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CONTENTS

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Epithelial-mesenchymal transition markers, proliferation markers, and cytokine secretion in breast tissue in malignant and benign breast diseases

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ABSTRACT

Aim. To develop methodological grounds for assessing the probability of breast malignancy in patients with non-cancerous breast diseases (NCBD) by the following parameters: expression of markers of epithelial – mesenchymal transition (EMT) and proliferation and production of cytokines by samples of the breast tissue.

Materials and methods. In breast samples (BS) of patients with invasive carcinoma of no special type (ICNT) and patients with NCBD, immunohistochemistry was used to determine the expression of E-cadherin (CDH1), integrin β1 (CD29), type II collagen (CII), and proliferation of Ki-67. Using the enzyme-linked immunosorbent assay, concentrations of interleukin (IL)-2, IL-6, IL-8, IL-10, IL-17, IL-18, IL-1β, IL-1Ra, tumor necrosis factor (TNF)α, interferon (IFN)γ, granulocyte colony-stimulating factor (G-CSF), granulocyte – macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF)-A, and monocyte chemoattractant protein (MCP)-1 were determined in the supernatant of the cultured breast tissue samples.

Results. It was shown that ICNT and NCBD differ in the expression of E-cadherin, CD29, Ki-67, and the production of IL-2, IL-4, IL-6, IL-17, IL-18, IL-1Ra, TNFα, IFNγ, and MCP-1.

The ROC analysis found that the models characterizing the differences between the ICNT and NCBD samples were formed by the parameters of CD29 and Ki-67 expression and IL-17, IL-18, TNFα, VEGF-A, and MCP-1 production. The neural network analysis revealed that CD29, IL-1Ra, TNFα, and VEGF-A had the greatest normalized importance for assessing the differences between the ICNT and NCBD samples. Clustering of the combined database of patients with NCBD and ICNT by the expression of E-cadherin, CD29, Ki-67 and by the production of IL-17, IL-18, TNFα, MCP-1, and VEGF-A resulted in a cluster which includes the parameters of 94.1% of patients with NCBD. The parameters of less than 10% of patients with NCBD who fell into other clusters practically coincided with the studied parameters of the ICNT group, which suggests that these patients may form a risk group with the malignancy probability of more than 90%.

Conclusion. The data obtained made it possible to develop methodological grounds for assessing the likelihood of breast malignancy in patients with NCBD.

Keywords: non-cancerous breast diseases, invasive carcinoma of no special type, proliferation marker, markers of epithelial – mesenchymal transition, cytokines

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the

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Маркеры эпителиально-мезенхимального перехода, пролиферации и продукция цитокинов в ткани молочной железы при злокачественных и незлокачественных заболеваниях молочной железы

Аутеншлюс А.И.[1, 2], Архипов С.А.[1, 2], Михайлова Е.С.[1, 2], Архипова В.В.[1], Проскура А.В.[2], Вараксин Н.А.[3], Ляхович В.В.[2]

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РЕЗЮМЕ

Цель. На основе изучения экспрессии маркеров пролиферации, эпителиально-мезенхимального перехода (ЭМП) и цитокинового профиля суперталантов образцов ткани молочной железы (МЖ) при раке МЖ и незлокачественных заболеваниях (НЗМЖ) разработать методологические основы оценки вероятности малигнизации МЖ при НЗМЖ.

Материалы и методы. В образцах МЖ больных с инвазивной карциномой неспецифического типа (ИКНТ) и пациентов с НЗМЖ иммуногистохимическим методом определяли экспрессию Е-кадгерина (CDH1), интегрина β1 (CD29), коллагена II типа (CII) и маркера пролиферации Ki-67. С помощью иммуноферментного анализа в супернатанте культивируемых образцов МЖ определяли концентрацию интерлейкина (IL) 2, IL-6, IL-8, IL-10, IL-17, IL-18, IL-1β, IL-1Ra, фактора некроза опухоли-альфа (TNFα), гамма-интерферона (IFNγ), гранулоцитарного колониестимулирующего фактора, гранулоцитарно-макрофагального колониестимулирующего фактора, фактора роста эндотелия сосудов (VEGF-A) и моноцитарного хемотаксического белка 1 (MCP-1).

Результаты. Показано, что ИКНТ и ДЗМЖ отличаются по экспрессии Е-кадгерина, CD29, Ki-67 и продукции IL-2, IL-4, IL-6, IL-17, IL-18, IL-1Ra, TNFα, IFNγ, MCP-1. При помощи ROC-анализа установлено, что модели, характеризующие различия между образцами ИКНТ и ДЗМЖ, формируются по параметрам экспрессии CD29, Ki-67 и продукции IL-17, IL-18, TNFα, VEGF-A и MCP-1. При помощи нейросетевого анализа выявлено, что наибольшую «нормализованную важность» для оценки различий образцов ИКНТ и ДЗМЖ имеют параметры CD29, IL-1Ra, TNFα и VEGF-A. При кластеризации объединенной базы данных пациентов с ДЗМЖ и ИКНТ по экспрессии Е-кадгерина, CD29, Ki-67 и по показателям продукции IL-17, IL-18, TNFα, MCP-1 и VEGF-A формируется кластер, в который входят показатели 94,1% пациентов с ДЗМЖ. Параметры менее 10% пациентов с ДЗМЖ, попавших в другие кластеры, практически совпадали с исследованными параметрами ИКНТ. Это дает основание предположить, что эти пациенты могут составить группу риска с вероятностной малигнизацией более 90%.

Заключение. Полученные данные позволили сформировать методологическую основу для оценки вероятности малигнизации МЖ у пациентов с ДЗМЖ.

Ключевые слова: незлокачественные заболевания молочной железы, инвазивная карцинома неспецифического типа, маркер пролиферации, маркеры эпителиально-мезенхимального перехода, цитокины

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

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом Института молекулярной биологии и биофизики Федерального исследовательского центра фундаментальной и трансляционной медицины (протокол № 2016-3 от 15.03.2016).

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INTRODUCTION

It is known that the pathogenetic background for the development of cancer may be non-cancerous breast diseases (NCBD) [1, 2]. According to the classification of the International Agency for Research on Cancer, ductal carcinoma *in situ* is included in the group of precancerous breast lesions [3]. However, according to the literature, precancerous breast lesions encompass sclerosing adenosis [4, 5], radial scar [6], and intraductal proliferative lesions that increase the risk of developing breast cancer from 1.27 to 10.35 times, depending on the form of pathology [7–10]. These data determine the relevance of research aimed at searching for new markers to detect precancerous changes in the breast tissue, which may reflect the mechanisms of breast tissue malignancy in NCBD.

One of the processes characterizing the onset of malignant transformation is epithelial – mesenchymal transition (EMT) [11, 12]. It is known that EMT is characterized by activation of the expression of mesenchymal markers, such as integrin β1 (CD29) and type II collagen (CII), as well as by a decrease in the expression of E-cadherin (CDH1) [13–17]. The most widely used marker of cell proliferation in breast cancer is Ki-67 due to its reliable correlation with the proliferative activity of cancer cells [18].

Detection of EMT in the breast tissue can be considered as the first sign of developing cellular atypia, and the expression of a number of molecules associated with EMT can be seen as a marker indicating the onset of malignant transformation in NCBD, induced by a number of inflammatory mediators, including cytokines [12, 13]. In turn, production of cytokines that stimulate EMT can be caused by activation of certain signaling pathways in cells. Thus, induction of EMT under the effect of interleukin (IL)- β1 and TNFα is due to activation of the NF-kB signaling pathway [14]. These data indicate that malignancy of the breast tissue may depend not only on EMT, but also on specific changes in the cytokine profile of the tumor that determine a tumor microenvironment, which includes various immunocompetent cells, fibroblasts, fibrocytes, epithelial cells, and other cells that produce various cytokines. Some of the cytokines, which are produced by cells of the tumor microenvironment, facilitate progression of breast cancer [19, 20]. However, the role of cytokines in the formation of cellular atypia and breast malignancies has not yet been sufficiently studied for them to be considered as markers indicating a high risk of malignancy.

Aim of the study: to develop methodological grounds for assessing the probability of breast malignancy in patients with NCBD by studying the expression of EMT and proliferation markers and cytokine profile in the supernatants of breast tissue samples in breast cancer (BC) and NCBD.

MATERIALS AND METHODS

The material of the study was samples of breast tumors obtained from 79 women who were treated at

the oncology department No. 1 of Novosibirsk City Hospital No. 1. Of them, 62 people had stage II invasive carcinoma of no special type (ICNT) and 17 people had NCBD, including 8 people with fibroadenoma, 6 people with fibrocystic breast disease, including fibroadenomatosis, 2 people with ductal hyperplasia with areas of sclerosing adenosis, and 1 person with focal fibrosis with microcalcifications. The average age of patients with ICNT was 53.9 ± 1.8 (23–76 years), with NCBD – 45.4 ± 5.1 (19–67 years). The exclusion criteria were signs of distant metastasis and concomitant hormonal, chronic, inflammatory, and infectious diseases.

The study and all research protocols were approved by the Ethics Committee at the Institute of Molecular Biology and Biophysics (Protocol No. 2016-3) of the Federal Research Center for Fundamental and Translational Medicine (Novosibirsk, Russia). All procedures performed in this study were carried out in accordance with the Declaration of Helsinki of 1964 and its subsequent amendments (Brazil, Fortaleza, 2013). Each patient was informed about the study, its aim, and methods. An informed consent to participate in the study and to use tumor samples was signed by each patient and verified by the attending physician.

Tumor samples (8 mm^3) obtained by trepanobiopsy were washed with the DMEM-F12 culture medium three times, then placed in a glass vial with 1 ml of the DMEM-F12 medium, and incubated for 72 hours at 37 °C. After incubation, the test samples were removed from the medium and fixed in a 10% neutral buffered formalin solution for further immunohistochemical and histopathological studies. Concentrations of IL-2, IL-6, IL-8, IL-10, IL-17, IL-18, IL-1β, IL-1Ra, tumor necrosis factor (TNF) α, interferon (IFN) γ, granulocyte colony-stimulating factor (G-CSF), granulocyte – macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF)-A, and monocyte chemoattractant protein (MCP)-1 were determined in the supernatant of the cultured breast tissue samples by enzyme-linked immunosorbent assay (ELISA) using reagent kits manufactured by Vector-Best JSC (Russia).

The tissue samples fixed in 10% neutral buffered formalin were dehydrated and embedded in paraffin. Dewaxing and rehydration of paraffin sections were carried out according to the standard xylene / ethanol protocol.

The expression levels of Ki-67, E-cadherin (CDH1), integrin β1 (CD29), and CII in the ICNT and NCBD samples were determined using monoclonal antibodies, such as anti-Ki-67 (Leica Biosystems, Inc.), anti-E-cadherin (BD Biosciences, USA), anti-CD29 (BD Transduction Laboratories, USA), and anti-CII (Santa Cruz Biotechnology, Inc.), and VECTASTAIN ABC detection systems (Vector Laboratories, PK-7200, USA) in accordance with the manufacturers' instructions. The sections were additionally stained with hematoxylin and eosin and mounted with Canada balsam. The expression of the studied markers was analyzed using the MICROMED-6 microscope, the DSM 510 digital camera, and the ImageJ 1.42g software (NIH, USA). For each patient, 10 microphotographs (taken at x40) were evaluated. The expression data for Ki-67, CDH1, CD29, and CII were presented as percentage (% of cells expressing the marker).

The level of statistical significance of differences between the groups was determined using the nonparametric Wilcoxon – Mann – Whitney test. The data were presented as the median and the interquartile range Me (Q_{25}; Q_{75}). The calculations were performed using the Statistica v. 7 software.

The neural network analysis and ROC analysis of the obtained data were performed using the IBM SPSS software, v. 22. Normalized importance of various tumor sample characteristics for assessing the differences between the ICNT and NCBD samples was evaluated by the neural network analysis of the entire database, including parameters of ICNT and breast tissue in NCBD. The study used a neural network model generated on the basis of the Multilayer Perceptron model, with one hidden layer consisting of three hidden neurons.

The hidden layer activation function was hyperbolic tangent activation function, the output layer activation function was identity function. To verify the accuracy of the neural network analysis, the normalized importance of all model parameters was determined using two training methods – batch gradient descent method and interactive gradient descent method. The cluster analysis was performed using the Statistica v. 7 software.

RESULTS

Table 1 assesses the differences between the ICNT and NCBD samples by the expression of proliferation markers, EMT markers, and cytokine concentrations in the supernatant. Fig. 1 shows the ICNT and NCBD samples stained for Ki-67, E-cadherin, CII, and CD29.

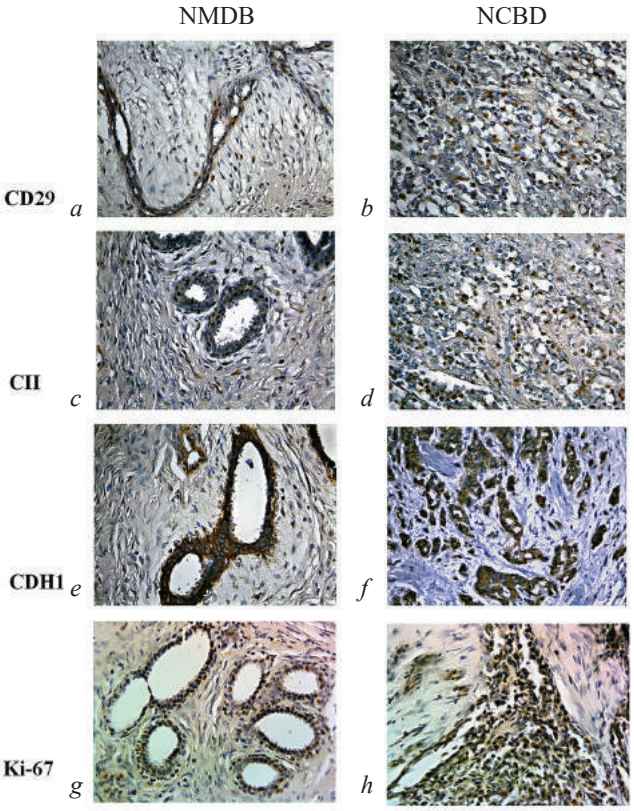

Fig. 1. Results of the immunohistochemical analysis of the tumor tissue: NCBD – non-cancerous breast disease (*a, c, e, g* – fibroadenoma); ICNT – invasive carcinoma of no special type (*b, d, f, h*). The brown – yellow coloration indicates the expression of EMT markers (CDH1 – E-cadherin; CD29 – integrin β1; CII – type II collagen) and the proliferation marker Ki-67. Counterstaining with hematoxylin and eosin, ×400

Ki-67 expression was 2.7 times higher in the ICNT samples compared to the NCBD samples. It was shown that the ICNT samples and the NMDB samples significantly differed in the expression of E-cadherin and CD29: the expression of E-cadherin was higher in the NCBD group than in the ICNT samples, while the expression of CD29 was higher in the ICNT samples than in the NCBD samples. There were no significant differences in the CII expression between the groups.

It was found that the ICNT and NCBD samples significantly differed in the production of IL-2, Il-6, Il-17, IL-18, IL-1Ra, TNFα, IFNγ, VEGF-A, and MCP-1. The concentration of IL-6 and MCP-1 in the breast tissue supernatant was higher in the NCBD samples than in the ICNT samples, and the concentration of IL-2, Il-17, IL-18, IL-1Ra, TNFα, IFNγ, and VEGF-A was higher in the ICNT samples compared to the NCBD samples.

Table 2 presents the results of the ROC analysis and the neural network analysis used to identify the differences between the ICNT and NCBD samples in the expression of proliferation and EMT markers and cytokine concentrations in the tumor tissue supernatant. The ROC analysis showing the quality of the models found that the best quality models characterizing the differences between the ICNT and NCBD samples were formed when CD29 and Ki-67 expression, as well as production of IL-17, IL-18, TNFα, VEGF-A, and MCP-1 were used as comparison parameters. According to the ROC analysis based on these parameters, the quality of models for detecting the differences between the ICNT and NCBD samples was good (AUC > 0.7) or very good (AUC > 0.8). The AUC values for CD29 and Ki-67, IL-17, IL-18, TNFα, VEGF-A, and MCP-1 were 0.750, 0.863, 0.732, 0.784, 0.722, 0.873, and 0.742, respectively (Table 2).

According to the data obtained using the neural network analysis, the highest normalized importance (more than 80%) in the neural network model used to detect the differences between the ICNT and NCBD samples was found for CD29 expression (100%), IL-1Ra production (> 90%), TNFα (> 90%), and VEGF-A (> 80 %). Relatively high normalized importance (more than 70%) in the neural network model used to identify the differences between the ICNT and NCBD samples was detected for E-cadherin and Ki-67 (Table 2). Table 2 shows that the neural network model training method (batch or interactive gradient descent method) did not have a significant impact on the results of the analysis for all variables.

Table 1

Expression of EMT-associated markers, Ki-67, and cytokine concentrations in the supernatant of ICNT and NCBD samples, *Me* (Q_{25}; Q_{75})

Parameter	Breast tissue samples		*p*
	ICNT	NCBD	
E-cadherin	64.2 (60.9; 91.7)	82.7 (79.7; 97.6)	0.020
CD29	19.6 (8.4; 19.7)	8.9 (1.3; 15.3)	0.001
CII	12.1 (6.5; 15.7)	10.5 (5.3; 14.9)	0.542
Ki-67	21.0 (12.0; 43.0)	8.3 (3.2; 19.8)	0.001
IL-2	2.8 (2.1; 5.4)	2.2 (2.1; 2.5)	0.005
IL-4	2.7 (1.7; 4.1)	3.2 (2.6; 4.4)	0.286
IL-6	297.8 (87.2; 482.7)	502.4 (279.6; 654.5)	0.027
IL-8	366.6 (203.1; 672.8)	378.7 (295.5; 1,360.0)	0.467
IL-10	6.1 (1.3; 11.8)	9.5 (1.7; 19.5)	0.404
IL-17	2.3 (1.0; 5.1)	6.0 (2.2; 7.4)	0.003
IL-18	42.4 (15.2; 180. 6)	5.0 (3.3; 26.5)	0.001
IL-1b	32.3 (14.7; 70.2)	17.0 (11.2; 38.8)	0.148
IL-1Ra	3,273.5 (2,172.6; 4,195.0)	2,070.6 (689.6; 3,003.2)	0.034
TNFα	2.9 (1.5; 5.2)	2.0 (1.1; 3.2)	0.046
IFN γ	11.5 (5.2; 26.0)	5.9 (2.0; 17.4)	0.027
G-CSF	61.1 (8.7; 424.6)	80.3 (41.1; 468.1)	0.745
GM-CSF	8.6 (3.1; 22.2)	3.2 (2.0; 13.4)	0.099
VEGF-A	1,359.2 (161.0; 2,144.0)	55.8 (18.4; 377.2)	0.001
MCP-1	560.9 (196.9; 1,556.0)	660.8 (259.6; 1,133.2)	0.046

Note: the expression of E-cadherin, CD29, and CII is presented as a percentage (% of expressing cells); cytokine values – in pg / ml.

Table 2

Evaluation of the differences between the ICNT and NCBD samples by the expression of the EMT and proliferation markers and cytokine concentrations in the breast tissue supernatant using the ROC analysis and the neural network analysis

Parameter	Normalized importance of a parameter in the NN-model; batch gradient descent method	Normalized importance of a parameter in the NN-model; interactive gradient descent method	Area under the curve (AUC) in the ROC analysis
E-cadherin	79.4%	78.6%	0.549
CD29	100.0%	100.0%	0.750
CII	36.3%	38.0%	0.162
Ki-67	78.0%	77.7%	0.863
IL-2	53.9%	53.5%	0.642
IL-4	22.5%	34.4%	0.415
IL-6	47.5%	49.7%	0.280
IL-8	45.5%	50.4%	0.441
IL-10	33.6%	40.4%	0.433
IL-17	68.3%	68.5%	0.732
IL-18	34.3%	23.1%	0.784
IL-1β	53.8%	40.6%	0.616
IL-1Ra	90.7%	95.7%	0.668
TNFα	91.3%	95.2%	0.722
IFNγ	12.3%	12.0%	0.676
G-CSF	9.7%	9.7%	0.473
GM-CSF	76.7%	71.7%	0.459
VEGF-A	81.6%	82.8%	0.873
MCP-1	72.1%	69.0%	0.742

Note: the NN-model – the neural network model. The results of the NN analysis are presented in terms of normalized importance of each parameter (%). The results of the ROC analysis are presented in AUC values.

Using the cluster analysis, we assessed the probability of cluster formation from the parameters of patients with NCBD with account of only the parameters with the highest normalized importance in the neural network analysis and the greatest AUC in the ROC analysis: CD29, Ki-67, IL-17, IL-18, TNFα, MCP-1, and VEGF-A. It was shown that when clustering combined data of patients with ICNT (sample No. 1–62) and NCBD (sample No. 63–79) by the specified parameters of breast tissue samples, 4 clusters were formed at the Euclidean distance of 15. One of the clusters – cluster III – included parameters of more than 90% (94.12%) of patients with NCBD (Fig. 2).

