table 6

The results of the classification in the training sample (predictors: CT_{max})

Parameter	DLCO ≥ 80%pred., *n* (predicted)	DLCO < 80%pred.., *n* (predicted)	Classified correctly, %
DLCO ≥ 80%pred., *n*	60	38	65.3
DLCO < 80%pred., *n*	27	127	81.8
Total			75.4

Table 6 shows that the sensitivity, specificity, and accuracy for the training sample using equation 3 were 81.8, 65.3, and 75.4%, respectively.

The ROC analysis showed (Fig. 3) that when using equation 3 in the training sample, the AUC value was 0.780 (95% CI 0.723–0.837), sensitivity and specificity (at the cut–off point of 0.171) were 80 and 67%, respectively. In the test sample, sensitivity and specificity were 79 and 70%, respectively.

At $Z \geq 0.171$, reduced DLCO was predicted, at $Z < 0.171$, DLCO was within the normal range.

Using equation 3, OR was calculated [16]:

$$OR = e^{-1,564}e^{0,046x}1 \quad (4),$$

where x_1 is CT_{max} (%).

Equation 4 evidences that OR > 1 is observed at $CT_{max} > 40\%$.

DISCUSSION

Predicting the state of medical systems depending on factors affecting them is an important task of statistical analysis. The use of mathematical models solves a lot of tasks, such as assessing the influence of factors on the response rate, changes in the parameter caused by changes in factors affecting the system, predicting the response rate for the given values of factors [17].

The methods for binary classification include both classical discriminant analysis and logistic regression analysis, which are used in various fields of medicine [18–20]. In this study, the created binary classifier model allows to predict reduced DLCO after COVID-19 with virus-associated lung damage.

The correlation analysis, data analysis depending on the DLCO value (normal or reduced), and the created binary classifier models showed that CT_{max} is an important predictor of reduced DLCO after

COVID-19 with virus-associated lung damage in patients without an underlying lung pathology.

Similar studies have been conducted abroad. W. Qin et al. [21] also did not reveal statistically significant differences in age, gender, and BMI between groups with reduced and normal DLCO 3 months after COVID-19. In addition, after exploring a wide range of possible abnormal DLCO predictors (demographic and clinical data, results of laboratory and instrumental research and X-ray, treatment regimens, the presence of acute respiratory distress syndrome) in 81 patients 3 months after COVID-19, the authors concluded that CT_{max} and acute respiratory distress syndrome affected DLCO after the acute phase of COVID-19.

In the present study, using the logistic regression analysis with the stepwise exclusion of the least significant predictors also demonstrated that CT_{max} contributed to a decrease in DLCO. The sensitivity and specificity of the created model were 80 and 67% for the training sample, respectively, and 79 and 70% for the test sample, respectively. The inclusion of additional predictors in the model, such as gender, age, BMI, and the time interval from the onset of COVID-19 to the DLCO test, did not significantly affect the quality of the decision rule. Moreover, it was shown that at $CT_{max} > 40\%$, reduced DLCO can be reliably expected after the end of the acute phase of COVID-19.

LIMITATIONS OF THE STUDY

To create a model for predicting reduced DLCO after COVID-19, we used the results of lung function tests obtained mainly in the first 6 months after the acute phase of the disease. It should be taken into account that CT_{max} reflects the volume, but does not characterize the depth and morphological features of lung tissue damage, which may subsequently affect DLCO. I.E. Tyurin et al. [22] pointed out that the clinical manifestation and prognostic value of radiological signs, such as ground-glass opacities and consolidation, appear to be completely different even for the same lesion volume.

CONCLUSION

A decision rule was obtained for predicting reduced DLCO after COVID-19 with virus-associated lung damage in patients without an underlying lung pathology. The analysis of chest CT scans in the acute phase of COVID-19 is essential for predicting impaired lung diffusion capacity. Patients with $CT_{max} > 40\%$ require more thorough clinical follow-up with obligatory DLCO monitoring after the acute phase of COVID-19.

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Authors' contribution

Savushkina O.I. – conception and design, selection and examination of patients, analysis and interpretation of the data, critical revision of the manuscript for important intellectual content, drafting of the manuscript. Muraveva E.S. – analysis and statistical processing of the data, drafting of the article. Zhitareva I.V. – analysis and statistical processing of the data, critical revision of the manuscript for important intellectual data. Davydov D.V. – critical revision of the manuscript for important intellectual data. Kryukov E.V. – final approval of the manuscript for publication, critical revision of the manuscript for important intellectual data.

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Intestinal microbiota in children with bronchial asthma

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ABSTRACT

Background. Intestinal microbiota is one of the most important factors determining the state of human health, including its influence on the immunological mechanisms regulating the development of allergic diseases in childhood. The role of intestinal microbiota and the gut – lung axis in the development of bronchial asthma (BA) is an important area of research.

Aim. To analyze the taxonomic composition of intestinal microbiota in children with BA using 16S rRNA gene sequencing.

Materials and methods. The study included patients with BA (n = 50, mean age 10.34 ± 2.99 years) and a group of apparently healthy individuals (n = 49, mean age 10.3 ± 2.8 years). For all patients, medical history was taken, and physical examination and stool test were performed. Patients with BA were assessed for the level of total and specific immunoglobulin (Ig) E and underwent spirometry. The microbiota composition was analyzed by 16S rRNA gene sequencing with subsequent bioinformatic and statistical analysis.

Results. Significant differences in the composition of the intestinal microbiota (beta diversity) and a decrease in taxonomic diversity (alpha diversity) were found in patients with BA compared to healthy controls. The intestinal microbiota of patients with BA was characterized by an increase in the abundance of *Bacteroides, Parabacteroides, Lachnospira, Roseburia, Akkermansia, Anaerostipes, Sutterella, Odoribacter, Phascolarctobacterium, Butyricimonas,* as well as unclassified bacteria from the Rikenellaceae families. The intestinal microbiota of children without BA was characterized by greater abundance of bacteria from *Blautia, Bifidobacterium, Dorea, Ruminococcus, Streptococcus, Eubacterium, Acinetobacter, Collinsella, Lactococcus, Catenibacterium* genera and unclassified bacteria from the Clostridiaceae and Coriobacteriaceae families. Significant differences in the quantitative abundance of bacteria were revealed depending on the type of sensitization, the level of total IgE, and the value of FEV1.

Conclusion. The results obtained indicate the differences in the intestinal microbiota composition in children with BA and healthy children.

Keywords: intestinal microbiota, bronchial asthma, 16S rRNA gene sequencing, children

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

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Conformity with the principles of ethics. Patients over 15 years of age signed an informed consent to participate in the study, for children aged 7–14, an informed consent was obtained from their legally authorized representatives. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 8946 of 24.01.2022).

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Микробиота кишечника у детей, больных бронхиальной астмой

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РЕЗЮМЕ

Введение. Микробиота кишечника является одним из важнейших факторов, определяющих состояние здоровья человека, в том числе оказывает влияние на иммунологические механизмы развития аллергических болезней в детском возрасте. Роль микробиоты кишечника и оси «кишечник – легкие» в развитии и течении бронхиальной астмы (БА) является актуальной областью исследований.

Цель – провести анализ таксономического состава кишечной микробиоты у детей с БА с использованием метода секвенирования 16S рРНК.

Материалы и методы. В исследование включены пациенты, страдающие БА (n = 50, средний возраст 10,34 ± 2,99) и группа условно здоровых детей (n = 49, средний возраст 10,3 ± 2,8). Для всех участников выполнен сбор анамнеза и физикальное обследование, сбор образцов стула. Пациентам с БА проводилась оценка уровня общего и специфического иммуноглобулина (Ig) E и спирометрия (измерение объема форсированного выдоха за первую секунду ($ОФВ_1$)). Определение состава микробиоты выполнено с помощью секвенирования гена *16S* рРНК с последующим биоинформатическим и статистическим анализом.

Результаты. Установлены значимые различия в составе микробиоты кишечника (бета-разнообразие) и снижение таксономического богатства (альфа-разнообразия) у пациентов с БА в сравнении с детьми без БА. У пациентов с БА увеличена представленность бактерий родов *Bacteroides, Parabacteroides, Lachnospira, Roseburia, Akkermansia, Anaerostipes, Sutterella, Odoribacter, Phascolarctobacterium, Butyricimonas*, а также неклассифицированные бактерии семейств Rikenellaceae. Кишечная микробиота детей, не страдающих БА, характеризовалась более высоким содержанием бактерий родов *Blautia, Bifidobacterium, Dorea, Ruminococcus, Streptococcus, Eubacterium, Acinetobacter, Collinsella, Lactococcus, Catenibacterium* и неклассифицированные бактерии семейств Clostridiaceae, Coriobacteriaceae. Выявлены значимые отличия в количественной представленности бактерий в зависимости от вида сенсибилизации, уровня общего иммуноглобулина IgE и значения $ОФВ_1$.

Заключение. Полученные результаты свидетельствуют о различиях состава микробиоты кишечника детей, страдающих БА, и условно здоровых детей.

Ключевые слова: кишечная микробиота, бронхиальная астма, секвенирование 16S рРНК, дети

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

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Соответствие принципам этики. Информированное согласие на участие в исследовании дети старше 15 лет подписывали самостоятельно, для детей 7–14 лет – законные представители. Исследование одобрено локальным этическим комитетом СибГМУ (протокол № 8946 от 24.01.2022).

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INTRODUCTION

Bronchial asthma (BA) is a multifactorial chronic respiratory disease that affects more than 250 million people of different ages worldwide [1]. Currently, BA remains a serious public health problem, which is associated with high prevalence, a significant decrease in the quality of life of patients and their families, and significant economic burden to the healthcare system [2]. In this regard, it is relevant to study the pathogenetic factors in the development of BA to elaborate new preventive technologies based on personalized medicine, which is of vital importance in pediatrics.

In recent years, a lot of data have demonstrated an association between the development of BA and the composition of respiratory microbiota in childhood [3–5]. However, in chronic lung diseases, respiratory microbiota changes, and an imbalance in the composition of intestinal microbiota is also noted. Molecular genetic methods for identifying microorganisms have expanded our understanding of the gut microbiome and contributed to the recognition that microbial communities influence host physiology beyond the gastrointestinal tract. According to the concept of the gut – lung axis, the gut microbiome has significant effects on immune regulation and lung function [6, 7].

Circulation through the circulatory and lymphatic systems transports regulatory cytokines and bacterial metabolites, such as short-chain fatty acids (SCFA), to the lungs, where they participate in immune and anti-inflammatory responses, thereby connecting the gut – lung axis [8]. The results of experimental and epidemiological studies demonstrate that the formation of the intestinal microbiota at an early age plays a significant role in the development of BA [9–11]. However, most studies on microbiota in childhood asthma are aimed at investigating a correlation between the composition of bacterial communities in early life and the risk of developing the disease in adulthood. In the Russian Federation, research on the intestinal and oropharyngeal microbiota in BA in adult population was carried out using molecular genetic methods, demonstrating modification of the microbiome against the background of the disease [12, 13].

The aim of the study was to analyze the taxonomic composition of intestinal microbiota in children with BA using 16S rRNA gene sequencing.

MATERIALS AND METHODS

A cross-sectional, case-control study included 50 children with BA and 49 apparently healthy children without acute or chronic diseases. Patients with BA were recruited at the Children's Clinic of Siberian State Medical University in Tomsk. Data from a sample of healthy participants from a previous epidemiological study were used as a control group [14].

Criteria for inclusion in the main group: children aged 7–16 years with persistent BA of mild to moderate severity; forced expiratory volume in 1 second (FEV_1) reversibility of more than 12% from the baseline value according to spirometry; basic therapy with inhaled glucocorticoids at a low daily dose as monotherapy or in combination with long-acting β2-agonists or leukotriene receptor antagonists within 12 months preceding the study. Criteria for inclusion in the control group: apparently healthy children aged 7–16 years without BA or other chronic diseases. Exclusion criteria for all study groups: intake of antibacterial drugs and systemic glucocorticoids within three months preceding the study; intake of eubiotics and/or intestinal infections within one month before the study; presence of clinically significant conditions or diseases that can prevent patients from participating in the study or affect any manipulations or interpretation of the study results; a lack of a signed informed consent.

All study participants were divided into two groups: patients with BA (n = 50, mean age 10.34 ± 2.99 years, the ratio of girls to boys 27 / 23) and a group of apparently healthy individuals (n = 49, mean age 10.3 ± 2.8 years, the ratio of girls to boys 21 / 28).

The study included history taking and a physical examination. To assess asthma control, we used the Asthma Control Test for children over 12 years of age and the c-ACT in children from 7 to 11 years of age. Patients with BA were assessed for the level of total and specific immunoglobulin (Ig) E (Alkor Bio, Russia) and underwent spirometry (MasterScreen, Germany). To assess the composition of the intestinal microbiota, stool samples were collected using a special stool collection kit with a transport medium (Stool Collection kit, Nobias Technologies).

Sample preparation and sequencing of the V3-V4 region in the 16S rRNA gene

To extract DNA, the Nobias DNA Extraction Kit was used with an extraction protocol that included

homogenization of the stool sample using the bead beating technique and precipitation of inhibitors. Sequencing of the V3-V4 region in the *16S* rRNA gene (341F-801R primers, Litech LLC, Russia) was carried out on the Illumina MiSeq Sequencing System (Illumina, USA).

Analysis of sequencing data

The composition of the samples was determined using the Knomics – Biota platform [15] and QIIME algorithms [16]. Rare and underrepresented bacteria (those that were found in less than half of the samples and did not account for more than 5% in any sample) were excluded from the analysis. The nearest balance method was used to analyze the differences between the groups [17].

The taxonomic abundance and α-diversity were assessed using the Chao1 and Shannon indices after rarefaction to 5000 reads. The Mann – Whitney test was used for statistical analysis. The correlation analysis was carried out using the Spearman's rank correlation coefficient.

Beta-diversity was assessed using the Aitchison distance (after excluding rare microbes) and the Bray – Curtis dissimilarity (after rarefaction to 5000 reads per sample). The PERMANOVA method was used for statistical analysis [18]. Qualitative variables were presented as absolute or relative (%) frequencies. Normally distributed quantitative variables were presented as the mean and the standard deviation $M \pm m$. Quantitative variables with non-normal distribution were presented as the median and the interquartile range Me (Q_{25}; Q_{75}). The differences were considered to be statistically significant at $p < 0.05$.

RESULTS

The ACT test revealed that 52% of patients had a well-controlled course of asthma (ACT score ≥ 20), while 48% of patients had the ACT score of 19 or less (20 (18; 24)). According to the laboratory tests, the total IgE level of more than 100 IU / ml was detected in 66% of children with BA. The median total IgE level was 293.5 (82.6; 705.8). Sensitization to household allergens was detected in 50% of patients with BA, to pollen – in 52% of children, to epidermal allergens – in 44% of patients with BA, and to food allergens – in 34% of the examined children. According to spirometry findings in patients with BA, the FEV_1 value was 100.7 (88.9; 111.1).

Taxonomic characteristics

The most abundant phyla in the intestinal microbiota of patients with BA were Firmicutes (71.1 ± 13.9%), Bacteroidetes (20.2 ± 14.8%), Proteobacteria (3.4 ± 6%), Verrucomicrobia (1.2 ± 2.7%), and Actinobacteria (1.1 ± 0.6%). The intestinal microbiota of children in the control group was dominated by Firmicutes (73.7 ± 13.6%), Actinobacteria (14.6 ± 13%), Bacteroidetes (6.1 ± 8.1%), Proteobacteria (4.7 ± 5. 9%), and Verrucomicrobia (0.4 ± 0.8%) phyla. The intestinal microbiota of children with BA was characterized by abundance of Ruminococcaceae (50.4 ± 13%), Bacteroidaceae (13 ± 9.6%), Clostridiaceae (5.5 ± 6%), Lachnospiraceae (5.3 ± 3.5%), and Prevotellaceae (4.4 ± 12.5%) families. The samples from the control group were characterized by abundance of Lachnospiraceae (30.6 ± 14%), Ruminococcaceae (16.6 ± 8.5%), Bifidobacteriaceae (11.3 ± 12.6%), and Clostridiaceae (4.4 ± 3.5 %) families. The most abundant bacterial families in the samples of the studied groups are shown in Fig. 1. Each column in the figure refers to one of the samples. Different colors mark proportions of different bacteria in the sample. The figure shows proportions of the 10 most abundant families, the remaining families are marked with the gray color.

The intestinal microbiota of children with BA at the genus level was dominated by Bacteroides (31.6 ± 12.6%), Prevotella (4.4 ± 12.5%), and unclassified bacteria from the Ruminococcaceae (16.1 ± 5.1%) and Clostridiaceae (5.5 ± 6%) families. The intestinal microbiota in the control group was represented by Blautia (14.4 ± 9.4%), Bifidobacterium (11.3 ± 12.6%), and unclassified bacteria from the Lachnospiraceae (11.7 ± 6.5%), Clostridiaceae (10.6 ± 5.2%), and Ruminococcaceae (7.4 ± 4.4%) families.

Assessing the taxonomic diversity of the intestinal microbiota

The assessment of α-diversity using the Shannon index revealed a decrease in microbiota diversity in children with BA compared to the controls (Shannon index: $p < 0.01$; Fig. 2, *a*). The assessment of the taxonomic abundance using the Chao1 index revealed no significant differences between the groups (Chao1 index: $p = 0.2$, Fig. 2, *b*).

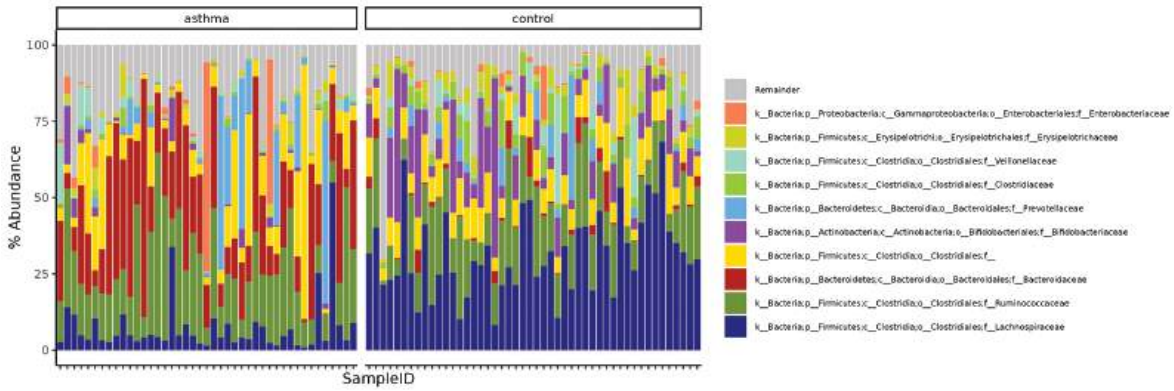
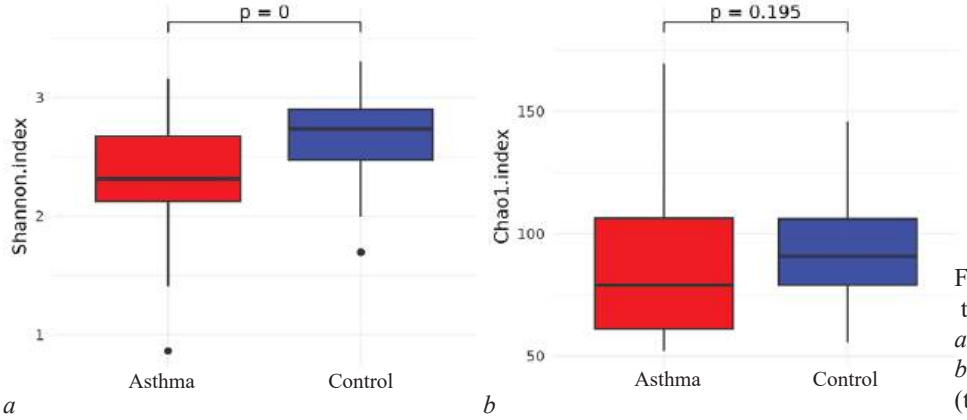


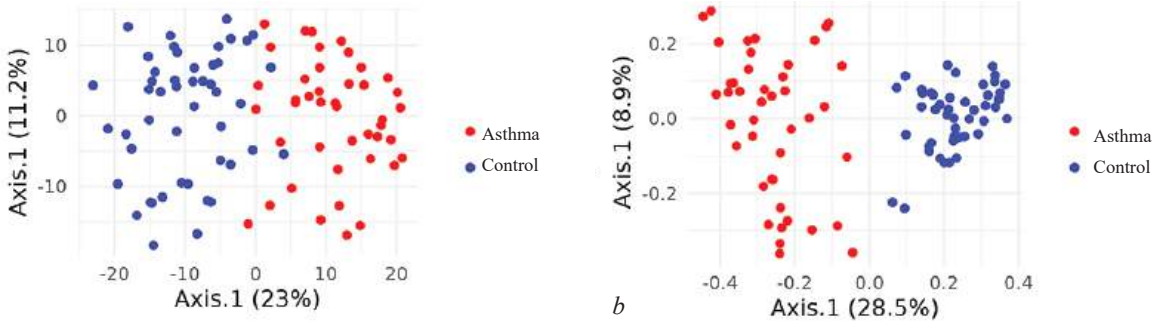
Fig. 1. Taxonomic composition of the intestinal microbiota samples at the family level



a *b*

Fig. 2. Differences between the groups in α-diversity: *a* – Shannon index; *b* – Chao1 index: $p < 0.05$ (the Mann – Whitney test)

Beta-diversity of the intestinal microbiota was analyzed using the PERMANOVA method. It revealed significant differences in the microbiota composition between the BA patients and controls (Aitchison distance: $p = 0.001$; Bray – Curtis dissimilarity: $p = 0.001$). Fig. 3 visualizes these differences using the principal coordinate analysis (PCoA). Each point in the figure corresponds to a sample, and the difference between them indicates β-diversity: the closer the points, the more similar their composition is.



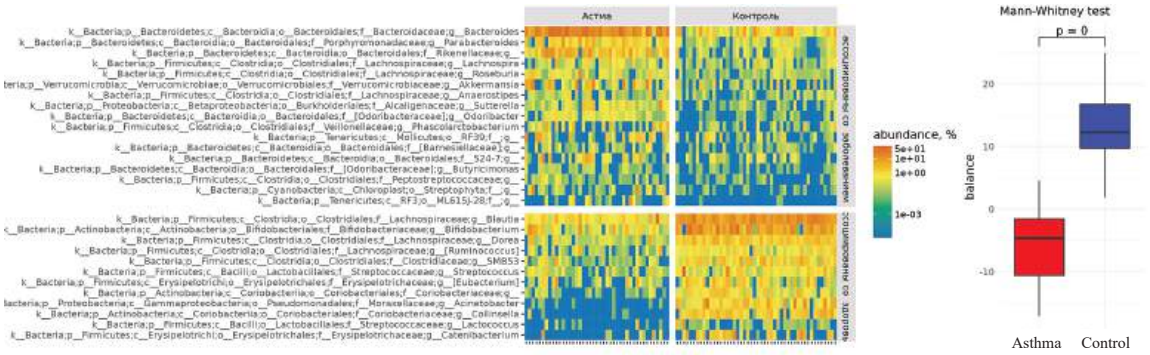
a *b*

Fig. 3. Visualization of the differences between the samples using the PCoA: **a** – Aitchison distance, which assesses similarity of proportions of prevailing bacteria; **b** – Bray – Curtis dissimilarity, which takes into account the proportions of all bacteria.

Features of intestinal microbiota in BA

The comparative analysis of the intestinal microbiota composition in patients with BA and the control group revealed significant differences in the abundance of 29 taxa ($p < 0.05$). The presence of BA was associated with increased abundance of the *Bacteroides, Parabacteroides, Lachnospira, Roseburia, Akkermansia, Anaerostipes, Sutterella, Odoribacter, Phascolarctobacterium, Butyricimonas* genera, as well as unclassified bacteria of the Rikenellaceae, Barnesiellaceae, and Peptostreptococcaceae families of the Mollicutes class, Bacteroidales and Streptophyta orders. The intestinal microbiota in children without BA was

characterized by a higher content of bacteria of the *Blautia, Bifidobacterium, Dorea, Ruminococcus, Streptococcus, Eubacterium, Acinetobacter, Collinsella, Lactococcus*, and *Catenibacterium* genera and the Clostridiaceae and Coriobacteriaceae families (Fig. 4).



a *b*

Fig. 4. Differences in the bacterial abundance between patients with asthma and controls: *a* – heatmap showing the abundance of bacteria associated with the presence or absence of the disease, *b* – proportion of bacteria associated with the presence or absence of the disease in the samples obtained from the BA patients and controls (the nearest balance method): each row corresponds to a microorganism, and each column corresponds to a sample; the color of the cell indicates the proportion of bacteria in the sample. The samples are grouped by the presence of the disease, and microorganisms are grouped by their association with the disease

The comparative assessment of the intestinal microbiota composition was performed in the samples obtained from children with BA depending on the clinical course of the disease, asthma control, the level of total IgE, and sensitization. It was revealed that sensitization to household allergens was associated with an increase in the abundance of the *Peptostreptococcus* genus, and sensitization to epidermal allergens – with increased abundance of the *Bacteroides fragilis* genus. In the intestinal microbiota samples obtained from children with sensitization to food allergens, increased abundance of the *Blautia* genus and a decrease in the *Ruminococcus* and *Dialister* genera were noted. Patients with total IgE levels greater than 100 IU / ml had decreased representation of the *Lachnospira* genus in their intestinal microbiota samples. The correlation analysis revealed a direct correlation of FEV_1 with the abundance of *Bifidobacterium* (0.36; $p = 0.04$), *Streptococcus* (0.39; $p = 0.02$), and *Ruminococcus gnavus* (0.37; $p = 0.03$). There were no significant differences in the taxonomic composition of bacterial communities in patients with ACT scores ≥ 20 and < 20. The assessment of α- and β-diversity also detected no significant differences between the indices.

DISCUSSION

The intestinal microbiota in patients with BA is characterized by a decrease in the taxonomic diversity, as well as by qualitative and quantitative changes in the bacterial composition compared to the controls. The taxonomic diversity indicates stability of the microbiota and its resistance to mutations, and a decrease in diversity usually evidences of the presence of a pathological process. Studies have revealed a decrease in the taxonomic diversity of the microbiota in early childhood; such children are subsequently diagnosed with BA [10, 11, 19]. When assessing the taxonomic diversity in children and adults with manifestations of BA, compared to healthy controls, no significant differences in α-diversity were identified [12, 20]. The absence or minimal differences in the taxonomic diversity at an older age suggests that microbial diversity might have a stronger effect on the formation of immunological tolerance and, consequently, the development of BA at an early age [9, 10, 20].

In general, the data obtained in the study groups on the most abundant bacteria at different taxonomic levels are in line with modern ideas about the composition of the intestinal microbiota [21]. We found that the intestinal microbiota composition in children with BA compared to the controls was characterized by a relatively greater abundance of *Bacteroides, Parabacteroides, Lachnospira, Roseburia, Akkermansia, Anaerostipes, Sutterella, Odoribacter, Phascolarctobacterium,* and *Butyricimonas* and unclassified bacteria of the Rikenellaceae, Barnesiellaceae, and Peptostreptococcaceae families, the Mollicutes class.

Prospective cohort studies showed that an increase in the abundance of *Bacteroides, Akkermansia, Roseburia*, and *Lachnospira* in the intestinal microbiota of infants, on the contrary, was associated with a decreased risk of developing BA [9, 11].

At the same time, it was shown that at the age of over one year, the differences in the microbiota composition in these cohorts were not significant [9, 11]. Studies revealed the anti-inflammatory potential of these microorganisms and that an increase in their abundance in children with BA might be a compensatory mechanism in response to inflammation of the respiratory mucosa [22]. The study using the Mendelian randomization identified an association between bacteria of the *Butyricimonas* genus and the development of BA [23]. According to the systematic review, in children with BA, the intestinal microbiota was characterized by an increase in the abundance of *Bacteroides* and a decrease in the abundance of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii, Clostridium,* and *Bifidobacterium* [24].

Our study also showed a decrease in the abundance of *Bifidobacterium* in the intestinal microbiota of children with BA compared to the controls. Bacteria of the *Bifidobacterium* genus have an immunomodulatory effect due to stimulation of regulatory T cells [25]. It is suggested that one of the mechanisms mediating interactions within the gut– lung axis is the production of SCFAs by intestinal bacteria [6, 7]. Children with BA are characterized by both an increase (*Anaerostipes, Roseburia, Phascolarctobacterium*) and a decrease (*Blautia Eubacterium*) in the abundance of SCFA-producing bacteria.

The patients with BA exhibited differences in the quantitative abundance of bacteria depending on the type of sensitization. Studies aimed at assessing microbiota and sensitization to various allergens are scarce. It was shown that sensitization to pet allergens in children with BA was associated with lower diversity of nasal microbiota and *Corynebacterium sp.* and *Staphylococcus epidermidis* compared to children without sensitization [26]. Another study found that the presence of *Ruminococcus* was positively associated with sensitization to casein [27].

CONCLUSION

Following the conducted study, we established that the intestinal microbiota in children with BA is characterized by the decreased taxonomic diversity and differs in the bacterial composition from the microbiota of apparently healthy children. A number of significant differences in the abundance of bacteria were revealed depending on the type of sensitization and functional parameters of children in BA.