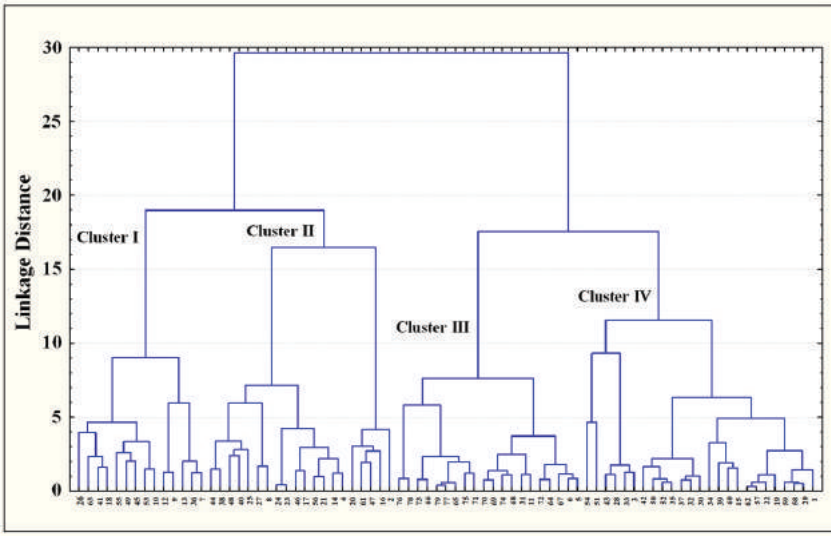

Fig. 2. Graphical representation of the results of the multidimensional cluster analysis of combined data from patients with ICNT (sample No. 1–62) and NCBD (sample No. 63–79). The dendrogram is constructed using the Ward's method. The horizontal axis represents encrypted numbers of samples obtained from different patients with ICNT (from 1 to 62) and NCBD (from 63 to 79), and the vertical axis represents the clustering distance (Euclidean distances). Clustering was performed on the basis of the simultaneous analysis of E-cadherin, CD29, and Ki-67 expression in the tumor samples and IL-17, IL-18, TNFα, MCP-1, and VEGF-A secretion by the tumor samples

The subcluster within cluster III, located at the Euclidean distance of 2.0, contained the parameters of three NCBD samples (sample No. 64, 67, 72) and two ICNT samples (sample No. 5, 6). The specified subcluster included samples from patients with NMDB with proliferative fibrocystic changes and atypical ductal hyperplasia (sample No. 64 and 72), as well as with fibroadenoma with severe ductal hyperplasia (sample No. 67) in the medical history. The parameters of one NCBD sample (assigned to other clusters) almost coincided with the parameters of ICNT. This sample was obtained from the patient diagnosed with fibroadenomatosis with pronounced proliferation (cluster I, sample No. 63).

DISCUSSION

The parameters of ICNT and NCBD samples obtained from different patients varied in terms of the expression of immunohistochemical markers of EMT and proliferation, as well as in cytokine production. In this regard, one of the main tasks was to develop a neural network model that would make it possible to predict and evaluate the probability of malignancy in non-cancerous diseases based on the assessment of EMT and proliferation markers and cytokine profile produced by tumor samples. It is known that if output parameters of a neural network model change, the importance of a particular tumor parameter also changes. The output of a neural network model may also depend on the way the model is trained. Therefore, when conducting the neural network analysis, we used two options for training the model.

With the help of the ROC analysis and neural network analysis, we found that some parameters of cytokine production by BS may have an even greater prognostic value for assessing the differences between malignant tumors and non-cancerous diseases than E-cadherin, CII, CD29, and Ki-67. Such cytokines include IL-17, IL-18, TNFα, MCP-1, and VEGF-A, as well as a number of others with a lower prognostic value. It was shown that when clustering the combined database of patients with NCBD and ICNT by a wide range of BS parameters, the expression of E-cadherin, CD29, and Ki-67 and the production of IL-17, IL-18,

TNFα, MCP-1, and VEGF-A allow to form a cluster which includes parameters of more than 90% of patients with NCBD. At the same time, the parameters of less than 10% of the NCBD samples that fell into other clusters practically coincided with the studied parameters of ICNT.

On the one hand, these data indicate that IL-17, IL-18, TNFα, MCP-1, and VEGF-A may play an important role in the formation of the microenvironment contributing to the onset of breast tissue malignancy in NCBD. On the other hand, at a certain level of their production, they can be considered as markers indicating the probability of malignancy in NCBD. According to the results of the study, patients with the following diagnoses were attributed to a group with a probable risk of malignancy in NCBD: fibroadenomatosis with pronounced proliferation, proliferative fibrocystic breast disease with atypical ductal hyperplasia, and fibroadenoma with pronounced ductal hyperplasia and with the presence of interductal proliferative lesions.

CONCLUSION

The data obtained make it possible to form a risk group of patients with NCBD with a probability of breast tissue malignancy of more than 90%. Thus, a more accurate prediction of probable malignancy in NCBD can be made taking into account not only the expression of E-cadherin, CII, CD29, and the proliferation marker Ki-67, but also the production of IL-17, IL-18, TNFα, MCP-1, and VEGF-A.

The data obtained can serve as methodological grounds for further study of cytokines that form the microenvironment in the breast tissue in non-cancerous diseases, which may contribute to breast tissue malignancy, and the level of cytokine production can serve as a marker for assessing the likelihood of this process.

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Authors' contribution

Autenshlyus A.I., Arkhipov S.A., Lyahovich V.V. – conception and design, analysis and interpretation of the data; justification of the manuscript and critical revision of the manuscript for important intellectual content; final approval of the manuscript for publication. Mikhaylova E.S., Arkhipova V.V., Proskura A.V., Varaksin N.A. – analysis and interpretation of the data; final approval of the manuscript for publication.

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A method to evaluate the functional state of the human brain after acute in-hospital stroke

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ABSTRACT

Acute in-hospital stroke is a severe complication of the early recovery period after cardiovascular surgery with a probability of up to 15%. Unfortunately, in-time diagnostic neuroimaging (computed tomography and magnetic resonance imaging) in cases of severe brain damages is considerably hindered increasing the risk of an adverse outcome.

The aim of the study was to develop a method to evaluate the functional state of the human brain in patients with severe in-hospital stroke measuring parameters of electrical activity in the central nervous system.

Materials and methods. The sample was composed of 20 anonymous archived electroencephalograms obtained from volunteers with no neurological disorders, 10 records of patients without neurological symptoms during general anesthesia, 17 records of patients with out-of-hospital strokes obtained from the UCLH Stroke EIT Dataset, and 18 records from patients with acute in-hospital stroke during neuromonitoring in the early postoperative recovery period. A new integral coefficient of the functional state was introduced, and an algorithm to calculate the proposed measure of the functional activity of the central nervous system was developed and implemented.

Results. The proposed method to evaluate the functional state of the human brain was applied to analyze neurophysiological records obtained from people with different activity of the nervous system: from resting state to deep coma. It was shown that the integral coefficient naturally reflects the functional state of the human brain and can be used for early detection of brain dysfunction and damages caused by cerebral hemodynamic impairment.

Conclusion. The introduced integral criterion to evaluate the functional state of the human brain can be used for long-term postoperative monitoring in cardiac patients who underwent surgical treatment.

Keywords: acute in-hospital stroke, electroencephalogram, functional state

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Способ оценки функционального состояния головного мозга при острых внутрибольничных нарушениях мозгового кровообращения

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РЕЗЮМЕ

Внутрибольничные нарушения мозгового кровообращения у пациентов кардиохирургического профиля являются тяжелым осложнением в раннем послеоперационном периоде с вероятностью появления до 15%. При развитии тяжелого поражения головного мозга проведение нейровизуализирующих диагностических исследований (компьютерной и магнитно-резонансной томографии) затруднено, что повышает вероятность неблагоприятного исхода.

Цель исследования заключается в разработке способа оценки функционального состояния головного мозга у пациентов с тяжелым течением внутрибольничного инсульта на основе неинвазивных измерений электрической активности центральной нервной системы.

Материалы и методы. Выборка составлена из 20 анонимизированных архивных записей электроэнцефалограммы добровольцев без неврологических нарушений, 10 записей пациентов без неврологических нарушений во время наркоза, 17 записей пациентов из банка данных UCLH Stroke Dataset с внебольничными инсультами и 18 записей, полученных в процессе нейрофизиологического мониторинга пациентов с тяжелым инсультом в раннем послеоперационном периоде. Для оценки функционального состояния разработан и реализован алгоритм вычисления интегрального показателя функционального состояния, характеризующего уровень функциональной активности центральной нервной системы.

Результаты. Предложенный способ оценки функционального состояния головного мозга был применен для анализа нейрофизиологических сигналов, полученных у людей с разной степенью активности нервной системы, от спокойного бодрствования до глубокой комы. Показано, что вычисляемый коэффициент закономерно отражает функциональное состояние головного мозга и может быть использован, в том числе, для раннего обнаружения нарушений, обусловленных церебральной гемодинамической недостаточностью при развитии острого инсульта.

Заключение. Интегральный критерий функционального состояния головного мозга может быть использован для длительного наблюдения за состоянием пациентов кардиохирургического профиля в ранний послеоперационный период.

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

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Ключевые слова: острый внутрибольничный инсульт, электроэнцефалограмма, функциональное состояние

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INTRODUCTION

Acute in-hospital stroke is a frequent adverse event in the early recovery period after cardiac surgery with a probability of up to 15% [1]. In the meantime, timely diagnosis of in-hospital stroke can be complicated particularly in coma patients. According to the study [1], in most cases, ischemic stroke is diagnosed within 3–6 hours from its onset. Taking into account a high risk of stroke within 3–7 days after cardiac surgery, neuromonitoring could be considered as a useful tool to detect early signs of cerebral hemodynamic impairment in the postoperative period [2]. Unfortunately, electrical activity of the human brain (electroencephalogram, EEG) is a complex noise-like signal characterized by low specificity in relation to causes of brain dysfunctions [3].

According to the published reviews, the diagnostic and prognostic potential of EEG strictly depends on EEG markers of stroke and qualification of a neurophysiologist. These facts along with complicated clinical interpretations and time-consuming neurophysiological studies (especially continuous round-the-clock neuromonitoring) may be the main reason for EEG not becoming a routine monitoring procedure in the early postoperative period.

Recently, a broadband system for multifrequency electrical impedance tomography (EIT) has been considered as a promising method for early stroke detection [4]. The EIT inherently features three-dimensional imaging options that makes it partly comparable with computed tomography (CT) and magnetic resonance imaging (MRI). Unfortunately, EIT has very low spatial resolution, preventing direct visualization of the damaged brain tissues, therefore, it cannot be used in clinical applications.

The study [5] suggests some EEG biomarkers which are highly reproducible in stroke patients. However, measuring these parameters implies active patient's participation, which is impossible in the early postoperative period. The alternative approach is based on mapping and evaluation of coherence of the cortical electrical activity in a resting state [6]. The authors showed that the proposed method is suitable for evaluating long-term brain function recovery, but is not as effective in continuous post-surgery monitoring.

Many formal EEG-based estimators of the functional state of the human brain rely on calculated frequencies and power of the dominant brain rhythms. In the study [7], many formal EEG parameters are used as independent variables to build partial regression models showing a relationship between the power of the dominant brain rhythms and NIHSS scores. Although the models demonstrated good performance, their practical usability is questionable because they require 256-channel EEG records.

A large number of publications focus on the attempt to use EEG-based methods for monitoring the depth of anesthesia (for example, bispectral index, entropy, information complexity of EEG, etc.) as a tool to monitor the functional state of the human brain in stroke patients. Although sensitivity, specificity, and information value of the known EEG-based methods still have to be confirmed, their relevance for continuous monitoring of the brain function in the early postoperative period is beyond doubt [8].

The aim of the study was to develop a method to evaluate the functional state of the brain in cardiac surgical patients with acute in-hospital stroke in the early postoperative period.

MATERIALS AND METHODS

Anonymized archived EEG records were obtained from Siberian State Medical University (20 records, healthy subjects with no neurological disorders), Cancer Research Institute of Tomsk National Research Medical Center (NRMC) (10 patients without neurological disorders during general anesthesia), 17 records from the UCLH Stroke EIT Dataset with out-of-hospital strokes [9], and 18 records from Cardiology Research Institute of Tomsk NRMC from cardiac surgical patients with acute stroke in the early postoperative period. All records were converted to the unified format: sampling rate 250 Hz, amplitude resolution 0.25 uV, frequency range from 0 to 100 Hz. The preprocessing included high-pass filter with the time constant of 0.16 sec and low-pass filter with the cut-off frequency of 100 Hz.

EEG signals were recorded using the Encephalan 131 EEG machine (Medicom, Russia, Taganrog), Neuron – Spectrum (Neurosoft, Russia, Ivanovo), and Biosemi Active Two system (Biosemi, the Netherlands, Amsterdam). All the EEG machines had comparable technical parameters. Electrode montage schemes were also identical and corresponded to the international 10 – 20 system. After preliminary calculations, it was found that the necessary and sufficient number of channels to evaluate an integral coefficient of the functional state of the brain was 8, including O1-A1, P1-A1, C1-A1, F3-A1, O2-A2, P2-A2, C2-A2, F4-A4. The raw signals were remounted

according to the monopolar montage scheme with ipsilateral ear electrodes.

Statistical analysis and signal processing were performed using free version of integrated development environment RStudio [10] with Signal and edfReader packages. The significance level of 0.05 was adopted.

RESULTS

To evaluate the functional state of the human brain, we proposed a new dimensionless index λ varying from 0 to 100 %, where 0 corresponded to zero electrical activity (brain death) and 100 % – to active state.

The algorithm to calculate λ includes the following steps.

Preprocessing of the multichannel EEG: removing baseline drift, components with frequencies above 100 Hz, and power line interference (50 Hz).

Independent component analysis (ICA) of the multichannel EEG:

$$Si(C_{1i},C_2) = ICA(EEG,ch_i,ch_{ref},$$

where S_i is signal decomposition; component C_{Ii} contains mainly EEG from channel *i*; C_2 represents artefacts with amplitude well above the typical EEG voltage. These artefacts are caused by electrical activity of the heart, eye movement, electrode dislocation, and muscle contraction.

Independent components are calculated based on paired channels with one channel always being the reference one (A1 or A2 for left and right hemisphere, respectively). This approach effectively removes artefacts common for both channels and, as a result, reduces uncertainty of bispectral parameters.

Bispectral analysis of the component C_{Ii} was followed by calculation of Gaussianity and linearity of the signal based on the Hinich method [11], as well as bicoherence and spectral power in the frequency range from 0.5 to 47 Hz. Fast Fourier transform with length $n = 512$ and Hamming window *w(n)* was used to build the bispectrum. The bispectrum was calculated for each component C_I independently.

Test for signal normality and linearity was performed according to the procedure given in [6]. The proposed integral index of the functional state λ will be calculated if the signal passes both the normality and linearity tests:

$$Sp(f,l) = FFT_n\big(w(n)\cdot C_1(l+n+m)\big); l \in (1, M-n-m)$$

$$B1 = \sum_{f1=0.5}^{8}\sum_{f2=0.5}^{8}\big(Sp(f1)Sp(f2)Sp^*(f1+f2)\big)^2$$

$$B2 = \sum_{f1=0.5}^{47}\sum_{f2=0.5}^{47}\big(Sp(f1)Sp(f2)Sp^*(f1+f2)\big)^2$$

$$\beta = \begin{cases}\|C_1\|, & \|C_1\| < 1\\ 1, & \|C_1\| \geq 1\end{cases}$$

$$\lambda = 100\cdot\left(1-\sqrt{\frac{B1}{B2}}\right)\cdot\beta$$

where *Sp* is Fourier transform of the component *C1*; *f1*, *f2* are frequency ranges; *B1* is the total power of the low-frequency bispectral components; *B2* is the total bispectral power in the range from 0.5 to 47 Hz; β is correction coefficient; λ is integral index of the functional state.

Steps 3 and 4 are repeated after moving the window by *m* samples, *m* should be chosen from 32, 64, 128, 256 as a a trade-off between computational complexity and required temporal resolution of λ.

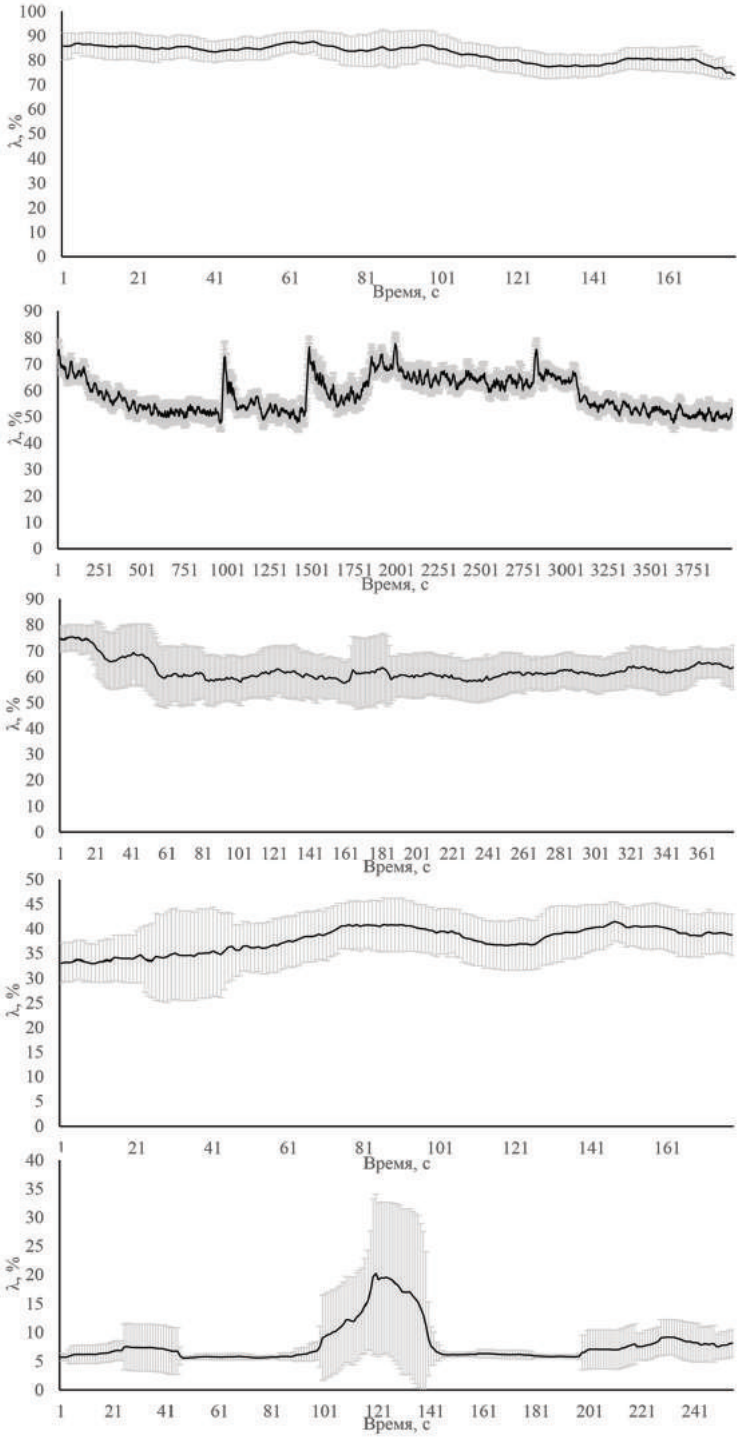
The frequency ranges were selected using commonly agreed neurophysiological bands: 8 Hz corresponds to the lower limit of alpha activity, and the upper limit of 47 Hz allows for effective removal of power line interference (50 Hz).

The proposed integral index of the functional state of the brain was calculated using archived EEG data obtained from healthy subjects in an active state and during normal sleep, from patients undergoing surgery, and from coma patients with acute in-hospital stroke (Figure). The solid line is an average value of λ for previous 30 sec, the confidence intervals were calculated for $\alpha = 0.05$ and $n = 30$.

It could be noticed (Fig. *b, c*), that the λ index reflects the functional state of the human brain with uncertainty of no more than 10 % throughout the recording intervals. This fact confirms that the proposed index follows the functional state of the human brain within physiological variations.

Not only does the average value of λ index decrease as patient's brain functions degrade, but also the confidence intervals dramatically expand (Fig. *d, f*). It could be indirect evidence that functional degradation of the brain manifests through gradual deceleration of dominant rhythms in parallel with disturbances in cortical interactions.

These results are in good agreement with the published clinical and neurophysiological findings [1]. The authors [1] showed that the key markers characterizing the severity of condition in stroke patients include typical neurological symptoms,



a) λ index in an active state

b) normal sleep

c) surgery, induced anesthesia

d) stage III coma

e) stage IV coma

Figure. Dynamics of the integral coefficient of the functional state of the brain: *a* – in an active state; *b* – during normal sleep; *c* – during surgery with induced anesthesia; *d* – stage III coma; *f* – stage IV coma

absence of the dominant EEG rhythm on the occipital electrodes, decreasing frequency of the electrical activity, and a lack of response to external stimuli.

Certainly, the selected examples are not quite typical of clinical practice, and the real cases are definitely more complicated; however, they allow to evaluate margins of the functional state of the brain. Unfortunately, the absolute values of λ can be used to differentiate only quite distinctive functional states of the brain, but its relative variations are sensitive enough to follow individual changes.

DISCUSSION

The proposed EEG-based method for evaluating the functional state of the human brain takes into account the commonly accepted neurophysiological concepts that attribute certain changes in electrical activity to functional degradation of the brain. One

of the most well-known parameters estimating the depth of anesthesia – bispectral index (BIS) – is based on the same concepts, but does not include cortical interactions. Our algorithm to calculate the λ index is different from the earlier described ones in the following:

Instead of raw EEG signals, independent components of multichannel EEG are used to calculate the λ index. This step significantly improves the reproducibility of the estimator.

Multichannel EEG must include at least 8 separate channels to reduce λ index uncertainty through spatial averaging.

The correction coefficient β was introduced to expand the dynamic range and numerical stability of the algorithm down to the lowest amplitude of EEG signals, which allows to evaluate even the most severe conditions of the brain.

The main drawback of the proposed index is low specificity with respect to the causes of brain dysfunction. As a result, it is almost impossible to diagnose the exact pathology that has caused the brain damage. One more limitation is a small size of the sample, which included only well distinguishable EEG records. Clinical ranges of the λ index can be found in expanded trails including more patients and a wider spectrum of functional states. Nevertheless, in the present form, the proposed integral index is sensitive enough to detect changes in the functional activity of the brain.

Taking into account the main features of the λ index, we would suggest two possible applications in clinical practice: control over the depth of anesthesia and continuous neurophysiological monitoring in the early postoperative period. We believe that the index will be the most effective for continuous monitoring of cardiac surgical patients in the early postoperative period.

CONCLUSION

The proposed integral index of the functional state of the human brain features some desirable properties, such as high reproducibility and clear physiological interpretation. The method is numerically stable and can deal even with very low-amplitude EEG. Sensitivity and specificity of this index in different pathologies require further expanded studies.

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The experimental study of dexamethasone effectiveness in a model of lipopolysaccharide-induced acute lung injury in rats

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ABSTRACT

Aim. To evaluate the efficacy and safety of dexamethasone at various doses in an experimental model of direct acute lung injury (ALI).

Materials and methods. The study was performed on 80 white outbred male rats, in which ALI was modeled by intratracheal administration of lipopolysaccharide. The animals were divided into 4 groups: the control group and three experimental groups (groups 1–3), where the animals were intraperitoneally administered dexamethasone at doses of 0.52, 1.71, and 8.00 mg / kg / day, respectively, for 3 days. A complete blood count, blood biochemistry test, and hemostatic tests were performed to assess the efficacy and safety of dexamethasone on day 3 of the experiment The severity of pulmonary edema was assessed by changes in the lung weight coefficient and the wet / dry weight ratio.

Results. The use of dexamethasone in the ALI model increased the survival of rats in groups 1 and 2 by 35% ($p < 0.05$), and in group 3 only by 20% compared with control animals. The rat lung weight coefficient and the wet / dry weight ratio when using dexamethasone at all doses studied were equally reduced by an average of 28% ($p < 0.05$) and 17% ($p < 0.05$), respectively ($p < 0.05$). The severity of side effects of dexamethasone (hyperglycemia, hyperproteinemia, hyperkalemia, hypercoagulability, increased activity of creatine phosphokinase in the blood) was dose-dependent and was maximum in group 3 (dexamethasone dose 8.00 mg / kg / day).

Conclusion. The effectiveness of both low (0.52 mg / kg / day) and high (8.00 mg / kg / day) doses of dexamethasone in an experimental model of ALI in rats is characterized by the same anti-edematous effect. Based on the results of the blood tests and the analysis of rat survival, the use of dexamethasone at the lowest dose (0.52 mg / kg / day) should be considered the safest.

Keywords: lipopolysaccharide, dexamethasone, acute lung injury, acute respiratory distress syndrome, biomodeling

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

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Экспериментальное исследование эффективности дексаметазона на модели липополисахарид-индуцированного острого повреждения легких у крыс

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РЕЗЮМЕ

Цель. Оценить эффективность и безопасность применения дексаметазона в различных дозах на экспериментальной модели прямого острого повреждения легких (ОПЛ).

Материалы и методы. Исследование выполнено на 80 белых беспородных самцах крыс, у которых моделировали ОПЛ посредством интратрахеального введения липополисахарида. Животные были разделены на четыре группы: контрольная группа и экспериментальные группы № 1–3, где животным по лечебной схеме (внутрибрюшинно, 1 раз/сут, в течение 3 сут) вводили дексаметазон в дозах 0,52; 1,71 и 8,00 мг/кг/сут соответственно. В целях оценки эффективности и безопасности дексаметазона на 3-е сут эксперимента проводили клинический, биохимический и гемостазиологический анализ крови, а также оценивали выраженность отека легких по изменению массового коэффициента и степени влагонасыщения органа.

Результаты. Использование дексаметазона в модели ОПЛ повышало выживаемость крыс в сравнении с контрольными животными в группах № 1 и 2 на 35% ($p < 0,05$), а в группе № 3 – только на 20%. Массовый коэффициент легких и степень влагонасыщения легких у крыс при использовании дексаметазона во всех исследованных дозах были одинаково снижены в среднем на 28% ($p < 0,05$) и 17% ($p < 0,05$) соответственно ($p < 0,05$). Степень выраженности побочных эффектов дексаметазона (гипергликемия, гиперпротеинемия, гиперкалиемия, гиперкоагуляция, повышение активности креатинфосфокиназы в крови) носила дозозависимый характер и была максимальной в группе № 3 (доза дексаметазона 8,00 мг/кг/сут).

Заключение. Эффективность как низких (0,52 мг/кг/сут), так и высоких (8,00 мг/кг/сут) доз дексаметазона на экспериментальной модели ОПЛ у крыс характеризуется одинаковым противоэдематозным эффектом. По совокупности результатов лабораторных исследований крови и анализа выживаемости крыс наиболее безопасным следует считать применение дексаметазона в минимальной дозе (0,52 мг/кг/сут).

Ключевые слова: липополисахарид, дексаметазон, острое повреждение легких, острый респираторный дистресс-синдром, биомоделирование

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

Over the past two decades, humanity has constantly faced challenges posed by outbreaks of infectious diseases caused by MERS-CoV, SARS-CoV, influenza A/H1N1, and SARS-CoV2. Severe forms of these infectious diseases often lead to significant damage to the lung tissue, up to the development of adult acute respiratory distress syndrome (ARDS) with 35–45% mortality [1].

One of the methods to find the best means of diagnosing, preventing, and treating ARDS in humans is experimental biomodeling of acute lung injury (ALI) in laboratory animals, which is as close as possible to the clinical manifestations of ARDS [2]. The best experimental model of ALI should reproduce the mechanisms and consequences of ARDS in humans and have the following features: clinical (acute onset, diffuse bilateral lung injury, acute exudative phase, proliferation and fibrosis in the end); pathophysiological (ventilation – perfusion mismatch, hypoxemia, decreased lung compliance, impaired alveolar fluid clearance); biochemical (increased concentration of proinflammatory cytokines, hemostatic disorders), and pathomorphological changes (neutrophil infiltration of lung tissue, damage to the alveolar epithelium and impaired permeability of the blood – air barrier, formation of hyaline membranes) [2]. The model of lipopolysaccharide-induced ALI in laboratory animals fully meets the above requirements and is widely used by researchers to search for and justify anti-inflammatory therapy regimens for ARDS using glucocorticoids [3, 4].

For example, M. Qin et al. [5] and J.W. Jang et al. [6] observed inhibition of NF-κB, p38, and NLRP3 inflammasome pathways when dexamethasone was administered at doses of 5 and 6 mg / kg in a model of ALI in mice, which led to a decrease in mortality, pulmonary edema, neutrophil tissue infiltration and microthrombosis in the lung vessels, as well as to a decrease in the levels of proinflammatory cytokines in the blood and bronchoalveolar lavage fluid. Another study using a similar experimental model showed that dexamethasone at a dose of 1 mg / kg exhibited antioxidant effects, inhibiting the functioning of inducible NO synthase in macrophages and polymorphonuclear leukocytes and increasing the expression of heme oxygenase-1 in the lung tissue [7]. Another positive effect of dexamethasone therapy revealed in the experiment was a decrease in the concentration of degradation products of autoantibodies in the peripheral blood flow and bronchoalveolar lavage fluid in mice [8].

In clinical practice, glucocorticoids are used as part of the complex therapy for ARDS, but their role in this therapy is still intensively discussed by the medical community. The results of meta-analyses on the evaluation of the effectiveness of glucocorticoids are often contradictory due to differences in patient selection, heterogeneity of ARDS causes, administration regimens (starting dates, pharmacological agents, dosage, treatment duration), and data processing [3].

The aim of the study was to assess the efficacy and safety of dexamethasone at various doses in an experimental model of direct ALI.

MATERIALS AND METHODS

The experiment was performed on 80 outbred male rats (age: 8–10 weeks, body weight: 310–320 g). The animals were kept in the vivarium in compliance with the basic hygienic requirements: temperature 20–24 °C, 12-hour light / 12-hour dark cycle, free access to food and water. The study was carried out in accordance with the requirements of the Order of the Ministry of Healthcare of Russia No. 199n of 01.04.2016 "On the Approval of the Rules of Good Laboratory Practice".

ALI was modeled by intratracheal (i/t) administration of lipopolysaccharide (LPS) of the *Salmonella enterica* cell wall (Sigma-Aldrich, USA) at a dose of 20 mg / kg. Before i/t administration, the animals were anesthetized with an intraperitoneal (i/p) injection of Zoletil 100 at a dose of 4.0 mg / kg. Intratracheal administration of LPS was performed using the MicroSprayer Aerosolizer device (model IA-1B, USA) 5 min after the animals were anesthetized.

The animals were divided into 4 groups: the control group with ALI and the experimental groups. Three hours after the modeling of ALI according to the treatment regimen (i/p, OD for 3 days), the animals were administered dexamethasone at doses of 0.52; 1.71 and 8.00 mg / kg. Doses of dexamethasone were calculated using the technique of interspecies dose conversion taking into account body surface area and were equivalent to daily doses of glucocorticoid for humans equal to 6 mg, 20 mg, and 94 mg, respectively [9].

Blood samples were taken for examination from the caudal vena cava on day 3 of the experiment. Complete blood count was performed on the automated veterinary hematological analyzer (Mythic 18 Vet, Switzerland), and blood biochemistry test was performed on the automated analyzer (ChemWell 2910, USA). Partial thromboplastin time (PTT), prothrombin time (PT), fibrinogen level, and antithrombin activity (in %) were determined on the semi-automated coagulation analyzer (Tcoag KC 4 Delta, Ireland). The level of soluble fibrin monomer complexes (SFMCs) was studied in the paracoagulation phenanthroline test (NPO Renam, Russia). Blood gas level and electrolyte composition were studied using the i-STAT automatic

analyzer (Abbott, USA). The following parameters of blood gas and electrolyte composition were determined: Na, K, Ca, pH, pCO_2, pO_2, TCO_2, HCO_3, BE, sO_2. After taking blood samples from the animals, the severity of pulmonary edema was assessed by conducting the morphometric analysis with the determination of the lung weight coefficient (LWC) and the wet / dry weight ratio (W / D ratio). LWC was calculated using the formula (lung weight, g / animal weight, g) × 1,000. The W / D ratio was calculated by the formula: wet lung weight, g / dry lung weight g. Before calculating the W / D ratio, the lungs were dried for 5 days in a thermostat at 37 ºC.

To test the hypotheses presented in this study, the statistical analysis of the results was carried out using the Graph Pad Prism 8.0 program. The results were presented as the median and the interquartile range *Me* [Q_1; Q_3]. The Kruskal – Wallis test was used for multiple comparisons of quantitative variables with further post hoc pairwise comparison using the Dunn's test. The relationship between qualitative variables (mortality) was assessed by constructing four-fold contingency tables and performing the Fisher's exact test based on them, followed by constructing a Kaplan – Meier survival curve. The differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Dyspnea and tachypnea were more often observed in rats of the control group than in comparison groups, in which rats were administered dexamethasone at various doses, which indicated the development of respiratory failure. The survival rate in the control group was 60%. The survival rate in groups 1 (dexamethasone at a dose of 0.52 mg / kg / day) and 2 (dexamethasone at a dose of 1.71 mg / kg / day) reached 95%, and in group 3 (dexamethasone at a dose of 8.00 mg / kg / day) was 80% (Fig.1).

In groups 1–3, statistically significant differences were observed in the values of LWC compared with the control group ($p = 0.002$, $p = 0.03$, $p = 0.0001$, respectively). The W /D ratio in the lungs also turned out to be significantly lower in all three experimental groups than in the control group ($p = 0.006$ and $p = 0.04$, $p = 0.02$) (Fig. 2).

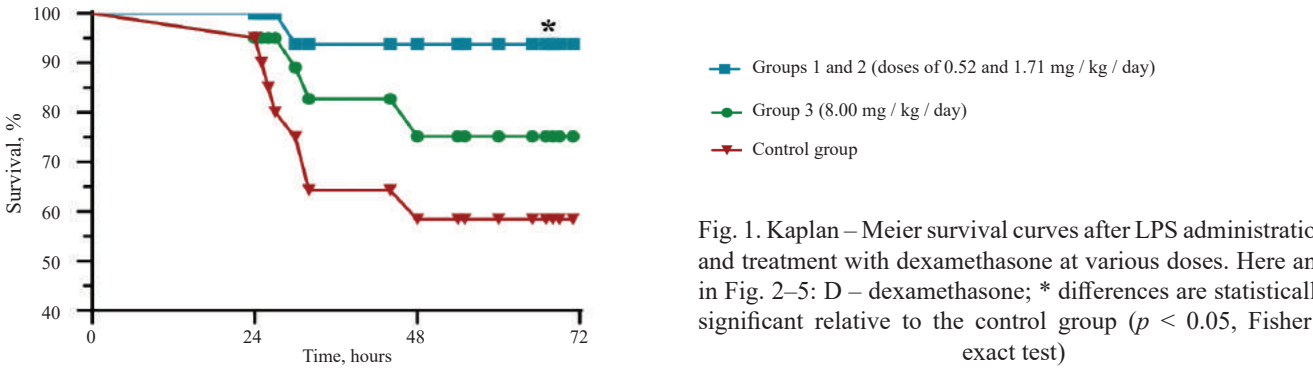

Fig. 1. Kaplan – Meier survival curves after LPS administration and treatment with dexamethasone at various doses. Here and in Fig. 2–5: D – dexamethasone; * differences are statistically significant relative to the control group ($p < 0.05$, Fisher's exact test)

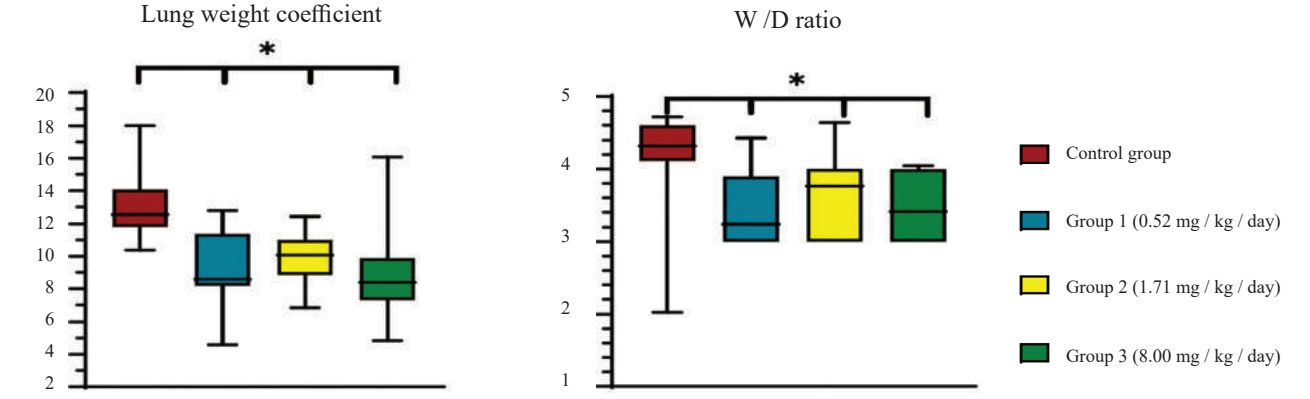

Fig. 2. Lung weight coefficient and the W / D ratio in rats on day 3 after modeling acute lung injury *Me* [Q_1; Q_3]. * differences are statistically significant relative to the control group ($p < 0.05$, Kruskal – Wallis test)

The use of dexamethasone led to typical changes in the leukocyte formula, which manifested by significantly lower absolute and relative lymphocyte count (p = 0.002, p = 0.02, p = 0.04). At the same time, an increase in the absolute and relative monocyte count (p = 0.03, p = 0.007, and p = 0.01, respectively) and granulocyte count (p = 0.08, p = 0.007, p = 0.09) was observed. The number of platelets was significantly smaller in group 3 when compared with groups 1, 2, and the control group (p = 0.006, p = 0.004, p = 0.02) (Table).

When comparing the concentrations of sodium and ionized calcium in the blood of rats in the experimental groups, no significant differences were found, although these groups showed changes depending on the doses of dexamethasone used. Groups 2 and 3 (Fig. 3) had higher potassium levels compared to the

Table

Parameters of complete blood count in rats on day 3 after modeling acute lung injury and treatment with dexamethasone at various doses, *Me* [Q_1; Q_3]

Parameter	Experimental groups			
	control group	group 1 (dexamethasone 0.52 mg /kg/mg)	group 2 (dexamethasone 1.71 mg/kg/mg)	group 3 (dexamethasone 8 mg/kg/mg)
Leukocytes, 10^9 / l	8.6 [7.0; 11.0]	7.9 [6.9; 8.0]	8.3 [6.7; 11.0]	8.5 [7.2; 10.0]
Lymphocytes,10^9 / l	5.4 [4.6; 5.8]	1.7* [0.8; 2.2]	2.1* [1.4; 2.8]	2.4* [1.6; 3.0]
Monocytes, 10^9 / l	0.4 [0.3; 0.5]	0.9* [0.7; 1.2]	1.0* [0.7; 1.3]	1.0* [1.0; 1.3]
Granulocytes, 10^9 / l	1.5 [1.3; 1.7]	4.4* [3.3; 5.0]	4.6* [3.6; 6.0]	4.1* [3.7; 4.5]
Lymphocytes, %	74.0 [73; 75]	19.0* [17.0; 23.0]	25.0* [20.0; 30.0]	31.0* [30.0; 34.0]
Monocytes, %	5.0 [5.0; 6.0]	14.0* [10.0; 15.0]	13.0* [11.0; 15.0]	15.0* [13.0; 16.0]
Granulocytes, %	21.0 [20.0; 21.0]	67.0* [55.0; 74.0]	62.0* [57.0; 68.0]	54.0* [50.0; 57.0]
Erythrocytes 10^{12} / l	7.5 [7.4; 7.7]	6.9 [6.5; 7.8]	7.5 [7.2; 8.1]	7.7 [7.3; 7.9]
Hemoglobin, g / l	154.0 [150.0; 160.0]	156.0 [147.0; 166.0]	153.0 [147.0; 166.0]	163.0 [155.0; 164.0]
Platelets, 10^9 / l	519.0# [515.0; 592.0]	548.0# [506.0; 598.0]	511.0# [443.0; 586.0]	410.0 [335.0; 449.0]

Differences are statistically significant: * relative to the control group (p < 0.05, the Kruskal – Wallis test); # relative to group 3 (p < 0.05, the Kruskal – Wallis test).

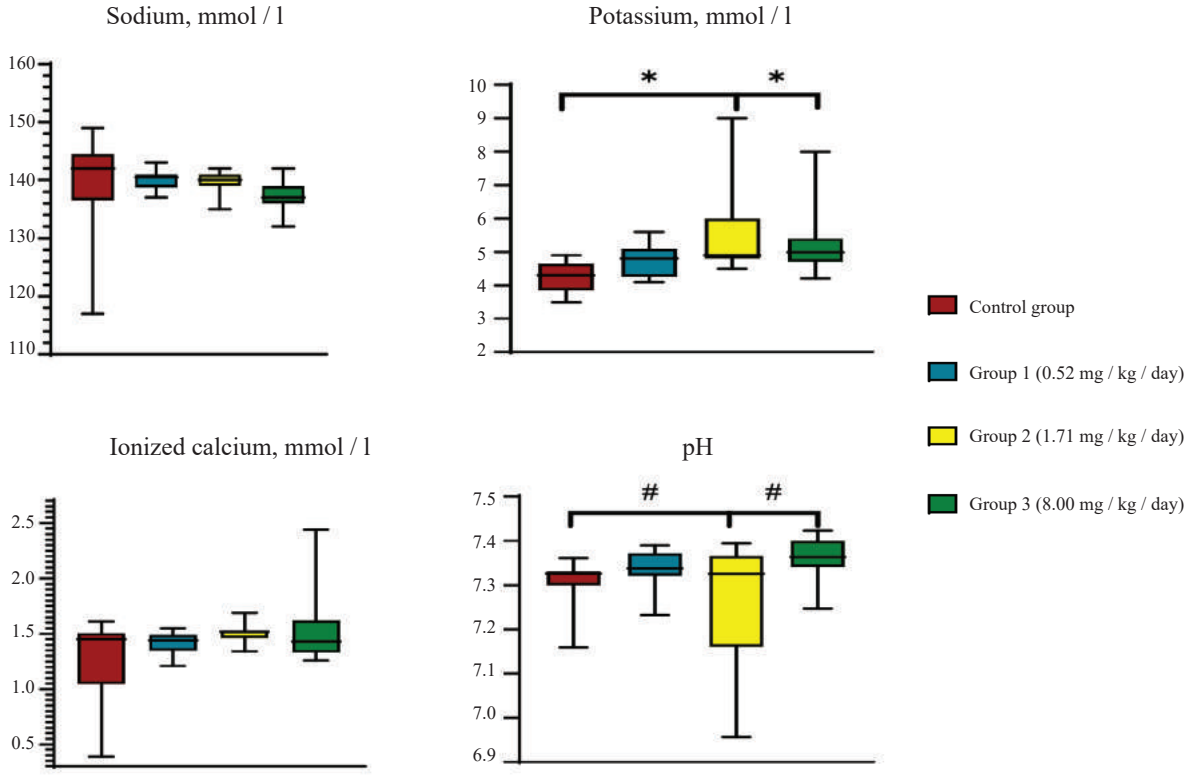

Fig. 3. The concentration of sodium, potassium, ionized calcium, and the pH level in the blood on day 3 after modeling acute lung injury and treatment with dexamethasone at various doses, *Me* [Q_1; Q_3]). * differences are statistically significant relative to the control group (p < 0.05, the Kruskal – Wallis test)

control group ($p = 0.002$, $p = 0.004$). The pH values were significantly higher in group 3 compared to the control group and group 2 ($p = 0.02$, $p = 0.04$) There were no significant differences among the groups when comparing pCO_2, pO_2, TCO_2, HCO_3, BE, sO_2 in venous blood.