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Authors' contribution

Sokolova T.S. – conception and design, carrying out of experiments, analysis and interpretation of the data, drafting of the manuscript. Malchuk V.N. – collection and processing of the material, carrying out of experiments, drafting of the manuscript. Fedorova O.S. – conception and design, critical revision of the manuscript for important intellectual content, final approval of the article for publication. Kulenich V.V. – statistical processing of the research results. Odintsova V.E., Koshechkin S.I. – bioinformatic analysis and processing of the research results.

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Liver damage in patients hospitalized with acute decompensated heart failure, depending on the degree of glucose metabolism disorder

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ABSTRACT

Aim. To study the frequency of cardiohepatic syndrome and steatosis by the value of controlled attenuation parameter (CAP), fibrosis, and their combination, depending on the degree of glucose metabolism disorder in patients with acute decompensated heart failure (ADHF).

Materials and methods. The study included 280 patients (53% men, average age 70.1 ± 10.8 years) with ADHF: 72.5% of patients had a history of arterial hypertension, 60% of patients had coronary heart disease. The HbA1c test was performed in all patients to assess the status of glucose metabolism. The patients were divided into groups depending on the results obtained: at HbA1c values < 5.7%, patients were included in the group without glucose metabolism disorders, at HbA1c 5.7–6.4% – in the prediabetes group, at HbA1c ≥ 6.5% – in the type 2 diabetes group. All patients underwent a standard physical examination at admission and at discharge. Clinical and comprehensive assessments of congestion were performed – NT-proBNP, lung ultrasound, liver fibroscan with CAP, and bioelectrical impedance analysis of body composition.

Results. The frequency of glucose metabolism disorders in patients hospitalized with ADHF was 57.5% ($n = 161$), while prediabetes was detected in 17.1% ($n = 48$) and type 2 diabetes – in 40.4% ($n = 113$) of patients. We revealed significantly higher incidence of steatosis by CAP value (69 vs. 42%, $p < 0.001$), fibrosis (80 vs. 64%, $p < 0.001$), and their combination (59 vs. 30%, $p < 0.001$), as well as cardiohepatic syndrome (87 vs. 61%, $p < 0.001$) in patients with ADHF and glucose metabolism disorders compared to individuals with ADHF without glucose metabolism disorders, respectively. The group of ADHF patients with glucose metabolism disorders and a combination of steatosis / fibrosis was characterized by more pronounced manifestations of metabolic syndrome, impaired kidney and liver function, and more pronounced manifestations (both clinical and laboratory) of congestion.

Conclusion. In patients with ADHF with glucose metabolism disorders, liver function test and liver fibroscan with CAP allow for identifying the most severe group of patients with a combination of steatosis/fibrosis and pronounced congestion.

Keywords: heart failure, liver fibroscan, steatosis, fibrosis, glucose metabolism disorder

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Поражение печени у пациентов, госпитализированных с острой декомпенсацией хронической сердечной недостаточности, в зависимости от степени нарушения углеводного обмена

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РЕЗЮМЕ

Цель: изучить частоту стеатоза по значению контролируемого параметра ослабления (CAP), фиброза и их сочетания, а также сердечно-печеночных синдромов в зависимости от степени нарушения углеводного обмена (НУО) у пациентов с острой декомпенсацией хронической сердечной недостаточности (ОДХСН).

Материал и методы. В исследование были включены 280 пациентов (53% мужчин, средний возраст 70,1 ± 10,8 лет) с ОДХСН. Артериальную гипертонию в анамнезе имели 72,5%, ишемическую болезнь сердца – 60% пациентов. Всем пациентам для оценки статуса углеводного обмена определяли уровень гликированного гемоглобина (HbA1c). Пациенты были разделены на группы в зависимости от полученных результатов: при значениях HbA1c < 5,7% – в группу без НУО; 5,7–6,4% – в группу предиабета; ≥6,5% – в группу сахарного диабета (СД) 2-го типа.

Пациентам проводили стандартное физическое обследование при поступлении и при выписке, а также клиническую и комплексную оценку застоя – исследования NT-proBNP, ультразвуковое исследование легких, фибросканирование печени, включая контролируемый параметр ослабления CAP, биоимпедансный анализ состава тела.

Результаты. Частота НУО у пациентов, госпитализированных с ОДХСН, составляет 57,5% (n = 161), при этом предиабет был выявлен в 17,1% (n = 48), СД 2-го типа – в 40,4% (n = 113) случаев. Выявлена достоверно более высокая частота стеатоза по значению CAP (69 против 42%, p < 0,001), фиброза (80 против 64%, p < 0,001) и их сочетания (59 против 30%, p < 0,001), а также сердечно-печеночного синдрома (87 против 61%, p < 0,001) у пациентов ОДХСН и НУО в отличие от пациентов ОДХСН без НУО соответственно. Группа пациентов ОДХСН с НУО и сочетанием стеатоза (фиброза) была наиболее тяжелой, характеризовалась более выраженными проявлениями метаболического синдрома, нарушениями функции почек, печени, более выраженными проявлениями застоя, как клиническими, так лабораторно-инструментальными.

Заключение. У пациентов ОДХСН с НУО определение уровня печеночных ферментов, а также проведение фибросканирования печени и определение CAP позволит выделить наиболее тяжелую группу пациентов с сочетанием стеатоза (фиброза) и выраженными явлениями застоя.

Ключевые слова: сердечная недостаточность, фибросканирование печени, стеатоз, фиброз, нарушение углеводного обмена

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 Медицинского института РУДН (протокол № 28 от 15.04.2021).

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INTRODUCTION

The problem of heart failure (HF) remains one of the most urgent in cardiology, and dysfunction of peripheral target organs makes a significant contribution to HF development. Carbohydrate metabolism disorders (CMD), such as type 2 diabetes mellitus (DM) and prediabetes, are common in patients hospitalized with acute decompensated heart failure (ADHF). Prolonged systemic congestion in HF contributes to the development of liver damage in patients with CMD and is associated with a poor prognosis and the formation of fibrosis and then liver fibrosis [1].

Non-alcoholic fatty liver disease (NAFLD), which is often detected in this group of patients, also contributes to this pathology to a certain extent. The presence of a combined pathology leads to significant deterioration in the prognosis, as well as an increased risk of death from cirrhosis. The overall prevalence of NAFLD in patients with type 2 DM is 55.5%, which is more than two times higher than in the general population [2]. The relationship between the presence of NAFLD and metabolic syndrome is beyond doubt, while insulin resistance, which is the main characteristic of metabolic syndrome, is a key factor in this relationship. There are few studies devoted to the assessment of structural liver disorders using elastography in patients with stable HF, ADHF, and CMD. The most common indices for the diagnosis of steatosis are the Fatty Liver Index (FLI) and the Hepatic Steatosis Index (HSI).

It has been shown that the severity of NAFLD is associated with HSI in patients with metabolic syndrome who had dyslipidemia and CMD regardless of the presence of obesity and insulin resistance [3]. It has been demonstrated that patients with ischemic heart disease and type 2 DM are characterized by the presence of pronounced structural and functional changes in the liver, which are manifested by elevated levels of liver enzymes and high values of liver HSI and fibrosis index compared to patients with HF without type 2 DM [4]. At the same time, data on the frequency of steatosis and liver fibrosis in patients with HF and prediabetes are not presented in the literature. To identify and measure steatosis, controlled attenuation parameter (CAP), a new parameter developed based on the ultrasonic properties of radiofrequency back-propagated signals received via elastography, is used.

In this regard, the aim of this research was to study the frequency of steatosis by the value of CAP as well as to study the frequency of fibrosis, their combination, and cardiohepatic syndrome depending on the degree of CMD in patients with ADHF.

MATERIALS AND METHODS

A prospective, observational study included 280 people hospitalized with ADHF. The main criteria for the diagnosis of ADHF were considered to be rapid deterioration of symptoms and signs of HF, requiring emergency hospitalization of the patient and intensive care in the presence of objective signs of heart damage, which included systolic and/or diastolic dysfunction, left ventricular hypertrophy, left atrial dilation according to echocardiography, and increased levels of NT-proBNP.

The exclusion criteria were the following: the presence of acute coronary syndrome, end-stage renal disease, liver failure, known hepatitis (cirrhosis), non-cardiogenic edema, active cancer, lung damage due to exacerbation of obstructive lung disease, bronchial asthma, pneumonia, COVID-19 or COVID-19 contact patients, type 1 diabetes mellitus, immobilization, and severe cognitive deficit.

To assess the status of carbohydrate metabolism, we determined the level of glycated hemoglobin (HbA1c). The patients were divided into groups depending on the results obtained: at HbA1c values < 5.7%, the patients were included in the group without CMD, at HbA1c 5.7–6.4% – in the prediabetes group, at HbA1c ≥ 6.5% – in the type 2 DM group [5].

Upon admission and discharge, a standard physical examination and routine laboratory and instrumental studies were performed, which included lung ultrasound, determination of NT-proBNP, liver fibroscan with CAP, and bioelectrical impedance vector analysis (BIVA) of body composition (Fig. 1).

All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at RUDN Medical Institute (Protocol No. 28 of 15.04.2021). The clinical and demographic characteristics of the patients are presented in Table 1.

Table 1

Clinical and demographic characteristics of patients included in the study, *n* = 280

Parameter	Value
Gender (male / female), *n* (%)	148 (53%) / 132 (47%)
Age, years, *M* ± *SD*	70.1 ± 10.8

Table 1 (continued)

Parameter	Value
Body mass index, kg / m^2, *M* ± *SD*	32.1 ± 5.7
HF functional class, NYHA, *n* (%) II III IV	 90 (32%) 123 (44%) 67 (24%)
Left ventricular ejection fraction, %, *M* ± *SD*	45.1 ± 11.9
Left ventricular ejection fraction, *n* (%): <40% 40–49% ≥50%	 84 (30%) 71 (25%) 125 (45%)
Arterial hypertension, *n* (%)	203 (72.5%)
History of stroke, *n* (%)	36 (13%)
Coronary heart disease, *n* (%)	167 (60%)
History of myocardial infarction, *n* (%)	106 (38%)
Atrial fibrillation / flutter, *n* (%)	185 (66%)
Chronic kidney disease, *n* (%)	73 (26%)
Chronic obstructive pulmonary disease (bronchial asthma), *n* (%)	47 (17%)

The composite congestion score (CCS) was used to assess clinical congestion. Orthopnea, swelling of the cervical veins, and peripheral edema were evaluated in points. Each clinical symptom and sign were evaluated at admission and at discharge. The total score ≥ 1 was considered as clinical congestion at admission and as residual congestion with clinical manifestations at discharge. The results on the assessment of the hydration status in patients hospitalized with ADHF, depending on the degree of CMD, were published earlier [5].

The level of NT-proBNP was determined by the enzyme-linked immunosorbent assay (ELISA) using the NT-proBNP-ELISA-BEST test systems and the A-9102 set of reagents (Vector-Best, Russia). Lung ultrasound was performed in 8 chest zones with the calculation of the sum of B-lines on the VIVID iq ultrasound system (GE Healthcare, USA). Indirect liver elastography (ILE) was performed using the FibroScan 502 Touch device (Echosens, France) according to the standard procedure. BIVA was performed using the Russian serial bioelectrical impedance analyzer ABC-01 Medass [5].

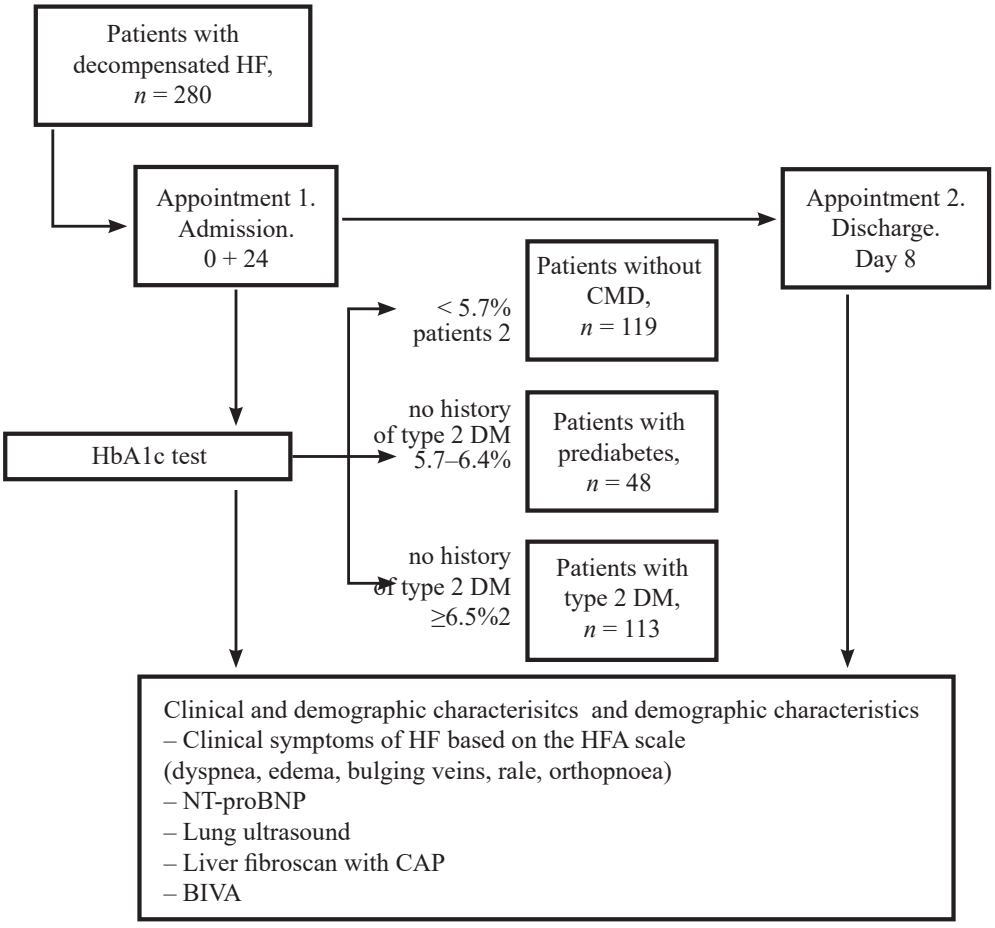

Fig. 1. Study design

If there was a deviation of at least one hepatic parameter from reference values, the patient was considered to have cardiohepatic syndrome. With an increase in markers of cytolysis syndrome (alanine transaminase (ALT), aspartate transaminase (AST)), the patient was diagnosed with hepatocellular cardiohepatic syndrome, with an increase in markers of cholestasis (alkaline phosphatase (ALP), total bilirubin) – with cholestatic cardiohepatic syndrome. A combined increase in markers of cytolysis and cholestasis and an increase in total bilirubin were designated as mixed cardiohepatic syndrome.

STATISTICAL ANALYSIS

Statistical data were processed using the MedCalc version 19.0 and IBM SPSS Statistics version 26.0 software tools. Quantitative variables were described as the arithmetic mean and the standard deviation of the mean (*M ± SD)* when the distribution was normal or as the median and the interquartile range (*Me; IQR*) in case of non-normal distribution. The nature of the data distribution was determined by the Kolmogorov – Smirnov test. With normal distribution of data, the statistical significance of the differences was assessed using the Student's *t*-test for related and unrelated samples. When the data distribution was not normal, the significance of the differences was analyzed using the Mann – Whitney test for unrelated samples and the Wilcoxon test for related samples. The differences were considered statistically significant at $p < 0.05$ (with account of the Bonferroni correction).

RESULTS

The frequency of CMDs in patients hospitalized with ADHF was 57.5% (*n* = 161), while prediabetes was detected in 17.1% (*n* = 48) and type 2 DM – in 40.4% (*n* = 113) of cases [**5**].

The incidence of steatosis in patients without CMDs was 42% (*n* = 50), while in patients with CMDs, it accounted for 69% (*n* = 111) of cases ($p < 0.001$). The incidence of fibrosis (F1–F4 > 5.8 kPa) was 64% (*n* = 77) in patients without CMDs and 80% (*n* = 129) in patients with CMDs ($p < 0.001$). The number of patients with a combination of steatosis / fibrosis among patients with ADHF and CMD was maximum and amounted to 59% (*n* = 95), which was 2 times greater than in the group of ADHF patients without CMDs – 30% (*n* = 36) (Fig. 2).

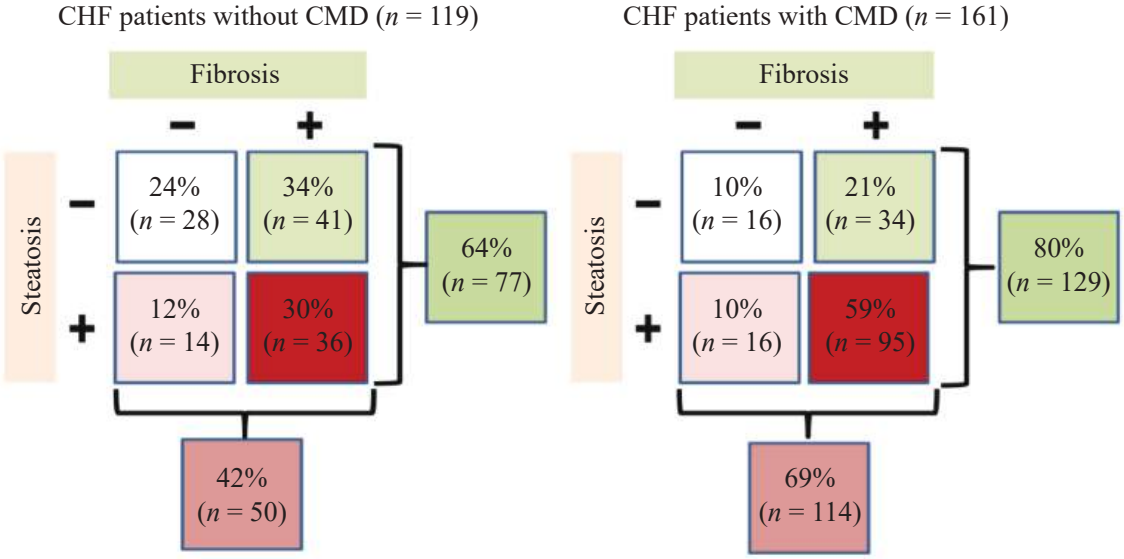

Fig. 2. The frequency of steatosis and fibrosis in patients with ADHF, depending on CMD, *n* = 280

The group of ADHF patients with CMDs and a combination of steatosis/fibrosis had the most severe course of the disease, characterized by high incidence of comorbid pathology, namely arterial hypertension, coronary heart disease, atrial fibrillation, and chronic kidney disease in the medical history, pronounced manifestations of metabolic syndrome, impaired renal and liver functions, manifestations of congestion, which were detected both in the clinical presentation and test results, the lowest values of the left ventricular ejection fraction and the 6-minute walk test (6MWT), and higher clinical rating scales (CRS).

The correlations were studied of CAP (Table 2) and liver density values (Table 3) with clinical (laboratory) parameters in patients with ADHF, depending on the degree of CMD. Significant positive correlations were found between CAP and liver enzymes (bilirubin, ALT, AST, lactate dehydrogenase (LDH)). Significant moderate positive correlations were determined between CAP and HSI, glycemic status parameters (Triglyceride – Glucose Index (TyG)), lipid metabolism parameters (total cholesterol, low-density lipoproteins (LDL), triglycerides (TG)), body mass index (BMI), waist circumference, and the frequency of coronary heart disease (CHD) in the medical history. Negative correlations were found between CAP and 6MWT and HDL in all groups of patients, regardless of the status of carbohydrate metabolism. At the same time, positive correlations of CAP with HbA1c and liver density were observed in patients with CMDs (prediabetes and type 2 DM) (Table 2).

Positive correlations were revealed between liver density index and CRS index, the level of NT-proBNP, and the content of creatinine. Negative correlations were found between liver density and glomerular filtration rate (GFR), LV ejection fraction (LVEF), and 6MWT, regardless of the status of carbohydrate metabolism (Table 3).

Table 2

Correlations of the CAP index with clinical and laboratory parameters in patients with ADHF depending on the degree of carbohydrate metabolism disorder, *r/p*

Parameter	ADHF without carbohydrate metabolism disorder, *n* = 119	ADHF and prediabetes, *n* = 48	ADHF and type 2 diabetes mellitus, *n* = 113
Clinical and demographic parameters			
BMI	0.21/ 0.02	0.40/ 0.004	0.34/ <0.001
Waist circumference	0.24/ 0.006	0.57/ <0.001	0.40/ <0.001
CHD	0.37/ <0.001	0.34/ 0.01	0.25/<0.001
6MWT	–0.23/ 0.009	–0.41/ 0.003	–0.37/ <0.001
Glycemic status			
Glucose	0.37/ <0.001	0.41/ 0.003	0.38/ <0.001
HbA1c	–	0.47/ <0.001	0.24/ 0.001
TyG	0.39/ <0.001	0.46/ <0.001	0.43/ <0.001
Lipid metabolism			
Cholesterol	0.27/ 0.002	0.42/ 0.002	0.28/ <0.001
LDL	0.30/ <0.001	0.41/ 0.003	0.31/ <0.001
HDL	–0.29/ <0.001	–0.34/ 0.01	–0.40/ <0.001
TG	0.32/ <0.001	0.45/ 0.001	0.30/ <0.001
Liver parameters			
Total bilirubin	0.59/ <0.001	0.62/ <0.001	0.54/ <0.001
ALT	0.57/ <0.001	0.56/ <0.001	0.74/ <0.001
AST	0.57/ <0.001	0.64/ <0.001	0.76/ <0.001
LDH	0.51/ <0.001	0.36/ =0.01	0.52/ <0.001
ALP	0.55/ <0.001	0.23/ <0.001	0.27/ <0.001
HSI index	0.31/ <0.001	0.33/ 0.02	0.37/ <0.001
Liver density	–	0.46/ <0.001	0.17/ 0.02

Table 3

Correlations of liver density with functional (laboratory) parameters in patients with ADHF depending on the degree of carbohydrate metabolism disorder, *r/p*

Parameter	ADHF without carbohydrate metabolism disorder, *n* = 119	ADHF and prediabetes, *n* = 48	ADHF and type 2 diabetes mellitus, *n* = 113
Functional status			
6MWT	–	–0.18/ 0.03	–0.18/ 0.04
CRS	0.22/ 0.01	0.28/ 0.005	0.26/ 0.005
Laboratory parameters			
LVEF	–0.29/ 0.001	–0.19/ 0.01	–0.40/ <0.001
NT-proBNP	0.31/ 0.001	0.26/ 0.04	0.32/ 0.002
Functional state of the kidneys			
Creatinine	0.29/ 0.001	0.45/ 0.001	0.35/ 0.001
GFR	–0.17/ 0.05	–0.21/ 0.05	–0.23/ 0.003

The frequency of cardiohepatic syndrome in patients was studied depending on the degree of CMD. The incidence of cardiohepatic syndrome in patients without CMDs was 61% (n = 72) and in patients with CMDs – 87% (n = 140) ($p < 0.001$). At the same time, the frequency of hepatocellular and cholestatic syndromes was comparable between the groups, and in ADHF patients with CMD, the frequency of the mixed type prevailed and was two times higher than in CHF patients without CMD (60% (n = 97) vs. 37% (n = 43), $p < 0.001$, respectively) (Fig. 3).

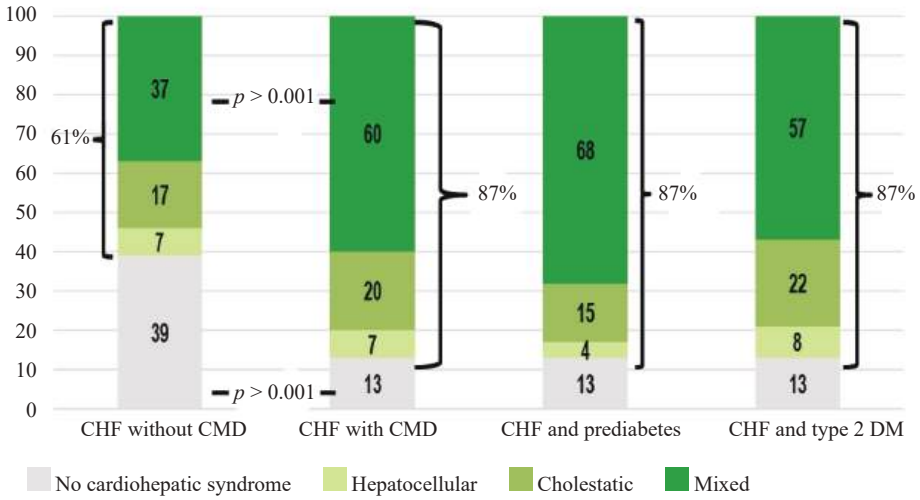

Fig. 3. The frequency of cardiohepatic syndromes in patients with ADHF depending on CMDs, n = 280

Significantly higher values of fasting glucose, total cholesterol, liver enzymes, liver density, and CAP were found in patients with ADHF and cardiohepatic syndromes, regardless of the degree of CMD.

DISCUSSION

Liver damage caused by a combined pathology, namely ADHF and CMD, was observed in a fairly large number of cases. Inflammation and fibrosis are the main factors determining the progression of liver damage. Fibrosis in patients with NAFLD is associated not only with the risk of morbidity and mortality from liver pathology, but also with the cardiovascular risk. It has been shown that an increase in liver enzymes can be observed with initial manifestations of HF caused by hemodynamic mechanisms [6]. Liver enzymes are associated with the class of HF, while the level of bilirubin according to the CHARM study [7] is the most important predictor of hospitalizations for ADHF and cardiovascular mortality. Venous congestion, decreased cardiac output, and arterial hypoxemia also play an important role in ischemic hepatitis [8]. At the same time, fibrosis has been shown to be the most critical factor in the progression of liver disease [9–12].

Given high prevalence of steatosis, its mainly benign course, and a lack of a clear association with changes in liver enzymes, it is important to use non-invasive methods to identify and quantify steatosis. CAP is a new parameter developed based on the ultrasonic properties of radiofrequency back-propagated signals receive via elastography to identify and measure steatosis. Based on CAP, the study established high frequency of steatosis (69 vs. 42%, $p < 0.001$), fibrosis (80 vs. 64%, $p < 0.001$), their combination (59 vs. 30%, $p < 0.001$), and cardiohepatic syndrome (87 vs. 61%, $p < 0.001$) in patients with ADHF and CMD, as opposed to ADHF patients without CMD, respectively. At the same time, the frequency of hepatocellular and cholestatic syndromes was comparable.

In a multicenter study involving 4,228 patients, more than 40% of patients hospitalized with acute heart failure had abnormal liver enzyme values. The multivariate analysis showed that only an increase in total bilirubin was independently associated with deterioration in clinical outcomes after both 30 and 180 days and may represent an important prognostic marker [13].

A retrospective study involving 1,032 patients with CHF of Caucasian origin demonstrated high incidence of liver dysfunction, which was characterized by a

predominant increase in cholestasis enzymes (total bilirubin, gamma-glutamyltransferase (GGT), and ALP). The frequency of elevated cholestasis enzymes was 19.2%, the frequency of elevated transaminases was 8.3% [14]. In our study, the incidence of cholestatic syndrome was 20% in patients with ADHF and CMD and 17% in ADHF patients without CMD. Hepatocellular syndrome and cardiohepatic syndrome were detected in 7 and 7% of cases, respectively.

Patients with ADHF and cardiohepatic syndrome, regardless of the degree of CMD, had higher values of BMI, waist circumference, glycemia, total cholesterol, liver enzymes, liver density, and CAP. In addition, patients with prediabetes and type 2 DM and cardiohepatic syndrome had significantly higher HSI values, a history of coronary heart disease and arterial hypertension, lower HDL values, as well as unreliably lower 6MWT results, which confirms the presence of more pronounced metabolic disorders in these patients.

The work by M.E. Statsenko et al. showed that patients with ischemic HF and type 2 DM had more pronounced structural and functional changes in the liver, manifested by high incidence of GGT, AST, and ALT, high HSI and liver fibrosis index compared to HF patients without type 2 DM [4].

In our work, the group of ADHF patients with CMD and a combination of steatosis/fibrosis was characterized by the most severe manifestations of congestion, lower values of LVEF, more pronounced functional disorders of the kidneys and liver, as well as glycemic and lipid profiles, which were detected both in the clinical presentation and test results, compared to all other groups.

Significant positive correlations of CAP with liver enzymes (bilirubin, ALT, AST, LDH) and negative correlations of CAP with 6MWT results were revealed in all groups of patients, regardless of the status of carbohydrate metabolism. At the same time, positive correlations were revealed between CAP and HbA1c and liver density in patients with CMD (prediabetes and type 2 DM). The literature also describes a strong correlation between CAP and the HOMA-IR insulin resistance index [15]. Our work also revealed the relationship between CAP and TyG index, regardless of the presence of CMD.

In our work, positive correlations were revealed between CAP and all parameters of lipid metabolism, regardless of the presence of CMD in patients with ADHF, which is consistent with the literature data [1, 3, 16]. In addition, positive correlations were noted between liver density and the values of CRS, NT-proBNP level, and creatinine; negative correlations were identified between liver density and GFR, LVEF, and 6MWT results, regardless of the status of carbohydrate metabolism. Inflammation and fibrosis are the main factors determining the progression of liver disease. Fibrosis in patients with NAFLD is associated not only with the risk of morbidity and mortality from liver pathology, but also with the cardiovascular risk.

CONCLUSION

Taking into account the presence of positive correlations between CAP and liver enzymes in all groups of CHF patients, regardless of the status of carbohydrate metabolism, on the one hand, and positive correlations between CAP and HbA1c and liver density in patients with ADHF and CMD, on the other hand, determining the level of liver enzymes and CAP and performing liver fibroscan testing will allow to identify a group of patients with a combination of steatosis/fibrosis and pronounced congestion.

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Authors' contribution

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Parameters of leukopoiesis and thrombocytopenia in early urosepsis as potential predictors of a lethal outcome in hospitalized patients

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ABSTRACT

Aim. To perform a comparative analysis of leukopoiesis parameters and platelet count in peripheral blood with evaluation of their changes in the first 48 hours from urosepsis (US) verification in hospitalized patients depending on the outcome of the disease.

Materials and methods. A retrospective comparative study included 40 patients with US divided into a group of deceased (n = 10) and a group of recovered (n = 30) individuals. Along with a full clinical and paraclinical examination, which is a routine practice in the urology clinic in case of suspected (confirmed) sepsis, we performed a differentiated assessment of leukopoiesis and platelet count in peripheral blood at baseline (at the moment of US verification) and 48 hours after US verification. The assessment included determination of the immature granulocyte count, investigation of neutrophil granularity intensity (NEUT-GI) and neutrophil reactivity intensity (NEUT-RI), and measurement of the mean platelet volume (MPV).

Results. The baseline level of organ dysfunction graded by the SOFA (Sequential Organ Failure Assessment) score was significantly higher in deceased patients than in survivors (6 points vs. 3 points, respectively; p = 0.001). The group of the deceased was characterized by lower platelet and monocyte levels. The ROC analysis with the calculation of area under the curve (AUC) identified the following potential predictors of a lethal outcome in US: proportion of monocytes from the total leukocyte count at baseline ≤ 5.5% (AUC 0.732, p = 0.032), proportion of eosinophils from the total leukocyte count at baseline ≤ 0% (AUC 0.756, p = 0.011), absolute eosinophil count at baseline ≤ 0.01 × 10^9 / l (AUC 0.802, p = 0.009), absolute basophil count at baseline ≤ 0.03 × 10^9 / l (AUC 0.718, p = 0.028), NEUT-GI at baseline ≤ 153.2 scatter intensity (SI) units (AUC 0.754, p = 0.021), NEUT-RI at baseline ≤ 59.3 SI units (AUC 0.737, p = 0.024) and their increase after 48 hours by > 0.9 SI units (AUC 0.852, p = 0.001) or by > 1.34% (AUC 0.844, p = 0.003), platelet count at baseline ≤ 144 × 10^9 / l (AUC 0.762, p = 0.007) and after 48 hours ≤ 174 × 10^9 / l (AUC 0.769, p < 0.007).

Conclusion. The assessment of the platelet count and leukopoiesis parameters, including the ones characterizing neutrophil maturation (NEUT-RI, NEUT-GI), in the first 48 hours from US verification, can be effective predictors of a lethal outcome in patients with US.

Keywords: urosepsis, lethal outcome, NEUT-GI, NEUT-RI, immature reticulocytes, MPV, lymphopenia, thrombocytopenia, anemia

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Показатели лейкоцитарного ростка кроветворения и тромбоцитопения в ранние сроки развития уросепсиса как потенциальные предикторы летального исхода у госпитализированных пациентов

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РЕЗЮМЕ

Цель. Выполнение сравнительного анализа показателей лейкоцитарного ростка гемопоэза и уровня тромбоцитов в периферической крови с оценкой характера их изменений в первые 48 ч от момента верификации уросепсиса (УС) у госпитализированных пациентов в зависимости от исхода болезни.

Материалы и методы. Проведено ретроспективное сравнительное исследование 40 пациентов с УС, разделенных на группу умерших (n = 10) и выздоровевших (n = 30). Наряду с полным клиническим и параклиническим обследованием, принятым в урологической клинике при подозреваемом (подтвержденном) сепсисе, исходно в момент верификации УС и через 48 ч проводилась дифференцированная оценка в периферической крови форменных элементов лейкоцитарного гемопоэтического ростка и тромбоцитов, включающая подсчет числа незрелых гранулоцитов, исследование интенсивности нейтрофильной зернистости (NEUT-GI) и реактивности (NEUT-RI) нейтрофилов, а также среднего объема тромбоцитов (MPV).

Результаты. Исходно уровень органной дисфункции, оцененный по шкале SOFA (Sequential Organ Failure Assessment), у умерших пациентов был значимо выше, чем у выживших (6 баллов vs 3 баллов соответственно; p = 0,001). Группа умерших отличалась более низкими уровнем тромбоцитов и моноцитов. ROC-анализ с расчетом AUC (площадь под кривой) позволил выявить следующие потенциальные предикторы летального исхода при УС: доля моноцитов от общего числа лейкоцитов исходно ≤5,5% (AUC 0,732; p = 0,032), доля эозинофилов от общего числа лейкоцитов исходно ≤0% (AUC 0,756; p = 0,011) и абсолютное число эозинофилов исходно ≤0,01 × 10^9/л (AUC 0,802; p = 0,009), абсолютное число базофилов исходно ≤0,03 × 10^9/л (AUC 0,718; p = 0,028), NEUT-GI исходно ≤153,2 единицы интенсивности флуоресценции (ИФ) (AUC 0,754; p = 0,021), NEUT-RI исходно ≤59,3 ИФ (AUC 0,737; p = 0,024) и их увеличение через 48 ч на более чем 0,9 ИФ (AUC 0,852; p = 0,001) или на более чем 1,34% (AUC 0,844; p = 0,003), уровень тромбоцитов исходно ≤144 × 10^9/л (AUC 0,762; p = 0,007) и через 48 ч ≤174 × 10^9/л (AUC 0,769; p < 0,007).

Заключение. Оценка уровня тромбоцитов, а также показателей лейкоцитарного ростка гемопоэза, включая параметры, характеризующие активацию нейтрофилов (NEUT-RI, NEUT-GI), в первые 48 ч от момента диагностики септического состояния может быть полезной при прогнозировании летального исхода у пациентов с УС.

Ключевые слова: уросепсис, летальный исход, NEUT-GI, NEUT-RI, незрелые ретикулоциты, MPV, лимфопения, тромбоцитопения, анемия

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.

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INTRODUCTION

Urosepsis (urogenic sepsis, US) is characterized by clinical manifestations of urinary tract infection (UTI) and / or male genital tract infection, which are complicated by the development of acute systemic organ dysfunction. The prevalence of US among all sepsis cases varies significantly and ranges from 9 to 31% [1].

Regardless of the lesion intensity, urinary tract obstruction is the main risk factor for US development. According to the retrospective study by R.S. Hotchkiss et al. (2003), this risk factor was associated with the development of US in men in 78% and in women – in 54% of cases [2]. At the same time, this complication most often develops in women (approximately in 2/3 of cases) [3, 4].

In addition to high prevalence, the problem of US is also relevant due to high mortality of patients which can be as high as 30–49% [5].

The diagnosis of US is based on the detection of UTI and / or male genital tract infection in combination with acute organ dysfunction, as determined by the Sequential Organ Failure Assessment (SOFA) score. The SOFA score is an important tool for assessing the risk of death and predicting the duration of hospitalization and stay in the intensive care unit (ICU) [6]. Thus, the SOFA score ≥ 2 is associated with a mortality risk of > 10% [4]. However, in real clinical practice, the search for reliable biomarkers and criteria for a dynamic assessment of the patient's condition at an early stage (e.g., in the first 24–48 hours) of US development remains relevant. It will allow to more accurately identify patients at high risk of a lethal outcome and timely adjust the pharmacotherapeutic approach to their treatment.

The **aim of the study** was to perform a comparative analysis of leukopoiesis parameters and platelet count in peripheral blood with evaluation of their changes in the first 48 hours from urosepsis (US) verification in hospitalized patients depending on the outcome of the disease.

MATERIALS AND METHODS

Based on the study protocol approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 8616/1 of 29.03.2021).), a retrospective comparative study was performed that included data of 40 patients with US hospitalized to Siberian State Medical University clinics via the ICU with acute infection or via planned admission to the urology unit with subsequent development of infectious complications from 01.01.2019 to 30.04.2023 (continuous sampling). Within the study, two comparison groups were formed depending on the outcome of hospitalization (discharge from the hospital or a fatal outcome) for a dynamic assessment of clinical, anamnestic, and laboratory parameters in early US (first 48 hours) in order to determine their relationship with the outcome of the disease.

The study included data obtained from patients with confirmed bacterial UTI and the quick SOFA (qSOFA) score of at least 2, as well as with the presence of comprehensive information about the disease and the clinical and laboratory parameters of interest, as stated in the medical record of the inpatient and in the medical information system used by the medical institution.

Data on the nature, timing, and outcome of hospitalization, as well as anthropometric data were analyzed. The qSOFA and SOFA scores, duration of US, and data on the patient's stay in the ICU were recorded for all patients. We performed a dynamic assessment (at the moment of US verification and after 48 hours) of standard leukopoiesis parameters and platelet count in peripheral blood, including the differential assessment of blood cell count and the neutrophil-to-lymphocyte ratio (NLR). The use of the extended version of the Sysmex XN-1000 hematology analyzer software (Sysmex, Germany) allowed to additionally evaluate such parameters as immature granulocyte (IG) count, neutrophil granularity intensity (NEUT-GI), neutrophil reactivity intensity (NEUT-RI), mean platelet volume (MPV), platelet crit (PCT), and the

proportion of reactive lymphocytes (RE-LYMP) and antibody-synthesizing lymphocytes (AS-LYMP).

Statistical analysis was performed using the StatSoft STATISTICA 12.5 program. Quantitative variables were presented as the median and the interquartile range (*Me* (Q_1; Q_3)). Qualitative variables were presented as absolute and relative frequencies (*n* (%)). Quantitative and qualitative variables in independent samples were compared using the Mann – Whitney *U*-test and the Fisher's exact test. Quantitative variables in dependent samples were compared using the Wilcoxon test. The ROC analysis was performed using the MedCalc software package, Version 18.9.1. The area under the curve (AUC) with 95% confidence interval (CI), the cut-point according to the Youden's index, as well as sensitivity and specificity for this point were evaluated. The results were considered statistically significant at $p < 0.05$.

RESULTS

Clinical and anamnestic characteristics of the comparison groups

In accordance with the protocol, 40 patients of both sexes were included in the study: 21 (53.0%) women and 19 (47.0%) men. Depending on the outcome of the disease (discharge from the hospital or a fatal outcome), two comparison groups were formed: group 1 (*n* = 30) included hospitalized patients with a favorable outcome (survivors), group 2 (*n* = 10) encompassed hospitalized patients with a fatal outcome (deceased).

The majority of the patients (*n* = 38, 95.0%) included in the study were hospitalized in the urology unit of Siberian State Medical University clinics via the ICU. Two patients (5.0%) were hospitalized following planned admission for surgical treatment of urolithiasis and benign prostatic hyperplasia, but subsequently developed infection complicated by US.

The age of the patients was 64.5 (48.0; 75.0) years. It was noted that the patients in the deceased group belonged to an older age group (77 (65; 83) years) than the patients discharged from the hospital with a favorable outcome (60 (34; 75) years, $p = 0.026$).

The groups of patients did not differ in the time of US detection, which was 3.0 (1.0; 6.0) days in the group of survivors and 2.5 (1.0; 6.0) days in the group of deceased patients ($p = 0.775$). Twenty patients (50% of the total number) required transfer to the ICU, of whom 50% of patients died (*n* = 10).

Analyzing the causes of US development, we found that 24 patients had acute nonobstructive (*n* = 17) or obstructive (*n* = 8) pyelonephritis, 7 patients had renal carbuncle, 3 patients were followed up for chronic bilateral pyelonephritis, 2 patients were hospitalized with a renal abscess, and 1 patient was hospitalized with a renal and related retroperitoneal abscess. Surgery was performed in 30 (75%) patients, with 9 patients out of 10 (90%) in the group of deceased patients undergoing surgery. The data analysis identified 5 patients with infection directly caused by medical manipulations. Thus, catheter-associated UTI was verified in 3 patients, and UTI caused by surgical interventions was verified in 2 patients.

When taking a history, the presence of comorbidity was recorded. Ischemic heart disease was registered in 12 (30%) patients, history of acute myocardial infarction – in 6 (15%) patients, history of stroke – in 6 (15%) cases, diabetes mellitus – in 7 (17.5%) patients, stage 2–3 chronic heart failure – in 9 (22.5%) patients, bronchial asthma – in 2 (5%) cases, chronic obstructive pulmonary disease – in 3 (7.5%) patients. HIV infection was observed in 2 (5%) patients, chronic kidney disease – in 8 (20%) patients, intravenous drug abuse – in 1 (2.5%) patient, and alcohol abuse – in 2 (5%) patients. Also, 7 (17.5%) patients included in the study had active cancer. The groups differed significantly only in the presence of hypertension, which was registered in 15 (50%) survivors and in 9 (90%) deceased patients ($p = 0.032$).

Chest radiography revealed that infiltrative changes in the lung parenchyma during hospitalization emerged in 7 (23.3%) hospitalized patients with a favorable outcome and in 2 (20%) hospitalized patients who died. These radiographic changes with corresponding clinical manifestations were registered ≥ 48 hours from the moment of hospitalization. Therefore, hospital-acquired pneumonia was diagnosed, the development of which was associated with the progressive course of the underlying disease and / or invasive mechanical ventilation [7].

Progression of UTI was characterized by leukocyturia and bacteriuria according to urinalysis in 100% of patients. At the time of US verification, all patients underwent bacteria culture tests of blood and urine. It was found that UTI in all examined patients was caused by one pathogen, most often

from the *Enterobacteriaceae* family. The dominant pathogen was *Escherichia coli* (in 73% of patients) and *Enterococcus faecium* (in 13% of cases); much less frequently (not more than in 1 case), the infection was caused by *Klebsiella pneumoniae*, *Acinetobacter baumannii*, etc. Positive blood culture test was obtained in 3 (7.5%) patients: *Klebsiella pneumoniae* was identified in 1 patient, *Escherichia coli* – in 1 patient, and *Enterococcus faecium* – in 1 patient.

The comparison groups did not differ significantly in the choice of drugs for initial antibiotic therapy (ABT), which was based on the stratification of patients by the presence of antibiotic resistance and complied with current clinical guidelines [8]. Thus, amoxicillin + clavulanic acid was received by 5 (12.5%) patients, cefuroxime – by 1 (2.5%) patient, cefotaxime – by 12 (30%) patients, ceftriaxone – by 3 (7.5%) patients, cefepime – by 1 (2.5%) patient, cefoperazone + sulbactam – by 1 (2.5%) patient, ciprofloxacin – by 6 (15%) patients, levofloxacin – by 6 (15%) patients, ertapenem – by 3 (7.5%) patients, and meropenem – by 2 (5%) patients. In 33 (82.5%) patients, 1 (1; 2) substitution of the anti-infective drug / modification of ABT regimen was required due to aggravation of the clinical course of the disease (in 15 (37.5%) patients) or following the results of the microbiological examination and antibiotic sensitivity test in case of doubtful efficacy of initial therapy – in 18 (45%) patients.

At the time of US verification in patients with a lethal outcome, the SOFA score was 6 (4; 7) points, while in the survivors, it was significantly lower – 3 (1; 5) points ($p = 0.001$).

Results of blood tests

The detailed analysis of the hemogram did not reveal significant differences in most of the studied parameters. In contrast to the recovered patients, the group of fatal patients differed in thrombocytopenia at both time points: 235 (178; 392) $\times 10^9$ / l vs. 105 (82; 194) $\times 10^9$ / l, respectively, at baseline ($p = 0.019$) and 262 (203; 358) $\times 10^9$ / l vs. 101 (97; 174) $\times 10^9$ / l, respectively, after 48 hours ($p = 0.058$). At the same time, the comparison groups did not differ significantly in MPV and platelet distribution width (PDW).

Changes in the most significant leukopoiesis parameters of peripheral blood are presented in the Table. The comparison groups were characterized by a steady increase in the neutrophil count in the first 48 hours from the moment of US verification without significant differences in the number of mature and immature granulocytes, lymphocytes, and NLR value (Table).

Table

Changes in the leukopoiesis parameters of peripheral blood in the first 48 hours after US verification, *Me* (Q_1; Q_3)

Parameter	Group 1 – hospitalized patients with a favorable outcome	Group 2 – hospitalized patients with a lethal outcome	p_{1-2}
Leukocytes, 10^9 / l, at baseline	11.74 (8.15; 21.60)	10.27 (5.90; 17.80)	0.391
Leukocytes, 10^9 / l, after 48 hours	10.86 (6.14; 15.63)	12.17 (10.10; 12.70)	0.942
Neutrophils, %, at baseline	85.4 (75.2; 88.9)	88.0 (69.7; 93.2)	0.571
Neutrophils, %, after 48 hours	79.7 (71.3; 85.1)	85.2 (84.2; 89.3)	0.215
Neutrophils, 10^9 / l, at baseline	9.27 (5.40; 18.96)	8.94 (5.20; 9.57)	0.524
Neutrophils, 10^9 / l, after 48 hours	7.91 (4.61; 13.45)	11.06 (10.25; 12.12)	0.616
NEUT-GI, SI, at baseline	158.6 (154.1; 161.2)	152.9 (148.2; 159.2)	0.065
NEUT-GI, SI, after 48 hours	157.3 (151.3; 160.1)	148.4 (144.4; 154.9)	0.160
NEUT-RI, SI, at baseline	59.2 (49.1; 62.6)	48.3 (46.9; 56.8)	0.086
NEUT-RI, SI, after 48 hours	56.9 (50.8; 60.5)	54.8 (47.8; 61.7)	0.670
Changes in NEUT-RI (T2–T1), SI units	–1.65 (–3.85; 0.35)	4.15 (–0.20; 9.70)	**0.033**
Changes in NEUT-RI (T2–T1), SI, %	–3.24 (–6.92; 0.69)	7.44 (–0.49; 19.24)	0.038
Lymphocytes, %, at baseline	7.3 (4.7; 16.3)	7.4 (5.5; 13.1)	0.941
Lymphocytes, %, after 48 hours	12.4 (8.2; 16.6)	8.4 (4.7; 9.0)	0.197
Lymphocytes, 10^9 / l, at baseline	1.12 (0.76; 1.64)	0.65 (0.60; 1.06)	0.058
Lymphocytes, 10^9 / l, after 48 hours	1.42 (0.81; 2.01)	0.80 (0.51; 1.02)	0.175
NLR	12.84 (4.52; 21.46)	14.54 (7.39; 19.86)	0.658
NLR	6.91 (4.49; 11.62)	15.87 (5.34; 65.93)	0.345
Monocytes, %, at baseline	6.0 (4.5; 7.7)	4.4 (3.1; 5.2)	**0.038**
Monocytes, %, after 48 hours	6.4 (4.7; 9.0)	4.3 (3.6; 6.7)	0.185
Monocytes, 10^9 / l, at baseline	0.80 (0.41; 1.12)	0.55 (0.21; 0.59)	0.132

Table (continued)

Parameter	Group 1 – hospitalized patients with a favorable outcome	Group 2 – hospitalized patients with a lethal outcome	p_{1-2}
Monocytes, 10^9 / l, after 48 hours	0.69 (0.37; 0.88)	0.44 (0.44; 0.46)	0.161
Eosinophils, %, at baseline	0.4 (0.1; 1.4)	0.0 (0.0; 0.2)	**0.021**
Eosinophils, %, after 48 hours	0.7 (0.3; 1.6)	0.0 (0.0; 0.0)	0.062
Eosinophils, 10^9 / l, at baseline	0.065 (0.030; 0.125)	0.010 (0.010; 0.040)	0.055
Eosinophils, 10^9 / l, after 48 hours	0.085 (0.050; 0.155)	0.105 (0.090; 0.120)	0.531
Basophils, %, at baseline	0.3 (0.2; 0.5)	0.2 (0.1; 0.2)	0.067
Basophils, %, after 48 hours	0.3 (0.2; 0.4)	0.6 (0.3; 0.7)	0.236
Basophils, 10^9 / l, at baseline	0.040 (0.020; 0.070)	0.025 (0.015; 0.030)	0.062
Basophils, 10^9 / l, after 48 hours	0.030 (0.020; 0.040)	0.040 (0.010; 0.090)	0.716
IG, %, at baseline	0.90 (0.50; 2.65)	1.70 (0.50; 2.70)	0.562
IG, %, after 48 hours	0.75 (0.40; 2.55)	0.50 (0.15; 4.10)	0.509
IG, 10^9 / l, at baseline	0.11 (0.04; 0.60)	0.49 (0.08; 0.77)	0.349
IG, 10^9 / l, after 48 hours	0.08 (0.03; 0.25)	0.17 (0.06; 0.57)	0.693

Note. SI – scatter intensity.

It should be noted that the group of patients with a lethal outcome was characterized by smaller relative monocyte and eosinophil counts in the blood at baseline. At the same time, the eosinophil count in this group at the time of US verification was actually close to zero, amounting to 10 kl / μl (Table).

Despite the fact that NEUT-RI in the comparison groups did not differ significantly at baseline and 48 hours after US verification, the nature of changes in this parameter in the groups in the first two days was multidirectional ($p < 0.05$). In the group of patients with a lethal outcome, NEUT-RI increased by almost 7.5%, while in the group with a favorable outcome, it decreased by more than 3% (Table).

Verification of potential early predictors of mortality in US by the ROC analysis

Predictors of an unfavorable outcome in US can be:

– proportion of monocytes from the total leukocyte count at baseline ≤ 5.5% with the sensitivity of 88.9% and specificity of 57.1% (AUC 0.732, 95% CI (0.561; 0.864), $p = 0.032$);

– proportion of eosinophils from the total leukocyte count at baseline ≤ 0% with the sensitivity of 66.67% and specificity of 82.14% (AUC 0.756, 95% CI (0.587; 0.882), $p = 0.011$) and absolute eosinophil count ≤ 0.01 × 10^9 / l with the sensitivity of 75.0% and specificity of 83.3% (AUC 0.802, 95% CI (0.609; 0.927), $p = 0.009$);

– absolute basophil count at baseline ≤ 0.03 × 10^9 / l with the sensitivity of 87.5% and specificity of 66.7% (AUC 0.718, 95% CI (0.540; 0.856), $p = 0.028$);

– NEUT-GI at baseline ≤ 153.2 SI units with the sensitivity of 66.7% and specificity of 79.0% (AUC 0.754, 95% CI (0.543; 0.903), $p = 0.021$);

– NEUT-RI at baseline ≤ 59.3 SI units with the sensitivity of 100.0% and specificity of 47.4% (AUC 0.737, 95% CI (0.524; 0.891), $p = 0.024$) and an increase in this index after 48 hours by > 0.9 SI units with the sensitivity of 75.0% and specificity of 87.5% (AUC 0.852, 95% CI (0.623; 0.969), $p = 0.001$) or by > 1.34% with the sensitivity of 75.0% and specificity of 87.5% (AUC 0.844, 95% CI (0.614; 0.965), $p = 0.003$);

– platelet count at baseline ≤ 144 × 10^9 / l with the sensitivity of 66.7% and specificity of 82.8% (AUC 0.762, 95% CI (0.597; 0.885), $p = 0.007$) and after 48 hours ≤ 174 × 10^9 / l with the sensitivity of 80.00% and specificity of 75.86% (AUC 0.769, 95% CI (0.593; 0.896), $p < 0.007$). The identified critical values of factors that increase the probability of a lethal outcome are presented in the Figure.

DISCUSSION

Regardless of the localization of the source of infection, sepsis is a life-threatening condition characterized by systemic inflammation with the development of dysfunction of various organs, hemodynamic disorders, systemic hypotension, and tissue hypoxia. The mechanisms of sepsis cannot but affect the blood system, which is most often associated with the development of coagulation disorders and thrombocytopenia, as well as multidirectional changes in the leukocyte formula [9].

In sepsis, low platelet count is a well-known biomarker of disease severity. Recently, researchers

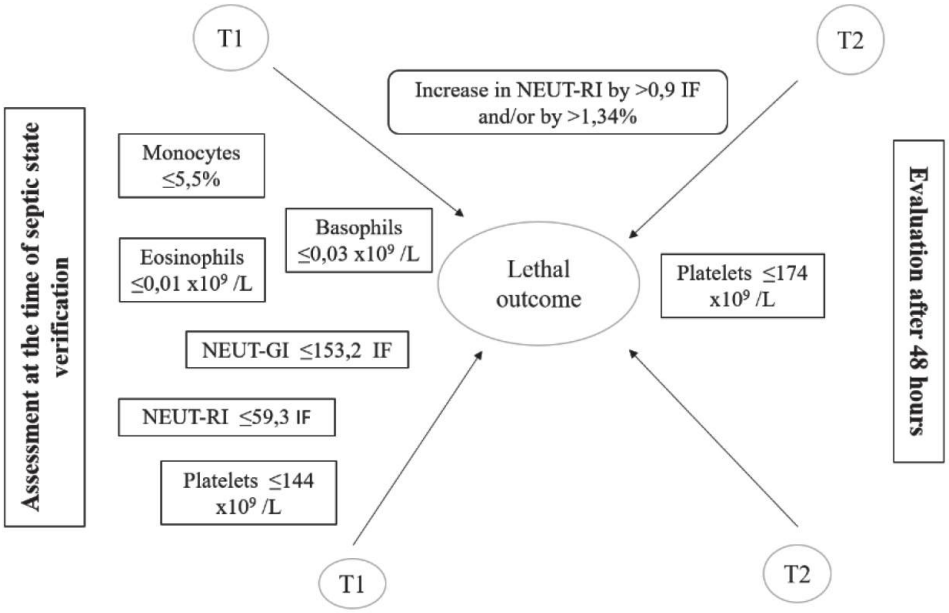

Figure. Factors associated with mortality in urosepsis: T1 – baseline value; T2 – evaluation after 48 hours

have focused on the role of platelets in the pathogenesis of multiorgan failure and have considered them as a potential therapeutic target in sepsis [10]. It is assumed that the predominant processes here include peripheral platelet consumption, determined by platelet activation, chemotaxis, and isolation in the microcirculation. Immune destruction and disseminated intravascular coagulation are also discussed [11]. In the present study, the group of deceased patients was characterized by decreased platelet counts at both time points. It is worth noting that the platelet counts at the time of US verification and after 48 hours were ≤ 144 × 10^9 / l and ≤ 174, respectively, and were associated with a lethal outcome.

In sepsis, infection is known to trigger a complex and prolonged host response involving both innate and adaptive immunity. An imbalance in the production of pro- and anti-inflammatory immunoregulatory molecules and inadequate involvement of effector cells impair the host response to infectious agents and cause tissue damage. Recent studies including patients with US have confirmed that significant depletion of circulating CD4+ and CD8+ lymphocytes was associated with a lethal outcome in this group of patients [12].

In our study, a significant increase in the absolute neutrophil count was recorded in the first 48 hours in both groups. In the group of patients with an unfavorable outcome of US treatment, a trend toward absolute lymphocytopenia (< 1.0 × 10^9 / l) with lower baseline monocyte counts was noted. The eosinophil count in this group of patients approached zero.

Eosinopenia is often observed in severe non-parasitic infections characterized by a shift in hematopoiesis toward an increase in the number of neutrophil granulocytes in peripheral blood. According to H. Shaaban et al. (2010), eosinophil count < 50 cells / μl with the sensitivity of 81% and specificity of 65% was associated with the presence of sepsis in adults [13]. A systematic literature review noted that of 39 analyzed studies on the role of eosinophils in sepsis, 11 studies demonstrated an association between eosinopenia and sepsis, and 8 studies found persistent eosinopenia > 48 hours after admission to the ICU. The authors concluded that persistent peripheral eosinopenia was a marker of bacterial sepsis and was independently associated with adverse outcomes, such as death or re-hospitalization [14].

In our study, the baseline eosinophil count (values at the time of US verification) of 0% and monocyte count of ≤ 5.5% in the leukocyte formula, as well as the absolute eosinophil count of ≤ 10 cells / μl and the absolute basophil count ≤ 30 cells / μl were registered as predictors of a lethal outcome. The obtained data regarding the prognostic value of the basophil count in peripheral blood in US patients

correlate with the data obtained by X. Chen et al. (2023), who found that the absence of basophils in blood in patients with sepsis in ICU was associated with critical progression of the disease, positively correlated with 28-day mortality, and served as an independent predictor of an unfavorable outcome for this group of patients (odds ratio (OR) 3.425, 95% CI (3.717–3.165), $p < 0.001$) [15].

Monocytes play an important role in the development of sepsis. However, the diagnostic and prognostic value of changes in the monocyte count is controversial. Some authors report an increase in the number of monocytes in peripheral blood, while others describe monocytopenia associated with increased mortality [16, 17].

Modern capabilities of hematology analyzers allow to additionally assess such parameters as NEUT-GI and NEUT-RI, which, in our opinion, have a prognostic potential in US. These parameters characterize the innate immune response: an increase in NEUT-GI reflects intensification of the inflammatory process, and NEUT-RI reflects the metabolic activity of the neutrophil population [18]. NEUT-RI can potentially correlate with the development of sepsis [19]. Thus, NEUT-RI can predict the emergence of IGs in the peripheral blood, thereby acting as an early marker of bacterial infection. At the same time, an increase in the NEUT-RI levels correlated with an increase in the concentration of immunoglobulins in peripheral blood within 72 h from the development of infection [20]. Similarly, in the study by R.J. Dinsdale et al. (2017), the NEUT-RI value was significantly greater in patients with sepsis after burn injury compared to patients without sepsis, indicating the possibility of early diagnosis of sepsis [18]. The study by E. Mantovani et al. (2023) demonstrated that NEUT-RI showed AUC > 0.80 and better prognostic value of a negative result than procalcitonin and C-reactive protein in patients in ICU for the diagnosis of US (87.4 vs. 83.9% and 86.6%, $p = 0.038$) [20].