Compared with the values in the control group, the concentration of glucose in the blood of experimental animals was significantly higher ($p = 0.01$, $p = 0.0009$, and $p = 0.0002$ for groups 1, 2, and 3, respectively), and the dose of dexamethasone determined how much the parameter increased. When analyzing the content of total protein, its concentration in animals of group 3 was significantly higher than in the control group ($p = 0.01$), while the level of albumin was increased in all experimental groups ($p = 0.02$, $p < 0.0001$, $p = 0.005$ for groups 1, 2, and 3, respectively). Activity of CPK was significantly higher in animals treated with dexamethasone at doses of 1.71 and 8.00 mg / kg / day (groups 2 and 3) compared to the control group ($p = 0.006$, $p = 0.02$, respectively) (Fig. 4).

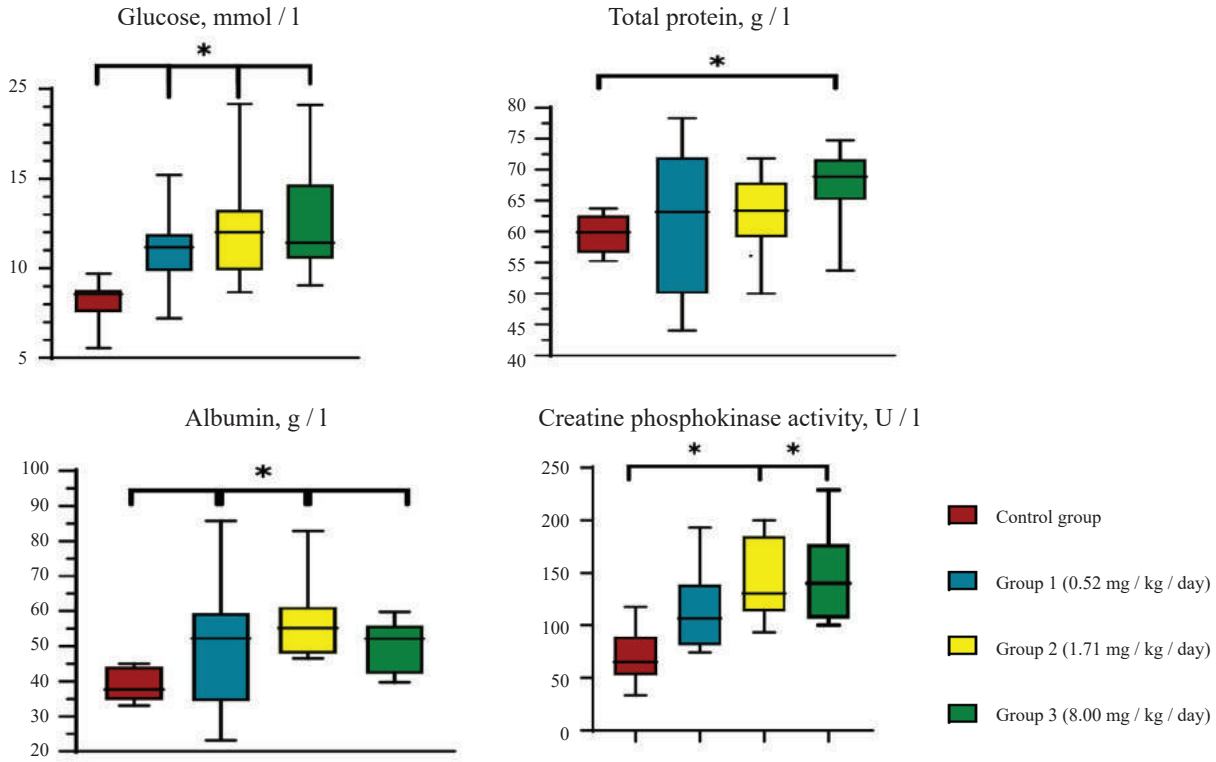

Fig. 4. Concentration of glucose, total protein, albumin, and creatine phosphokinase activity in blood on day 3 after modeling acute lung injury and treatment with dexamethasone at various doses, *Me* [Q_1; $Q3$]. Differences are statistically significant: * relative to the control group ($p < 0.05$, the Kruskal – Wallis test); # relative to the dexamethasone 8 mg / kg / day group ($p < 0.05$, the Kruskal – Wallis test)

The concentration of SFMCs in blood plasma of the animals in group 3 (dose of dexamethasone 8 mg / kg / day) was significantly higher not only when compared with the control group ($p < 0.0001$), but also when compared with groups 1 and 2 ($p = 0.008$, $p = 0.01$, respectively). Significant differences in PT were observed when comparing groups 2 and 3 with the control group. In these groups, the parameter was significantly lower than in the control group ($p = 0.03$, $p = 0.0009$, respectively). Antithrombin III activity was significantly lower in groups 2 and 3 ($p = 0.003$, $p = 0.0002$) compared to the control group. In addition, antithrombin activity was lower in group 3 compared to group 1 ($p = 0.02$). No significant difference was found in the concentration of fibrinogen when comparing groups 1–3 with the control group (Fig. 5). There were no significant differences between the groups when comparing the PTT values in venous blood.

It was shown that after i/t administration of LPS, the rats developed bilateral diffuse lung damage, leading to edema with high LWC and W /D ratio, as well as to death of 40% of the animals in the group. The obtained data are consistent with the results of previous studies using the selected experimental ALI model [2, 6, 10, 11].

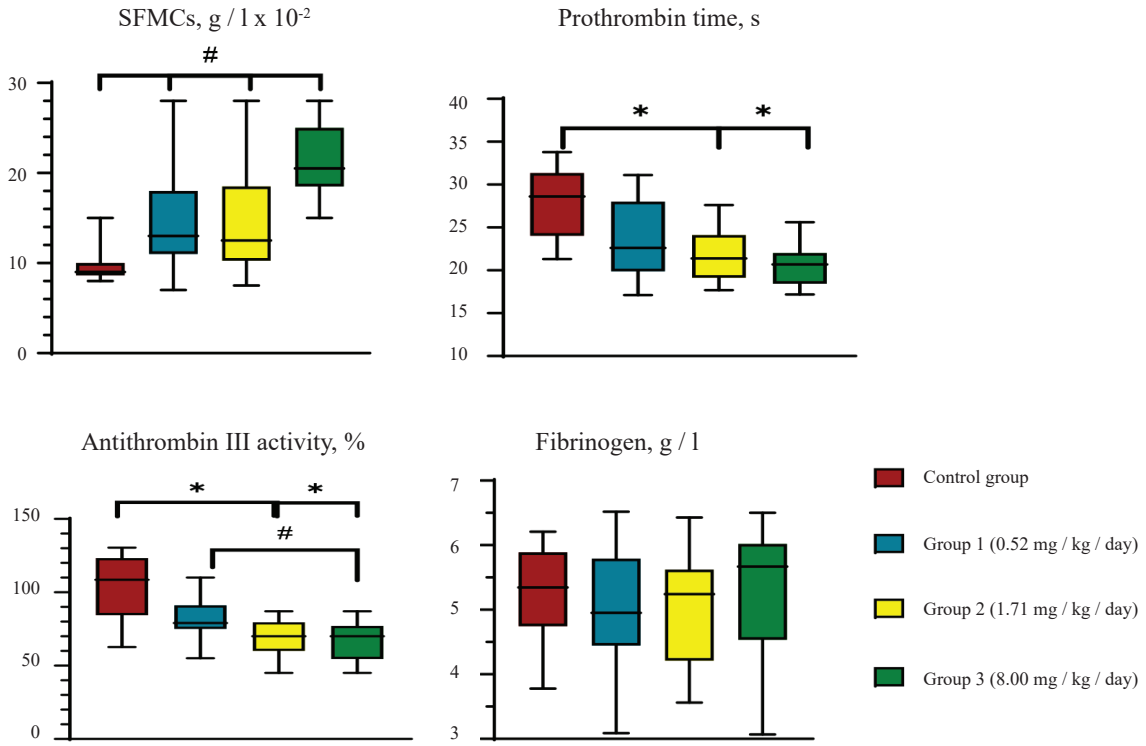

Fig. 5. Coagulation parameters in rats on day 3 after modeling acute lung injury and treatment with dexamethasone at various doses, *Me* [Q_1; Q_3]. Differences are statistically significant: * relative to the control group ($p < 0.05$, the Kruskal – Wallis test); # relative to group 3 ($p < 0.05$, the Kruskal – Wallis test).

The use of dexamethasone in all studied doses led to positive therapeutic effects including a decrease in the volume of lung tissue damage, which was accompanied by correction of respiratory failure (normopnea, normalization of blood pH) and decreased mortality. At the same time, significant dose-dependent side effects of glucocorticoid therapy developed including hyperglycemia, hyperproteinemia, hyperalbuminemia, hyperkalemia, and increased CPK activity in the blood. In addition, coagulation disorders were observed including high values of SFMCs in combination with low PT and low antithrombin III activity, which, in combination with hyperproteinemia, aggravated hypercoagulability that progressed while accompanying inflammation. In rats receiving dexamethasone therapy, the analysis revealed a decreased lymphocyte and platelet count, as well as an increase in the number of granulocytes and monocytes in the blood.

In group 1, where dexamethasone was administered at a dose of 0.52 mg / kg / day, the lowest mortality among all groups and the lowest values of LWC and the W/D ratio were observed. The glucose concentration was higher than in the control group, but was the lowest among all comparison groups. Despite the large number of SFMCs, no significant decrease in PT was observed, and antithrombin III activity was the highest among all groups, which may indicate a balanced state of coagulation and anticoagulation systems, as well as a low risk of thrombotic complications.

In group 2, in which dexamethasone was administered at a dose of 1.71 mg / kg / day, the survival rate was similar to that in group 1. At the same time, when dexamethasone was administered at this dose, the highest values of LWC, W/D ratio, and monocyte count in the blood were observed among all experimental groups in which dexamethasone was used. High concentrations of glucose and potassium in the blood similar to those in group 3 were detected. SFMC parameters were significantly higher, while PT and antithrombin III activity were lower than in the control group.

The highest mortality in animals was observed in group 3 (dexamethasone dose was 8.00 mg / kg / day), despite low values of LWC and W / D ratio. The concentration of glucose, potassium, total protein, albumin, and CPK activity were the highest in this group, and PT was the shortest. The parameters characterizing the blood coagulation system indicated a high risk of thrombosis as the maximum values of

SFMCs, the minimum values of PT, and the minimal activity of antithrombin III were recorded. A low platelet count and severe hypercoagulability might indicate increased platelet consumption.

CONCLUSION

As a result of the study, it was found that the effectiveness of both low (0.52 mg / kg / day) and high (8.00 mg / kg / day) doses of dexamethasone in an experimental model of LPS-induced ALI is characterized by a similar antiedematous effect. Based on the results of blood tests and analysis of animal survival, the use of dexamethasone at the minimum dose (0.52 mg / kg / day) should be considered the safest. Despite significant anti-inflammatory effects on the lung tissue, the administration regimen of dexamethasone at the maximum dose (8.00 mg / kg / day), which is equivalent to pulse therapy, was accompanied by the occurrence of the most pronounced adverse events, namely: hyperglycemia, hyperproteinemia, hyperkalemia, hypercoagulation, and increased CPK activity. The identified side effects of dexamethasone therapy most likely contributed to higher mortality in this group of animals.

When prescribing glucocorticoid therapy, especially at high doses, it is necessary to monitor the level of glucose, electrolytes, and the state of the blood coagulation system and timely correct the identified pathological changes, since these changes can significantly contribute to the development of ARDS.

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Authors' contribution

Salukhov V.V., Tyunin M.A. – conception and design, final approval of the manuscript for publication. Voloshin N.I., Levchuk E.V., Minakov A.A. – carrying out of the experiment, analysis and interpretation of the data. Voloshin N.I., Pugach V.A., Ilyinskiy N.S. – justification of the manuscript or critical revision of the manuscript for important intellectual content.

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Prediction of a poor ovarian response in assisted reproductive technology programs in patients after surgical interventions on the ovaries

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ABSTRACT

The aim. To conduct a comparative analysis of clinical and anamnestic data in women of reproductive age after ovarian cyst surgery and with occult premature ovarian insufficiency (POI) to predict a poor ovarian response to stimulation.

Materials and methods. We conducted a retrospective study of medical records of women (aged 18–40 years) with infertility at the Assisted Reproductive Technology Center of Siberian State Medical University from 2017 to 2020. The main group consisted of 84 patients who underwent ovarian cyst surgery. The comparison group consisted of 33 patients with biochemical signs of POI (follicle stimulating hormone (FSH) 10–12 mMU / ml) who did not undergo ovarian cyst surgery. Anti-Mullerian hormone (AMH), FSH, estradiol, the antral follicle count (AFC), and the ovarian response to stimulation were compared.

Results. A correlation was established between AFC and a poor ovarian response both in the main group ($r = –0.7$; $p = 0.004$) and in the comparison group ($r = –0.620$; $p = 0.000$) in women under 35 years of age. A correlation was found between the concentration of estradiol and a poor ovarian response in the comparison group in women over 35 years of age ($r = –0.707$; $p = 0.001$). A moderate negative correlation between AMH and a poor ovarian response was revealed only in the main group of women under the age of 35 years ($r = –0.589$; $p = 0.021$). A moderate negative correlation between AMH and a poor ovarian response was revealed in the comparison group in women under the age of 35 years ($r = –0.648$; $p = 0.000$), a weak negative correlation was found for women at the age of 35 years ($r = –0.500$; $p = 0.004$). In both groups, the level of FSH did not determine the ovarian response to stimulation.

Conclusion. The determination of AFC and AMH is more significant in predicting a poor ovarian response in women after ovarian surgery and in women with occult signs of POI under the age of 35 years, compared with FSH. In the group of women over 35 years with occult signs of POI, the concentration of estradiol may matter in predicting a poor ovarian response, which requires further research.

Keywords: poor ovarian response, ovarian surgery, assisted reproductive technologies, ovaries, ovarian reserve

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Siberian State Medical University (Protocol No. 9308 of 15.12.2022).

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Прогнозирование «бедного ответа» в программах вспомогательных репродуктивных технологий после оперативных вмешательств на яичниках

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РЕЗЮМЕ

Цель. Провести сравнительный анализ клинико-анамнестических данных у женщин репродуктивного возраста с оперативными вмешательствами на яичниках и с оккультными признаками преждевременной недостаточности яичников (ПНЯ) для прогнозирования «бедного ответа» на стимуляцию.

Материалы и методы. Проведено ретроспективное исследование медицинских карт женщин (18–40 лет) с бесплодием Центра вспомогательных репродуктивных технологий Сибирского государственного медицинского университета с 2017 по 2020 г. Основная группа – 84 пациентки с оперативными вмешательствами на яичниках. Группа сравнения – 33 пациентки с биохимическими признаками ПНЯ (фолликулостимулирующий гормон (ФСГ) 10–12 мМЕ/мл) без оперативного вмешательства на яичниках. Проводилось сравнение антимюллерова гормона (АМГ), ФСГ, эстрадиола, количества антральных фолликулов (КАФ), ответ яичников на стимуляцию овуляции.

Результаты. Установлена корреляционная связь между КАФ и «бедным ответом» как в основной группе (r = –0,7; p = 0,004), так и в группе сравнения (r = –0,620; p = 0,000) у женщин младше 35 лет. Выявлена корреляционная связь между концентрацией эстрадиола и «бедным ответом» в группе сравнения у женщин старше 35 лет (r = –0,707; p = 0,001). Отрицательная зависимость средней силы между АМГ и «бедным ответом» выявлена только в основной группе в возрасте младше 35 лет (r = –0,589; p = 0,021). Средняя отрицательная связь между АМГ и «бедным ответом» выявлена в группе сравнения у женщин в возрасте младше 35 лет (r = –0,648; p = 0,000), слабая отрицательная взаимосвязь – в возрасте старше 35 лет (r = –0,500; p = 0,004). В обеих группах уровень ФСГ не предопределял ответ яичников на стимуляцию.

Заключение. Определение КАФ и АМГ являются более значимыми при прогнозировании «бедного ответа» у женщин как с оперированными яичниками, так и у женщин с оккультными признаками ПНЯ в возрасте младше 35 лет по сравнению с ФСГ. В группе с оккультными признаками ПНЯ у женщин старше 35 лет при прогнозировании «бедного ответа», вероятно, имеет значение концентрация эстрадиола, что требует дальнейших исследований.

Ключевые слова: «бедный ответ» яичников, операции на яичниках, вспомогательные репродуктивные технологии, яичники, овариальный резерв

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

Источник финансирования. Проект поддержан конкурсной комиссией СибГМУ (протокол заседания от 27.06.2022) в соответствии с положением от 16.05.2022 № 51 «О поддержке научно-исследовательских проектов, выполняемых молодыми учеными "SibMed.Scholar".

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом СибГМУ (протокол № 9308 от 15.12.2022).

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INTRODUCTION

Predicting the outcomes of assisted reproductive technologies (ART) is relevant not only for fertility specialists, but also for women with infertility themselves. An accurate diagnosis increases the effectiveness of ART and meets patients' expectations. To accomplish this task, using available clinical and laboratory parameters is reasonable, which will not contradict medical examination guidelines.

According to the Bologna criteria adopted in 2011 by the ESHRE, a poor ovarian response (POR) is a failure of woman's ovaries to respond to the selected stimulation protocol. The diagnosis of POR can be established after at least one cycle of ovulation induction and if at least two of the following three features are present: maternal age > 40 years or risk factors for a poor ovarian response; less than three oocytes retrieved with the standard stimulation protocol; an abnormal ovarian reserve test (antral follicle count (AFC) less than 5–7, anti-Mullerian hormone (AMH) less than 0.7–1.1 ng / ml) [1]. Women at risk of POR are those with clinical and anamnestic signs of diminished ovarian reserve: structural chromosome aberrations and gene mutations that can lead to primary ovarian insufficiency, including Turner syndrome and FMR1 premutation [2]; a history of pelvic inflammatory diseases, including chlamydiosis [3, 4]; cyst or endometrioma removal [5, 6]; past chemotherapy [2, 7].

Thus, while POR is already implied in case of a surgical intervention on the ovaries, predicting POR in women with hypergonadotropism is fairly challenging. Women with infertility due to ovarian aging, sometimes referred to as primary ovarian insufficiency, account for a substantial proportion of patients seeking treatment in ART centers. This group of patients is growing, since a large number of women postpone childbearing until 30-40 years of age, and it is impossible to establish a clear cause in more than a half of them [1].

In addition to the 2011 Bologna criteria of POR, fertility specialists also use the POSEIDON stratification proposed in 2016 for low prognosis patients in ovulation induction. The literature data show a need for further clinical research to confirm the effectiveness of the ESHRE and POSEIDON approaches for better ART outcomes [8].

The aim of this study was to conduct a comparative analysis of clinical and anamnestic data in women of reproductive age after ovarian cyst surgeries and with occult primary ovarian insufficiency (POI) to predict a poor ovarian response to stimulation.

MATERIALS AND METHODS

A retrospective study included women who underwent infertility treatment in the ART Center of Siberian State Medical University from 2017 to 2020. The main group encompassed women of reproductive age with infertility and past ovarian surgery (n = 84). The main group was divided into two subgroups according to age: subgroup 1 – women under 35 years (n = 51), subgroup 2 – women aged 35 years and older (n = 33). The comparison group consisted of women of reproductive age with infertility and serum follicle-stimulating hormone (FSH) concentration of 10–12 mIU / ml before ovulation stimulation (which is typical of occult POI) (n = 33) [9]. The comparison group was also divided into two subgroups: subgroup 1 – women under 35 years (n = 15), subgroup 2 – women aged 35 years and older (n = 18). The groups were divided by age according to the current understanding of the hormonal function of the ovaries and the size of ovarian reserve, as well as according to the current POSEIDON stratification assessing diminished ovarian reserve [10].