Despite the fact that in our study, the comparison groups with US did not differ significantly in the NEUT-RI value at baseline and after 48 hours, the nature of changes in this parameter in the first two days in the groups was multidirectional ($p < 0.05$). Thus, in the group of patients with a lethal outcome, the NEUT-RI value continued to grow from lower values, while in the group with a favorable outcome, a trend toward a decrease in this parameter was noted. The ROC analysis revealed that the baseline value of NEUT-RI ≤ 59.3 SI units and the increase in this parameter by > 0.9 SI units after 48 hours predicted the onset of a lethal outcome. It is worth noting that baseline NEUT-GI ≤ 153.2 SI units was also associated with a lethal outcome in US patients. It is possible that delayed activation of neutrophils with relatively low baseline granularity in US reflects a delayed and inadequate response to infection, which may be associated with an increased risk of a lethal outcome in US.

CONCLUSION

The results of the study showed that certain blood parameters can serve as predictors of a lethal outcome in US. For example, it was found that the low platelet counts at the time of US verification and after 48 hours, as well as changes in the values of some parameters in the leukocyte formula may be associated with a lethal outcome.

Such parameters as NEUT-GI and NEUT-RI may also play an essential role in predicting the outcome of sepsis. Their changes in the first days of the disease can be evidence of the disease severity and indicate an inadequate immune response to infection.

Therefore, the hematologic parameters analyzed in this study may be effective for assessing the risk of a lethal outcome in US and may be used as predictors of its development. Further studies and clinical observations may help to clarify their role in prognosis and treatment of this group of patients.

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Authors' contribution

Fedosenko S.V. – conception and design, coordination of the study, drafting of the article, review of literature, final approval of the manuscript for publication. Rodionova Yu.O. – compilation of the database, acquisition and interpretation of clinical data. Ivanova A.I. – statistical processing of the data, interpretation of the data, drafting of the article. Arzhanik M.B. – conception and design, statistical processing of the data, interpretation of the data, critical revision of the manuscript for important intellectual content. Semenova O.L. – statistical processing of the data. Nesterovich S.V. – coordination of the study, critical revision of the manuscript for important intellectual content. Starovoitova E.A. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Zima A.P. – interpretation of the laboratory data, critical revision of the manuscript for important intellectual content. Vinokurova D.A., Kamaltynova E.M. – critical revision of the manuscript for important intellectual content, drafting of the manuscript. Kalyuzhin V.V. – review of literature, interpretation of the data, final approval of the manuscript for publication.

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Prognostic value of elevated transaminase levels as predictors of adverse outcomes in patients with acute myocardial infarction

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ABSTRACT

Aim. To assess the prevalence of elevated serum liver transaminases (LTs), including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and their impact on in-hospital and long-term mortality in patients with acute myocardial infarction (AMI).

Materials and methods. The prospective observational study included 416 consecutive AMI patients (median age 65 years, 40.9% female, 46.9% with ST elevation) without prior liver diseases, who underwent coronary angiography within 24 hours after hospitalization. AST and ALT levels were measured upon admission. LTs were considered as abnormal when their levels exceeded the local upper limit of normal. Clinical endpoints were all-cause in-hospital and 18-month mortality. Associations between clinical endpoints and various risk factors, including LT levels, were assessed by the multivariate logistic regression analysis.

Results. Elevated LT levels were seen in 28.6% of AMI patients: an isolated increase in ALT was noted in 17.8% of patients, while an isolated increase in AST was registered in 25% of cases. In-hospital and 18-month mortality was 5.8 and 11.3%, respectively. Abnormal LT levels were associated with the presence of ST elevation (odds ratio (OR) 1.873, 95% confidence interval (CI) 1.218–2.881, $p = 0.004$), lower systolic and diastolic blood pressure (OR 0.993, 95% CI 0.986–1.0, $p = 0.04$ and 0.979, 95% CI 0.964–0.994, $p = 0.007$, respectively), higher Killip class (OR 1.510, 95% CI 1.142–1.999, $p = 0.004$), and higher creatinine level (OR 1.010, 95% CI 1.003–1.016, $p = 0.004$). In the multivariate analysis, elevated LT levels were independently associated with in-hospital and 18-month mortality (OR 3.607, 95% CI 1.199–10.848, $p = 0.022$ and 2.182, 95% CI 1.011–4.708, $p = 0.047$, respectively).

Conclusion. Elevated LT levels were present in about a third of patients with AMI. They were associated with specific clinical, biological, and prognostic features, including in-hospital and long-term mortality in AMI patients.

Keywords: acute myocardial infarction; alanine transaminase; aspartate transaminase; in-hospital mortality; long-term mortality, prognosis

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

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study was approved by the Ethics Committee at RUDN University.

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Прогностическая ценность повышенного уровня трансаминаз в качестве предиктора неблагоприятного исхода у больных с острым инфарктом миокарда

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РЕЗЮМЕ

Цель. Оценить распространенность повышенного уровня аланинаминотрансферазы (аланинтрансаминазы, АЛТ) и аспартатаминотрансферазы (аспартаттрансаминазы, АСТ) у больных с острым инфарктом миокарда (ОИМ) и их возможное прогностическое влияние на госпитальную и долгосрочную смертность.

Материалы и методы. Проспективное наблюдательное исследование включало 416 последовательных пациентов с ОИМ без известного на момент госпитализации заболевания печени (медиана возраста 65 лет, 40,9% женщин, 46,9% с подъемом сегмента ST), которым выполняли коронарографию в течение первых 24 ч после поступления в стационар. Сывороточные показатели АСТ и АЛТ определялись сразу при поступлении. Значения сывороточных трансаминаз считались повышенными, если их уровень превышал верхнюю границу нормы, определенной для локальной лаборатории. Конечными клиническими точками обсервационного исследования были определены внутрибольничная и 18-месячная смертность. Связь между клиническими конечными точками и вероятными факторами риска, включая уровень сывороточных трансаминаз, оценивались с применением многофакторного логистического регрессионного анализа.

Результаты. Повышенные значения трансаминаз наблюдались у 28,6% пациентов с ОИМ: изолированное повышение АЛТ отмечалось у 17,8% больных, изолированная гиперферментемия АСТ – в 25% случаев. Внутрибольничная и 18-месячная смертность в исследовании составили 5,8 и 11,3% соответственно. Повышение уровня трансаминаз было связано с регистрацией подъема сегмента ST на электрокардиограмме (отношение шансов (ОШ) 1,873; 95%-й доверительный интервал (ДИ) 1,218–2,881; p = 0,004), более низким систолическим и диастолическим артериальным давлением (ОШ 0,993; 95%-й ДИ 0,986–1,0; p = 0,04 и 0,979; 95%-й ДИ 0,964–0,994; p = 0,007 соответственно), высоким классом острой сердечной недостаточности по шкале Killip (ОШ 1,510; 95%-й ДИ 1,142–1,999; p = 0,004) и повышением уровня креатинина (ОШ 1,010; 95%-й ДИ 1,003–1,016; p = 0,004). В многофакторном анализе повышение трансаминаз независимо было ассоциировано с внутрибольничной и 18-месячной смертностью (ОШ 3,607; 95%-й ДИ 1,199–10,848; p = 0,022 и 2,182; 95%-й ДИ 1,011–4,708; p = 0,047 соответственно).

Заключение. Повышенный уровень трансаминаз обнаружен у трети пациентов с ОИМ. Он ассоциирован с определенными клиническими, биологическими и прогностическими особенностями, включая отрицательное прогностическое влияние на внутрибольничную и долгосрочную смертность пациентов с ОИМ.

Ключевые слова: аланинтрансаминаза, аспартаттрансаминаза, внутрибольничная смертность, долгосрочная смертность, острый инфаркт миокарда, прогноз

Конфликт интересов. Авторы заявляют об отсутствии явного или потенциального конфликта интересов, связанного с публикацией данной статьи.

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

Соответствие принципам этики. Все участники подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом Российского университета дружбы народов им. Патриса Лумумбы.

Для цитирования: Хоанг Ч.Х., Майсков В.В., Мерай И.А., Кобалава Ж.Д. Прогностическая ценность повышенного уровня трансаминаз в качестве предиктора неблагоприятного исхода у больных с острым инфарктом миокарда. *Бюллетень сибирской медицины.* 2024;23(3):126–135. https://doi.org/10.20538/1682-0363-2024-3-126-135.

INTRODUCTION

Acute myocardial infarction (AMI) is a critical manifestation of coronary artery disease (CAD), characterized by obstruction of coronary artery, leading to myocardial ischemia and subsequent necrosis of myocardial tissue [1]. AMI causes acute heart failure, resulting in reduction of cardiac output, tissue perfusion, and passive venous congestion [2]. These hemodynamic alterations significantly affect the liver, which receives about one-quarter of the total cardiac output [3]metabolism, clearance, and host defense are tightly dependent on an adequate microcirculation. To guarantee hepatic homeostasis, this requires not only a sufficient nutritive perfusion and oxygen supply, but also a balanced vasomotor control and an appropriate cell-cell communication. Deteriorations of the hepatic homeostasis, as observed in ischemia/reperfusion, cold preservation and transplantation, septic organ failure, and hepatic resection-induced hyperperfusion, are associated with a high morbidity and mortality. During the last two decades, experimental studies have demonstrated that microcirculatory disorders are determinants for organ failure in these disease states. Disorders include 1.

In clinical practice, serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) are routinely assessed to evaluate liver function [4]. ALT, primarily localized in hepatocytes with minimal distribution in cardiac, renal, and muscular tissues, serves as a specific marker for hepatic dysfunction [4]. In contrast, AST is derived not only from hepatic tissue but also from various other tissues, including the heart, erythrocytes, skeletal muscles, kidney, and brain. Elevated AST levels are observed following ischemic cell death in these tissues [4]. While previous studies have demonstrated an association between abnormal transaminase levels and cardiovascular outcomes [5–8]as a proxy marker of NAFLD, and death from cardiovascular disease (CVD, the prevalence of elevated transaminase levels and related outcomes in patients with AMI remain understudied.

The aim of the study was to assess the prevalence of elevated serum liver transaminases and factors associated with them in a cohort of AMI patients and to evaluate their impact on in-hospital and long-term all-cause mortality.

MATERIALS AND METHODS

A single-center, prospective, observational study was conducted in the Vinogradov Municipal Clinical Hospital (Moscow, Russia) from January 2021 to December 2022. The study included patients aged >18 years presenting with AMI who underwent coronary angiography < 24 hours from symptom onset. The exclusion criteria were: patients diagnosed with type 3, 4, and 5 myocardial infarction (MI), as well as those who developed MI during hospitalization. Additionally, individuals with elevated liver transaminases (LTs) indicative of hepatitis B or C, cirrhosis of the liver, fatty liver disease, hepatobiliary obstructive disease, bone disease, pancreatitis, infectious diseases or individuals after a known episode of alcohol consumption prior to the index event (the AST / ALT ratio of more than 2) were excluded from the study [9]. The AMI diagnosis was established following the Third Universal Definition of MI [10].

We collected baseline demographic and clinical characteristics, cardiovascular risk factors, comorbidities, physical examination data, as well as blood test and imaging findings (including electrocardiography, echocardiography, and

coronary angiography). Patients with incomplete medical history were excluded from the dataset. All blood samples obtained upon admission were analyzed in the core laboratory of Vinogradov Municipal Clinical Hospital.

Cardiac troponin I levels were measured using the Access 2 Immunoassay System (Beckman Coulter, USA) with a 99th-percentile upper reference limit of 0.02 ng / l. LTs were measured using the Beckman Coulter Clinical Chemistry Analyzer (AU 680) and considered abnormal when the levels exceeded 50 U / l for ALT and 50 U / l for AST. Furthermore, liver injury was classified according to the extent of liver enzyme elevation: mild (1–2 times higher than the upper limit of normal (ULN)), moderate (≥ 2–5 times higher than the ULN), and severe (≥ 5 times higher than the ULN) [11, 12]. Risk stratification of MI patients was assessed using the GRACE (Global Registry of Acute Coronary Events) 2.0 score [13].

The primary endpoint was in-hospital mortality, which was obtained from medical records. The secondary endpoint was 18-month mortality. Mortality was defined as all-cause death that was recorded in patient medical records and death registers. Long-term mortality was evaluated using structured telephone interviews at 1, 3, 6, 12, 15, and 18 months after discharge. At the study closing date, all follow-up information was available. The study complied with the principles of the Declaration of Helsinki and was approved by the local Ethics Committee at the Institute of Medicine, RUDN University. All patients signed an informed consent to participate in the study.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics 25.0 software package (SPSS Inc., Chicago, IL, USA). Categorical variables were described as frequencies and percentages, while continuous variables were presented as the median and the interquartile range (*Me* (Q_1; Q_3)). The Chi-square test or the Fisher's exact test was used to compare categorical variables, and the Kruskal – Wallis test was used for to compare continuous variables between the groups. The univariate and multivariate logistic regression models were used to identify risk factors associated with elevated LTs in AMI patients, as well as factors associated with in-hospital and 18-month mortality. Odds ratio (OR) and 95% confidence interval (CI) were calculated. The differences were considered statistically significant at two-tailed $p < 0.05$.

RESULTS

Baseline clinical characteristics

The study included a total of 411 patients, 170 (40.9%) patients were female, 195 (40.9%) patients presented with ST-elevation. The median age was 65.0 years. The group of patients with elevated ALT and (or) AST levels differed significantly from the general sample and the controls by the incidence of ST-elevation, Killip class II–IV heart failure, higher creatinine levels, chest pain intensity, lower diastolic blood pressure, and higher troponin levels. Other that that, no significant differences between the groups were noted.

Table 1

Baseline characteristics of MI patients

Parameter	Patients, *n* = 416	Normal AST and ALT values, *n* = 297	Elevated AST and ALT, *n* = 119	*p*
Age, years, *Me* (Q_1; Q_3)	65 (56; 74)	65 (55; 74)	65 (57; 76)	0.595
Women, *n* (%)	170 (40.9)	126 (42.4)	44 (37)	0.322
ST-elevation, *n* (%)	195 (46.9)	126 (42.4)	69 (58)	0.005
History of cardiovascular diseases				
Arterial hypertension, *n* (%)	370 (88.9)	259 (87.2)	111 (93.3)	0.084
CAD, *n* (%)	177 (42.5)	132 (44.4)	45 (37.8)	0.229
Previous MI, *n* (%)	85 (20.4)	67 (22.6)	18 (15.1)	0.106
Previous myocardial revascularization, *n* (%)	49 (11.8)	40 (13.5)	9 (7.6)	0.096
Previous HF, *n* (%)	33 (7.9)	20 (6.7)	13 (10.9)	0.163
Diabetes mellitus, *n* (%)	85 (20.4)	57 (19.2)	28 (23.5)	0.347
Previous stroke, *n* (%)	32 (7.7)	20 (6.7)	12 (10.1)	0.308
Previous atrial fibrillation, *n* (%)	43 (10.3)	30 (10.1)	13 (10.9)	0.859
CKD, *n* (%)	32 (7.7)	24 (8.1)	8 (6.7)	0.839
PVD, *n* (%)	12 (2.9)	7 (2.4)	5 (4.2)	0.336

Table 1 (continued)

Parameter	Patients, *n* = 416	Normal AST and ALT values, *n* = 297	Elevated AST and ALT, *n* = 119	*p*
Chronic lung disease, *n* (%)	60 (14.4)	40 (13.5)	20 (16.8)	0.440
Peptic and duodenal ulcer, *n* (%)	39 (9.4)	30 (10.1)	9 (7.6)	0.464
Anemia, *n* (%)	107 (25.7)	72 (24.2)	35 (29.4)	0.321
Chest pain, *n* (%)	380 (91.3)	277 (93.3)	103 (86.6)	0.034
Dyspnea, *n* (%)	81 (19.5)	56 (18.9)	25 (21)	0.681
Killip class II–IV, *n* (%)	98 (23.6)	61 (20.5)	37 (31.1)	0.029
Systolic BP, mm Hg., *Me* (Q_1; Q_3)	138 (120; 160)	140 (120; 160)	130.5 (111.5; 160)	0.063
Diastolic BP, mm Hg., *Me* (Q_1; Q_3)	80 (74; 90)	80 (77; 90)	80 (67.7; 83.2)	0.005
Troponin I, ng / ml, *Me* (Q_1; Q_3)	0.39 (0.10; 2.88)	0.25 (0.09; 1.69)	1.83 (0.30; 7.45)	<0.001
Hemoglobin, g / l, *Me* (Q_1; Q_3)	137 (123; 146)	136 (123; 146)	138 (122; 148)	0.734
ALT, U / l, *Me* (Q_1; Q_3)	25 (18; 40)	21 (15; 28)	53.3 (36; 87)	<0.001
AST, U / l, *Me* (Q_1; Q_3)	29 (23; 49.2)	25 (21; 31)	77 (55; 129.9)	<0.001
Creatinine, μmol / l, *Me* (Q_1; Q_3)	95 (82; 109)	94 (81.2; 108)	96 (84; 121)	0.029
GFR < 60 ml / min / 1.73 m^2, *n* (%)	160 (39.5)	110 (37.2)	54 (45.4)	0.149
LVEF, %, *Me* (Q_1; Q_3)	45 (40; 55)	45 (40; 55)	44 (40; 53)	0.288
No lesion (stenosis) < 50% CA, *n* (%)	55 (13.2)	38 (12.8)	17 (14.3)	0.749
Three-vessel CAD, *n* (%)	203 (48.8)	153 (51.5)	50 (42)	0.084
PCI, *n* (%)	328 (78.8)	235 (79.1)	93 (78.2)	0.894
GRACE score, *Me* (Q_1; Q_3)	117 (97.2; 142.7)	116 (95.5; 140.5)	119 (99; 152)	0.081
Mortality				
In-hospital, *n* (%)	24 (5.8)	11 (3.7)	13 (10.9)	0.009
18-month, *n* (%)	47 (11.3)	27 (9.1)	20 (16.8)	0.038

Note. BP – blood pressure; PVD – peripheral vascular disease; CAD – coronary artery disease; CCI – Charlson comorbidity index; MI – myocardial infarction; CA – coronary artery; HF – heart failure; LVEF – left ventricular ejection fraction; CKD – chronic kidney disease; PCI – percutaneous coronary intervention.

Assessing the results of transaminase tests

The increase in LT levels was detected in 119 (28.6%) patients (ALT alone or AST alone in 17.8 and 25% of cases, respectively). Most of transaminase alterations were mild elevations (Table 2). In the ST-elevation subgroup, 35.4% (*n* = 69) of patients had elevated AST and ALT, 30.8% (*n* = 60) of patients had elevated AST, and 19% (*n* = 37) of the study population had elevated ALT. In non-ST-elevation subgroup, 22.6% (*n* = 50) of patients had elevated AST and ALT, 18.6% (*n* = 41) of patients had elevated AST, and 14% (*n* = 31) of the study population had elevated ALT.

Table 2

Transaminase levels in patients upon admission

Parameter	ALT, *n* = 416	AST, *n* = 416
Normal range, *n* (%)	342 (82.2)	312 (75)
1–2 times higher than ULN, *n* (%)	54 (13)	64 (15.4)
≥2–5 times higher than ULN, *n* (%)	17 (4.1)	28 (6.7)
≥5 times higher than ULN, *n* (%)	3 (0.7)	12 (2.9)

Factors associated with abnormal transaminases at baseline

The univariate analysis showed that abnormal ALT and (or) AST levels at baseline were associated with higher prevalence of ST-elevation, a higher Killip class, higher creatinine levels, and higher systolic and diastolic blood pressure.

Table 3

Univariate logistic regression analysis to assess predictors of abnormal ALT and (or) abnormal AST in patients with acute myocardial infarction

Parameter	OR	95% CI	*p*
ST-elevation (yes / no)	1.873	1.218–2.881	0.004
Systolic BP (per mm Hg)	0.993	0.986–1.0	0.04
Diastolic BP (per mm Hg)	0.979	0.964–0.994	0.007
Killip class (per class)	1.510	1.142–1.999	0.004
Creatinine (per *μmol* / l)	1.010	1.003–1.016	0.004

Abnormal liver function and outcome

All-cause mortality rates were 5.8 and 11.3% for in-hospital and 18-month mortality, respectively. Elevated ALT and AST levels were three times more common in the group of in-hospital mortality compared to patients with normal LT levels (10.9 vs. 3.7%; *p* = 0.009) and two times more common in the 18-month mortality group (16.8 vs. 9.1%, *p* = 0.038) (Table 1). Table 4 and Table 5 present

the results of the multivariate logistic regression analysis, indicating that abnormal LT levels were a significant and independent predictor of in-hospital (OR 3.607; 95% CI: 1.199–10.848; $p = 0.022$) and long-term mortality (OR 2.182; 95% CI: 1.011-4.708; $p = 0.047$). Additionally, factors associated with increased OR for in-hospital and long-term mortality included the presence of anemia, three-vessel coronary artery disease (CAD), and Killip class ≥ II. It is worth noting that older age was independently associated only with the long-term outcome.

Table 4

Multivariate logistic regression analysis of predictors of in-hospital mortality in acute myocardial infarction

Parameter	Univariate regression model OR (95% CI)	*p*	Multivariate regression model OR (95% CI)	*p*
Age (per year)	1.098 (1.051–1.147)	<0.001	1.030 (0.975–1.088)	0.287
Diabetes mellitus (yes / no)	2.495 (1.052–5.916)	0.038	1.084 (0.349–3.368)	0.889
Atrial fibrillation (yes / no)	3.198 (1.195–8.556)	0.021	2.217 (0.659–7.460)	0.198
Anemia (yes / no)	8.149 (3.277–20.268)	<0.001	3.977 (1.313–12.051)	0.015
GFR < 60 ml / min / 1.73 m² (yes / no)	4.722 (1.821–12.246)	0.001	1.439 (1.443–4.677)	0.545
Three-vessel CAD (yes / no)	4.296 (1.573–11.734)	0.004	4.572 (1.346–15.530)	0.015
Killip class ≥II (yes / no)	45.737 (10.526–198.727)	<0.001	26.432 (5.621–124.287)	<0.001
Elevated ALT and (or) AST (yes / no)	3.189 (1.386–7.337)	0.006	3.607 (1.199–10.848)	0.022

Note. GFR – glomerular filtration rate.

Table 5

Multivariate logistic regression analysis of predictors of long-term mortality in acute myocardial infarction

Parameter	Univariate regression model OR (95% CI)	*p*	Multivariate regression model OR (95% CI)	*p*
Age (per year)	1.102 (1.066–1.139)	<0.001	1.060 (1.018–1.104)	0.005
Diabetes mellitus (yes / no)	2.002 (1.028–3.898)	0.041	1.103 (0.488–2.493)	0.814
Atrial fibrillation (yes / no)	2.334 (1.041–5.234)	0.040	1.170 (0.434–3.158)	0.756
Anemia (yes / no)	5.410 (2.871–10.192)	<0.001	2.722 (1.291–5.739)	0.009
GFR < 60 ml / min / 1.73 m² (yes / no)	2.781 (1.488–5.196)	0.001	1.233 (0.549–2.739)	0.611
Three-vessel CAD (yes / no)	3.959 (1.955–8.017)	<0.001	3.260 (1.431–7.426)	0.005
Killip class ≥II (yes / no)	6.295 (3.326–11.915)	<0.001	3.397 (1.634–7.062)	0.001
Sex (female)	2.605 (1.395–4.865)	0.003	1.353 (0.592–3.091)	0.474
Previous stroke (yes / no)	4.263 (1.876–9.686)	0.001	2.333 (0.802–6.218)	0.124
Previous MI (yes / no)	2.002 (1.028–3.898)	0.041	1.237 (0.540–2.837)	0.615
Elevated ALT and (or) AST (yes / no)	2.020 (1.084–3.764)	0.027	2.182 (1.011–4.708)	0.047

DISCUSSION

The results of this study demonstrate that elevated LT levels were observed in approximately one-third of patients with AMI. Furthermore, the presence of ST-elevation, higher Killip class, lower systolic and diastolic BP, and elevated creatinine levels exhibited associations with abnormal LT levels. In addition, abnormal serum LT levels were independently associated with a worse clinical prognosis – increased risks of both in-hospital and long-term all-cause mortality.

The prevalence of elevated LT levels among AMI patients is known to vary depending on the defined cut-off values and the studied population sample. For instance, using cutoff values recommended by local guidelines (ALT > 50 U / l for men and ALT > 40 U / l for women; AST > 40 U / l for men and AST > 35 U / l for women), M. Gao et al. found elevated ALT in 38.9% and elevated AST in 71.9% of 2,417 consecutive ST-elevation MI (STEMI) patients [14]. These values are higher than the ones in our study: 19% for ALT and 30.8% for AST.

Similarly, J. Moon et al. reported the hypoxic liver injury prevalence of 22% among 456 STEMI patients undergoing primary PCI [15]. Another study reported the prevalence of increased LT of 19.5% among 1,176 STEMI patients [16]. Both studies used serum LT levels twice higher than ULN as cut-off values (> 80 U / l for ALT and > 80 U / l for AST). Using hypoxic liver injury criteria, which define hepatic injury as a sudden, transient ten-fold or

greater rise in ULN values in two or more consecutive samples for lactate dehydrogenase, ALT, and AST within 48 hours from the acute coronary event, R. Birrer et al. revealed the prevalence of hepatic injury of 27% among 87 AMI patients admitted to intensive care units [17]. Such variations underscore the importance of standardized diagnostic criteria and the need to consider patient population heterogeneity in assessing elevated LT levels in AMI.

In our study, we found that elevated LT levels were associated with signs of hypoperfusion, such as hypotension, and signs of kidney dysfunction and heart failure, including Killip Classification. This observation is in line with with previous findings [11, 14], which suggest that hepatic cell damage is linked to reduced perfusion in the centrilobular region of the liver, where blood flow is less robust due to its more distant location from the hepatic artery and portal veins. This reduction in blood flow may stem from the rapid deterioration of cardiovascular function, leading to liver ischemia.

Furthermore, the interaction between liver and heart pathophysiology shares common mechanisms with cardiorenal syndromes, such as increased venous congestion and (or) reduced cardiac output, which can exacerbate kidney function impairment [18], as demonstrated by the independent association between increased creatinine levels and elevated LTs in our study . The results of our study further support the association between elevated LT levels and unfavorable short- and long-term prognoses.

Recent studies have shown that the presence of ST-elevation is associated with elevated serum LT levels. They have also demonstrated an association between elevated serum LT levels and adverse clinical outcomes in patients with AMI [14–16, 19–21]. J. Li et al. analyzed 712 AMI patients without known liver disease, revealing the in-hospital mortality rate of 27% (n = 192). The multivariate logistic regression analysis identified that ALT ≥ 2 times higher than the ULN was an independent predictor of in-hospital mortality (OR 2.240, 95% CI 1.331–3.771; p = 0.002), while the AST level did not exhibit such an association [19].

Similar findings were reported by A. Huseynov et al., assessing the prognostic value of elevated LTs in predicting in-hospital major adverse cardiac events (MACE), defined as a composite endpoint in AMI: a need for repeated target vessel revascularization by PCI,coronary artery bypass grafting among STEMI patients, and all-cause mortality [20]. The study revealed the total in-hospital MACE rate of 9.8%, primarily driven by all-cause mortality (8.4%). In the logistic regression model, both ALT (OR 1.0018, 95% CI: 1.0008–1.0028, p = 0.0003) and AST (OR 1.0011, 95% CI: 1.0005–1.0018, p = 0.0006) were independently associated with MACE after adjusting for age and cardiac enzymes (troponin I and creatine kinase) [20].

Additionally, M. Gao et al. investigated the association of serum transaminases with 2-year mortality in 2,417 STEMI patients and found that both ALT and AST levels ≥ 95th-percentile value were associated with an increased risk of adverse outcomes (OR 1.051, 95% CI: 0.302–3.652) and (1.796, 95% CI: 0.588–5.481), respectively, after adjusting for confounding factors [14].

Several potential mechanisms were proposed to clarify the link between the elevated LT levels and the increased risk of in-hospital and long-term all-cause mortality in patients with AMI. The liver, known for its high metabolic activity and perfusion rates, is directly affected by acute circulatory changes, such as hypotension resulting from AMI, which can lead to increased levels of ALT and AST [11].

Besides, elevated LTs may occur due to hepatic congestion resulting from acute right ventricular dysfunction [22]. Current research underscores the role of factors, such as venous congestion, reduced oxygen uptake by hepatocytes, and reperfusion injury, in contributing to this LT elevation [22, 23]. While elevated serum LTs may originate from ischemic myocardial tissue, their increase often indicates secondary hypoxic liver injury, particularly common during AMI.

Furthermore, a recent meta-analysis including data from over 9.24 million participants and 242,953 cases of all-cause mortality revealed a moderately significant correlation of AST with all-cause mortality, along with geographical variations in the correlation between ALT elevation and a risk of all-cause mortality in the general population [24]. Interestingly, additional studies have documented elevation of common markers, including ALT and AST, in patients with heart failure and cardiovascular disease, wherein liver injury resulted from ischemia or congestion [25–28]. These findings further corroborate the observed association between liver

LT elevation and increased in-hospital and long-term mortality in AMI patients.

In addition to the increased LT activity, our study identified several clinical parameters associated with increased in-hospital and 18-month mortality rates in patients with AMI, which is in line with findings of earlier research [29–35]. It is worth noting that the presence of anemia, three-vessel CAD, and higher Killip class upon admission were all found to be significantly associated with adverse outcomes.