The inclusion criteria were somatically healthy women of reproductive age (18–40 years) with normoprolactinemia and euthyroidism. The exclusion criteria were failure to meet the inclusion criteria; metabolic and endocrine disorders (diabetes mellitus, all classes of obesity); myoma that requires surgical treatment; endometriosis; premalignant and malignant diseases; extragenital diseases accompanied by immune and endocrine disorders; contraindications to *in vitro* fertilization (IVF) according to the order No. 803n of the Ministry of Health of the Russian Federation of 31.07.2020 "On the Procedure for the Use of Assisted Reproductive Technologies, Contraindications, and Restrictions on their Use".

The women were examined in accordance with the clinical guidelines "Assisted reproductive technologies and artificial insemination" (letter of the Ministry of Health of the Russian Federation of 5.03.2019 No.15-4/I/2-1908, of 05.03.2019 No.15-4/i/2-1908) and medical examination guidelines. The data analysis included the results of the following tests and procedures: 1) clinical procedures: analysis of medical records, study of past medical history, complaints, and physical exam data; 2) routine clinical laboratory tests; 3) diagnostic imaging and procedures:

pelvic ultrasound (AFC), data of past laparoscopic / laparotomic surgery; 4) measurement of serum FSH, luteinizing hormone (LH), estradiol, and AMH levels; 5) number of follicles before a transvaginal puncture, number of oocytes retrieved. In all the cases, multifollicular ovarian stimulation in the IVF program was performed according to the established protocol using gonadotropin-releasing hormone (GnRH) antagonists from day 6 of stimulation.

The obtained findings were processed using SPSS® 26.0 (© SPSS Inc.). The quantitative data were presented as the median and the interquartile range *Me* (Q_1–Q_3). The significance of the differences was estimated using the nonparameteric Kruskal – Wallis test for independent samples. The correlation between the parameters was studied using the Pearson's correlation coefficient $\chi 2$ and the Spearman's rank correlation coefficient. The differences were considered statistically significant at $p \leq 0.05$.

RESULTS

The clinical and anamnestic parameters of the patients with infertility in the main and comparison groups are presented in Table 1. There were no significant differences between the subgroups in the age, body mass index (BMI), age of menarche, history of IVF (the Kruskal – Wallis test; significant differences between the groups were considered at $p < 0.05$).

Significant differences were found in the duration of infertility in women of subgroup 2 of the main group and subgroup 2 of the comparison group, as well as when comparing subgroups 1 and 2 of the comparison group in the context of hypergonadotropism (Table 1).

The median age of the patients was comparable and was 34 (32–36) years. The duration of infertility ranged from 2 to 15 years and equaled to 6 (4-10) years. It was greater in the comparison group, namely, in the women of subgroup 2 (Table 1).

Table 1

Comparative characteristics of clinical and anamnestic data of the examined groups, *Me* (Q_1–Q_3)

Parameter	Main group		*p, subgroups of the main group	Comparison group		*p, subgroups of the comparison group	*p, subgroups 1 of the main group and comparison group	*p, subgroups 2 of the main group and comparison group
	Subgroup 1, $n = 51$	Subgroup 2, $n = 33$		Subgroup 1, $n = 15$	Subgroup 2, $n = 18$			
Age, years	32.0 (29.0–34.0)	36.0 (35.0–37.0)	<0.001*	33.0 (32.0–34.0)	38.0 (36.0–39.0)	<0.001*	0.100	0.060
BMI, kg / m^2	23.4 (20.5–27.5)	25.8 (21.8–31.4)	0.092	23.8 (22.2–25.7)	21.1 (19.8–24.9)	0.190	0.789	0.060
Age of menarche, years	13.0 (12.0–14.0)	13.0 (12.0–14.0)	0.432	13.0 (12.0–14.0)	14.0 (13.0–14.0)	0.325	0.524	0.282
Duration of infertility, years	6.0 (4.0–8.0)	6.0 (2.5–10.0)	0.928	4.0 (2.0–8.0)	10.5 (3.5–15.3)	0.008*	0.105	0.045*
Past IVF	1.0 (1.0–2.0)	1.0 (1.0–2.0)	0.742	2.0 (1.0–3.0)	1.5 (1.0–2.0)	0.656	0.462	0.957

Here and in Table 2: * Kruskal – Wallis test; the differences between the groups are significant at $p < 0.05$.

Past surgical interventions on the ovaries in the patients of the main group were performed for the following indications: ovarian apoplexy – 39 cases (46.45%), of which in 13 cases (33.33%), the affected ovarian tissue was sutured, while in 26 cases (66.67%), the ovary was resected; ovarian cystectomy due to a complicated follicular cyst on the ovary (hemorrhage, rupture) – 26 cases (30.95%); ovarian cystectomy due to a complicated corpus luteum cyst (hemorrhage, rupture) – 9 cases (10.71%); ovarian cystectomy due to benign ovarian tumors – 10 cases (11.9%) (serous cystadenoma (4), dermoid cyst (3), fibroma (3)). Four women underwent recurrent surgery: 1 – for ovarian fibroma, 2 – for a complicated follicular cyst, 1 – for ovarian apoplexy. Thus, surgical treatment was predominantly done via laparoscopy using organ-preserving techniques. A comparative analysis of the indications for ovarian surgery in the subgroups of the main group did not reveal any significant differences.

The data in Table 2 demonstrate that the level of FSH was predictably higher in women aged 35 years and older in both groups; a significant difference was identified in the estradiol levels in women aged 35 years and older compared to the women under 35 years in the main group.

Moreover, there were no significant differences in the AFC ($p > 0.05$). The number of oocytes retrieved showed no significant differences either ($p > 0.05$).

All the patients underwent multifollicular ovarian stimulation in the IVF program according to the established protocol using GnRH antagonists from day 6 of the stimulation.

Table 2

Characteristics of hormonal status and antral follicle count of the examined women with infertility before ovarian stimulation in IVF programs, *Me* (Q_1–Q_3)

Parameter	Main group		*p**	Comparison group		*p**	*p* subgroups 1 of the main group and comparison group	*p* subgroups 2 of the main group and comparison group
	Subgroup 1, *n* = 51	Subgroup 2, *n* = 33		Subgroup 1, *n* = 15	Subgroup 2, *n* = 18			
FSH, mIU / ml	6.7 (5.8–8.9)	8.5 (6.7–12.2)	0.039*	10.4 (10.1–10.9)	11.1 (10.3–11.6)	0.100	<0.001*	0.004*
LH, mIU / ml	5.1 (3.6–6.9)	4.7 (3.2–6.4)	0.166	5.4 (4.6–8.0)	5.9 (4.7–8.3)	0.708	0.375	0.013*
Estradiol, pmol / l	80.0 (36.7–164.8)	221.0 (105.0–351.0)	<0.001*	108.0 (67.1–179.1)	138.4 (65.9–226.0)	0.911	0.163	0.067
AMH, ng / ml	1.47 (0.66–3.82)	1.34 (0.57–2.22)	0.463	0.69 (0.46–4.73)	1.3 (0.66–1.79)	0.762	0.447	0.754
AFC	9.5 (4.0–14.0)	7.0 (3.5–13.5)	0.637	9.0 (4.8–14.0)	6.0 (5.0–15.0)	0.866	0.851	0.600
Oocytes	5.0 (2.0–9.5)	4.0 (2.0–8.5)	0.761	3.0 (1.0–10.0)	3.0 (2.0–5.0)	0.735	0.708	0.225

In the main group, a poor ovarian response accounted for 39.3% of cases and was distributed in the following way: 40.8% in subgroup 1 and 39.4% in subgroup 2 ($p = 0.465$, $\chi2 = 0.533$). In the comparison group, a poor ovarian response occurred in 57.6 % of cases. In patients under 35 years, a poor ovarian response to stimulation was recorded in 53.3% of cases; in women over 35 years, it was detected in 58.8% of cases ($p = 0.782$, $\chi2 = 0.077$). In subgroup 2 of the main group, a poor ovarian response to stimulation occurred less often than in subgroup 2 of the comparison group ($p = 0.000$, $\chi2 = 0.486$).

A subsequent correlation analysis revealed a phenomenon that had not previously been described by other researchers: a moderate negative correlation between AFC and a poor ovarian response in women under 35 years in the main group ($r = –0.7$, $p = 0.004$) and in the comparison group ($r = –0.620$, $p = 0.001$). Such correlation was not established in the main group of women older than 35 years ($r = –0.034$, $p = 0.894$). Only a weak negative linear relationship was found in the comparison group in women over 35 years ($r = –0.372$, $p = 0.033$). This fact suggests that it is possible to estimate the expected response to stimulation based on AFC only in the age group under 35 years.

No correlation was detected between the FSH concentration and a poor ovarian response in the subgroups of the main group ($r = 0.295$, $p = 0.042$; $r = 0.072$, $p = 0.692$) and in the subgroups of the comparison group ($r = 0.124$, $p = 0.659$; $r = 0.363$; $p = 0.139$).

The investigation of the relationship between a poor ovarian response to stimulation and the AMH concentration revealed a moderate negative correlation ($r = –0.648$, $p = 0.000$) in women younger than 35 years and a weak negative correlation ($r = –0.500$, $p = 0.004$) in women older than 35 years in the main group. In the comparison group, there was a weak negative correlation between a poor ovarian response to stimulation and the AMH concentration in women younger than 35 years ($r = –0.589$, $p = 0.021$) and no correlation in women over 35 years ($r = 0.154$, $p = 0.542$).

Interestingly, a positive correlation was established between the concentration of estradiol and a poor ovarian response in the comparison group in women over 35 years of age ($r = –0.707$, $p = 0.001$). However, such correlation was not found in the other subgroups ($p > 0.05$).

DISCUSSION

The review by Q.H.Y. Wong and R.A. Anderson (2018) presents studies estimating changes in the ovarian reserve marker, AMH, before and after gonadotoxic treatment [11]. Ovarian reserve was reported to decrease after cystectomy, which resulted in lower concentrations of AMH [12, 13]. The main mechanism through which ovaries are damaged during a surgical intervention (thus, the ovarian reserve is diminished) is believed to be excision of contact ovarian tissue by employing surgical energy devices [14]. Two systematic reviews and a meta-analysis demonstrated that the use of bipolar electrocoagulation is associated with a considerable decrease in the AMH level compared to non-thermal hemostasis techniques, including sutures or application of a hemostatic sealant [15, 16].

It is worth noting that most studies did not find a correlation between a reduced AMH concentration

and the size of retention cysts [17–20]. However, patients with follicular and endometrial cysts exhibited a significant decrease in the AMH level within 6 months after surgical treatment. It was also established that surgical removal of dermoid cysts and true ovarian neoplasms (including serous tumors) did not significantly change the AMH concentration [21].

According to the data of the conducted retrospective study, a poor ovarian response accounted for 39.3% in the main group and 57.6% in the comparison group. Yet, when dividing the groups according to the Bologna criteria and the POSEIDON stratification, no statistical significance was revealed. In this research, when the groups were stratified according to the POSEIDON classification, the risk of a poor ovarian response was higher in women under 35 years who underwent ovarian surgery than in women over 35 years, which is confirmed by the correlations obtained.

Thus, a correlation was detected between the AFC and a poor ovarian response in women under 35 years in the main and comparison groups. The study also found a correlation between the AMH concentration and a poor ovarian response in the main group in women under 35 years, which was not observed in patients over 35 years. In the comparison group, there was a moderate negative correlation between the AMH levels and a poor ovarian response only for women under 35 years, while for the subgroup of women over 35 years, the correlation was weak.

This research revealed that the FSH concentration was not associated with a poor ovarian response to stimulation in ART programs in women with a past surgical intervention on the ovaries, which is inconsistent with the previously published data. S. Salama et al. (2021) found that the basal FSH level in women under 35 years correlated more with the number of follicles and number of oocytes retrieved, which, in turn, determined the pregnancy rate [22]. G. Sahin et al. (2021) reported that at the concentration of FSH ≥ 10 IU / l, the frequency of pregnancy and live birth in younger women was higher despite elevated FSH levels [23].

Further research on the prediction of a poor ovarian response might explore the estradiol concentration that also displays a correlation in the analysis of the data from the comparison group of women aged 35 years and older with infertility and potential hypergonadotropism.

CONCLUSION

The findings indicate that AFC and AMH were significant markers for women under 35 years in both examined groups. The FSH level is not the main predictor of a poor ovarian response. In the group of women aged 35 years and older with potential hypergonadotropism, the estradiol level can serve as a predictor of a poor ovarian response. Further research with a greater sample size should be carried out to establish the significance of known and potential markers of a poor ovarian response in women after ovarian surgeries and occult primary ovarian insufficiency. This will allow to develop a predictive mathematical model using logistic regression.

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Authors' contribution

Dmitrieva M. L., Tikhonovskaya O.A., Petrov I.A. – conception and design. Dmitrieva M. L., Chernyshova M.A., Duma O.M., Kosimova Z.O., Timofeeva O.S., Zhdankina A.A., Gerasimov A.V., Yuriev S.Y. – collection and processing of the material. Dmitrieva M. L., Petrov I.A., Zhdankina A.A., Gerasimov A.V. – statistical analysis of the data. Dmitrieva M. L., Tikhonovskaya O.A., Petrov I.A., Logvinov S.V., Chernyshova M.A., Yuriev S.Y. – drafting of the article. Tikhonovskaya O.A., Logvinov S.V. – editing of the article.

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Study of wound-healing properties of humic substance – zinc complexes in the aseptic wound model *in vivo*

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ABSTRACT

The aim was to investigate wound-healing properties of zinc-containing biocomposites based on humic ligands (humic substance (HS) – Zn) in the *in vivo* experiment on the aseptic wound model and to evaluate their resorptive properties.

Materials and methods. The objects of the study were 5 samples of HS-Zn in the form of complex salts comprising fine black powders synthesized in the Laboratory for Natural Humic Systems of the Faculty of Chemistry at Moscow State University. The wound-healing effect of the substances was studied on 70 male Wistar rats using a traumatic model of an excisional aseptic skin wound. The degree of affected skin healing was evaluated during 21 days by the planimetric method. The resorptive properties of the HS-Zn samples were studied by inductively coupled plasma mass spectrometry (ICP-MS) in the biomaterial (blood serum, fur, skin from the wound surface).

Results. It was found that course application of zinc-containing HS-Zn biocomposites to the wound surface led to a decrease in the wound area in comparison with $ZnSO_4$ with the equivalent concentration of elemental Zn (1.67 mg / ml). Two samples FA-Zn and Peat1-Zn showed the most pronounced regenerating effect. We noted an increase in Zn level in the tested skin samples from the wound area, in fur, and in the blood serum, which indicates the resorptive effect of zinc-containing HS-Zn biocomposites during course application; however, the parameters did not exceed limiting permissible concentrations. The correlation between the tested samples was not equal, which indicates a significant impact of the initial HS matrix on the Zn bioavailability.

Conclusion. The observed reparative effect of zinc and HS complexes in the context of their low toxicity is of interest for further study to develop effective wound-healing preparations.

Keywords: zinc, humic substances, ligands, wound healing

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

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Исследование ранозаживляющих свойств комплексов цинка с гуминовыми веществами в эксперименте *in vivo* на модели асептической раны

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РЕЗЮМЕ

Цель – исследовать ранозаживляющие свойства цинксодержащих биокомпозиций на основе гуминовых лигандов (ГВ-Zn) в эксперименте *in vivo* на модели асептической раны и оценить их резорбтивные свойства.

Материалы и методы. Объекты исследования – пять образцов ГВ-Zn в форме комплексных солей, синтезированные в лаборатории природных гуминовых систем химического факультета МГУ, представляющие собой мелкодисперсные порошки черного цвета. Ранозаживляющее действие исследуемых веществ было изучено на 70 самцах крыс линии Wistar с использованием травматической модели плоскостной асептической кожной раны. Степень заживления пораженного участка кожи оценивали в течение 21 сут планиметрическим методом. Изучение резорбтивных свойств образцов ГВ-Zn проводилось методом масс-спектрометрии с индуктивно-связанной плазмой в биоматериале (сыворотка крови, шерсть, кожа с раневой поверхности).

Результаты. Установлено, что курсовое нанесение на раневую поверхность цинксодержащих биокомпозиций ГВ-Zn приводит к уменьшению площади раны в сравнении с площадью раны при нанесении $ZnSO_4$ с эквивалентной концентрацией элементарного Zn (1,67 мг/мл). Наиболее выраженный регенерирующий эффект проявили два образца: FA-Zn и Peat1-Zn. Отмечено увеличение уровня Zn в опытных участках кожи раневой поверхности, в шерсти и сыворотке крови, что указывает на резорбтивное действие цинксодержащих биокомпозиций ГВ-Zn при их курсовом применении, но показатели не превышали уровня допустимых предельных концентраций. Также отмечена неодинаковая зависимость между тестируемыми образцами, что свидетельствует о значительном влиянии исходной матрицы ГВ на биодоступность Zn.

Заключение. Обнаруженный репарационный эффект композиций цинка и гуминовых лигандов на фоне их низкой токсичности представляет интерес для дальнейшего изучения с целью разработки на их основе эффективных ранозаживляющих препаратов.

Ключевые слова: цинк, гуминовые вещества, лиганды, ранозаживление

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

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INTRODUCTION

Traumatism, and in particular, injuries of various etiologies are a priority and relevant issue in modern medicine, despite the introduction of state-of-the-art advances in high technology. Aseptic, infected, and purulent lesions of soft tissues are very likely to be obtained in domestic and industrial conditions, during the occurrence and elimination of the consequences of natural and man-made disasters [1]. Moreover, the aggravation of the issue is facilitated by a decrease in the activity of the body natural resistance systems and rapid changes in the morphophysiological organization of the wound area microflora [1–3]. As a result, people get temporary or lifelong disability, working capacity is significantly reduced, and a multiple increase in government spending is observed [4]. In view of this, the search for substances that have a positive effect on the main stages of the repair process and are more accessible in comparison with device-based treatment is relevant.

Advances in molecular biology, medical elementology, and a number of other natural sciences have proven that normal wound healing occurs with the participation of biometals, in particular zinc (Zn) [5–7]. Zinc is a cofactor of more than 300 matrix metalloproteinases, which have anti-inflammatory, antioxidant, immunomodulatory, and antibacterial effects and inhibit the proliferation and differentiation of keratinocytes by directly inhibiting / activating enzymes and influencing gene expression [6–8]. Today, in the form of oxide and salts (sulfate, acetate, gluconate, etc.), zinc is included in topical medications for the treatment of wounds, diaper rash, and skin defects [6, 9–11].

It is known that the ionic form of Zn^{2+} has low bioavailability, therefore, to achieve the optimal concentration locally, long-term administration is required, which is associated with the development of both local and systemic side effects [5, 12]. Recently, it has become possible to simultaneously reduce toxicity and increase biological activity, and sometimes achieve new types of pharmacological action in Zn (as well as in a number of other metals) and its derivatives, which are absent in the ionic form due to the formation of complexes whose ligands are polymers [3, 5, 13, 14].

Humic substances (HS), products of the transformation of plant matter under the influence of biotic and abiotic factors, are promising high-molecular compounds. Having a wide variety of oxygen- and nitrogen-containing functional groups in their structure, HS can interact with various compounds of living cells, forming bonds with them through exchange, donor – acceptor, and other mechanisms. As a result, it becomes possible to affect the activity of cells in many organs and systems and, thereby, realize pleiotropic biological effects: immunomodulatory, anti-inflammatory, antioxidant, antihypoxic, etc.

The effect of hepatitis B on cells of the immune system is one of the most studied types of their activity. It was proven that they increase the humoral immune response in mice, enhance the synthesis of tumor necrosis factor (TNF)α, interleukin (IL)-1β, IL-12 by animal peritoneal macrophages and the production of interferon (IFN)γ and TNFα by peripheral blood mononuclear cells of healthy donors [15]. Due to a common type of pharmacological action in zinc and HS, but a different mechanism for its implementation, it can be assumed that the creation of a complex coordination zinc compound containing HS as a ligand will allow to achieve synergism, which will make it possible to heal wound rapidly without developing complications. Therefore, the aim of this work was to investigate the wound-healing properties of zinc-containing biocomposites based on humic ligands (HS – Zn) in an *in vivo* experiment on an aseptic wound model and to evaluate their resorptive properties.

MATERIALS AND METHODS

The objects of research were 5 samples of zinc-containing biocomposites based on humic ligands (HS – Zn), synthesized in the Laboratory for Natural Humic Systems of the Faculty of Chemistry of Moscow State University. Their characteristics are presented in Table 1. In order to synthesize HS – Zn samples, a HS solution with a concentration of 15 g / l was prepared, which was centrifuged at 5,000 rpm to separate the ballast. Next, this solution was mixed with the prepared solution of zinc nitrate with a concentration of 4.84 g / l in a ratio of 1:5 ($Zn(NO_3)_2$:humate). The concentration of humates in

the solution was recalculated taking into account the mass of the separated ballast to maintain equal ratios of humate to zinc nitrate. The synthesis was carried out for 4 hours without heating while maintaining pH = 9, using sodium hydroxide. The samples were then freeze-dried. The samples were frozen at –50 °C and placed in the Scientz-18ND Top-Press multi-manifolds freeze-drying system. Drying was carried out in external flasks for 2 days. The HS – Zn samples are complex salts comprising fine black powders.