Anemia has long been recognized as a prognostic factor of poor outcomes in patients with cardiovascular diseases, including AMI. Its association with increased mortality rates is attributed to its role in exacerbating myocardial ischemia through decreased oxygen delivery to the myocardium and increased myocardial workload [31, 36, 37]. Similarly, the severity of CAD, particularly when characterized by three-vessel lesion, has consistently been linked to higher mortality rates in patients with AMI. This association is largely determined by extensive myocardial damage and an increased risk of adverse cardiac events associated with severe CA involvement [33, 38].

A higher Killip class at presentation, which reflects the severity of heart failure, has been identified as a significant predictor of mortality in AMI patients in our study. The higher Killip class was indicative of more extensive myocardial damage and hemodynamic compromise, leading to poorer outcomes [32, 39]. Additionally, age served as one of the most important risk factors for adverse outcomes in hospitalized patients with acute coronary syndrome, including AMI. Advanced age was associated with age-related physiological changes, comorbidities, and diminished physiological reserve, all of which contributed to an increased mortality risk [40]. Taken together, these findings underscore the multifactorial nature of mortality risk in patients with AMI and highlight the importance of comprehensive risk assessment incorporating clinical and demographic factors in guiding treatment strategies and improving patient outcomes.

This study had several potential limitations. Firstly, it was constrained by its single-center setting and a relatively small sample size. Secondly, despite efforts to exclude patients with various liver diseases based on medical records and risk factors potentially influencing LT levels, we admit that undiagnosed liver conditions or medications affecting liver function may have gone undetected. Additionally, the presence of AST in organs other than the liver complicates the interpretation of elevated serum LT levels, and the exact source of elevated enzymes cannot be discerned. Furthermore, liver enzyme levels were assessed only once, upon admission to the intensive care unit following revascularization, which presents another limitation.

CONCLUSION

Elevated LT levels upon admission were found in about one-third of patients presenting with AMI, which is significantly associated with ST-elevation, systolic and diastolic blood pressure, Killip class of HF, and creatinine levels. Our study showed that high LT levels were associated with an in-hospital and long-term mortality. These findings may be used for elaborating future personalized treatment strategies for AMI patients.

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Hormonal and metabolic disorders in connective tissue dysplasia

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ABSTRACT

The lecture considers the results of research conducted by Russian and foreign scientists concerning the characteristics of all types of metabolic disorders and hormonal imbalance in undifferentiated connective tissue dysplasia (CTD). Understanding the role of hormonal and metabolic disorders in the development and course of diseases associated with CTD is of great importance for the development of pathogenetically grounded algorithms for the diagnosis, treatment, and prevention of emergency conditions.

Keywords: connective tissue dysplasia, metabolic disorders, hormonal status

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Гормонально-метаболические нарушения при дисплазиях соединительной ткани

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РЕЗЮМЕ

В лекции обсуждаются результаты исследований отечественных и зарубежных ученых, касающиеся особенностей нарушений всех видов обмена и гормонального дисбаланса при недифференцированной дисплазии соединительной ткани (ДСТ). Понимание роли гормонально-метаболических нарушений в развитии и

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течении ассоциированных с ДСТ заболеваний имеет большое значение для разработки патогенетически обоснованных алгоритмов диагностики, лечения и профилактики неотложных состояний.

Ключевые слова: дисплазия соединительной ткани, нарушения обмена, гормональный статус

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.

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INTRODUCTION

The concept of connective tissue dysplasia (CTD) implies structural and functional disorders of connective tissue and their systemic manifestations in multiple organs. CTD syndrome is marked by a number of specific pathological conditions of connective tissue, which are designated as genetic disorders of collagen metabolism, since they are associated with congenital metabolic and structural defects of collagen. In addition to a number of hereditary syndromes described in detail (Marfan syndrome, Ehlers – Danlos syndromes, Stickler syndrome, etc.), pathological changes in the form of incomplete, suppressed, undifferentiated forms combined into a group of undifferentiated connective tissue diseases (UCTD) are of great social and medical significance [1]. They do not meet the diagnostic criteria of the above listed genetically determined syndromes, but at the same time they are characterized by a set of signs indicating the presence of connective tissue disease with multiple organ damage [2].

Due to the systemic nature of the manifestations, the symptoms of UCTD are of interest for various clinicians, including not only pediatricians and therapists, but also specialty physicians, such as cardiologists, surgeons, gynecologists, urologists, pulmonologists, etc. [3]. Researchers are interested in studying and making practical use of the knowledge obtained about this pathological process because of its high prevalence, the diversity of phenotypic characteristics, the trend toward a progressive course and adverse outcomes, and the relevance of the search for prognostically significant markers of a severe course and complications of associated pathology [4].

Currently, there is no generally accepted classification of UCTD that would be recognized by all researchers and clinicians. No consensus was reached regarding diagnostic criteria, terminology, or assessment of the severity of this condition. However, patients with UCTD represent a large group with multiple organ disorders of varying severity and require an interdisciplinary approach in their medical care [3–6].

METABOLIC DISORDERS IN CTD

The formation of this pathological process is based on a genetically determined defect in the development of the mesenchymal matrix of the body. It is accompanied by a decrease in the strength of the connective tissue framework supporting systems and organs, with subsequent morphofunctional disorders closely related to changes in the hormonal and metabolic state [7]. Hormonal and metabolic parameters can form a panel of biological markers of CTD at the preclinical stage, which determines the relevance of this literature review.

According to a number of authors, CTD is a hereditary metabolic anomaly, which is based on impaired formation and maturation of connective tissue structures in the body, which leads to the development of dysplastic changes in tissues and contributes to the occurrence of diseases of the musculoskeletal system and internal organs [8]. It is believed that impaired synthesis of collagen, the main structural element of connective tissue, underlies organ dysfunction in CTD. Proteins including

glycosaminoglycans – glycoproteins perform a structural function in various mucous secretions and cell membranes, being an important component of the intercellular matrix. Intermolecular bond disruption leads to collagen disorganization [9]. In practice, the state of collagen is assessed by the level of a number of amino acids – its structural components in the biological media of the body. Oxyproline is one of the main amino acids of collagen, a biomarker reflecting the catabolism of this protein. An increase in the concentration of oxyproline in a 24-hour urine test is associated with the severity of CTD and an increased level of other amino acids in the blood, namely hydroxyproline, lysine, and proline [3].

The presence of CTD significantly affects tolerance and adaptation to exercise. Patients with UCTD often suffer from exercise-related musculoskeletal disorders and are predisposed to re-injury [10]. In this regard, the presence of CTD implies a personalized approach to the exercise regime in these patients.

According to a number of authors, the asthenic body type associated with malnutrition is one of the most important phenotypic signs of CTD and is statistically significantly more often observed in this pathology than in the general population [11]. Protein – energy malnutrition in UCTD is registered in 40% of cases and is accompanied by all types of metabolic disorders, which makes active search for mechanisms that explain this association relevant [12]. There is a point of view that nutrient deficiency as an adaptation mechanism in the prenatal period leads to complex metabolic changes that determine collagenopathy and associated dysfunction of body systems. Since the hypothalamic – pituitary – adrenal axis (HPA axis) represents one of the adaptational systems of the body [13], the features of the gestational period can preset the state of the HPA axis and contribute to an unfavorable prognosis of developing diseases in adulthood.

This concept is highlighted by literature data that low body weight of newborns is associated with arterial hypertension in both children and adults, as well as with increased excretion of androgens and glucocorticoids. Increased activity of the HPA axis contributes to more active catabolic processes with progressive body weight loss in patients with hypoproteinemia and hypoalbuminemia. High sympathetic tone ensures an increase in the intensity of the basal metabolic rate and a sharp decrease in energy expenditure. Increased catabolism in malnutrition is characterized by depletion of glycogen and fat depots at the beginning. Then, with a significant deficiency of nutrients, protein breakdown occurs in muscle tissue, and the level of transport proteins in the blood decreases. Protein deficiency leads to immune suppression associated with both limited synthesis of immunoglobulins and ineffective antioxidant defense, which together contribute to deficiency of intracellular energy, disruption of adequate transport of micronutrients, and damage to cell membranes [12].

Protein metabolism disorders in CTD are also manifested by changes in the vascular and platelet component of hemostasis, as evidenced by the presence of platelet function disorders in most children [14]. The relationship between the severity of anemia and the number of clinical signs of CTD was found. This is confirmed by low levels of hemoglobin, red blood cells, ferritin, and serum iron [15]. Researchers consider different mechanisms of anemia development in CTD. However, most tend to believe that disruption of submembrane cytoskeleton in red blood cells plays a crucial role in reducing the life span of red blood cells, which significantly affects elasticity and reduces their elastic deformation when passing through the microvasculature [16, 17]. In particular, it has been shown that an imbalance of antioxidant enzymes (superoxide dismutase and catalase) is observed in adolescents with UCTD. In turn, this can lead to the accumulation of aggressive hydroperoxides in the blood plasma, which damage blood cells, and contribute to the development of lipid peroxidation in erythrocyte membranes [18].

Neonatal body weight deficiency was also found to be associated with insulin resistance and impaired glucose tolerance afterwards. Since skeletal muscles and adipose tissue are the main peripheral tissues affected by insulin in adults, body weight deficiency and malnutrition at birth can be considered as adaptive manifestations [19]. However, researchers from Donetsk National Medical University showed that in children with UCTD and functional dyspepsia (FD), insulin and cortisol levels were significantly lower than in children without signs of CTD and in healthy individuals [20]. Increased catabolism in people with body weight deficiency and related stress are also associated with a decrease in enzyme

activity and insulin synthesis, and an increase in the cortisol / insulin index is observed.

In malnutrition associated with CTD, energy metabolism changes significantly, mainly from carbohydrate to lipid. Moderate malnutrition is accompanied by increased fat breakdown, in which non-esterified fatty acids (NEFA) become an important source of energy. Under these conditions, the biosynthesis of fatty acids from cholesterol is activated, which is necessary for the satisfactory functioning of the digestive system and the complete synthesis of corticosteroids, which regulate adaptation reactions. With more severe protein – energy deficiency and grade III malnutrition in patients with slowing fat catabolism, the absorption of NEFA worsens, and the level of cholesterol and its fractions in the blood decreases, which contributes to disrupted structure and impaired function of cell membranes and a decrease in the concentrations of corticosteroids and fatty acids [12].

Impaired lipid metabolism in CTD is a key link in the pathogenesis of many diseases in people of any age. It is believed that the content of free and bound fatty acids in the blood serves as an additional biomarker of CTD [3]. Researchers discuss the significance of the pathology of cell membranes and lipid and fatty acid composition of blood plasma in the pathogenesis of arterial hypertension and other cardiovascular diseases [21]. It is now known that deficiency of polyunsaturated omega-3 fatty acids underlies the occurrence of cardiovascular pathology. Cardiovascular diseases in children are some of the most common childhood pathologies [22, 23]. Changes in the architectonics of the heart and great vessels in young people with CTD and their dysfunction are explained, among other things, by impaired lipid metabolism.

Impaired mineral metabolism is another important link in the pathogenesis of CTD, given the essential role of macro- and microelements in the formation of the connective tissue structure. According to the literature data, the majority of patients with CTD experience changes in the macro- and microelement composition of biological media. Thus, in persons with CTD, deficiency of the following elements has been described: silicon, selenium, potassium, calcium, copper, manganese, magnesium, and zinc. All of the above elements are more or less involved in bone mineralization, collagen synthesis, and maturation [24]. A comparative analysis of clinical and laboratory data from underweight children with and without CTD showed that in the absence of statistically significant differences in anthropometric parameters, including bioelectrical impedance analysis, in patients without CTD, the content of mineral substances in the body was within the reference values [25].

The analysis of the literature data showed that researchers mainly focus on studying the role of calcium and magnesium in the pathogenesis of morphofunctional disorders associated with CTD [26]. The deficiency of these elements is associated not only with a decrease in the strength of the bone skeleton according to densitometry, but also with a disruption of the vascular wall structure and impaired function of the nervous system. At the same time, low magnesium levels negatively correlate with the severity and number of phenotypic signs of CTD [27, 28]. It is also known that hypomagnesemia underlies the development of insulin resistance and increases the risk of impaired glucose tolerance. Often, children with CTD exhibit deficiency of not just one, but a number of microelements. As expected, the most frequently described combination is deficiency of calcium and magnesium, however, some researchers have described combined deficiency of microelements which actively participate in collagen formation, such as zinc, selenium, iodine, and copper [29].

HORMONAL DISORDERS IN CTD

The above mentioned metabolic disorders in CTD have complex hormonal regulation, which is confirmed by the results of research characterizing hormonal imbalance in CTD. Since diabetes mellitus (DM) is an extreme manifestation of carbohydrate metabolism disorders and the formation of vascular and neurological complications in this pathology is associated with the involvement of connective tissue in the pathological process, an attempt was made to study the features of the association between UCTD and DM. It was found that UCTD negatively affects the prognosis and course of type 1 diabetes [30] and type 2 diabetes, which is manifested by earlier depletion of regulatory mechanisms and decreased opportunities for rehabilitation and psychological adaptation. UCTD in combination with type 2 diabetes is considered as an independent

cardiovascular risk factor for cardiac arrhythmias, valvular dysfunction, and autonomic imbalance, contributing to sympathicotonia, electrical instability of the myocardium and prolonged PQ interval and increasing the risk of sudden cardiac death [31].

The analysis of the results of a large-scale study on the functional state of the main body systems demonstrated that patients with UCTD were more likely to have uncompensated DM and developed late complications two times more often and 2 to 3 years earlier than patients without CTD [32]. Symptoms of CTD were regarded as predictors of the development of comorbid pathology in patients with type 2 diabetes, which primarily implied cardiovascular and musculoskeletal diseases [32]. In children and adolescents with type 1 diabetes and CTD, complications, such as diabetic neuropathy and nephropathy, develop earlier from the onset of the disease and have a more severe course than in patients without dysplasia, which allows to consider them as a special risk group [33].

Thyroid pathology is considered to be the most frequently diagnosed pathology in patients with CTD. Autoimmune thyroiditis occurs in more than 37% of patients, which is significantly more common than in the control group [34]. Thyroid hormones are involved in the regulation of growth and skeletal maturation [4]. It is known that free thyroxine and triiodothyronine activate the synthesis of type I collagen and osteocalcin in bones. The biological effects of thyroid hormones and thyroid-stimulating hormone include inhibition of fibroblast proliferation and chondrocyte differentiation in various types of connective tissue, which underlies an increase in bone density in hypothyroidism [35].

Krasnodar researchers also showed that autoimmune pathology of the thyroid gland and basal and/or postprandial hyperinsulinemia are the most common of all endocrine diseases identified in patients with UCTD, diagnosed in 37.7% and 43.4% of cases, respectively, which is statistically significantly higher than in the control group [34]. Various research groups have shown that in young people with UCTD, autoimmune thyroiditis is diagnosed in 32.5% of cases and in women of fertile age – in 42.9% of cases, which is more often than in the general population. At the same time, the frequency and severity of visceral, osteoarticular, and skin phenotypic signs of UCTD are more significant than in individuals with UCTD who do not have thyroid pathology. Also, in this category of people, higher titers of antibodies to thyroid peroxidase and thyroglobulin are recorded, in contrast to healthy controls, which allows to consider all patients with UCTD as a risk group for the development of autoimmune thyroid pathology [36]. The high risk of developing autoimmune thyroiditis and hypothyroxinemia in joint hypermobility syndrome makes it reasonable to examine patients in order to diagnose thyroid dysfunction that would require subsequent targeted personalized therapy and iodine prophylaxis in this target group [35].

Impaired development of the reproductive system in adolescents is a consequence of UCTD. In girls, the most common problem is menstrual dysfunction, characterized by early menarche, hypomenorrhea and amenorrhea, as well as by uterine bleeding during puberty. In turn, hormonal and metabolic changes accompanied by menstrual dysfunction aggravate the course of CTD. The assessment of the hormonal status of teenage girls with UCTD and menstrual disorders showed that younger girls (11–13 years old) with UCTD had an increased level of follicle-stimulating hormone (FSH) and decreased content of luteinizing hormone (LH), the LH / FSH index was reduced up to 0.7, which corresponds to gonadotropin function in infants and luteal phase deficiency.

In a group of adolescent girls with UCTD (14–16 years old), 55% were also diagnosed with immaturity of the reproductive system, which may be associated with UCTD (pubertal maturity index < 1.0). However, 45% of patients had high sexual maturity rating (2.0–2.5) [37]. The hormonal status of adolescents with UCTD of both sexes was characterized by a higher level of gonadotropins than in healthy peers. Moreover, in patients with minimal manifestations of dysplasia, the FSH level was significantly higher than in the control group and in patients with moderate and severe dysplasia, and the LH level was significantly higher than in the control group.

However, in severe dysplasia, gender-specific hormonal status was revealed. Thus, in the group of girls with severe CTD, FSH levels were significantly reduced and had an inverse correlation with the severity of dysplasia. In the group of boys, elevated FSH and LH levels were noted in mild UCTD, while

patients with severe UCTD had low concentrations of the two hormones [37]. This indicates that in severe cases, inhibition of the gonadotropin function of the pituitary gland occurs, whereas with minimal dysplastic changes, gonadotropin activity is high. Researchers explain hyperprolactinemia in adolescents with UCTD as a reaction caused by an inadequate response of the body to stress. It is known that patients with UCTD have a cluster of emotional, personal and temperament characteristics, which also requires in-depth study [38, 39].

The presence of CTD in boys may also underlie delayed puberty, including symptoms that require surgery, such as micropenis, hypermobility, testicular hypoplasia, and varicocele [40]. Varicocele can also be one of the manifestations of CTD in men. During the examination of 721 adolescent patients diagnosed with varicocele, it was found that patients who had recurrent varicocele had more than seven verified diagnostic signs of CTD, which makes it possible to consider UCTD as a cause of varicocele recurrence. A 10–100 increase in the level of sex hormones (estradiol and testosterone) in the blood of the pampiniform plexus was characteristic of such patients, which may indicate hormonal regulation in this pathology [40].

The presence of UCTD in patients is associated with a high risk of gestational complications, such as threatened miscarriage and incipient abortion, cervical incompetence, premature rupture of membranes, and retrochorial hematoma [41, 42]. The examination of 80 pregnant women with threatened preterm labor and cervical incompetence revealed the prevalence of phenotypic signs of UCTD in more than 90% of cases [43]. The above-described features of hormonal imbalance and sexual development in adolescents may partially explain the data obtained on the negative impact of UCTD on the reproductive function of both women and men.

Malnutrition is a symptom complex of great medical and social significance due to a high risk of adverse prognoses associated with the presence of this syndrome. It is believed that a large part of metabolic disorders is genetically determined, and mutations of genes encoding adipokines and their receptors are pivotal. Adipokines are hormone-like substances, secreted by adipose tissue, an imbalance of which can lead to metabolic disorders. The biological effects of adipokines in abdominal obesity, metabolic syndrome, and associated pathologies have been well studied [44, 45]. Since there is a lack of data on the pathogenesis of malnutrition in patients with CTD and high frequency and severity of nutritional status disorders, an attempt was made to assess the level of a number of adipokines and their receptors in patients with CTD and malnutrition. It was found that in such patients, the adipokine profile was characterized by a decrease in the levels of leptin and resistin in the blood and an increase in the concentration of soluble leptin receptors and adiponectin [19].

CONCLUSION

Thus, CTD contributes to the formation of pathology of various systems and organs and has a wide variety of manifestations. Metabolic disorders and hormonal imbalance are not only a manifestation of this pathology, but also underlie the progression of associated clinical conditions.

Understanding the role of hormonal and metabolic disorders in the development and course of diseases associated with CTD is of great importance for the development of diagnostic algorithms based on pathogenetics and targeted treatment, including metabolic therapy and effective personalized algorithms for preventing the progression and complications of the pathological process which would improve the quality of life.

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On the pathogenesis of COVID-19: the role of transforming growth factor beta

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ABSTRACT

Proteins of the transforming growth factor beta (TGF-β) family regulate numerous cellular processes that are essential in the pathogenesis of acute respiratory distress syndrome (ARDS), contributing to increased alveolar epithelial permeability, activation of fibroblasts, and extracellular matrix remodeling. TGF-β is involved in the pathogenesis of inflammatory respiratory diseases during the development of COVID-19. SARS-CoV-2 leads to complex immune responses that include the release of inflammatory cytokines, increased activity of mast cells, and the release of mast cell secretome, in particular profibrotic enzymes and cytokines, including TGF-β.

Tryptase- and chymase-positive mast cells play a major role in pulmonary fibrosis and embolism in COVID-19. Mast cell chymase is angiotensin-converting enzyme 2-independent due to extracellular formation of angiotensin II in the interstitium; it also activates TGF-β and other molecules, thereby playing a role in tissue remodeling. Mast cell β-tryptase increases the secretion of TGF-β1 by airway smooth muscle tissue and the expression of α-smooth muscle actin (α-SMA). TGF-β also induces the generation of mitochondrial reactive oxygen species (ROS), which enhances the production of ROS in lung fibroblasts. TGF-β is crucial for induing the synthesis of extracellular matrix components by fibroblasts.

The review is devoted to the structure of TGF-β, the sources of its secretion and functions, the mechanism of its involvement in the pathogenesis of COVID-19, and the possibility of its use as a prognostic marker of COVID-19 severity.

Keywords: transforming growth factor beta, mast cells, COVID-19, acute respiratory distress syndrome, inflammation

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К вопросу о патогенезе COVID-19: роль трансформирующего фактора роста бета

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РЕЗЮМЕ

Белки семейства трансформирующего фактора роста бета (TGF)β регулируют многочисленные клеточные процессы, которые играют важную роль в патогенезе острого респираторного дистресс-синдрома (ОРДС), способствуют повышению проницаемости альвеолярного эпителия, активации фибробластов и ремоделированию внеклеточного матрикса. Трансформирующий фактор роста бета участвует в патогенезе воспалительных заболеваний дыхательной системы при развитии COVID-19. SARS-CoV-2 приводит к сложным иммунным реакциям, которые включают высвобождение воспалительных цитокинов, повышение активности тучных клеток и высвобождение продуктов их секретома, в частности профибротических ферментов и цитокинов, в том числе TGF-β.

Триптаза- и химаза-положительные тучные клетки играют большую роль в легочном фиброзе и эмболии при COVID-19. Химаза тучных клеток является независимым от ангиотензинпревращающего фермента 2-го типа путем образования ангиотензина II внеклеточно в интерстиции, а также активирует TGF-β и другие молекулы, тем самым играя роль в ремоделировании тканей. Бета-триптаза тучных клеток увеличивает секрецию TGF-β1 гладкой мышечной тканью дыхательных путей и экспрессию α-гладкомышечного актина – α-SMA. TGF-β также индуцирует генерацию митохондриальных активных форм кислорода (АФК), что усиливает выработку АФК в фибробластах легких. TGF-β играет ключевую роль в индукции синтеза компонентов внеклеточного матрикса фибробластами.

Настоящий обзор посвящен рассмотрению структуры TGF-β, особенностям его секреции и функции. Представлен механизм его участия TGF-β в патогенезе COVID-19, а также возможности его использования в качестве прогностического маркера степени тяжести течения COVID-19.

Ключевые слова: трансформирующий фактор роста бета, COVID-19, тучные клетки, острый респираторный дистресс-синдром, воспаление

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.

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INTRODUCTION

The COVID-19 pandemic, which ended in May 2023, has lasted for more than three years. During this time, research and discoveries in the field of immunology made a breakthrough. COVID-19 affected more than 700 million people around the world causing more than 6.9 million confirmed deaths [1]. One of the long-term manifestations of COVID-19 that follows an acute phase of infection is post-COVID-19 syndrome.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19, can trigger complex immune responses, such as activation of proinflammatory cytokines, an increase in mast cell (MC) degranulation, and release of MC compounds. [2, 3].

The receptor for SARS-CoV2 is angiotensin-converting enzyme 2 (ACE2), which binds to spike protein on the surface of SARS-CoV2. MCs synthesize ACE2 and tryptase contributing to the development of cytokine storm with

high levels of proinflammatory cytokines, including tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), IL-1β, thymic stromal lymphopoietin (TSLP), and others. [1, 4]. MCs are activated by SARS-CoV-2 ribonucleic acid (RNA) through the retinoic acid induced 1 (RAI-1) protein, toll-like receptor (TLR)-3, TLR-7, and TLR-8 (TLR). High concentrations of TNFα have been reported both in plasma and tissues of patients with COVID-19 [1, 4].

The baseline expression of ACE2 mRNA in lungs is quite low compared to other organs, but it increases in alveolar cells after SARS-CoV-2 infection. [5]. ACE2 is cleaved by transmembrane serine protease 2 (TMPRSS2) and a disintegrin and metalloprotease 17 (ADAM17), facilitating COVID-19 penetration into cells of the patient. [5]. SARS-CoV-2 increases the level of ACE2 mRNA, transforming growth factor beta (TGF-β), connective tissue growth factor (CTGF), and fibronectin (FN) [5]. Consequently, binding of SARS-CoV-2 to ACE2 leads to an increase in transcription of genes associated with lung fibrosis [5].

A rise in the number of MCs influences rather high frequency of COVID-19-associated pulmonary embolism, since MCs were reported to induce thrombosis through activation of coagulation factors and thrombocytes. Tryptase- and chymase-positive MCs play a significant role in pulmonary fibrosis and embolism in COVID-19 [3]. Chymase is ACE2-independent due to formation of angiotensin II (AngII); it also activates TGF-β and other molecules and induces tissue remodeling. Chymase-associated formation of AngII occurs extracellularly in the interstitium [6].

FEATURES OF THE STRUCTURE AND SYNTHESIS OF TGF-β

Proteins of the TGF-β family are present in all multicellular organisms and play an essential role in development of organs, wound healing, immune responses, and oncogenesis [7–11]. Each of TGF-β-1, -2, and -3 (hereinafter collectively referred to as TGF-β, unless indicated otherwise) are secreted either as the small latent complex (SLC) which consists of a bioactive homodimer non-covalently associated with its pro-peptide (latency-associated protein; LAP) or as a large latent complex (LLC) in which the SLC is covalently bound to LTBP-1, -3, or -4. Whereas LTBP-1 and - can associate with all TGF-β isotypes, LTBP-4 binds only to TGFβ-1 and at a lower affinity than the other LTBPs [10, 12–14].

The LTBPs form latent complexes with TGF-β by covalent bonds to the TGF-β pro-peptide (LAP) through disulfide bonds in the endoplasmic reticulum [9]. LAP in turn is cleaved from the mature TGF-β precursor in the trans-Golgi network, but LAP and TGF-β remain strongly bound through non-covalent interactions [9, 15]. It is important to note that each monomer of LAP consists of a number of relatively rigid α-helices and β-sheets, but the active cytokine is held in place by multiple contacts with a single flexible loop, which has been termed the latency lasso and effectively holds the active TGF-β [8]. As long as this lasso maintains close interactions with the active cytokine, TGF-β can be stored in the extracellular space with almost no evidence of active TGF-β signaling or cellular responses [8].

LTBP were originally considered to play a role in maintaining TGF-β latency and directing the latent growth factor to the extracellular matrix. However, LTBP-1 also participates in TGF-β activation by integrins and may also regulate its activation by proteases and other factors [9]. LTBP-3 is involved in skeleton formation, including tooth formation. LTBP-2 and LTBP-4 are identified as TGF-β-independent activators that have important functions in the regulation of TGF-β and stabilize bundles of microfibrils and regulate the assembly of elastic fibers [9]. Genetic ablation of both short and long isoforms of LTBP-4 leads to an increase in TGF-β activity in lungs, which is consistent with the previous observation that LTBP-4L interacts with TGF-β in a more effective way [12]. However, significant amounts of LTBP-1 and LTBP-4 are secreted without SLS, just like LTBP-2, which cannot interact with SLC [12]. TGF-β together with LAP can form complexes with LTBP-1, which are generated by fibroblasts [16].

Activation of TGF-β1 and LAP cleavage are induced by an acidic environment or are implemented by extracellular proteases, including thrombospondin-1, plasmin, cathepsin D, matrix metalloproteinase (MMP)-2 and -9, furin convertase, and integrins [10]. The LAP domain of TGF-β has the RGD sequence (arginine – glycine – aspartic acid), which mediates binding to integrins [13].

Alpha V beta 6 (avβ6) integrin was demonstrated to activate TGF-β1, and alpha V beta 8 (avβ8) integrin was shown to activate TGF-β1 via membrane-bound MMPs [10]. The molecules of the integrin family, which interact with RGD, can activate latent TGF-β1 and TGF-β3 [17]. The αvβ1 heterodimer has recently been shown to be a regulator of latent TGF-β in fibrosis, and the integrins avß5 and α51 participate in the activation of myofibroblasts [18]. Alpha V beta 8 integrin is a cell surface receptor for the LAP of the multifunctional cytokine TGF-β [19]. Altered expression of the integrin subunit β8 (ITGB8) is found in human chronic obstructive pulmonary disease, cancers, and cerebrovascular malformations [19]. The inflammatory process can change the level of TGF-β through specific mediators that influence gene transcription [19]. Thus, IL-1β increases the accessibility of transcription factors to the ITGB8 promoter in lung fibroblasts through chromatin remodeling [19].

Signaling of the TGF-β superfamily can be mediated through the Smad- and non-Smad pathways, in particular through the activation of mitogen activated protein kinase (MAPK), including extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK); as well as through the phosphoinositide 3-kinase (PI(3)K)/Akt pathway, and the NF-κB pathway [10].

In addition to the non-proteolytic latent TGF-β activation mechanism, proteolytic mechanisms also take part in that process. They include two types of proteases: glycoside-containing proteases (N-glycanase and neuraminidase) and serine proteases (plasmin, cathepsin D, and MMPs) [20, 21].