Table 1

Experimental samples of zinc-containing biocomposites based on humic ligands (HS – Zn)

HS sample code (base ligand)	Description of HS samples	HS – Zn sample code
CHP	Humic acids of Powhumus coal (Humintech, Germany)	CHP-Zn
FA	Peat fulvic acids Fulvagra (Humintech, Germany)	FA-Zn
CHS	Coal humic substances (Sakhalin humates, Russia)	CHS-Zn
Peat1	Humic acids of high Angustifolium peat (Tomsk, Russia)	Peat1-Zn
Peat2	Humic acids of high-moor sphagnum-hollow peat (Tomsk, Russia)	Peat2-Zn

The wound-healing effect of the studied substances was studied on 70 male Wistar rats weighing 250–300 g. All manipulations and euthanasia were carried out in mandatory compliance with the rules of the "European Convention for the Protection of Vertebrate Animals used for Experimental or for other Scientific Purposes." The keeping of animals and the design of the experiment complied with the ethical standards and principles of biomedical research and were approved by the committee for control of the care and use of laboratory animals ((IACUC of the Center for Preclinical Research of Siberian State Medical University (Report No. 02/21 of 02.02.21)).

Before the experiment began, the animals were randomly divided into 7 groups (n = 10): group 1 – intact, saline solution (SS) was injected into the wounds; group 2 – control, the wounds were treated with a reference listed drug, $ZnSO_4$ solution; group 3 – the wound surface was treated with CHP-Zn solution; in group 4, FA-Zn was used; in group 5, CHS-Zn was used; in group 6, Peat1-Zn was applied, and in group 7, Peat2-Zn was used. To reproduce the traumatic model of an excisional skin wound, each rat was anesthetized by the complex administration of Zoletil-100 and XilaVeta under aseptic conditions, the area of the shoulder blades was depilated, followed by the formation of a round wound (d = 20 mm) through excision of the skin and a subcutaneous tissue layer. The operative field was treated once with 70 % ethyl alcohol. All substances (SS, $ZnSO_4$ solution, HS – Zn) were applied to the wounds daily for 21 days in a volume of 0.5 ml (Zn concentration in terms of elemental Zn 1.67 mg / ml). The condition of the animals involved in the experiment was assessed daily. A comprehensive assessment of the course of the wound process was conducted using the planimetric analysis of wounds on day 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21 with a digital camera under the same conditions, followed by image analysis using the ImageJ software.

The study of the resorptive properties of the HS – Zn samples was carried out using inductively coupled plasma mass spectrometry (ICP-MS) in order to establish the ability of zinc to overcome cellular barriers and accumulate in tissues and biological substrates. After the intravital course application of HS – Zn to the wounds, blood was taken from the animals, then serum was obtained. Then the rats were euthanized by CO_2 asphyxia and necropsy with the collection of biomaterial for subsequent determination of the Zn content in animal tissues, in particular, fur and skin were taken from the wound surface. To carry out ICP-MS, the studied samples were dried and subject to incineration at 500 °C for 2 hours. The resulting ash residues were converted into solution. Conversion into solution was carried out using pre-purified concentrated nitric acid (special purity), hydrogen peroxide, and the Milestone Start D microwave digestion system (200 °C, 700 W). After that, the samples were dried at 100–110 °C to the state of wet salts, then quantitatively transferred into disposable 50-ml polypropylene tubes using a background solution, i.e. 15% nitric acid with traces of hydrofluoric acid.

A blank experiment was prepared along with the samples. Before the analysis, an internal standard, an indium solution, was added to each test tube with samples and a blank sample. After that, all samples were diluted to the same volume. The calculation of the final results included taking into account the dilution factor, the internal standard, and the blank experiment. The analysis was performed on the

low-resolution inductively coupled plasma mass spectrometer Agilent 7500cx (Agilent Technologies, USA).

Statistical analysis of the data obtained during *in vivo* experiments was carried out using the Statistica 8.0 program. The Shapiro – Wilk test was used to check the data for normality of distribution, followed by the assessment of equality of variances using Levene's test. If the distribution in the experimental groups was normal and intergroup equality of variances was observed, further data processing was carried out using the analysis of variance (ANOVA, parametric method) followed by post-hoc comparison using the Bonferroni method and the Dunnett's test. When the distribution was different from normal and the intergroup equality of variances was not observed, the Kruskal – Wallis and Friedman tests were used (nonparametric statistics).

All results were presented as the mean and the error of the mean ($M \pm SE$). In the study of resorptive properties, statistical data was processed using the Statistica 8.0 and GraphPad Prism 8.0 programs. The level of statistical significance of differences between the samples was analyzed using the Kruskal – Wallis test. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The results of a planimetric study on the effect of the zinc-containing biocomposites based on HS when applied daily to aseptic wounds are presented in Tables 2 and 3. Starting from the 5th day of applying SS, as well as samples of CHS-Zn, Peat1-Zn and Peat2-Zn, a statistically significant decrease in the proportion of the initial area of the wound surface was observed. A statistically significant decrease in the proportion of the initial wound surface area when applying CHP-Zn and FA-Zn samples was observed later (from day 7) ($p < 0.05$). It is important to note that when Peat1-Zn (on day 5, 7 and 11), FA-Zn (on day 7, 11 and 17) and CHP-Zn (on day 21) were administered there was a statistically significant decrease in the proportion of the initial wound surface area in comparison with the comparison group $ZnSO_4$ ($p < 0.05$).

Table 2

Effect of zinc-containing biocomposites (HS – Zn) on the healing of aseptic wounds, $M \pm SE$

Group	Proportion of the initial wound surface area, %									
	day 3	day 5	day 7	day 9	day 11	day 13	day 15	day 17	day 19	day 21
SS	94.02±4.97	90.81±5.61^	69.43±1.74^	42.00±5.33	27.83± 1.93^	19.48±3.05^	12.42±1.83^	6.94± 1.03^	3.09±0.69^	1.63±0.63^
$ZnSO_4$	95.14±4.70	91.75±5.19	74.59±4.09^	46.57±6.76^	28.25± 2.32^	19.48±1.82^	14.17±1.65^	7.31± 0.97^	3.13±0.81^	2.04±0.35^
CHP-Zn	93.00±4.09	85.17±3.84	65.23±3.19^	39.24±3.65^	20.44± 1.27^	15.12±1.76^	7.59±1.32^	3.22± 1.09^	0.15±0.15^	0.00±0.00#^
FA-Zn	86.37±4.58	79.56±4.71	59.66±2.46#^	34.03±2.06^	17.05± 1.47#^	11.50±1.03^	6.91±0.94^	2.68± 0.79#*^	0.56±0.28^	0.27±0.17^
CHS-Zn	108.05±4.02	95.07±3.01^	81.13±3.47^	48.66±5.13^	24.73± 1.76^	22.85±2.14^	11.53±1.63^	8.37± 1.10^	2.55±0.94^	1.56±0.65^
Peat1-Zn	95.50±6.45	77.29±5.51#^	58.06±4.53#^	33.09±2.86^	16.05± 1.90#^	12.69±1.65^	8.14±1.40^	3.55± 0.65^	1.61±1.01^	0.56±0.56^
Peat2-Zn	104.69±6.17	90.25±3.40^	68.06±4.34^	41.82±2.68^	22.31± 1.35^	17.42±1.59^	9.82±0.76^	3.63± 0.73^	1.25±0.53^	0.46±0.33^

The differences are statistically significant: * with the SS group, $p < 0.05$; # with the $ZnSO_4$ group, $p < 0.05$; ^ with the Day 3 group, $p < 0.05$.

Table 3

Changes in excisional wound healing in rats under the influence of zinc-containing biocomposites (HS – Zn)

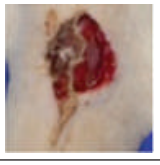
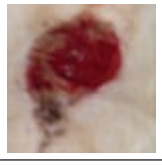
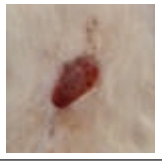
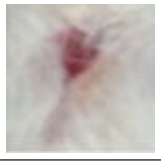
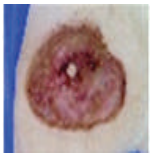
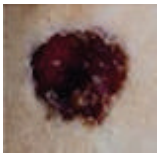
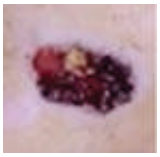
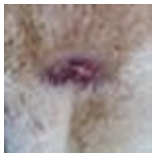
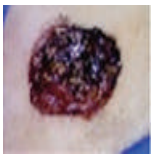
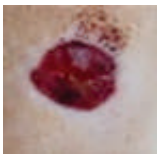
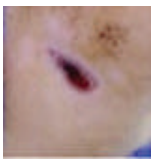
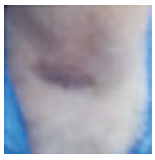
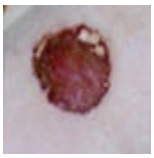
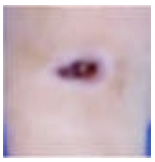
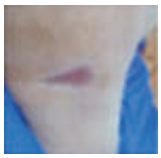
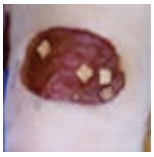
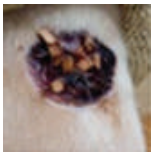
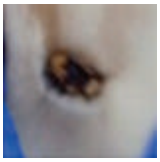
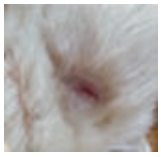
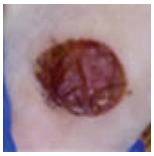
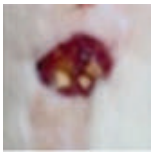
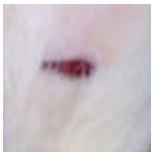
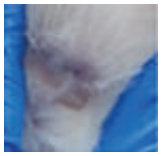
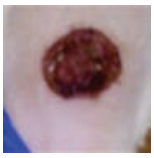
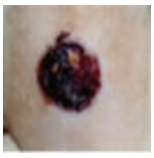
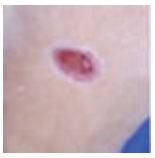
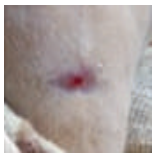
Group	Injected substance	Duration of treatment, days			
		3	7	13	21
1	SS				

Table 3 (continued)

Group	Injected substance	Duration of treatment, days			
		3	7	13	21
2	$ZnSO_4$				
3	CHP-Zn				
4	FA-Zn				
5	CHS-Zn				
6	Peat1-Zn				
7	Peat2-Zn				

The results of assessing Zn content in the wound surface area of the skin, fur, serum, and in intact areas when zinc-containing samples (HS-Zn) were applied externally to the wound surface are presented in Figure 1. It was established that Zn content in the fur of rats whose wound surface areas were treated with SS was comparable with the literature data: 149.23 ± 9.04 μg / ml [16]. In rats treated with the reference listed drug $ZnSO_4$ (the studied samples CHP-Zn and FA-Zn), no significant differences from the control group were observed in terms of zinc content in fur (Fig. 1, *a*).

When studying CHS-Zn, Peat1-Zn, and Peat2-Zn, there was a significant increase in Zn content in rat fur by 82, 78, and 73%, respectively, compared to the controls, which may indicate the presence of a resorptive effect when they were applied to the area treated. Zn content in the wound surface of the skin is consistent with the physiological indices of Zn in the skin [17]. It was shown that all skin samples to which HS – Zn was applied had a higher Zn concentration compared to the control group (Fig. 1, *b*).

The maximum Zn concentration in the skin was noted for the Peat2-Zn sample (70.2 ± 5.4 μg / g), in the remaining samples CHP-Zn, FA-Zn, CHS-Zn, Peat1-Zn, including the reference listed drug $ZnSO_4$, there was an increase in Zn concentration compared to the controls by 36, 51, 36, 54, and 45%, respectively, but no significant differences were found between the samples. Based on the results of assessing Zn content in the blood serum of rats, it was shown that an increase in Zn concentration was observed only in the CHS-Zn, Peat1-Zn, and Peat2-Zn samples compared to the control group (3.2 ± 0.37; 2.9 ± 0.2, and 3.1 ± 0.3 μg / g). No significant differences were

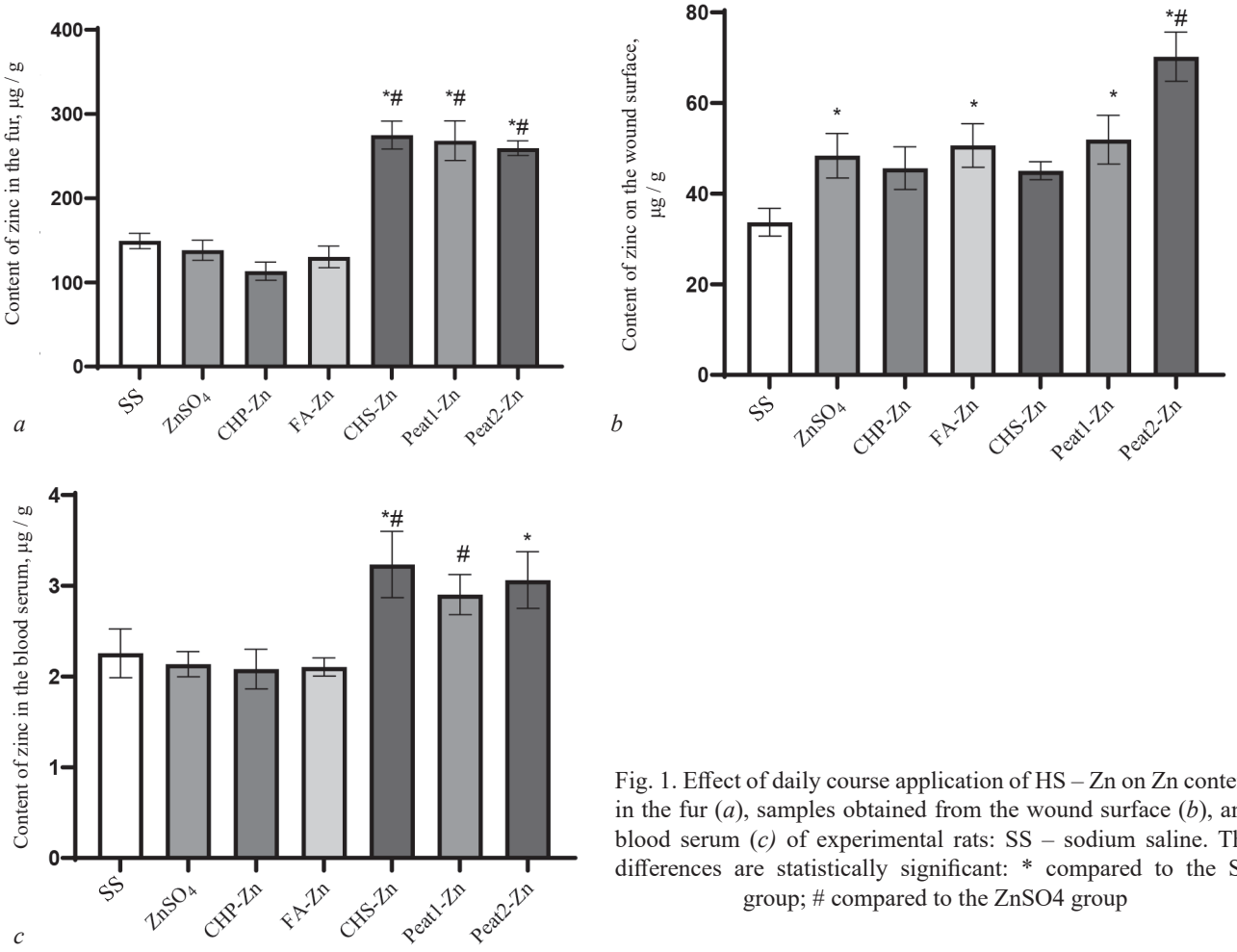


Fig. 1. Effect of daily course application of HS – Zn on Zn content in the fur (*a*), samples obtained from the wound surface (*b*), and blood serum (*c)* of experimental rats: SS – sodium saline. The differences are statistically significant: * compared to the SS group; # compared to the ZnSO4 group

noted in the remaining $ZnSO_4$, CHP-Zn, and FA-Zn samples compared to the control group.

CONCLUSION

The course application of zinc-containing biocomposites FA-Zn and Peat1-Zn, containing elemental zinc at a concentration of 1.67 mg / ml, accelerates the healing of aseptic wounds, as evidenced by a decrease in the wound surface area in comparison with the wound area when applying $ZnSO_4$ with an equivalent concentration of elemental zinc. It was established that course application of zinc-containing HS – Zn biocomposites provides a resorptive effect, but Zn content in the biomaterial does not exceed the level of limiting permissible concentrations.

It was also noted that the base ligands of HS affect the bioavailability of Zn. Thus, the CHP-Zn and FA-Zn samples do not affect the bioavailability of Zn, while the CHS-Zn, Peat1-Zn, and Peat2-Zn samples increase the bioavailability of Zn compared to the control group. The discovered reparative effect of HS – Zn biocomposites, in addition to their low toxicity, is of interest for further study in order to develop effective wound-healing drugs.

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Authors' contribution

Zykova M.V., Perminova I.V., Ivanov V.V. – conception and design, interpretation of the data, critical revision of the manuscript for important intellectual content. Larionov K.S. – synthesis of zinc-containing biocomposites based on humic ligands. Bratishko K.A., Azarkina L.A., Ufandeev A.A., Rabtsevich E.S., Mikhalev D.A., Kopnov I.S. – studies of biological activity and resorptive action, analysis of the data. Azarkina L.A. – drafting of the manuscript. Perminova I.V., Belousov M.V. – fundraising, final approval of the manuscript for publication.

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Prevalence of some internal diseases depending on the adipokine level in people under 45 years of age

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ABSTRACT

The aim was to study the prevalence of some common internal diseases in young people of working and childbearing age, depending on the levels of adipokines.

Materials and methods. The study included 1,340 people aged 25–44 years. The levels of leptin, adiponectin, adipsin, lipocalin-2, plasminogen activator inhibitor-1 (PAI-1), and resistin were determined by the multiplex analysis. Low-density lipoprotein hypercholesterolemia (LDL hypercholesterolemia), coronary artery disease (CAD), type 2 diabetes mellitus (T2DM), arterial hypertension (AH), renal dysfunction (RD), and chronic bronchitis (CB) were studied.

Results. With an increase in the level of adiponectin, the prevalence of CAD increased by 8.6 times. The highest quartile of the adipsin level was characterized by an increase in the prevalence of LDL hypercholesterolemia by 12.9%, AH by 3.9%, and RD by 17.9%. The quartiles of lipolkalin-2 showed higher prevalence of LDL hypercholesterolemia, AH, and RD in Q_4 compared to Q_1. The prevalence of CB was associated with a decrease in the level of lipocalin-2 and was higher by 35.9% within Q_1 compared to Q_4. In the quartiles of PAI-1, the prevalence of T2DM and LDL hypercholesterolemia was 2 and 1.5 times higher, respectively, and the prevalence of RD was 2.5 times lower in Q_4 than in Q_1. In quartiles of resistin, the prevalence of LDL hypercholesterolemia, AH, and RD increased by 13–38%, while the prevalence of CB decreased by 20% in Q_4 compared to Q_1. The prevalence of LDL hypercholesterolemia and RD was higher within Q_4 of leptin.

Conclusion. The results indicate the need for further research aimed at studying the molecular mechanisms underlying the effects of adipokines. This will allow to find a combined approach to restoring normal physiological levels of adipokines, which can have a positive effect in the studied internal diseases.

Keywords: internal diseases, adipokines, adiponectin, lipocalin-2, resistin

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

Source of financing. The article was written within the state assignment “Epidemiological monitoring of the population’s health and study of molecular genetic and molecular biological mechanisms underlying the development of common internal diseases in Siberia to improve approaches to their diagnosis, prevention, and treatment” (No. 122031700094-5_) and funded by the Russian Science Foundation grant (No. 21-15-00022).

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study and to personal data processing. The study was approved by the local Ethics Committee at the Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (Protocol No. 16 of 26.11.2019).

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Распространенность некоторых терапевтических заболеваний в зависимости от уровней адипокинов у людей до 45 лет

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РЕЗЮМЕ

Цель – изучить встречаемость некоторых распространенных терапевтических заболеваний у молодых людей трудоспособного и детородного возраста в зависимости от уровней адипокинов.

Материалы и методы. В исследование включено 1 340 человек в возрасте 25–44 лет. Методом мультиплексного анализа определены уровни лептина, адипонектина, адипсина, липокалина-2, ингибитора активатора плазминогена-1 (ИАП-1) и резистина. Изучены: гиперхолестеринемия липопротеинов низкой плотности (гиперХС-ЛНП), ишемическая болезнь сердца (ИБС), сахарный диабет 2-го типа (СД2), артериальная гипертензия (АГ), почечная дисфункция (ПД), хронический бронхит (ХБ).

Результаты. С увеличением уровня адипонектина распространенность определенной ИБС возрастает в 8,6 раз. Самый высокий квартиль уровня адипсина характеризуется увеличением распространенности гиперХС-ЛНП на 12,9%, АГ на 3,9% и ПД на 17,9%. Квартили липолкалина-2 показали более высокую распространенность гиперХС-ЛПН, АГ и ПД в Q_4 по сравнению с Q_1. Распространенность ХБ ассоциирована со снижением уровня липокалина-2 и выше в Q_1 на 35,9%, в сравнении с Q_4. В квартилях ИАП-1 встречаемость СД2 и гиперХС-ЛНП в 2 и 1,5 раза соответственно выше, а ПД в 2,5 раза ниже в Q_4, чем в Q_1. В квартилях резистина на 13–38% увеличивается распространенность гиперХС-ЛНП, АГ, ПД. На 20% снижается распространенность хронического бронхита в Q_4 по сравнению с Q_1. Встречаемость гиперХС-ЛНП и ПД была выше в Q_4 лептина.

Заключение. Результаты свидетельствуют о необходимости дальнейших исследований, направленных на изучение молекулярных механизмов, лежащих в основе эффектов адипокинов, что позволит найти комбинированный подход, направленный на восстановление нормальных физиологических уровней адипокинов. Это может дать положительный эффект при изученных терапевтических заболеваниях.

Ключевые слова: терапевтические заболевания, адипокины, адипонектин, липокалин-2, резистин

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

Источник финансирования. Статья подготовлена в рамках государственного задания «Эпидемиологический мониторинг состояния здоровья населения и изучение молекулярно-генетических и молекулярно-биологических механизмов развития распространенных терапевтических заболеваний в Сибири для совершенствования подходов к их диагностике, профилактике и лечению» (№ 122031700094-5_) и при финансовой поддержке гранта Российского научного фонда (№ 21-15-00022).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании и обработку персональных данных. Исследование одобрено локальным этическим комитетом НИИТПМ – филиал ИЦиГ СО РАН (протокол № 16 от 26.11.2019).

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INTRODUCTION

Adipokines are important circulating biomolecules mediating intertissue interactions throughout the body and thus playing a key role in maintaining endocrine homeostasis. The most studied adipokines are adiponectin, leptin, resistin, monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6, IL-1ß, and IL-10. Adipokines are involved in various functions and can affect many processes, including energy and appetite modulation, lipid and glucose metabolism, insulin and endothelial cell functions, inflammation, etc. [1].

To date, numerous associations of adipokines with widespread noncommunicable diseases have been identified, including cardiovascular diseases, type 2 diabetes mellitus, hypertension, and others [2, 3], although the functions and molecular mechanisms underlying the effects of adipokines have not been fully elucidated. The aim of this study was to investigate the prevalence of some common internal diseases in young people of working age and childbearing age, depending on adipokine levels.

MATERIALS AND METHODS

The study was conducted on a population sample of residents of Novosibirsk aged 25–44 years, formed in 2013–2017 in Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (IITPM – Branch of IC&G SB RAS). To build a sample, the database of the Territorial Compulsory Health Insurance Fund for the Novosibirsk Region was used. Using a random number generator, persons of both sexes aged 25–44 years were selected from this database. Throughout this time frame, 1,512 people were examined within a single-stage population screening; their biological material was collected, and a database was compiled.

All patients signed an informed consent to examination and processing of personal data. The study was approved by the local Ethics Committee at IITPM – Branch of IC&G SB RAS (Protocol No. 16 of 26.11.2019). The study included 1,340 people (618 men, 720 women) – all of them were persons whose samples of biological material were housed in the biological collection of the IITPM – Branch of IC&G SB RAS at the time of the study. Two people did not fill out a questionnaire including demographic and social data, but their serum was included in the work.

A clinical examination of patients was carried out at IITPM – Branch of IC&G SB RAS. The survey program included collection of demographic and social data, a survey on smoking habits, two measurements of blood pressure (BP), spirometry, anthropometric measurement (measurement of height, body weight, waist circumference (WC), hip circumference (HC)), functional tests, etc.

The serum content of total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (LDL-C), and glucose was determined by the enzymatic method using the reagent kit manufactured by Thermo Fisher Scientific (Finland) on the Konelab Prime 30i biochemical analyzer (Finland). The concentration of LDL-C was calculated according to the Friedewald equation. Conversion of serum glucose into fasting plasma glucose (FPG) was carried out according to the formula (EAST, 2005): FPG (mmol / l) = –0.137 + 1.047 = serum glucose (mmol / l). The levels of leptin, adiponectin, adipsin, lipocalin-2, plasminogen activator inhibitor-1 (PAI-1), and resistin were determined by the multiplex analysis using the Human Metabolic Hormone Panel V3 (USA) and the Human Adipokine Panel 1 (USA) on the Luminex MAGPIX system (USA). Concentrations were expressed in ng / ml for lipocalin-2, PAI-1, leptin, and resistin and in mcg / ml for adiponectin and adipsin.

Hypercholesterolemia was established at the level of LDL-C > 3.0 mmol / l [4]. The diagnosis of coronary heart disease (CHD) (according to epidemiological criteria - “Definite CHD”) was made in the presence of the following criteria: past large myocardial infarction (ECG), angina pectoris (Rose Angina Questionnaire), ischemic-like ECG changes without left ventricular hypertrophy, rhythm and conduction disturbances (ECG). Type 2 diabetes mellitus was established in the presence of FPG ≥ 7 mmol / l [5]. Arterial hypertension (AH) was observed with an average systolic blood pressure (SBP) greater than 140 mm Hg and / or diastolic blood pressure (DBP) greater than 90 mm Hg, according to the clinical guidelines “Arterial hypertension in adults” approved by the Ministry of Health of Russia in 2020 [6]. The presence of renal dysfunction was recorded at a glomerular filtration rate (GFR) < 90 ml / min / 1.73 cm². The GFR was calculated using the CKD-EPI equation. The diagnosis of chronic bronchitis (CB) was established on the basis of anamnestic data: cough with sputum for 3 months within a year and more often for 2 years or more, no signs of bronchial obstruction [7].

Baseline clinical, anamnestic, and biochemical characteristics of the studied sample are summarized in Table 1.

Table 1

Clinical, anamnestic, and biochemical characteristics of the studied sample, Me [Q_1; Q_4]

Parameter	Value
Age, years	37.08 [31.5; 41.83]
SBP, mm Hg	120.0 [110.5; 130.0]
DBP, mm Hg	78.5 [71.6; 87.0]
BMI	25.23 [22.16; 29.14]
Fasting plasma glucose, mmol / l	5.73 [5.31; 6.04]
TC, mmol / l	4.99 [4.32; 5.68]
HDL-C, mmol / l	1.29 [1.09; 1.50]
LDL-C, mmol / l	3.15 [2.51; 3.76]
TG, mmol / l	0.97 [0.69; 1.44]
Creatinine, mcmol / l	74.0 [67.0; 82.0]
GFR	101.37 [90.36; 110.05]

Note: SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, GFR – glomerular filtration rate

Statistical processing of the results was carried out in the SPSS 20.0 software. Normality of the distribution of variables was checked by the Kolmogorov – Smirnov test. Quantitative variables whose distribution was different from normal were presented as the median and the interquartile range *Me* [Q_1; Q_4]. Categorical variables were presented as relative values (%). The Pearson's $\chi 2$ criterion was used to compare the proportions. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The levels of the studied adipokines are presented in Table 2. In the study sample (n = 1,340), serum concentrations of adiponectin, adipsin, lipocalin-2, PAI-1, resistin, and leptin were measurable in 58, 82, 98, 61, 77, and 98% of samples, respectively, which is associated with obtaining too low levels in some samples, which did not allow to detect the biomarker.

To study the prevalence of internal diseases depending on the blood concentrations of the studied adipokines, the entire population sample was divided into quartiles based on the content of the studied parameters (Table 2).

The prevalence of the studied diseases in the quartiles of adipocytokines is presented in Table 3. The results of the study showed that with an increase in the level of adiponectin, the prevalence of a distinct coronary heart disease increases by 8.6 times.

Table 2

Levels of the studied adipokines in quartiles, *Me* [Q_1; Q_4]

Parameter	Adiponectin,mcg / ml	Adipsin, mcg / ml	Lipocalin-2, g /ml	PAI-1, ng / ml	Resistin, ng / ml	Leptin, ng / ml
Entire sample	37.14 [25.78; 114.47]	11.69 [7.59;14.1]	385.69 [198.36; 1,133.02]	21.66 [13.18; 32.41]	152.84 [25.74; 596.22]	4,524.61 [1,743.14; 8,513.13]
Q_1	16.1 [11.18; 22.31]	4.35 [2.79; 6.04]	125.7 [82.46; 166.44]	9.41 [6.27; 11.10]	13.93 [6.74; 19.57]	959.23 [435.82; 1,299.6]
Q_2	32.15 [28.06; 34.21]	9.75 [8.81; 10.55]	297,.62 [242.92; 343.81]	17.85 [15.95; 19.71]	51.98 [35.13; 101.08]	2,994.16 [2,123.5; 3,793.3]
Q_3	54.19 [41.04; 98.83]	13.03 [12.42; 13.50]	668.27 [503.57; 976.42]	26.25 [23.81; 29.43]	502.83 [414.56; 551.92]	6,280.95 [5,375.41; 7,112.79]

Table 3

Prevalence of the studied diseases in the quartiles of adipocytokines, %

Group	Q_1	Q_4	p
Adiponectin			
Hypercholesterolemia	58.1	59.7	0.940
CHD	0.5	4.3	0.050
T2DM	46.7	36.0	0.867
CB	55.6	66.7	0.529
AH	9.9	8.3	0.312
Renal dysfunction	22.4	22.4	0.276
Adipsin			
Hypercholesterolemia	48.7	61.6	0.009
CHD	1.1	2.3	0.490
T2DM	31.3	35.1	0.968
Bronchitis	50.0	56.9	0.230
AH	5.6	9.5	<0.0001
Renal dysfunction	17.2	35.1	<0.0001
Lipocalin-2			
Hypercholesterolemia	48.1	61.0	0.005
CHD	1.0	1.9	0.516
T2DM	37.5	30.0	0.958
Bronchitis	40.9	5.0	<0.0001
AH	3.7	11.6	0.001
Renal dysfunction	12.2	24.0	<0.0001
PAI-1			
Hypercholesterolemia	44.1	58.9	0.012
CHD	1.0	0.0	0.075
T2DM	30.0	64.3	0.043

Table 3 (continued)

Group	Q_1	Q_4	p
Bronchitis	57.1	56.5	0.564
AH	4.9	5.9	0.696
Renal dysfunction	20.6	8.0	0.010
Resistin			
Hypercholesterolemia	48.0	61.3	0.010
CHD	0.8	3.3	0.180
T2DM	40.0	31.0	0.808
Bronchitis	20.0	0	0.001
AH	1.2	14.1	<0.0001
Renal dysfunction	3.4	41.2	<0.0001
Leptin			
Hypercholesterolemia	50.9	59.5	0.038
CHD	2.2	2.2	0.881
T2DM	18.8	48.1	0.108
Bronchitis	41.7	57.9	0.536
AH	6.4	10.1	0.394
Renal dysfunction	14.0	31.3	<0.0001

Note: CHD – coronary heart disease, T2DM – type 2 diabetes mellitus, CB – chronic bronchitis, AH – arterial hypertension.

The highest quartile of adipsin was characterized by an increase in the prevalence of hypercholesterolemia by 12.9%, AH – by 3.9%, and renal dysfunction – by 17.9%. The analysis of the quartiles of lipocalin-2 showed higher prevalence of hypercholesterolemia, AH, and renal dysfunction in Q_4 compared to Q_1. The prevalence of CB was associated with a decrease in the level of lipocalin-2; it was 35.9% higher in Q_1 compared to Q_4. In the quartiles of PAI-1, the prevalence of T2DM and hypercholesterolemia was 2 and 1.5 times higher, respectively, and the prevalence of renal dysfunction was 2.5 times lower in Q_4 than in Q_1.

When considering the quartiles of resistin, we noted a 13–38% increase in the prevalence of hypercholesterolemia, AH, and renal dysfunction and a 20% decrease in CB in Q_4 compared to Q_1. The analysis of leptin quartiles showed significant changes in the prevalence of only hypercholesterolemia and renal dysfunction. The prevalence of these diseases was higher in Q_4.

DISCUSSION

Adipokines in the human blood serum are found in a wide dynamic range, from pg / ml to mcg / ml. Changes in the expression and secretion of adipokines correlate with such internal diseases as T2DM, AH, CB, and cardiovascular diseases, which pose a serious health problem worldwide.

Adiponectin is secreted mainly by the adipose tissue and exists in cells and plasma in three main forms: a low-molecular-weight trimer, a medium-molecular-weight hexamer, and a high-molecular-weight (HMW) multimer, which is the main bioactive isoform contributing to its insulin-sensitizing and cardiovascular protective effects [8].

Studies show that plasma adiponectin levels decrease in patients with obesity [9], T2DM [10, 11], atherosclerosis [12], and AH [13]. Along with the inverse correlation with the total mass of the adipose tissue, adiponectin secretion is also regulated by the quality of adipose tissue [9]. Metabolically healthy but obese people tend to have higher levels of adiponectin compared to unhealthy people with similar adipose tissue mass [14]. In addition, disorganized formation of adiponectin isoforms may be associated with cardiometabolic disorders. Patients with CHD have a lower proportion of the HMW multimeter as opposed to a higher proportion of the trimeric form. Similarly, only HMW adiponectin form increases after weight loss in obese patients [15]. A number of studies have shown that high levels of adiponectin are associated with adverse cardiovascular and other metabolic outcomes [16–18]. Our study showed that in the quartile with a high adiponectin level, the prevalence of CHD was significantly higher.

Leptin is synthesized mainly by the adipose tissue and in a small amount – by the gastric mucosa. The structure of leptin is similar to that of proinflammatory cytokines, such as IL-6 and granulocyte colony stimulating factor (G-CSF). Leptin mediates its effects by binding to specific receptors (ObR) expressed in the brain and peripheral tissues (nervous tissue, liver, pancreas, heart, and intestines). The main target organ of leptin is the arcuate nucleus of the hypothalamus, which plays an important role in regulating appetite and energy homeostasis. Leptin suppresses food intake and promotes energy expenditure. Regardless of these effects, leptin improves the sensitivity of peripheral tissues (liver and skeletal muscles) to insulin and modulates the function of beta cells in the pancreas. In most cases, people with obesity, despite the high level of leptin circulating in the bloodstream, do not lose weight, which reflects the presence of leptin resistance [19].

It is known that many factors, such as free fatty acids, estrogen, tumor necrosis factor (TNF)α or impaired renal clearance, stimulate leptin secretion [20]. In our study, in the quartile with high leptin levels, the prevalence of renal dysfunction (GFR 90) was 2 times higher than in the quartile with low leptin values. Thus, circulating leptin levels are elevated in

the early stages of chronic kidney disease (CKD) [21] and increase with the progression of the disease [22].

Adipsin, also called complement factor D, is mainly secreted by adipocytes, monocytes, and macrophages. Adipsin maintains adipose tissue homeostasis and increases insulin secretion in response to glucose. Studies show that in the presence of T2DM, there is a decrease in adipsin levels and there is an independent negative relationship between adipsin and HOMA-IR [23]. The works of other authors indicate a direct relationship between obesity, adipose tissue, and adipsin [24]. In our study, the prevalence of T2DM depending on the level of adipsin in Q_1 and Q_4 did not differ.

Adipsin is associated with various pathophysiological processes underlying atherosclerosis, including low-grade inflammation, endothelial dysfunction, and lipid metabolism [25, 26]. The study on the prevalence of AH depending on the level of adipsin revealed a significant increase in the incidence of AH in the quartile with high values of this adipokine. As for the association of adipsin with lipid metabolism disorders, in our study, the incidence of hypercholesterolemia was higher in the quartile with the highest adipsin values. The prevalence of renal dysfunction was 2 times higher in the quartile with the highest adipsin values, which may be associated with the activation of an alternative complement pathway in patients with CHB [27].

Lipocalin-2 is a secreted glycoprotein involved in a wide range of pathophysiological processes and energy metabolism. Some researchers consider lipocalin-2 as a biomarker of cardiometabolic and chronic kidney diseases [28–30], although there are conflicting reports about the use of this molecule as a biomarker for early diagnosis or prognosis of these diseases [31, 32]. In our study, the prevalence of hypercholesterolemia, AH, and renal dysfunction were associated with high levels of lipocalin-2, and the prevalence of CB was associated with a decrease in lipocalin-2.

Resistin is a polypeptide belonging to the family of resistin-like molecules, a group of proteins that initiate inflammatory processes. Resistin in humans is mainly produced by macrophages, granulocytes, monocytes, and bone marrow cells. Resistin levels are elevated in T2DM [33]. High concentrations of resistin are also found in cardiovascular complications. S. Niaz et al. demonstrated a progressive increase in serum resistin levels in patients with AH and CHD compared to the control group [34].

The mechanism underlying the association between resistin levels and AH is still unclear. One of the possible mechanisms may be mediated via TLR4. Resistin is believed to alter the renin – angiotensin pathway and vascular remodeling [35]. Another potential mechanism is that resistin can reduce the expression of endothelial nitric oxide synthase and increase the expression of endothelin-1, as well as its release in human endothelial cells [36]. In our study, the incidence of AH was 11.7 times higher in the quartile with high levels of resistin than in the quartile with its lowest values. The study on the prevalence of CHD among young people did not show a significant difference between quartiles with the highest and lowest resistin values.

Our results regarding the prevalence of renal dysfunction are consistent with the data of a number of studies that show that high concentrations of resistin in CHB are due to a decrease in GFR and, as a consequence, low elimination of resistin through the kidneys [37, 38]. On the other hand, it has been proven that increased concentrations of resistin are associated with a higher risk of renal dysfunction. The mechanism via which resistin can accelerate renal dysfunction is not yet well studied, but the probable reason is that resistin enhances synthesis of proinflammatory cytokines and increases oxidative stress, which, consequently, causes glomerular dysfunction [39].

In our study, the prevalence of CB was high in the quartile with the lowest values of resistin. O. Pérez-Bautista et al. showed that in patients with chronic obstructive pulmonary disease, compared to the control group, the content of C-peptide, ghrelin, glucagon-like peptide-1, and leptin was higher, and the levels of glucagon and resistin were lower [40].

PAI-1, a member of the serine protease inhibitor superfamily, can be produced by various cells, such as platelets, adipocytes, vascular endothelial cells, endometrial cells, and liver cells. PAI-1 is one of the most powerful antifibrinolytic proteins that binds to a tissue plasminogen activator or a urokinase-type plasminogen activator, inhibiting their function and reducing plasmin production. Studies show that in patients with metabolic syndrome and (or) T2DM, plasma concentrations of PAI-1 are elevated, which contributes to the hypofibrinolytic environment [41]. In our study, the prevalence of T2DM was 2 times higher in the quartile with the highest values of PAI-1, compared to the quartile with the lowest values. The study on the prevalence of renal dysfunction (GFR 90) revealed the difference between the first and fourth

quartile of PAI-1. In the quartile with the lowest values of PAI-1, the incidence of renal dysfunction was 2.5 times higher.

CONCLUSION

In this study, we investigated the prevalence of some socially sensitive internal diseases (CHD, AH, T2DM, CB, renal dysfunction) and hypercholesterolemia, a risk factor in young people aged 25–44 years, depending on adipokine levels. The highest prevalence of internal diseases was found for lipocalin-2 and resistin. The prevalence of CB was associated with low values of these adipokines, while hypercholesterolemia, renal dysfunction, and AH were more common at high values of lipocalin-2 and resistin. The incidence of hypercholesterolemia was significantly higher in quartiles with the highest values of all studied adipokines. The prevalence of CHD was associated only with adiponectin levels.

Further studies aimed at investigating the molecular mechanisms underlying the effects of adipokines will allow to find a combined approach to restoring normal physiological levels of adipokines, which can have a positive effect in the studied internal diseases.

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Authors' contribution

Kashtanova E.V., Polonskaya Ya.V., Stakhneva E.M. – conception and design of the study, interpretation of the research data. Shcherbakova L.V. – statistical processing and analysis of the data, editing of the article. Shramko V.S. – carrying out of biochemical studies, analysis and interpretation of the research data. Sadovski E.V. – compilation of the database, analysis and interpretation of the research data. Khudyakova A.M., Denisova D.V. – analysis and interpretation of the research data. Ragino Yu.I. – final approval of the manuscript for publication.

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Clinicopathological features of colon cancer depending on the dMMR status of the tumor

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ABSTRACT

Aim. To conduct a clinical and morphological assessment of the characteristics of colon cancer depending on the dMMR / pMMR status of the tumor.

Materials and methods. A retrospective study included 66 patients with operable colorectal cancer (CRC) ($T_{1-4b}N_{0-2b}M_1$), who were treated at Cancer Research Institute of Tomsk National Research Medical Center (NRMC). The average age of the patients was 64.4 ± 12.8 years. All patients underwent hemicolectomy or colon resection, as well as intraoperative resection of distant metastases, if present.

Results. We determined that in CRC patients with pMMR tumors, hematogenous metastases were detected in 27.3% of cases, while in patients with dMMR tumors, hematogenous metastases were detected only in 6.1% of cases ($p = 0.021$). A comparative analysis of dMMR and pMMR tumors also allowed to establish higher frequency of perineural invasion among the pMMR subgroup of carcinomas ($p = 0.039$). The sign of tumor budding was found both in dMMR carcinomas (36%) and in pMMR tumors (45%). This sign was associated with damage to regional lymph nodes ($p = 0.0017$). A more detailed analysis of the tumor budding phenomenon showed that in dMMR tumors, Bd1 low-grade budding (83%) predominated. In pMMR tumors, Bd2 intermediate-grade budding (33%) and Bd3 high-grade budding (26.7%) prevailed. Bd2 and Bd3 tumor budding types were associated with hematogenous metastasis ($p < 0.001$).

Conclusion. The obtained data demonstrate the differences in such pathomorphological parameters as perineural invasion and the degree of tumor budding depending on the dMMR / pMMR status of the tumor. These histologic parameters in tumor tissue are also associated with higher incidence of distant metastasis in patients with pMMR carcinomas as opposed to patients with dMMR tumors.