LTBPs are structurally related to the fibrillin family of proteins, which includes fibrillin-1 and fibrillin-2. Fibrillin-1 is an inhibitor of TGF-β signaling and an integral component of microfibrils [22]. Microfibrils cover the core of elastic fibers and contribute to the temporal and spatial regulation of TGF-β activation [16]. Microfibrils consist of fibrillins, which are associated with glycoproteins and protein microfibrils. These microfibrils bind TGF-β, and fibrillins are the key components that provide scaffolds for elastic fiber formation [23].

Fibrillin-1 expression persists throughout life, while the expression of fibrillin-2 is limited to the development of fetal tissues [13]. Furthermore, fibrillin-1 together with the bound molecules, masks fibrillin-2 epitopes, blocking its bioactivity [16]. Fibrillins function as scaffolds for the elastic fiber formation, that contribute to the maintenance of tissue homeostasis and regulate growth factor signaling in the extracellular space [14]. Fibrillin-1 is a modular glycoprotein that consists of 7 latent TGF-β-binding domains and mediates cell adhesion through integrin binding to the RGD motif in its 4th domain [14]. The activation of integrin-mediated TGF- is quite important for immune system, oncogenesis, and functioning of fibroblasts [11]. Both avβ6 and avβ8 integrins regulate TGF-β signaling by binding to a linear tripeptide depending on the tensile force created by the actin cytoskeleton [11].

L. Zilberberg et al. in their research in 2012 analyzed both *in vitro* and *in vivo* the role of fibrillin microfibrils in the synthesis of LTBP-1, -3 and -4 [22]. The authors showed that fibrillin-1 is important for integration of LTBP-3 and LTBP-4 into the extracellular matrix (ECM), but not for LTBP-1, because the presence of fibronectin is important for the association of LTBP-1 with ECM [22].

TGF-β AS A REGULATOR OF NUMEROUS CELL FUNCTIONS

TGF- β is produced by fibroblasts, epithelial, endothelial and smooth muscle cells in the airways [24]. In addition, TGF-β induces the synthesis of various ECM proteins by fibroblasts, such as collagens, elastin, proteoglycans, and fibronectin [24]. TGF-β is expressed by infiltrated eosinophils, lymphocytes, and MCs [24]. Lymphocytes and thrombocytes also contain TGF-β [24].

Latent TGF-β s highly expressed in airway and alveolar epithelia, alveolar macrophages, and ECM of the lungs, where the inhaled microorganisms face host body defense mechanisms during their pulmonary phase [26]. Its maximum concentration in the respiratory system is observed in the bronchial epithelium [24]. Enhanced TGF-β1 signaling during inflammation initially leads to local suppression of the host immune system via direct inhibition of effector immune cells like T cells and dendritic cells [26].

TGF-β plays a key role in inducing the production of ECM components by fibroblasts [23]. TGF-β itself plays a significant and complex role in the immune response of the respiratory tract, mediating various proinflammatory or immunosuppressive effects [27].

TGF-β1 stimulates the attraction of inflammatory cells in the airways and stimulates the secretion of additional growth factors and proinflammatory cytokines [27]. And vice versa, TGF-β1 can suppress both proliferation of lymphocytes and production of particular cytokines [27]. Thus, IL-13 interacts with IL-4, inducing alternative activation of M2 macrophages. That interaction promotes the release of TGF-β1 platelet-derived factor [28]. This phase is characterized by short-term expansion of resident fibroblasts, formation of a temporary matrix, and proliferation of airway progenitor cells and type 2 pneumocytes [28].

TGF-β plays an essential role in regulating ECM components in the airways: it increases synthesis of proteins, such as fibronectin, enhances antiprotease activity, stimulates expression of desmosomal proteins by bronchial epithelial cells, which promotes activation of proteins involved in intercellular junctions and contributes to the formation of the basement membrane in alveolar epithelial cells [29]. Finally, TGF-β contributes to the mechanisms involved in the control of epithelial cell growth and differentiation. For example, TGF-β induces differentiation of normal bronchial epithelial cells, regulates growth of cells via inhibition of cell proliferation, and induces apoptosis of epithelial cells in the lungs [29].

TGF-β regulates the expression of proteins involved in the fibrous cascade, including the regulation of expression and activity of extracellular remodeling proteases, including MMPs, tissue inhibitors of metalloproteinases, and endothelin-1 expression [10]. Endothelin-1 contributes to persistent fibrosis by inducing fibroblasts to produce and secrete TGF-β by ECM and also regulating the activity of the Rho family of GTPases, which take part in TGF-β-induced fibrosis [10]. In general, TGF-β is a key regulator of ECM production and, therefore, enhanced TGF-β signaling is a crucial factor in progression of fibrosis [10].

TGF-β has been shown to suppress the proliferation of CD4+ T cells via inhibition of the autocrine production of IL-2 [15]. In CD8+ cytotoxic T cells (TC cells), TGF-β-activated Smad proteins cooperate with activating transcription factor 1 (ATF1), suppressing the expression of interferon-γ and cytolytic factors, such as perforin, granzyme, and Fas-ligand, thus inhibiting the anti-tumor activity of these cells. TGF-β also modulates proliferation and other functions of B cells, natural killer cells (NK-cells), monocytes, macrophages, and granulocytes. TGF-β plays a pleiotropic role in lymphocyte regulation, and this is especially apparent in T cells [15].

TGF-β1 induces alpha-smooth muscle actin (α-SMA) and collagen synthesis in fibroblasts both *in vivo* and *in vitro* and also plays a significant role in tissue repair and the development of fibrosis [30]. During these processes, fibroblasts differentiate into fibromyoblasts characterized by increased expression of α-SMA [30].

TGF-β1 suppresses plasmin and plasmin-mediated MMP activity in flexor tendon cells cultured *in vitro* via activation of PAI-1[21]. It is possible that TGF-β1 also suppresses MMP and plasmin activity in other ways, such as through the suppression of tissue-type plasminogen activators (tPA), urokinase-type plasminogen activators (uPA), and / or membrane-bound MMP expression in tenocytes [21]. TGF-β is aberrantly activated during the degradation of ECM because of high activity of MMP [12].

TGF-β1 and IL-6 have opposite effects on regulation of cathepsin B and L activity in A-549 human lung epithelial cells [29]. The proinflammatory cytokine IL-6 induced an upregulation of cathepsin L, whereas TGF-β1 suppressed cathepsin B and L expression. Cathepsins B and L are expressed cysteine proteinases that play an important role in lysosomal bulk proteolysis, protein processing, matrix degradation, and tissue remodeling [29].

M. Jain et al. in 2013 demonstrated that TGF-β triggers the production of mitochondrial reactive oxygen species (ROS), which in turn activates the transcription of nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4). That enhances the production of ROS in lung fibroblasts in patients with idiopathic and scleroderma-induced lung fibrosis [31].

FEATURES OF MOLECULAR INTERACTION OF TGF-β AND IMMUNOCOMPETENT CELLS IN COVID-19

The anatomy of the lungs is vulnerable to external stimuli and pathogens, and, as a result, inflammation, allergic reactions, and carcinogenesis take place. Increased regulation of TGF-β ligands is

observed in the majority of lung diseases, including pulmonary fibrosis, emphysema, bronchial asthma, and lung cancer [32]. Based on the published data, patients with COVID-19 or post-COVID syndrome may develop pulmonary fibrosis. The prognosis for this serious complication also deserves attention of medical professionals and the scientific community. The morphological characteristics of pulmonary fibrosis include thickening of the alveolar septa, collagen deposition, and fibroblast proliferation, as well as, diffuse inflammation.

After infection with COVID-19, the TGF-β pathway inhibits cell apoptosis and induces fibroblast proliferation and myofibroblast differentiation, developing pulmonary fibrosis [33]. In the early stage of SARS-CoV-2 infection, a number of inflammatory responses and dysregulation of the fibrinolytic and coagulation pathways can promote activation of the latent form of TGF-β in the blood and lungs [33].

TGF-β causes fibrosis, which is a common complication in patients with severe forms of COVID-19. Acute respiratory distress syndrome (ARDS) in COVID-19 patients can lead to terminal pulmonary fibrosis [34]. TGF-β activation promotes the differentiation of fibroblasts into myofibroblasts and also regulates remodeling of the fibrous component in ECM [34].

With the development of COVID-19, SARS-CoV-2 penetrates into the upper respiratory tract through ACE2 receptors, which are located on the surface of the pulmonary epithelium [35]. The virus infects type II pulmonary alveolar cells during their migration into the lower respiratory tract [35]. In patients with COVID-19, interstitial pulmonary edema occurs in the acute phase of ARDS, and the formation of hyaline membranes results from diffuse damage to the alveoli, while fibrosis of the interalveolar septa and fibroblast proliferation develop in the phase of chronic remodeling [28]. SARS-CoV-2 is supposed to increase the TGF-β concentration by reducing the regulation of the ACE2 receptor through its interaction with the spike protein. Consequently, as the regulation of ACE2 is decreased, the level of angiotensin II increases, and that enhances the activity of the TGF-β intracellular signaling pathway [36–39].

TGF-β has been shown to suppress the number of circulating NK-cells in patients with COVID-19 [26]. The activation of TGF-β increases the permeability of the endothelial and epithelial cells, which leads to an influx of fluids and proteins into the alveoli. This process worsens gas exchange in the lungs and results in arterial hypoxemia and respiratory failure [40]. Superficial proteins of epithelial and endothelial cells are surrounded by a dense mesh of glycans, called the epithelial / endothelial glycocalyx layer. Disruption of this layer is mediated by SARS-CoV2 [41].

Our research team showed the increase in MC activity and changes in their phenotype in lung tissues of patients who died from COVID-19 [42]. MC, located in close proximity to fibroblasts, are sensitive to mechanical stress in fibrotic lungs and, therefore, can be stimulated by breathing process in rigid and fibrous lungs [43, 44]. MC granules may contain large amounts of profibrotic enzymes and cytokines, such as TGF-β, IL-4, IL-10, IL-13, chymase, tryptase, and growth factors [43], which promote fibroblast activation [45].

Mast cell β-tryptase increases the production of TGF-β1 by airway smooth muscle and stimulates α-SMA secretion, leading to functional changes in muscle tissues [46]. TGF-β1 mediates the expression of genes involved into fibrotic process, including collagen, proteases, and fibronectin, through a Smad-dependent mechanism [27]. Airway smooth muscle cells (ASMCs) contain a lot of TGF-β1–3 [28]. Plasmin and serine protease regulate the production of a biologically active form of TGF-β by ASMCs as well as the release of extracellular TGF-β [24]. The release of TGF-β activated by plasmin induces autocrine synthesis of type I collagen by ASMC [24]. Macrophages are also important agents that contribute to lung fibrosis. The activation of TGF-β / Smad and IL-6 / STAT3 signaling pathways can be involved in the M1– M2 polarization transition of macrophages in cases of COVID-19 [47].

SERUM TGF-β LEVELS IN COVID-19

The most severe complication of SARS, caused by coronavirus infection, is pulmonary fibrosis, which develops in 45% of patients within 3 to 6 months after infection [48]. Increased expression of TGF-β in COVID-19 patients may be the cause of pulmonary fibrosis [49]. It was found that patients with ARDS with lower levels of TGF-β in the bronchoalveolar lavage fluid had fewer days

spent in intensive care units and mechanically ventilated [50].

Patients with COVID-19 have an increased level of TGF-β1 in their serum and plasma, and this correlates with the severity of their condition [26]. The level of immunosuppressive cytokines, such as TGF-β and IL-10, increased after SARS-CoV-2 infection and there was no correlation with gender [33]. Increased serum levels of TGF-β in the early days of COVID-19 infection may indicate the potential development of severe bleeding complications, including the need for intensive care and a risk of death.

S. Frischbutter et al. (2023) noted a significant increase in serum levels of TGF-β within the first two weeks after the onset of symptoms in COVID-19 patients [51]. This early increase of serum TGF-β impairs the ability of NK-cells to eliminate the cells infected with the virus [51]. Thus, patients with mild COVID-19 (n = 27) had concentrations of 165.4 pg / ml ± SD 105.4, patients with moderate COVID-19 (n = 11) had slightly higher levels of 240.6 pg / ml ± SD 150.2, and patients with severe COVID-19 (n = 25) had greater serum TGF-β levels of 416.3 pg / ml ± SD 161.7 ($p < 0.0001$) compared to patients with mild or moderate COVID-19 [51]. The authors suggest that patients with serum TGF-β levels of above 254.75 pg / ml are at high risk (92%) of progression of a severe form of COVID-19 infection [50]. TGF-β can be considered and recommended as a predictive marker of COVID-19 severity [51, 52].

A MODEL OF TGF-β INVOLVEMENT IN THE PATHOGENESIS OF COVID-19

A model of TGF-β activation in the development of COVID-19 is presented in the authors' scheme (Figure).

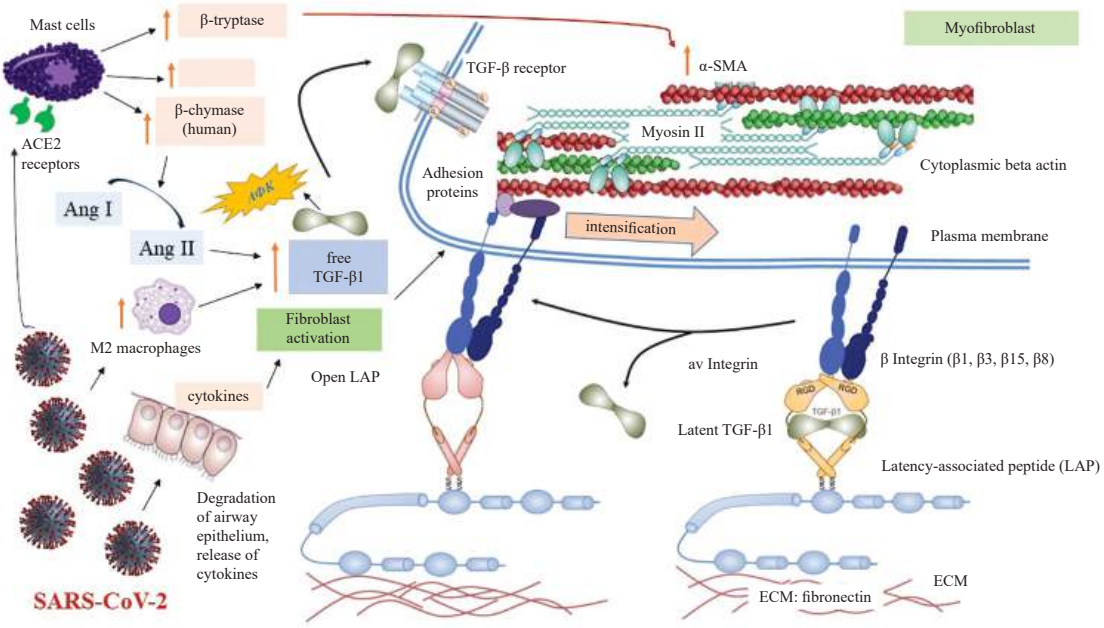

Figure. A model of TGF-β activation in the development of COVID-19 (authors' scheme modified according to [8])

Explanation: тhe receptor for SARS-CoV2 is ACE2, which binds to Spike protein on the surface of coronavirus. This interaction causes the release of cytokines by various cellular agents. With the development of COVID-19, the activity of MC and synthesis of their products increase, including profibrotic mediators, such as tryptase, chymase, and TGF-β. TGF-β induces the differentiation of fibroblasts into myofibroblasts. TGF-β also induces the production of mitochondrial ROS, which enhances their production in pulmonary fibroblasts.

MC chymase is the ACE2-independent pathway of Ang II formation in ECM. It also activates TGF-β and other molecules, thus contributing to tissue remodeling. Beta-tryptase of MC increases the production of TGF-β1 by fibroblastic differon and rises the expression of α-SMA.

TGF-β is produced in airway fibroblasts, endothelial and smooth muscle cells, then it is

accumulated in ECM as latent TGF-β (LTBP). LTBPs form latent complexes with TGF-β by covalently binding to the pro-peptide TGF-β, LAP, via disulfide bonds in the endoplasmic reticulum. Latent TGF-β1 activation from ECM is initiated by integrins through binding to the RGD sequence (arginine – glycine – aspartic acid) in the LAP – TGF-β domain. This activation is also induced by acidic pH or is carried out by extracellular proteases, such as thrombospondin-1, plasmin, cathepsin D, MMP-2, MMP-9, and furin convertase.

CONCLUSION

There is no doubt that TGF-β is involved in the pathogenesis of inflammatory diseases of the respiratory system in COVID-19. TGF-β regulates numerous cell processes, which play an important role in ARDS development, increases the permeability of the alveolar epithelium, activates fibroblasts, and promotes ECM remodeling. SARS-CoV-2 causes prolonged activation of immune cells, development of complex immune responses, and production of a large number of cytokines, including profibrotic enzymes, in particular, TGF-β.

Tryptase- and chymase-positive MCs play a significant role in the development of pulmonary fibrosis and pulmonary embolism in COVID-19, including through activation of the TGF-β pathway. MC chymase is the ACE2-independent pathway of Ang II formation in ECM. It also activates TGF-β and other molecules, thus contributing to tissue remodeling. Beta-tryptase of MC increases the production of TGF-β1 by airway smooth muscle cells and rises the expression of α-SMA. TGF-β also induces the production of mitochondrial ROS, which enhances their production in pulmonary fibroblasts.

TGF-β plays a crucial role in the regulation of airway ECM components: it increases the synthesis of proteins, such as fibronectin, enhances antiprotease activity, and stimulates the expression of desmosomal proteins by bronchial epithelial cells, which promotes the activation of proteins involved in intercellular junctions and the formation of the basement membrane of alveolar epithelial cells. Thus, the blood TGF-β level, considered by many scientists as a predicting marker of COVID-19 severity, has the potential to be used as a marker of fibrotic processes in other respiratory diseases.

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Authors' contribution

Budnevsky A.V. – research supervision, final approval of the manuscript for publication, critical revision of the manuscript for important intellectual content. Ovsyannikov E.S. – critical revision of the manuscript for important intellectual content, analysis of the data, editing of the article. Shishkina V.V. – drafting of the article, critical revision of the manuscript for important intellectual content, analysis of the data. Alekseeva N.G. – collection and processing of the material, drafting of the article, editing of the article for publication. Perveeva I.M., Kitoyan A.G., Antakova L.N. – collection and processing of the material, drafting of the article.

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Galectins: a potential pharmacological target

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ABSTRACT

Aim. To consider the use of galectin-1 and galectin-3 inhibitors as potential pharmacological targets in antitumor and antifibrotic therapy.

The lecture includes the analysis of experimental research and review articles presented in the PubMed database. A brief description of the structure of galectins is given. Their generally accepted classification and features of the structure of the carbohydrate recognition domain in galectin-1 and galectin-3 are presented. The main part of the lecture describes the results of research on the development of carbohydrate-based (β-galactoside derivatives or analogues) and non-carbohydrate-based (peptide-based, carboxamide derivatives) inhibitors capable of interacting with galectin-1 and galectin-3.

The results of experiments performed on animal models and tumor cell cultures demonstrate that the antitumor effect of galectin antagonists is realized through the suppression of proliferation and metastasis, activation of tumor cell apoptosis, and modulation of the antitumor immune response. Antagonists of galectin-1 and galectin-3 potentiate the effect of antitumor drugs and have an antifibrotic effect. Some of the compounds discussed in the lecture are undergoing clinical trials. The data presented in the lecture open up opportunities for the development and synthesis of new molecules of potential galectin-1 and 3 inhibitors.

Keywords: galectin-1, galectin-3, galectin inhibitors, antitumor immunity, tumor, fibrosis

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Галектины: потенциальная фармакологическая мишень

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РЕЗЮМЕ

Целью работы является рассмотрение использования ингибиторов галектина-1 и галектина-3 как потенциальных лекарственных средств противоопухолевой и антифибротической терапии.

Лекция разработана на основе анализа экспериментальных и обзорных статей, представленных в базе данных PubMed. Дана краткая характеристика строения галектинов, представлены их общепринятая классификация и особенности структурной организации углевод-распознающего домена галектина-1 и галек-

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тина-3. В основной части лекции описаны результаты исследований по разработке молекул-ингибиторов углеводной (производные или аналоги β-галактозида) и неуглеводной (на основе пептидов, производные карбоксамида) структуры, способных взаимодействовать с галектином-1 и галектином-3.

Результаты экспериментов, выполненных на лабораторных животных и культурах опухолевых клеток, демонстрируют, что противоопухолевое действие антагонистов галектинов реализуется через подавление пролиферации, метастазирования, активацию апоптоза опухолевых клеток и модуляцию противоопухолевого иммунного ответа. Антагонисты галектина-1 и галектина-3 потенцируют действие противоопухолевых лекарственных средств и оказывают антифибротический эффект. Ряд рассмотренных соединений проходит фазу клинических исследований. Представленные в лекции данные открывают возможности для разработки и синтеза новых молекул – потенциальных ингибиторов галектина-1 и галектина -3.

Ключевые слова: галектин-1, галектин-3, ингибиторы галектина, противоопухолевый иммунитет, опухоль, фиброз

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.

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INTRODUCTION

Studies of antitumor immunity molecular mechanisms substantiate the rationality of target approaches to cancer therapy. Galectins are one of the promising targets that support the immunosuppressive microenvironment of tumor cells [1–7]. Galectin-mediated mechanisms dysregulating immune responses that promote tumor growth and metastasis include inhibition of activation and induction of apoptosis of T lymphocytes, expansion of $Foxp3^+$ T-regulatory cells and their immunosuppressive activity, stimulation of differentiation of tolerogenic dendritic cells, suppression of natural killer pool, and polarization of macrophages towards M2 phenotype [3, 8–11].

Involvement of galectins in proliferation of fibroblasts and their high expression in lung, liver, and myocardium tissue fibrosis also allows considering galectins as targets for antifibrotic therapy [12–15]. The role of galectin-3 in fibroblast activation includes synthesis of type I collagen and a decrease in matrix metalloproteinase activity with suppression of extracellular matrix element degradation [12]. Overexpression of galectin-1 enhances angiogenesis and production of extracellular matrix elements through activation of the PI3K/Akt pathway leading to keloid tissue formation [16].

The lecture aims to consider the current state of developing galectin-1 and galectin-3 inhibitors as potential drugs for antitumor and antifibrotic therapy.

GALECTINS

Galectins, carbohydrate-binding proteins, are expressed by a wide range of cells. They have nuclear, cytoplasmic or extracellular localization and play a major role in inflammation, tumorigenesis, angiogenesis, and fibroblast differentiation. The structural component common for galectin molecules is the carbohydrate recognition domain (CRD) with a highly conserved amino acid sequence (about 135 amino acid residues), which binds β-galactosides as part of glycoprotein and glycolipid receptors. Depending on CRD number and structural organization, galectins are classified into prototype galectins which contain one CRD and can be monomers or homodimers (galectin-1, -2, -5, -7, -10, -11, -13, -14 and -15); chimeric galectins consisting of an N-terminal collagen-like domain and a C-terminal domain containing a single CRD (galectin-3); tandem-repeat galectins containing two CRDs connected by a linker peptide (galectin-4, -6, -8, -9, -12) [1, 3, 10, 17–20].

Among the galectin family, special attention should be paid to galectin-1 and galectin-3, the high expression of which is associated with fibrosis and poor prognosis in various types of cancer including colon, thyroid, pancreatic, bladder, stomach, kidney

carcinomas, squamous cell carcinoma, melanoma, lymphomas, and neuroblastoma [2, 10, 21–24].

Galectins specifically recognize the Gal-GlcNAc (LacNAc) branches of N-glycans bound to cell surface glycoproteins [19, 24]. The galectin-1 CRD preferentially recognizes galactose-β1-4-*N*-acetyl-glucosamine sequences on *N*- or *O*-linked glycans [25]. The galectin-1 CRD includes five subsites (A–E). Subsite C is highly conserved being the main recognition site for β-galactopyranoside residues. The tryptophan residue (Trp68) that establishes hydrophobic interactions between the aromatic ring and CH groups of galactose is highly important in recognizing the galactose part of molecules. Specific hydrogen bonds are formed between the hydroxyl groups of the carbohydrate ligand and amino acid residues (His44, Arg48, Asn46, Asn61, and Glu71) of subsites C and D [26, 27]. The CRD of galectin-3 consists of eight amino acids such as Arg144, His158, Asn160, Arg162, Asn174, Trp181, Glu184, and Arg186 conditioning its interaction with carbohydrates. The interaction of galectin-3 with natural disaccharide ligands (Lac/LacNAc) occurs through hydrogen bonds and van der Waals forces. Hydrogen bonds are formed between OH groups of galectin (C-4 and C-6) and Glc/GlcNAc (C-3) through His158, Asn160, Arg162, Glu184, and Asn174; van der Waals forces bond galectin and Glc/GlcNAc residues via Trp181 and Arg186 [28, 29].

The immunoregulatory activity of extra- and intracellular galectins makes it possible to develop therapeutic approaches based on eliminating effects of these molecules by altering their expression or direct blocking by specific inhibitor molecules.

GALECTIN INHIBITORS

Studies aimed at developing potential inhibitors of different galectin subtypes are focused on obtaining selective compounds with high bioavailability [17]. The main parameters of synthesized molecules conditioning their prospects are as follows: high affinity for the target galectin (values of the dissociation constant (K_d) in a low nanomolar range, the ability to compete with endogenous ligands in biologically significant concentrations), selectivity for various carbohydrate recognition domains of the target galectin, and cellular uptake and stability in biological media [30].

Most known galectin antagonists are glycomimetics and are β-galactoside derivatives or analogs that target the canonical carbohydrate binding site of galectins. These include aryl-*O*- and *S*-galactosides and lactosides, carbohydrate-based triazoles and isoxazoles, *O*-galactosylaldoximes, phenylthio-β-d-galactopyranoside analogues, *N*-acetyllactosamine thioureido derivatives, talosides, and various polyvalent sugar-based compounds [31–33]. Monoclonal antibodies, galactose-based polymers, synthetic multivalent and small ligands usually have low affinity and limited bioavailability when administered orally [17].

B.A.Salameh et al. succeeded in synthesizing a collection of stable 3-(1H-[1,2,3]-triazol-1-yl)-1-thio-galactosides containing galectin-3 inhibitors (K_d=107 μm), comparable in effect to natural disaccharide inhibitors such as lactose and N-acetyllactosamine [34]. Hydroxyl groups are known to impart polarity to thiodigalactoside molecules. Replacement or removal of any hydroxyl group not involved in interaction with galectin-3 may increase ligand affinity and oral bioavailability [35].

In a transplantable melanoma model (B16/F10 melanoma cell culture) and an orthotopic breast tumor model (breast tumor cell line 4T1), intratumoral administration of thiodigalactoside was accompanied by an increased count of $CD8^+$ T-lymphocytes infiltrating the tumor, a decreased count of $CD31^+$ endothelial cells, and proliferation of tumor cells [21, 36]. Thiodigalactosides were found to inhibit antioxidant protective effect of galectin-1 on hydrogen peroxide-induced apoptosis of endothelial cells [36].

K. Peterson et al. synthesized fluorinated derivatives of phenyltriazolylthiodigalactoside and investigated their inhibitory effect on galectin-1 and galectin-3. Symmetrically substituted phenyl-triazolylthiodigalactosides showed high affinity for galectin-3 (K_d up to 1-2 nm), asymmetric thiodigalactosides containing one triptrophenyl-triazole and one coumaryl fragments showed high affinity (K_d =7.5 nm) and 46-fold higher selectivity for galectin-3 versus galectin-1 [37].

An experiment on xenografts of a lung adenocarcinoma syngeneic model in C57/Bl/6 mice showed that oral administration of low-molecular-weight galectin-3 inhibitor GB1107 (3,4-dichlorophenyl-3-deoxy-3-[4 (3, 4, 5-trifluoro-phenyl))-1H-1, 2, 3-triazol-1-yl]-1-thio-α-D-galactopyranoside) suppressed the growth of adenocarcinoma and blocked metastatic spread.

Compound GB1107 promoted the polarization of tumor stroma macrophages towards the M1 phenotype and infiltration of tumor tissue with $CD8^+$T cells. Additional block of PD-L1 (programmed cell death ligand) with monoclonal antibodies promoted increased expression of IFNγ, granzyme B, perforin 1, and Fas ligand by cytotoxic $CD8^+$T lymphocytes and tumor cell apoptosis, assessed by increased expression of caspase 3 [22].

Using competitive NMR spectroscopy, F. Hőgye et al. assessed K_d of three symmetric derivatives of thiodigalactoside, bis-(β-D-galactopyranosyl)-sulfane modified by different aromatic substituents. According to the results obtained, bis-{3-*O*-[(naphthalene-2-yl) methyl]-β-D-galactopyranosyl}-sulfane, bis-{3-*O*-[(quinolin-2-yl)methyl]-β- D-galactopyranosyl}-sulfan and bis-(3-*O*-benzyl-β-D-galactopyranosyl)-sulfan bind to galectin-3 94, 30 and 24 times stronger than the reference compound bis-(β-D-galactopyranosyl)-sulfan. The authors highlighted the major importance of cation-π interactions in binding of aralkylthiodigalactoside derivatives to the ligand [38].

D. Vrbata et al. synthesized multivalent analogues of C-3 aryl-substituted thiodigalactoside inhibitors based on *N*-(2-hydroxypropyl) methacrylamide. Using enzyme immunoassay and bio-layer interferometry, 4 compounds were selected - with the substitution of 4-acetophenyl, 4-cyanophenyl, 4-fluorophenyl, and thiophen-3-yl, which possessed high affinity for galectin-3. Experiments on tumor cell cultures showed that the cyanophenyl-substituted glycopolymer demonstrated the greatest antiproliferative, antimigration, antiangiogenic and immunoprotective activity [6].

1,4-disubstituted triazoles were found to be high-affinity inhibitors of galectin-3. Conformational analysis of 1,4-di- and 1,4,5-trisubstituted galactose C3-triazoles showed that the triazole C5 substituent interfered with galectin proteins and, thereby, caused their lower affinity versus corresponding 1,4-disubstituted triazoles. The introduction of two 1,4-disubstituted triazole fragments into thiodigalactoside was accompanied by a lower affinity for galectin-3 [39].

Using surface plasmon resonance (SPR) technology, M.F. Marchiori et al. found that synthetic glycoconjugates of methyl 3-*O*-methyl-{[(3-phenyl)-2-propane-1-oic]-1H-1,2,3-triazol-4-yl}-α-d-galactopyranoside and methyl 3-*O*-methyl-[(6-aminohexan-2-oic)-1H-1,2,3-triazol-4-yl]-α-d β-galactopyranoside bind with high affinity (K_d=7.96 μm, K_d=4.56 μm, respectively) to galectin-3 through specific cation-π (Arg144) and ionic (Asp148) interactions. By connecting two independent CRDs of galectin-3 and creating a non-covalent cross-link between the two monomers, glycoconjugate methyl-{1-(1H-1,2,3-triazol-4-yl)-2-[2-(2-ethoxy)ethoxy]ethyl-4-*O*-(β-d-galactopyranosyl)-β-d-glucopyranoside}-4-*O*-(β-d-galactopyranosyl)-β-d-glucopyranoside achieves a submicromolar affinity for galectin-3 (K_{d1}= 0.15 μm, K_{d2}=19 μm) [28].

J. Stegmayr et al. assessed the absorption of previously synthesized galetin-3 inhibitors – a 1H-1,2,3-triazol-1-ylthiodigalactoside derivative and an α-d-galactopyranoside derivative *in vitro* on a colon adenocarcinoma cell culture (Caco-2). The authors showed that the 1H-1,2,3-triazol-1-ylthiodigalactoside derivative was only slightly absorbed by cells and likely exerted its effect in the extracellular compartment. The α-d-galactopyranoside derivative is characterized by high permeability through cell membranes [30].

Modification of glycomimetic molecules by introducing benzyl substituents into the 3-hydroxyl groups of β-d-galactopyranosyl-(1→1)-thio-β-d-galactopyranoside (TDG) made it possible to obtain compounds that inhibit binding of galectin-1 and galectin-3 on the cell surface [40].

F. Zetterberg et al. developed a relatively new class of promising drug structures of potential galectin-3 inhibitors, namely 1,3-substituted α-d-monogalactopyranosides. Higher affinity of monosaccharide molecules for the ligand was achieved through a combination of interactions of orthogonal multipolar fluoroamide, phenylarginine, sulfur-π- and halocarbonyl bonds [35]. Compound GB1490 (galectin-1: K_d=0.4 μm; galectin-3: K_d=2.7 μm) is a galectin-1 inhibitor obtained by replacing six-membered aryltriazolyl substituents in the α-d-thiogalactoside molecule. It prevents galectin-1-induced apoptosis of Jurkat cells and it demonstrated high bioavailability ($F\%$ > 99%) in experiments on mice when taken orally [41].

Doubly 3-*O*-coumarylmethyl-substituted thiodigalactosides demonstrated high affinity for galectin-3 in a mouse model of bleomycin-induced

pulmonary fibrosis [42]. Thiodigalactosides GB0139 and GB1211 obtained from disubstituted monogalactosides and having a high affinity for galectin-3, reduce the expression of profibrotic genes in liver myofibroblasts and exhibit antifibrotic activity in a model of carbon tetrachloride-induced liver fibrosis and bleomycin-induced lung fibrosis in C57BL/6 mice. Thiodigalactoside GB0139 (NCT03832946) is currently in phase IIb clinical trials for the treatment of idiopathic pulmonary fibrosis for inhaled use. Compound GB1211 (5-bromopyridin-3-yl-3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1H-1,2,3-triazol-1-yl]-1-thio-α-d-galactopyranoside) is undergoing phase IIa clinical trials as a potential drug for the treatment of liver cirrhosis (NCT03809052) and cancer (NCT05240131) [17].

M. Filipová et al. synthesized multivalent glycopolymer inhibitors of extra- and intracellular galectin-3 by combining poly-LacNAc-derived oligosaccharides (Galβ4GlcNAc) with copolymers of N-(2-hydroxypropyl) methacrylamide. Authors demonstrated that the synthesized glycopolymers significantly suppressed galectin-3-induced apoptosis of T-lymphocytes and migration of tumor cells in melanoma, colon, breast and prostate cancer [43].

Multivalent glycan ligands synthesized from β-cyclodextrin demonstrated a 153-fold higher affinity for galectin-3 versus monomeric glycan ligand. Maximum affinity for galectin-3 was found for the heptavalent ligand containing GalNAc (Tn antigen). Synthetic multivalent ligands based on β-cyclodextrin were shown to inhibit the binding of galectin-3 to human airway epithelial cells [44].

A typical disaccharide ligand of galectins is *N*-acetyllactosamine (LacNAc, Galβ4GlcNAc). A structure-affinity relationship study based on enzyme-linked immunosorbent assay of a series of fifteen *N*-(2-hydroxypropyl) methacrylamide-based glycopolymers with varying numbers of LacNAcs showed that the architecture and type of presentation of LacNAc (individual or clustered on di- or trivalent linkers) provided 300-fold increase in avidity for galctin-1 versus galectin-3 [45].

M. Raics et al. studied binding of two selenium-containing galectin-3 inhibitors such as di(β-D-galactopyranosyl) selenide in which two galactose rings are linked by one selenium atom, and di(β-D-galactopyranosyl) diselenide with a diselenic bond between two sugar units. Using NMR spectroscopy and fluorescence anisotropy titration, the studied compounds were found to bind to canonical S-shaped site of galectin-3. Di(β-D-galactopyranosyl) selenide demonstrated a stronger affinity for galectin-3 than di(β-D-galactopyranosyl) diselenide, but lower than thiodigalactoside which is the known inhibitor of galectin-3 [46].

Galectin inhibitors were found to be able to potentiate the effect of antitumor drugs. Thus, galectin-3 inhibitor of GCS-100 (NCT01843790) induces p53-mediated apoptosis of acute myeloid leukemia cells (myeloma cell lines U266 and RPMI8226) and enhances the effect of BH3-mimetics (drugs that inhibit anti-apoptotic proteins of the Bcl-2 and Mcl-1 family promoting the survival and chemoresistance of tumor cells [47]. Inhibition of galectin-3 by the antagonist GCS-100 increases apoptosis of prostate adenocarcinoma cell line (PC3) induced by cisplatin [48]. In a mixed culture of acute lymphoblastic leukemia (BCP-ALL) cells and bone marrow stromal cells (OP9), galectin-1 and galectin-3 inhibitors GM-CT-01 and GR-MD-02 increase sensitivity of tumor cells to vincristine and nilotinib, which was assessed by inhibition of proliferation and a lower number of viable cells [24].

One of the promising inhibitors of galectin-3 is a compound obtained from natural carbohydrate polymers that is the complex polysaccharide of belapectin (GR-MD-02). In a transplantable model of sarcoma (MCA-205 cells), prostate adenocarcinoma (TRAMP-C1 cells) and breast carcinoma (4T1 cells) in C57BL/6 and BALB/c mice, it was established that belapectin in combination with aOX40 (monoclonal antibody against OX40 (CD134)) reduces the content of myeloid derived suppressor cells (M-MDSC) in tumor tissue, proliferation of regulatory $Foxp3^+$ $CD4^+$ T lymphocytes and increases the density of effector $CD8^+$ T cells more effectively than with aOX40 monotherapy, which is accompanied by suppression of tumor growth and increased survival of experimental animals [49]. In phase I of the clinical trial, it was found that intravenous belapectin in combination with pembrolizumab (anti-PD-1 mAb) in patients with metastatic melanoma and squamous cell carcinoma of the head and neck leads to elevation of proliferating activated effector memory $CCR7^-$ $CD45RA^-CD4^+$ T cells and lower blood levels of monocytic myeloid suppressor cells [50].

An alternative to carbohydrate-based galectin antagonists are non-carbohydrate inhibitor molecules such as heterocyclic compounds, peptide-based inhibitors and peptidomimetics (OTX008/PTX008 and Anginex (β-pep25)) [33]. PTX008, allosteric inhibitor of galectin-1, suppresses aggregation, adhesion, and migration of acute lymphoblastic leukemia cells (early B-cell precursor ALL, BP-ALL) and increases their sensitivity to vincristine [51]. OTX008 (PTX008), galectin-1 inhibitor, suppresses the growth and increases oxygenation of tumor cells of human squamous cell carcinoma of the larynx (SQ20B) in an experimental mouse model (Athymic Nude, *Nu/Nu*) [31], enhances inhibitory effect of sorafenib on proliferation of hepatocellular carcinoma cells (MHCC97L) [52]. In combination with the chemotherapeutic agent of irofulven, PTX008 causes regression of ovarian tumor growth experimentally induced in mice (athymic nude, *Nu/Nu*) by introducing a human epithelial ovarian carcinoma cell line (MA148) [32].

Recently, new non-carbohydrate compounds binding C-terminal domains of galectin-3 and galectin-8C have been proposed. They are derivatives of *N*-arylsulfonyl-5-aryloxy-indole-2-carboxamide which are Cpd53 (galectin-3: K_d=4.12 μm, galectin-8C: K_d=6.04 μm) and Cpd57 (galectin-3: K_d=12.8 μm, galectin-8C: K_d=2.06 μm) compounds. Using molecular docking, the amino acids Arg144 of galectin-3 and Ser213 of galectin-8C were found to contribute to increased selectivity [5].

CONCLUSION

Galectin involvement in tumor cell transformation, metastasis, stimulation of angiogenesis and suppression of antitumor immune responses allows considering these carbohydrate-binding proteins as multifunctional targets for cancer therapy. Results of numerous studies assessing the effect of molecules with different structures on galectin-mediated effect indicate prospects of research in development of selective antagonists of galectin family individual members, and rationality of their combined use with antitumor drugs in order to enhance the chemotherapeutic effect. The high risk of tissue fibrosis of various localizations associated with increased expression of galectin-1 and galectin-3 indicates potential modulating effect on fibroblast proliferation by eliminating the effect of galectins using antagonists.

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Intracranial arachnoid cyst in a 28-year-old man. A clinical case with a fatal outcome

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ABSTRACT

Most intracranial arachnoid cysts are thought to be non-tumorous, congenital, intra-arachnoid cerebrospinal fluid collections that account for about 1% of all intracranial space-occupying lesions. In children, the prevalence of this pathology is 2.6%; in adults, it reaches 1.4%. The disease is more often registered in men. Most often arachnoid cysts are supratentorial. Their most common locations are in the middle cranial fossa and the retrocerebellar cistern. Less often they can be detected on the convexity of the brain hemispheres; however, cases of arachnoid cysts at more unusual sites have also been described, including in newborns. The pathology is often characterized by an asymptomatic course, while certain symptoms may have an acute onset, which is due to compression of brain structures caused by the large cyst size.

This article describes a clinical case of a large intracranial arachnoid cyst in a 28-year-old man. It was not verified in the antemortem diagnosis, but was revealed according to the autopsy findings (macroscopic features of the cyst, histologic presentation with specific morphological changes, and findings of computed tomography of the cerebral hemispheres).

Keywords: intracranial arachnoid cyst, morphology

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Арахноидальная внутримозговая киста у мужчины 28 лет. Клинический случай с летальным исходом

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РЕЗЮМЕ

Арахноидальные внутримозговые кисты представляют собой чаще врожденные, отграниченные листками паутинной оболочки пространства (образования кистозного строения), заполненные спинномозговой жидкостью, распространенность их составляет до 1% от всех объемных образований данной локализации, процесс в большинстве случаев имеет доброкачественный характер течения. У детей частота выявления патологии составляет 2,6%, этот показатель у взрослых пациентов соответствует значению 1,4%, несколько чаще заболевание регистрируется среди мужчин. Наиболее часто подобные кисты имеют супратенториальное расположение, преимущественно они диагностируются в средней черепной ямке, в ретроцеребеллярной области, реже их можно обнаружить в конвекситальных отделах больших полушарий, также описаны случаи более редких особых локализаций, в том числе у новорожденных. Патология часто характеризуется бессимптомным течением, при этом возникновение определенной симптоматики может иметь острое начало, обусловлено крупными размерами кист со сдавлением структур головного мозга.

В статье представлено описание случая внутримозговой арахноидальной кисты больших размеров у мужчины 28 лет, не верифицированной прижизненно и выявленной лишь посмертно на основании данных патологоанатомического исследования (макроскопические особенности образования, гистологическая картина с характерными морфологическими изменениями и данные компьютерной томографии больших полушарий головного мозга).

Ключевые слова: арахноидальная внутримозговая киста, морфология

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.

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INTRODUCTION

Intracranial arachnoid cysts (IAC) are most often congenital collections of cerebrospinal fluid delimited by the layers of the arachnoid mater. They account for about 1% of all intracranial space-occupying lesions. The process in most cases is benign. There is a classification according to which primary (developmental cysts) and secondary cysts are distinguished. The former are caused either by splitting of the arachnoid membrane during fetal development with the subsequent formation of sacs and abnormal accumulation of cerebrospinal fluid in them or by a failure of the frontal and temporal embryonic meningeal merging, resulting in a duplication within the Sylvian fissure [1]. Secondary cysts are more likely to be a consequence of previous infections or traumatic damage to brain structures. They can also result from surgical interventions and intracranial hemorrhages, while the communication with the ventricular system of the brain can be preserved but is often absent [1–4].

The literature presents data on cases of multiple IACs in one patient [5]. There is also information that congenital IACs can be associated with a mutation of the *FOXC2* gene on chromosome 16 [6] and can also cooccur with Arnold – Chiari malformation [7]. Medical cases of IACs were first described by R. Bright in 1831 [8]. The research results obtained by W.N. Al-Holou et al. report that the incidence of the pathology is about 2.6 and 1.4% in children and adult patients, respectively [5, 9]. It is known that the disease is detected somewhat more often in men [5, 8].

Most often arachnoid cysts are supratentorial. Their most common locations are in the middle cranial fossa (34%) and the retrocerebellar cistern (33%). Less often they can be detected on the convexity of the brain hemispheres (14%) [5]. However, cases of arachnoid cysts at more unusual sites have also been described, in particular, in prepontine cisterns, quadrigeminal plate, and between the cerebral hemispheres [10–12].

This pathology is registered in various age groups. Such cysts are often asymptomatic for a long time and become an incidental finding during structural and functional neuroimaging for unrelated symptoms and complaints [1]. A symptomatic IAC is a rare case and more often recorded in children [13]. However, researchers have described giant arachnoid cysts in young men who had acute neurological symptoms caused by severe compression of brain structures; the sizes of the cysts were 12.3 × 16. × 7.9 cm and 6.5 × 11.5 × 12.5 cm [14, 15].

There have been few studies addressing the morphology of arachnoid cysts. The main studies date back to 1972–2000. So, K. Rabiei et al. (2014) presented a detailed description of the morphological features of these formations and showed possible differences in the structure of the epithelial lining and extracellular components in the walls of the studied cystic brain lesions, including the presence of squamous and ciliated epithelium, as well as glial and neuronal components in typical arachnoid tissue. The discovered structural features of IACs allowed the authors to suggest that they have different barrier properties and characteristics of fluid flow, which may be a determining factor in assessing growth rates and susceptibility to relapse [16].

This article describes a clinical case of a large intracranial arachnoid cyst in a 28-year-old man. It was not verified in the antemortem diagnosis, but was revealed according to the autopsy findings (macroscopic features of the cyst, histologic presentation with specific morphological changes, and findings of computed tomography of the cerebral hemispheres).

CLINICAL CASE

On May 26, 2021, Patient F., 28 years old, was admitted to the University Clinics of Siberian State Medical University (Tomsk) via the emergency room. According to the medical history, the patient complained of the body temperature rising to 37.2˚C, severe weakness, and headache. These symptoms appeared acutely on the day of hospitalization. He came to the pharmacy, where his condition deteriorated significantly. The pharmacy staff called an ambulance.

The medical history reports that the patient had several tick bites in May. The patient was not examined for this reason and did not take any preventive measures from tick-borne encephalitis and Lyme disease (tick-borne borreliosis). Taking into account the symptoms and medical history, the patient was transported to the Infectious Disease Clinic of Siberian State Medical University. Upon admission, the clinical examination revealed that the patient was in a severe condition, body temperature was 37.3˚C, blood pressure – 100/60 mm Hg, heart rate – 88 bpm, and saturation rate (SpO_2) – 98%. The patient was conscious but had difficulty communicating. Facial hyperemia and sweating were noted. Neurological status during the examination in the emergency room revealed hyperesthesia, nuchal rigidity (four fingerbreadths), severe pain at the cranial nerve exits, palpebral fissure S > D, tongue deviation to the right, and decreased muscle strength.

Given such symptoms as the acute onset of the disease, the presence of severe weakness, fever, and headaches, high tick activity, and episodes of tick bites in history, upon admission to the clinic, the patient was diagnosed with A84.0 Far Eastern tick-borne encephalitis (Russian spring – summer encephalitis), form of neuroinvasive encephalitis, acute onset, severe condition. In accordance with this diagnosis, an examination and treatment plan were determined. Given the severe condition, the patient was immediately transported to the intensive care unit.

On May 27, 2021, the neurological examination showed that the patient was in a poor condition, which deteriorated gradually; neurological deficit increased; there was a trend toward hypotension. The patient was unconscious. He did not answer questions and did not follow commands, which made it impossible to conduct coordination and other tests. The following was observed: palpebral fissures and nasolabial folds D = S, eye pupils D = S narrowed, gaze fixed in the center. There were nuchal rigidity (four fingerreadths) and positive Kernig and Brudzinski signs. Reflexes and tone of

the upper and lower extremities were preserved. Abdominal reflexes were preserved. Pathological foot and hand signs were not detected. There were no reliable signs of pelvic disorders. On May 27, 2021, mechanical ventilation was used to support the patient's breathing. The patient started receiving inotropic therapy with a gradual increase in the dose of administered drugs.

Upon emergency admission, a lumbar puncture, magnetic resonance imaging (MRI) of the brain, as well as an enzyme-linked immunosorbent assay (ELISA) of blood were conducted to detect antibodies (Ab) and antigens (Ag) to tick-borne encephalitis and borreliosis virus in order to clarify the diagnosis and chose the treatment strategy. The result of cerebrospinal fluid examination (lumbar puncture): colorless, slightly turbid liquid (volume 2.5 ml); protein 0.2 g / l (reference 0.22–0.33 g / l); cytosis 26 in 1 μl (reference 4–5 in 1 μl); glucose 3.4 mmol / l. Cerebrospinal fluid (CSF) analysis: neutrophils 89%, lymphocytes 8%, monocytes 3%, plasma cells 0, eosinophils 0, basophils 0. Blood ELISA result was as follows: anti-tick-borne encephalitis IgM (–), IgG 160 U / ml; Lyme IgM (–) and IgG (–) antibodies.

MRI of the brain revealed the presence of an oval-shaped intracranial cystic lesion in the left frontal lobe (66 × 48 × 55 mm). There was expansive growth of a heterogeneous structure with signs of high protein content (the doctor speculated about the dysembryogenetic origin of the identified neoplasm). The contours of the cystic tumor were uneven and unclear. The cyst had a capsule of 2–5 mm thick. Areas of calcification were identified in the lower part of this formation. The anterior horn of the lateral ventricle was deformed and compressed. In the anterior sections, the interventricular septum was displaced to the right up to 6.8 mm. The sella turcica had a regular shape and size. The suprasellar cistern prolapsed into the sellar cavity, the pituitary gland was pushed to the bottom and flattened to 22 mm (changes similar to the empty sella syndrome). The intracranial segment of the right vertebral artery was narrowed to 1 mm; the left vertebral and basilar arteries were 2.5 mm.

The MRI protocol described the presence of areas of acute brain ischemia in the cortical parts of the right parietal lobe and in the cortical parts of the left parasagittal parietal lobe; their dimensions were up to 4 × 6 mm. The convexity subarachnoid space in the left parietal – occipital region was narrowed and was not distinct during the examination. Figure 1 shows MRI images of the brain. The changes in blood parameters, including those with a significant deviation from the reference values, are presented in Table. The patient's coagulogram and other blood biochemistry and general urinalysis parameters were within the normal range. The electrocardiogram (ECG) dated May 28, 2021 revealed the vertical axis of the heart, sinus rhythm of 96 beats per min, incomplete right bundle branch block; increased load on the right atrium; disruption of ventricular repolarization in leads 3 and aVF; changes in the ST segment and T wave with weak elevation in the precordial leads (ECG data suggest the possible presence of pericarditis, myocarditis, and / or cerebrovascular disorders); QT interval was within the normal range.

After conducting brain MRI and receiving an impression about the presence of a volumetric neoplasm in the left frontal lobe, a telemedicine consultation with a neurosurgeon was requested. Taking into account the symptoms, MRI results, and results of additional laboratory and instrumental research, the neurosurgeon diagnosed an abscess of the frontal lobe of the left hemisphere as the

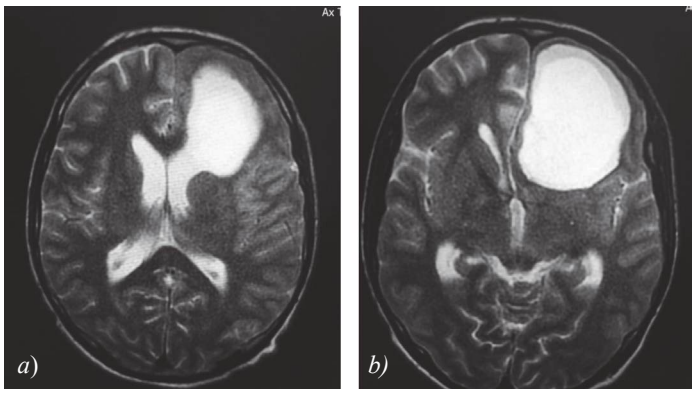


Fig. 1. Brain MRI. A cystic lesion in the left frontal lobe pressing against the adjacent brain tissues and adjacent structures (*a*, *b*)

underlying disease. It was recommended to transfer the patient for further treatment to the Neurosurgery Department. However, it was impossible to transport the patient and perform surgery due to the severity of the patient's condition. Therefore, it was decided to hold a second consultation when the hemodynamics stabilized.

Table

Patient's blood parameters

Blood counts	Changes in blood parameters		
	May 26, 2021	May 28, 2021	May 29, 2021
Red blood cells	4.24×10^{12} / l	4.86×10^{12} / l	4.9×10^{12} / l
Hemoglobin	133 g / l	155 g / l	162 g / l
White blood cells	9×10^{9} / l	19.2×10^{9} / l	18.5×10^{9} / l
Hematocrit	40%	45%	45%
Platelets	245×10^{9} / l	339×10^{9} / l	268×10^{9} / l

Over the next 3 days, the patient's condition was assessed as severe with negative dynamics (stage 3 coma). Heart rate was 126 beats per minute. Eye pupils D = S did not respond to light. On May 28, 2021, the body temperature rose to 37.6˚C; leukocytosis dramatically increased (19.2×10^{9} / l); a pronounced trend toward hypotension appeared; blood pressure was 80–60 / 50–40 mm Hg; hypernatremia of 160.2 mmol / l and hypokalemia of 2.32 mmol / l were detected. According to the progress notes, the condition was assessed as extremely severe and terminal with a pronounced trend toward hypotension.

On May 30, 2021, asystole was recorded. Medical staff started to perform cardiopulmonary resuscitation (CPR). Although CPR was effective and the heart rhythm restored in 5 minutes, blood pressure remained extremely low and unstable. Fifteen minutes later, with a newly emerging episode of asystole and signs of severe hypotension, medical staff started performing CPR which lasted for 30 minutes. CPR was not successful. The biological death was pronounced. The final clinical diagnosis was formulated as follows:

Underlying disease: an abscess in the frontal lobe of the left brain hemisphere.

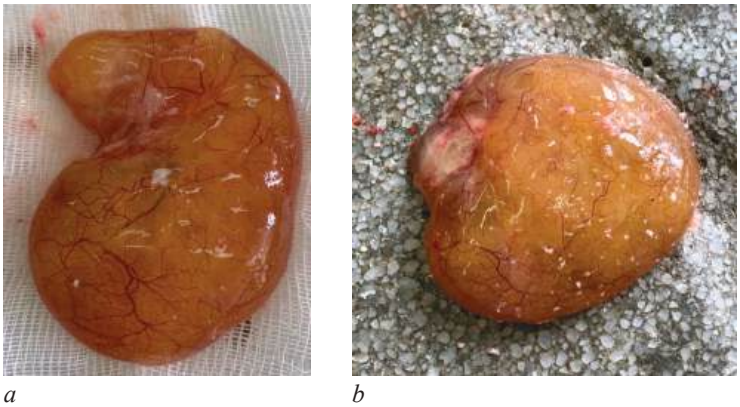
Complications: cerebral edema. Multiple organ dysfunction syndrome. Asystole. Cardiopulmonary resuscitation: mechanical ventilation, indirect heart massage, norepinephrine injection.

Concomitant diseases: incomplete bundle branch block.

The body of the deceased was sent for autopsy to the Anatomic Pathology Department of Siberian State University clinics.

PATHOLOGY EXAMINATION

The gross examination showed significant morphological changes in the brain and lungs. The meninges had a typical anatomical structure. The brain was enlarged due to edema (1,600 g). The brain was soft. The furrows and convolutions were unevenly smoothed. On the surface of the cerebellar hemispheres there was a distinct occlusal groove caused by the herniation into the foramen magnum. The cerebellum was flabby and watery; the structure was preserved on sections. The pons and brainstem were characterized by soft, flabby consistency; the anatomical structure remained intact. In the horizontal section of the white matter of the frontal lobe of the left hemisphere, a large (6.8 × 5 × 5 cm), clearly demarcated cystic structure with a thin transparent smooth capsule was found. It was located subcortically and had an oval shape. This cyst was represented by jelly-like masses of soft and elastic consistency of a yellowish-brown color with the presence of thin, dense layers of whitish tissue (Fig. 2). In the areas adjacent to the cyst, the brain



a *b*

Fig.2. Arachnoid cyst in the brain (macroscopic changes). The cyst appears as an oval-shaped, soft, elastic, yellowish-brown jelly-like masses with a thin, lucent capsule (*a*, *b*)

was flabby. The boundary between the white and the gray matter was not distinct. The cyst compressed the brain tissue. It made the shape of the anterior horn of the lateral ventricle slit-like; its cavity was not visually determined.

During the autopsy and examination of the chest organs, it was noted that the layers of the pleura were not changed; the lungs had the correct anatomical shape and were of normal size. The rest of the lung tissue was homogeneous, elastic, dark red in color; foamy, slightly pinkish liquid and liquid dark cherry-colored blood flowed abundantly from the surface of the sections. Gross autopsy did not reveal any significant pathological changes in other organs and tissues. Tissue fragments of internal organs were taken for the microscopic examination. The brain tissue fragments and fragments of the cystic lesion in the left frontal lobe of the brain and lungs were taken with specific marking.

The microscopic examination of brain tissue specimens revealed pronounced edema with the formation of areas of pronounced rarefaction of the neuropil, congestion in intra-organ vessels, diapedesis-type perivascular hemorrhage in single fields of vision, and leukostasis in the lumen of some capillaries. Pronounced edema was also detected throughout tissue fragments taken from the cerebellum and brain stem. The histologically described cystic formation in the frontal lobe of the left hemisphere was represented by abundance of chaotically located small-cell fibrous eosinophilic structures. The structures resembled multiple cystic cavities with deformed lumens and thin walls. Some cavities had homogeneous eosinophilic content.

In the peripheral areas of this cyst, an unevenly expressed inflammatory infiltrate was found with the presence of lymphoid cells, large cells like monocytes and macrophages with abundant cytoplasm; segmented leukocytes predominated in the cellular composition. The cyst was covered with one layer of closely adjacent columnar epithelial cells, on the apical part of which, multiple cilia were clearly detected. It also contained a large number of blood vessels, such as arteries, veins, as well as capillary-like vessels with thin walls in a state of pronounced congestion. In some fields of vision, only few large cells were identified, whose cytoplasm contained multiple round nuclei (multinucleate cells); small basophilic calcifications were also found in the cyst wall (Fig. 3). In the sections of the brain tissue adjacent to the cyst in the left frontal lobe, foci of acute ischemic damage were found with disruption of the matter structure. Signs of edema were significantly more pronounced; unchanged red blood cells were minimally detected in the foci.

Plethora and pronounced alveolar edema were observed throughout the fragments of lung tissue. In sections of the lower lobe of the left lung, the structure of the lung tissue was disrupted due to the presence of abundant purulent inflammation foci with a large number of segmented leukocytes and purulent bodies; erythrocytes were also detected in the exudate. Numerous red blood cells were also detected in the exudate. In some fields of vision, the interalveolar septa were not differentiated due to the described purulent exudates. In some fields of vision, the alveolar septa were not differentiated due to this exudate, which was most pronounced and had a predominant localization around the bronchi. The walls of the bronchi were unevenly thickened due to edema and pronounced inflammatory infiltrate. The boundaries of the bronchi in some fields of vision were not clearly differentiated, the walls were destroyed, the bronchial epithelium was absent, diffuse necrotic areas of the epithelium were identified. Masses of detritus and abundance of neutrophils with purulent bodies were found in the lumens of the bronchi. Leukostasis was detected in the capillaries of the lungs.

The morphological changes in the lung tissue described in the microscopic examination corresponded to focal purulent hemorrhagic bronchopneumonia of the indicated localization accompanied by alveolar edema and severe acute congestion with the presence of leukostasis. It should be noted that only microscopy revealed significant histologic changes in the myocardium: unevenly expressed stromal edema, partial fragmentation of muscle fibers with the presence of inflammatory infiltrate with lymphocytes, single plasma cells, and larger cells like macrophages and segmented leukocytes in the stroma between the myocytes. This infiltrate was detected in the interstitium and extended to cardiomyocytes in many fields of vision. In the zones with a more pronounced inflammatory infiltrate, the contours of myocytes were unclear, foci with partial destruction of muscle fibers and leukostasis in the capillaries were detected. In the

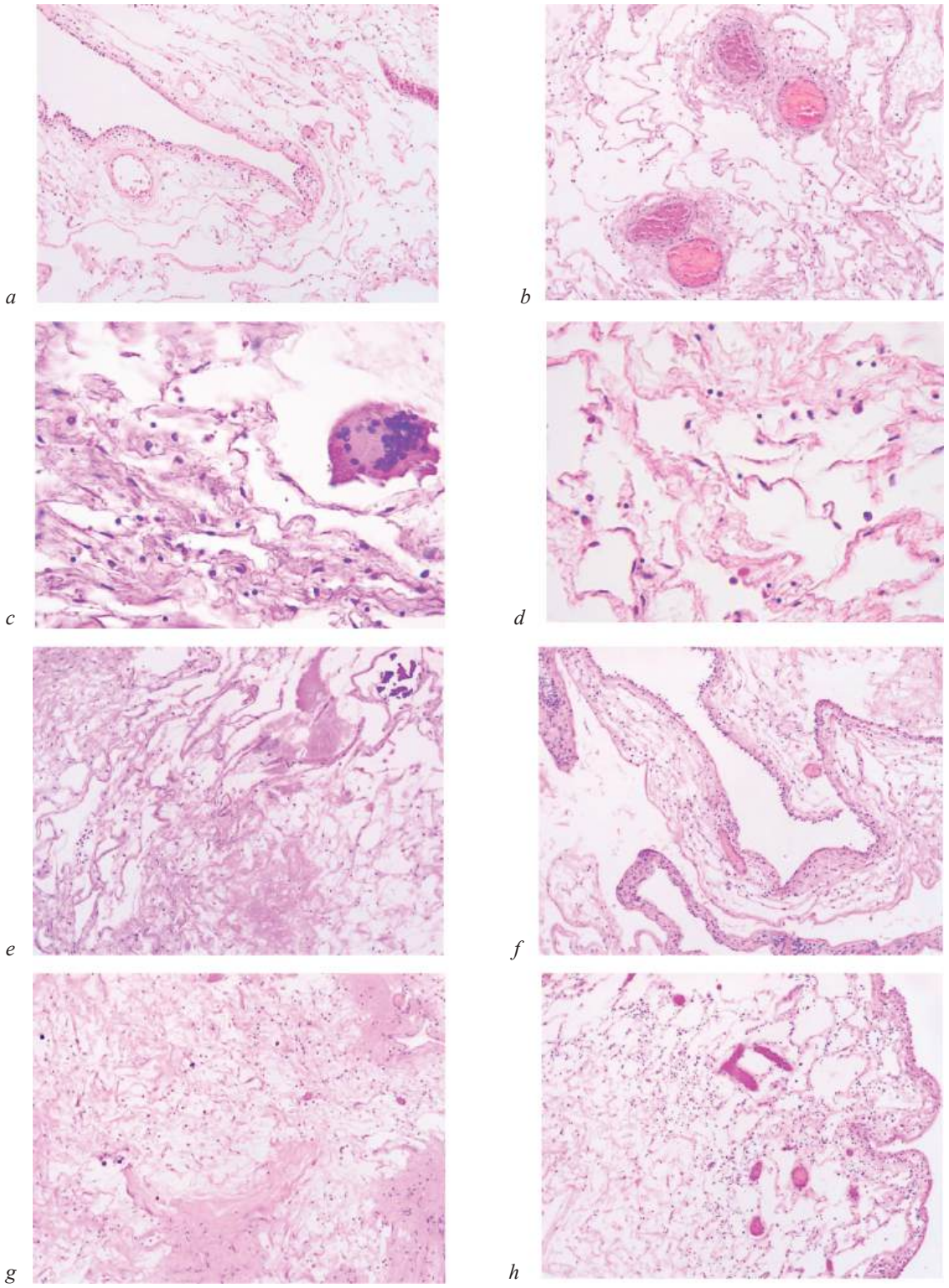


Fig.3. Arachnoid cyst in the brain (microscopic images). Staining with hematoxylin and eosin;
a–b, *e–h* ×10/0.22; *c–d* ×40/0.65

myocardium, uneven blood supply to the vessels, mild arteriosclerosis, and small foci of adipose tissue growth in the stroma were also identified.

The described pathomorphological changes corresponded to acute diffuse focal serous myocarditis with the morphological signs of acute heart failure. Based on the analysis of the medical history and taking into account the clinical presentation, gross changes, and microscopy results of organ tissue fragments, a pathology diagnosis was formulated.

PATHOLOGY DIAGNOSIS

Underlying disease: intracranial arachnoid cyst of the brain (cyst size 6.8 × 5 × 5 cm), type III according to the Galassi classification, with predominant localization in the frontoparietal region of the left hemisphere with foci of acute cerebral ischemia.

Complications: cerebral edema with dislocation and herniation of the cerebellar trunk into the foramen magnum. Coma. Hospital-acquired left-sided lower lobe focal purulent hemorrhagic bronchopneumonia. Sepsis (leukocytosis 19.2 × 10^9 /l, neutrophils 87.6%; lymphopenia 1.1 × 10^9 / l, lymphocytes 5.9%; leukostasis in the capillaries of the myocardium, lungs, brain). Acute diffuse focal serous myocarditis. Acute heart failure. Pulmonary edema.

Concomitant diseases: incomplete right bundle branch block (based on clinical data). Atherosclerosis of the aorta with the presence of type II–IV plaques, prevalence 10%.

CONCLUSION

In our article, we presented a clinical case of a 28-year-old male patient with IAC in the anterior horn of the left lateral ventricle. The cyst was discovered in a young man only posthumously. The diagnosis was established based on the results of a pathology examination. The case shows the presence of certain difficulties in diagnosing the pathology, including differential diagnosis in the presence of various factors in a particular patient.

In this case, the following aspects were of particular importance: no history of previous neurological symptoms, the acute onset of the disease during the peak seasonal incidence of tick-borne encephalitis and Lyme disease in the endemic area, extremely rapid development of another pathology in a hospital setting, in particular, pneumonia with changes in laboratory parameters typical of acute inflammation, (leukocytosis). The factors described above in the clinic resulted in an incorrect diagnosis of the pathological process, but the outcome of the disease most likely could not have been changed.

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Myopathy in glycogen storage disease type IV: case report of a family

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ABSTRACT

Aim. To study the clinical presentation and differential diagnosis of a rare hereditary disease glycogen storage disease type IV with progressive skeletal myopathy in a case report of a family.

Materials and methods. Two patients were followed up in the specialized neurology unit of the regional clinical hospital and in the outpatient setting.

Results. Long-term follow-up and examination in two clinically similar cases of myopathy in siblings allowed us to diagnose a hereditary metabolic disease. The congenital muscular form of glycogen storage disease type IV was manifested by myopathy and peripheral tetraparesis with the development of bone deformities. Difficulty in the diagnosis was due to isolated myopathy progression with no signs of liver involvement. The diagnosis was established with account of clinical manifestations, the progressive course of the disease, electromyography findings, and the results of molecular genetic testing for pathogenic mutations associated with hereditary neuromuscular diseases.

Conclusion. Glycogen storage disease type IV can clinically manifest itself by progressive myopathy without liver involvement and changes in blood biochemistry. The presented clinical cases in siblings are identical. Myopathy does not have clinical features that are significant for the differential diagnosis with other hereditary neuromuscular diseases. Genetic testing identified a mutation in the *GBE1* gene and is considered as the main diagnostic criterion of the disease.

Keywords: glycogen storage disease type IV, myopathy, neuromuscular diseases

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Миопатический синдром при болезни накопления гликогена IV типа на примере семейного случая

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РЕЗЮМЕ

Цель. Исследование клинической картины и дифференциальная диагностика редкого наследственного заболевания – болезни накопления гликогена IV типа с поражением скелетной мускулатуры на примере семейного случая.

Материалы и методы. Наблюдение двух пациентов в условиях специализированного неврологического отделения областной клинической больницы и амбулаторно.

Результаты. Длительное наблюдение и обследование в двух клинически схожих случаев миопатии у сибсов позволило установить диагноз наследственной болезни обмена веществ. Врожденная мышечная форма болезни накопления гликогена IV типа проявлялась синдромом миопатии, периферическим тетрапарезом с развитием костных деформаций. Сложность диагностики обусловлена прогрессированием изолированного миопатического синдрома при отсутствии симптомов поражения печени. Диагноз установлен с учетом клинических проявлений, прогрессирующего течения заболевания, данных электромиографии, результатов молекулярно-генетического исследования патогенных мутаций, ассоциированных с нервно-мышечными болезнями.

Заключение. Болезнь накопления гликогена IV типа может клинически манифестировать в виде прогрессирующей миопатии без патологии печени и изменений биохимических показателей крови. Представленные клинические случаи у сибсов идентичны. Миопатический синдром не имеет клинических особенностей, значимых для дифференциальной диагностики с другими наследственными нервно-мышечными болезнями. Генетическое исследование выявило мутацию в гене *GBE1* и рассматривается как основной диагностический критерий заболевания.

Ключевые слова: болезнь накопления гликогена IV типа, миопатия, нервно-мышечные болезни

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

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

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INTRODUCTION

Advances in the field of genetics in recent years have expanded the understanding of the diversity of storage diseases and the prospects for their early diagnosis, which determines the relevance of research and systematization of clinical aspects of this pathology. Glycogen storage diseases are a group of hereditary enzymopathies that occur due to genetically determined defects in enzymes involved in glycogen metabolism. Metabolic disorders lead to changes in the structure of glycogen and its accumulation in organs and tissues, which underlies the formation of clinical manifestations. Glycogen storage diseases are characterized by a wide range of clinical phenotypes [1, 2].

Currently, more than 20 types of glycogen storage diseases, including subtypes, are known.

Nine types have been studied the most, differing in the characteristics of enzyme deficiency, clinical manifestation, and prognosis variability – from a favorable course to severe progressive forms with a fatal outcome in childhood. As the study of various aspects of this pathology proceeds, their classification is improved [1–3].

Glycogen storage diseases are attributed to the group of orphan diseases, their prevalence in the population is 1 : 20,000–1 : 43,000 [1, 3]. Low prevalence of hereditary storage diseases in the population, clinical polymorphism, and a large number of phenocopies determine the difficulty in diagnosing them [4–7]. Glycogen storage disease type IV is an autosomal recessive disorder manifested by amylo-1,4:1,6-glucan transferase deficiency caused by a mutation in the *GBE1* gene encoding this enzyme, which leads to the accumulation of glycogen with an amylopectin-like structure in various organs and tissues including liver and muscles [1, 2].

Glycogen storage disease type IV is located on 3p12.2 chromosome [1, 2, 6]. The prevalence of this type of glycogen storage disease is between 1 : 600,000 and 1 : 800,000 [7]. There are several known clinical types of glycogen storage disease type IV: classic hepatic, non-progressive hepatic, fatal perinatal neuromuscular, congenital neuromuscular, childhood neuromuscular, adult neuromuscular with isolated myopathy. Various and topically heterogeneous syndromes of diffuse damage to the nervous system are possible [2, 6, 7]. The accumulation of genotype and phenotype correlations in this rare disease is now important.

CLINICAL CASE 1

Patient M., 7 years old, was examined in the neurological department of the regional hospital with complaints of periodic pain in the back and lower extremities. The parents noted that the child had weakness in the muscles of the back and limbs, impaired gait and posture. The child has been ill since birth and has a disability. It is known from the medical history that this was the first pregnancy, at the time the mother had anemia and threatened miscarriage in the first trimester. The boy was born at 40 weeks, spontaneous vaginal delivery. Birth weight was 3,340 g, body length was 56 cm. The Apgar score was 9/9 points.

After birth, diffuse hypotonia and hyporeflexia were noted. At an early age, the child was followed up with the diagnosis of "Spinal cord ischemia at the lumbar level, movement disorder." Weakness in the limbs, hypotension, and delayed motor development were noted in the patient. The parents were healthy, the mother was 34 years old, and the father was 43 years old. The patient's five-year old brother had similar symptoms; his three-year old sister was healthy. The child was repeatedly examined and received courses of treatment in multidisciplinary hospitals. The general condition was not affected, and no somatic symptom pathology was detected. The child's condition was interpreted as a movement disorder with delayed motor development due to perinatal pathology of the nervous system and subsequently as a possible hereditary neuromuscular disease.

Taking into account the slowly progressing motor disorders and the ineffectiveness of the treatment (metabolic therapy combined with exercise therapy, massage and physical therapy), myopathy was considered in the differential diagnosis. The level of creatine phosphokinase (CPK) in the blood throughout the entire follow-up period was within the normal range. The results of electromyography (EMG) revealed vague signs of primary muscle damage. Repeated EMG revealed symptoms of an anterior horn lesion, and, therefore, spinal muscular atrophy type 1 was included in the differential diagnosis. Magnetic resonance imaging (MRI) of the brain and lumbar spine and spinal cord did not reveal any pathology.

Neurological status. The child was active, mental and speech development was age-appropriate. The functions of the cranial nerves were not impaired. Muscle tone in the extremities was reduced and symmetrical. Tendon reflexes were of medium intensity in the arms, while in the legs, they were low and symmetrical. Muscle strength in the extremities was 3–4 points; it was decreased in the distal parts. The patient had muscle hypotrophy in the limbs and back; pronounced lumbar hyperlordosis, thoracolumbar scoliosis, and flat valgus feet. The patient rose from sitting and lying positions supporting himself. The gait was waddling. Sensitivity, statics, and coordination were regular.

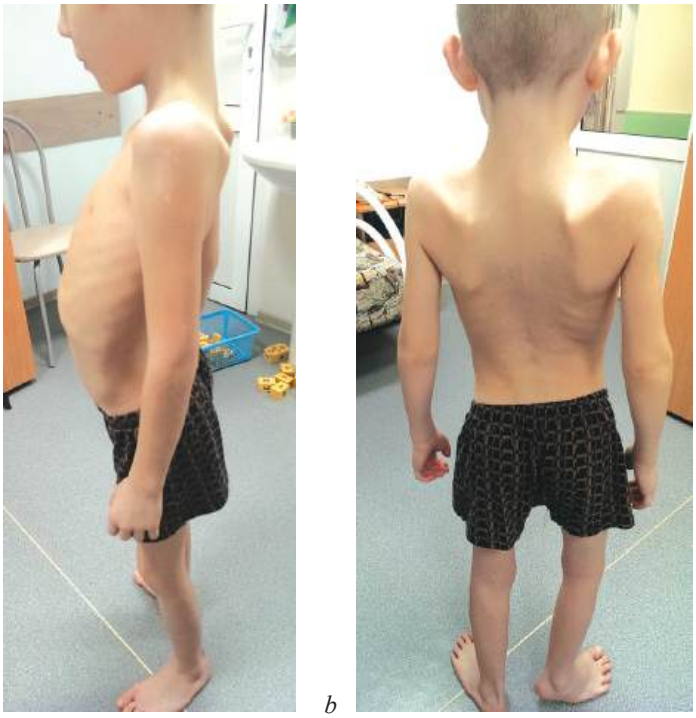
To clarify the type of myopathy, whole exome sequencing was performed. A search was conducted for pathogenic mutations associated with muscle dystrophies, as well as other hereditary diseases with similar phenotypic manifestations. A previously

described heterozygous mutation in intron 5 of the *GBE1* gene (chr3:81698005A>G, rs192044702) leading to disruption of the canonical splice site (c.691+2T>C, NM_000158.3) was identified.

The mutation has been described in compound heterozygous form along with other mutations in patients with glycogen storage disease type IV. Based on the data obtained, it should be regarded as pathogenic. In the same *GBE1* gene, a previously undescribed heterozygous mutation in exon 7 (chr3:81692139C>T, rs369574719) leading to an amino acid substitution in position 262 of the protein (p.Arg262His, NM_000158.3) was identified. Homozygous and compound heterozygous mutations in the *GBE1* gene have been described in patients with glycogen storage disease type IV (OMIM: 232500). Pathogenicity prediction algorithms evaluate this mutation as likely pathogenic (SIFT: 0.000, Polyphen2_HDIV: 1.000, Polyphen2_HVAR: 1.000, MutationTaster: 1.000, PROVEAN: -4.760, LRT: D). A mutation leading to amino acid replacement in the same position of the protein (p.Arg262Cys) was described in a compound heterozygous form together with another mutation in a patient with glycogen storage disease type IV (OMIM: 232500.0016). According to all the information obtained, the identified mutation should be regarded as likely pathogenic.

Upon further follow-up, progression of myopathy and secondary skeletal complications were noted (Fig. 1). Over time, the following symptoms progressed in the patient: the hypotrophy of the skeletal muscles of the limbs and back, thoracolumbar scoliosis to the left, lumbar hyperlordosis, flat back syndrome, flat valgus feet, retraction of the Achilles tendons, shortening of the right lower limb by 2 cm, secondary contracture of the right knee joint, first degree joint dysfunction, secondary extension contracture of the ankle joints, second degree joint dysfunction, weakness in the extremities, which was more pronounced in the proximal parts – up to 3 points, symmetrical tendon hyporeflexia.

The gait was waddling, involving extra muscles. The patient could not jump, run, or walk on his heels. Blood biochemistry test did not reveal any abnormalities. Clinically and following the results of additional examinations, no somatic symptom pathology was identified. Based on clinical manifestations, the progressive course of the disease, EMG data, and the results of molecular and genetic testing, a clinical diagnosis was established: “Congenital metabolic disease. Glycogen storage disease type IV, congenital muscular form, myopathy syndrome, peripheral tetraparesis, bone deformities”.



a *b*

Fig. 1. Patient M.: *a* – hypotrophy of the muscles of the extremities and back, lumbar hyperlordosis, flat back syndrome, flat valgus feet; *b* – curvature of the spine to the left in the lower thoracic and lumbar regions, shortening of the right lower limb

CLINICAL CASE 2

Patient G. is a 5-year-old brother of patient M. presented above (Fig. 2). The parents noted weakness in the muscles of the back and limbs, impaired gait and posture. The child has been ill since birth and has a disability. The child was born from the 2nd pregnancy, during which the mother was diagnosed with anemia and had threatened miscarriage in the first trimester. The child was born at 37–38 weeks of gestation by C-section due to breech presentation. Birth weight was 2,860 g, body length was 55 cm. Apgar score was 7/8 points. There was a delay in motor, mental and speech development.

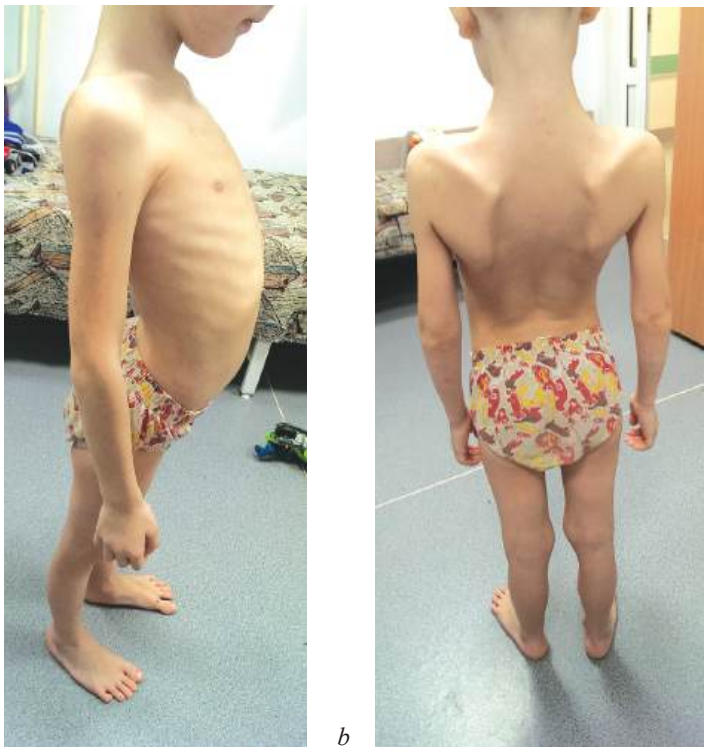


Fig. 2. Patient G.: *a* – hypotrophy of the muscles of the extremities and back, lumbar lordosis, flat back syndrome, flat valgus feet; *b* – curvature of the spine to the right in the lower thoracic and lumbar regions, pelvic asymmetry

He was followed up by a neurologist from an early age due to perinatal pathology of the nervous system, myopathy syndrome, and delayed motor and speech development. Courses of outpatient and inpatient treatment were conducted 2–3 times a year, no effect was observed. The patient was examined in a regional hospital. The blood level of CPK and aminotransferases was within the normal range. MRI of the brain, lower thoracic and lumbar spine did not reveal any pathological changes. EMG confirmed myopathy without any signs of anterior horn lesion. Abdominal ultrasound revealed hepatomegaly.

Neurological status. The mental and speech development were age-appropriate. The functions of the cranial nerves were not impaired. Muscle hypotrophy of the limbs and shoulder girdle, diffuse hypotonia, pterygoid shoulder blades, and a decrease in muscle strength in the arms and legs to 3–4 points were noted. Tendon hyporeflexia was noted in the limbs, without asymmetry or pathological reflexes. The gait was waddling, involving extra muscles. The patient used myopathy-specific movements when standing up (Gower's sign). Sensitivity was not impaired. Thoracolumbar scoliosis to the right, lumbar hyperlordosis, flat back syndrome, and pelvic asymmetry were noted. The patient had flat valgus feet with flattening of the longitudinal arch. The clinical diagnosis was established: "Congenital metabolic disease. Glycogen storage disease type IV, muscular form (clinically), myopathy syndrome, peripheral tetraparesis, secondary bone deformities".

CONCLUSION

The presented clinical cases of glycogen storage disease type IV reflect the diversity of clinical forms of this pathology and demonstrate the complexity of differential diagnosis in cases of skeletal muscle damage without manifestations of hepatic pathology. Myopathy

dominates in the clinical presentation and does not have specific features that make it possible to distinguish this disease from other hereditary myopathies. The absence of an increase in the blood CPK level characteristic of a primary muscle lesion during the entire follow-up period increased the diagnostic value of EMG for making the diagnosis. The ambiguity of the interpretation of EMG results determines the relevance of the differential diagnosis of muscular dystrophy with spinal muscular atrophy. The similarity of clinical symptoms in siblings, the progressive course of the disease, symmetrical and systemic muscle tissue damage, and development of the secondary bone deformities served as the reason for a genetic test. Clarifying the diagnosis was possible only with the use of DNA diagnosis and identification of mutations in the *GBE1* gene. Thus, genetic testing is an effective method in the differential diagnosis of neuromuscular diseases, the results of which can be used as a reliable guideline for medical genetic counseling.

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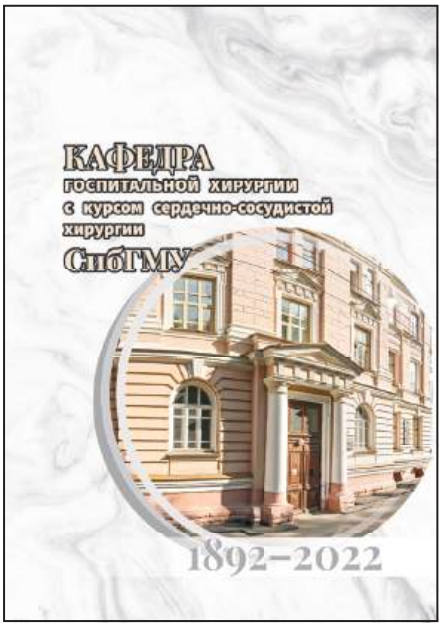
Издательский дом Сибирского государственного медицинского университета представляет серию книг

«Наследие томской медицины»



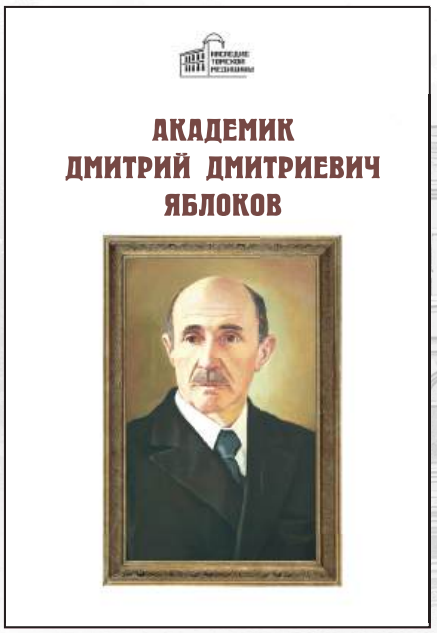
Книга посвящена 130-летнему юбилею кафедры госпитальной хирургии СибГМУ. Приведены биографические данные 79 сотрудников клиники и кафедры госпитальной хирургии в период с 1892 по 2022 г. Им предшествует подробная статья, характеризующая основные научно-практические достижения коллектива на каждом историческом 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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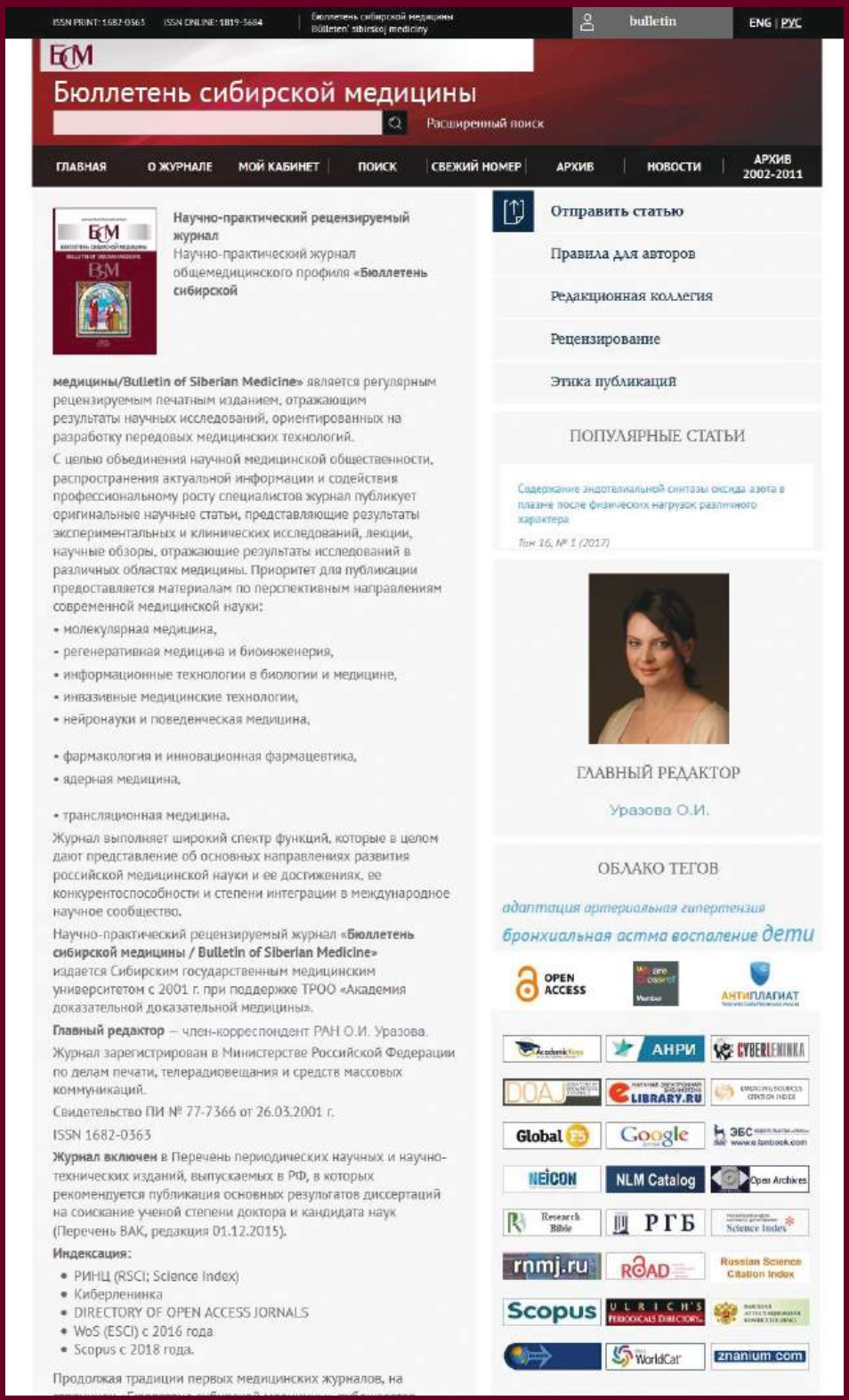

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