Keywords: mismatch repair system proteins, dMMR / pMMR status, colon cancer

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 9291 of 28.11.2022).

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Клинико-патологические особенности рака толстой кишки в зависимости от dMMR статуса опухоли

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РЕЗЮМЕ

Цель. Провести клинико-морфологическую оценку особенностей рака толстой кишки (РТК) в зависимости от dMMR/pMMR статуса.

Материалы и методы. В исследование ретроспективно включено 66 пациентов с операбельным РТК T1-4bN0-2bM1, прошедших лечение в НИИ онкологии Томского НИМЦ. Средний возраст больных составил 64,4 ± 12,8 года. Всем пациентам выполнено оперативное лечение в объеме гемиколэктомии или резекции кишки, а также интраоперационная резекция отдаленных метастазов при их наличии.

Результаты. Установлено, что у пациентов с РТК и pMMR статусом гематогенные метастазы определялись в 27,3% случаев, в то время как у пациентов с dMMR статусом гематогенные метастазы были обнаружены лишь в 6,1% случаев (p = 0,021). Сравнительный анализ опухолей с dMMR и pMMR статусом также позволил установить большую частоту наличия периневральной опухолевой инвазии среди pMMR подгруппы карцином (p = 0,039). Признак «опухолевого почкования» был обнаружен как в карциномах с дефицитом белков мисматч репарации (36%), так и в профицитных опухолях (45%). Данный признак был сопряжен с поражением регионарных лимфатических узлов (p = 0,0017). Более детальный анализ феномена «опухолевого почкования» показал, что в опухолях с дефицитом белков мисматч репарации преобладал первый тип почкования (Bd1) low grade – низкой степени – (83%), в то время как профицитные опухоли характеризовались преобладанием почкования второго типа (Bd2) – intermediate grade – умеренной степени (33%) и (Bd3) – high grade – высокой степени (26,7%). Феномен «опухолевого почкования» Bd2 и Bd3 был сопряжен с гематогенным метастазированием (p < 0,001).

Заключение. Полученные данные демонстрируют различия по таким патоморфологическим параметрам, как периневральная инвазия и степень «опухолевого почкования» в зависимости от dMMR/pMMR статуса карциномы. Данные гистологические параметры в опухолевой ткани также связаны с большей частотой встречаемости отдаленных метастазов у пациентов с карциномами со статусом pMMR в отличие от пациентов с dMMR статусом опухоли.

Ключевые слова: белки системы мисматч репарации, dMMR/pMMR статус, колоректальный рак

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

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INTRODUCTION

According to the World Health Organization (WHO), colorectal cancer (CRC) is one of the most common malignant neoplasms in the world. It is the 5th most common cancer. Over one million newly diagnosed tumors are reported among both sexes annually. The number of deaths exceeds 570,000 per year [1]. In 2021, over 18,000 new cases of CRC were registered in the Russian Federation, which accounts for 7.1% of all

malignant neoplasms. The increase in the incidence in 2011–2021 was 25.3% [2]. In 2021, more than 23,000 deaths from CRC were registered, making this disease the third leading cause of mortality from malignant neoplasms in the Russian Federation [2].

Only 30–50% of CRC patients achieve 5-year survival, since the disease in most cases is detected at late clinical stages [3]. Despite this, if the tumor is diagnosed at an early stage (T_{1-} T_2N_0), the 5-year survival rate is more than 90%. It is worth noting that among stage II CRC patients, the 5-year relapse rate is extremely variable, ranging from 12 to 38%. Moreover, in stage III CRC patients, the 5-year relapse-free survival rate does not exceed 50% [3].

The key stage in the diagnosis of CRC is morphological verification of the diagnosis followed by the assessment of pathomorphological signs. Currently, the main parameters are: the size of the primary tumor, histologic grade, degree of malignancy, pT and pN criteria, the condition of the resection margins and lymph nodes, extramural and peritumoral vascular invasion, perineural invasion, as well as the assessment of the invasive tumor front area for the presence of morphological manifestations of tumor budding [3]. This phenomenon is detected when there are clusters of single tumor cells at the invasive margin of the tumor (from 2 to 4 cells in one tumor bud).

Currently, depending on the number of tumor cell clusters at the invasive margin per 1 field of view, three budding grades are distinguished: 0–4 buds indicate low-grade budding (Bd1); 5–9 buds indicate intermediate-grade budding (Bd2): ≥ 10 buds indicate high-grade budding (Bd3) [4]. This phenomenon is based on epithelial – mesenchymal transition (EMT) [5]. EMT is the process by which the cell changes its phenotype from epithelial to mesenchymal, which ultimately leads to the possibility of tumor invasion, as well as to lymphogenous and hematogenous metastasis, which correlates with an unfavorable prognosis for the disease course in the presence of this morphological sign in the tumor tissue [6].

Despite the existing pathohistological criteria used in diagnosing surgical material to assess the objective prognosis of the disease course and elaborate an individual approach to treatment, morphological studies should be supplemented with the results of molecular genetic methods. To date, the most common and recommended methods are determination of mismatch repair deficiency proteins (dMMR status of the tumor – mismatch repair deficient status) or microsatellite instability (MSI) by immunohistochemistry using polymerase chain reaction [7, 8]. According to clinical guidelines, including the Russian Society of Clinical Oncology RUSSCO, these techniques can be mutually exclusive, since their concordance in CRC is more than 95% [9].

It should be noted that dMMR / MSI status of the tumor depending on the clinical stage of the disease can serve as both a prognostic and a predictive factor for the response to drugs, such as checkpoint inhibitors [9], as well as to the effectiveness of fluoropyrimidines [10], which are the main therapeutic drugs in both postoperative treatment and subsequent lines. This significantly influences the treatment strategy of a patient for both local and diffuse tumor processes.

Despite widespread testing of dMMR / MSI in clinical practice, studying the clinical and pathological characteristics of the primary tumor and their relationship with parameters of regional and distant metastasis is of interest in CRC.

MATERIALS AND METHODS

The study included 66 patients diagnosed with stage $T_{1-4b}N_{0-2b}M_{1a}$ CRC. The inclusion criterion for the study was histologically verified CRC. The exclusion criterion was preoperative chemotherapy. The scope of surgical treatment corresponded to hemicolonectomy (right / left) or radical resection of the colon with simultaneous removal of distant metastases, if present. A morphological study of the surgical material was carried out, assessing the following parameters: histologic tumor grade, density of immune cell infiltrate in the tumor stroma (in 10 fields of view at × 400), signs of lymphovascular and perineural invasion, the presence and number of tumor buds at the invasive tumor margin, presence / absence of metastases in the lymph nodes (at least 12 regional lymph nodes).

The microscopic examination was carried out using the Eclipse Ci-L upright microscope (Nikon, Japan). The histotype and stage of the disease were determined according to the 2019 WHO Classification of Tumors of the Digestive System. The density of the immune cell infiltrate in the tumor stroma was quantified. The results were converted into a point scale (1 point – mild infiltration, up to 300 immune cells; 2 points – moderate infiltration, 300–600 cells; 3 points – pronounced infiltration, more than 600 cells).

The assessment of tumor budding was carried out as follows: counting of tumor buds in hotspots at the invasive tumor margin per unit area of

0.785 mm^2 (corresponding to a ×20 eyepiece lens with a field of view diameter of 20 mm). The obtained quantitative values were also converted into a point scale, where 0–4 buds corresponded to Bd1 (low-grade budding); 5–9 buds corresponded to Bd2 (intermediate-grade budding); ≥10 buds and more corresponded to Bd3 (high-grade budding). The dMMR status of carcinoma was assessed on paraffin sections of the tumor tissue (surgical material) by immunohistochemistry. Staining was performed on the Bond RX Fully Automated IHC and ISH Staining System (Leica Biosystem) using such antibodies as MLH1 (Clone ES05, RTU, Dako An Agilent Technologies Company, RTU); MSH2 (Clone FE1, RTU, Dako An Agilent Technologies Company), MSH6 (Clone EP49, RTU, Dako An Agilent Technologies Company); and PMS2 (Clone EP51, RTU, Dako An Agilent Technologies Company) (Fig. 1–3). In the absence of nuclear IHC staining of at least one marker, mismatch repair protein deficiency was diagnosed (tumor with microsatellite instability – dMMR status). In the study group, distant metastases were detected in 16.67% of cases (n = 11 / 66). The presence of metastatic lesions and the number of metastatic lymph nodes (N) are presented in Table.

Statistical data analysis was performed using the SPSS 23.0 software package (IBM SPSS Statistics, USA). Quantitative variables were presented as the median and the interquartile range Me (Q_{25}; Q_{75}). Qualitative variables were described by absolute and relative frequencies n (%). The quantitative and qualitative variables in independent samples were compared using the Mann – Whitney U test and the $\chi 2$ criterion. The results were considered statistically significant at a $p < 0.05$.

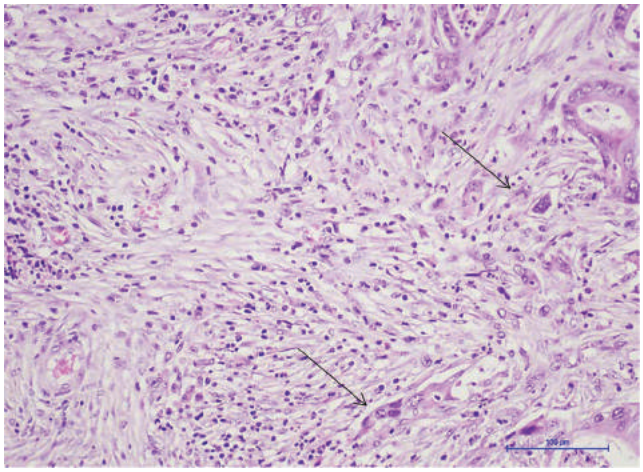


Fig. 1. Tumor budding at the invasive tumor front margin (marked by arrows) in colorectal adenocarcinoma. Here and in Fig.2 – H&E staining, × 200

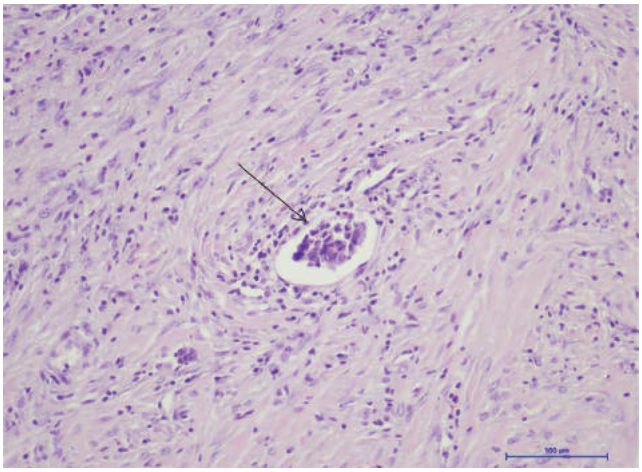
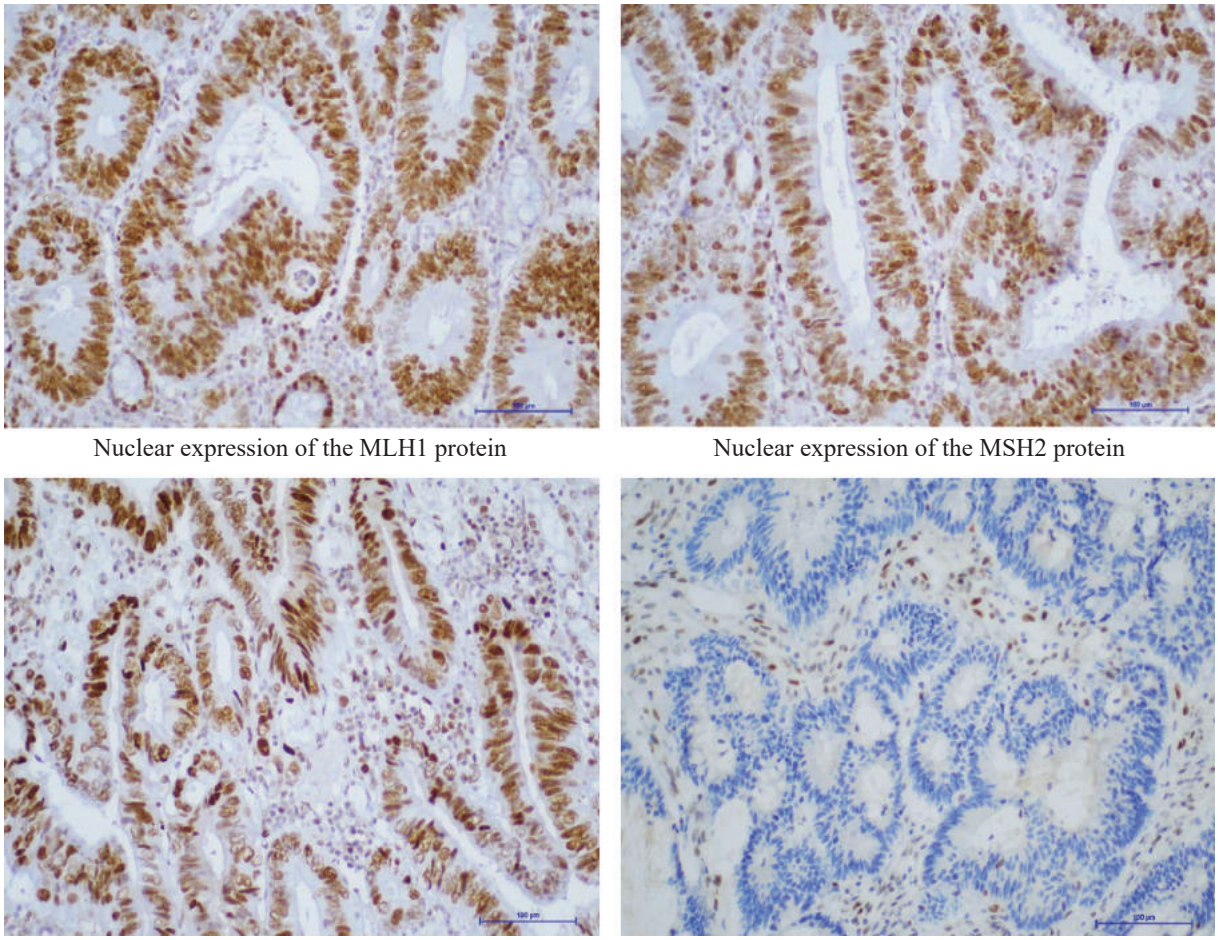


Fig. 2. Lymphovascular invasion (the arrow indicates a tumor embolus in the lumen of the vessel)



Nuclear expression of the MLH1 protein

Nuclear expression of the MSH2 protein

Nuclear expression of the MSH6 protein

Absence of PMS2 protein expression

Fig. 3. Immunohistochemical determination of the dMMR status of the tumor

RESULTS

Two groups of patients were formed depending on the immunohistochemically verified dMMR / pMMR status of the tumor. Group 1 included 50% of cases (n = 33 / 66) with mismatch repair protein deficiency (dMMR status) in the tumor. An equal number of cases (50%; n = 33 / 66) were included in the control group: tumors with mismatch repair protein proficiency (pMMR status). The assessment of existing correlations in the selected groups with tumor histotype, localization of the tumor in the colon, and the stage of the disease did not show significant differences (Table). The comparative analysis of cases with lymphogenous metastases in the groups of dMMR / pMMR tumors did not reveal significant differences. We did not find any differences in the number of metastatic lymph nodes.

Next, we assessed the relationship between the dMMR / pMMR status of carcinoma and the presence of hematogenous metastases. The results of the analysis allowed to establish that in the group of patients with dMMR tumors, hematogenous metastases were detected in 6.1% of cases (n = 2 / 11). Moreover, in cases with pMMR tumors, distant metastases were detected in 27.3% of cases (n = 9 / 11) (χ^2 = 5.3; p = 0.02) (Fig.4). As a result of this work, we did not see any significant differences between the dMMR / pMMR status and the density of immune cell infiltrate in the tumors (Table).

The assessment of tumor budding at the invasive tumor margin showed its presence in both groups. However, significant differences were found between tumor budding grades (χ^2 = 6.0; p < 0.04). Thus, we observed Bd1 (n = 10) and Bd2 (n = 2) in dMMR tumors, with the presence of morphological manifestations of tumor budding at the invasive margin. No morphological manifestations of this phenomenon were found in other dMMR tumors (n = 21).

In the group of pMMR tumors, we identified Bd3 (n = 4) along with Bd1 (n = 6) and Bd2 (n = 5). No manifestations of this phenomenon were found at the

invasive margins of other tumors ($n = 18$). The additional analysis of the results obtained allowed to establish that tumors with Bd2 and Bd3 budding were characterized by the presence of hematogenous metastases ($\chi^2 = 17.2$; $p < 0.001$). The assessment of perineural tumor invasion revealed that in dMMR tumors this morphological feature was detected less frequently compared to pMMR carcinomas ($\chi^2 = 4.2$; $p = 0.03$).

Table

Clinical data and morphological parameters of the tumor in CRC patients

Parameter		dMMR		pMMR		Criterion value	*p*
		n	%	*n*	%		
Sex	male	23	69.7	19	57.6	1.048	0.433
	female	10	30.3	14	42.4		
Age, years, *Me* (Q_{25}; Q_{75})		63 (54.5; 63) 63.3±13.5		66 (59.5; 74) 65.6±12.1		481.5	0.419
Tumor histotype	high-grade adenocarcinoma	10	30.3	9	27.3	0.635	0.728
	low-grade adenocarcinoma	14	42.4	12	36.4		
	mucinous adenocarcinoma	9	27.3	12	36.4		
Tumor localization	cecum	5	15.2	3	9.1	10.094	0.183
	ascending colon	6	18.2	3	9.1		
	hepatic flexure	4	12.1	4	12.1		
	transverse colon	2	6.1	2	6.1		
	splenic flexure	4	12.1	2	6.1		
	descending colon	5	15.2	1	3.0		
	sigmoid colon	6	18.2	17	51.5		
	rectosigmoid colon	1	3.0	1	3.0		
Cancer stage (T)	T2	5	15.2	1	3.0	4.333	0.228
	T3	6	18.2	8	24.2		
	T4a	19	57.6	23	69.7		
	T4b	3	9.1	1	3.0		
Lymphogenous metastasis (N)	N0	17	51.5	15	45.5	3.869	0.568
	N1a	6	18.2	3	9.1		
	N1b	2	6.1	1	3.0		
	N1c	1	3.0	4	12.1		
	N2a	3	9.1	5	15.2		
	N2b	4	12.1	5	15.2		
Density of the immune cell infiltrate	mild	6	18.2	3	9.1	1.310	0.519
	moderate	13	39.4	16	48.5		
	pronounced	14	42.4	14	42.4		
Lesion side	right	17	51.5	13	39.4	0.978	0.459
	left	16	48.5	20	60.6		

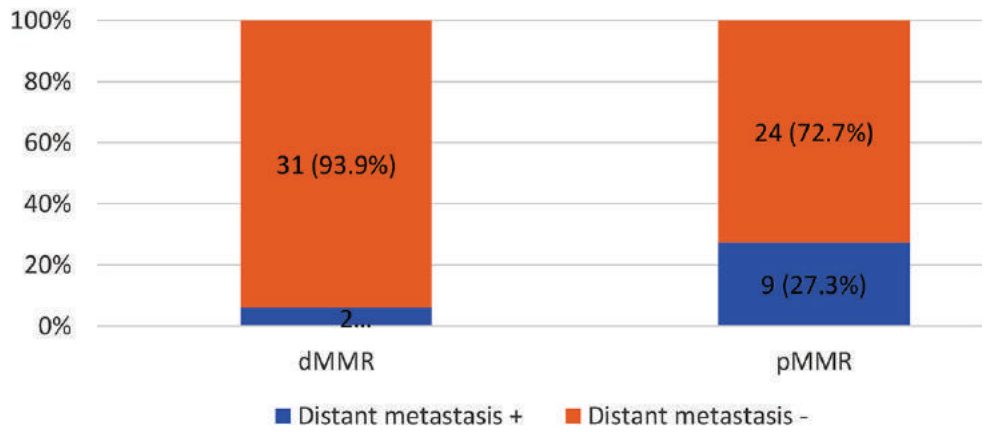


Fig.4. Frequency of distant metastasis in CRC patients depending on the dMMR / pMMR status of the primary tumor

DISCUSSION

Our research showed that in CRC, various clinical and morphological features can be simultaneously characteristic of both dMMR and pMMR tumors. In this work, we identified a number of differences that may determine the nature of the disease course. Thus, the comparative analysis of dMMR and pMMR tumors depending on tumor morphology and its localization did not show significant differences. The most important morphological features that were characteristic of dMMR carcinomas were lower frequency of perineural invasion and predominance of Bd1 and Bd2 tumor budding grades.

In the analyzed literature, there was no description of similar morphological characteristics depending on the molecular status of the tumor in CRC. The most indicative parameter that we detected was low hematogenous metastatic potential of dMMR tumors compared to pMMR carcinomas. One of the reasons explaining the discovered relationship may be different biological behavior of tumors due to differences in the volume of mutational load [10]. This hypothesis is also supported by the fact that no significant differences in the density of the immune cell infiltrate (which, according to one of the existing hypotheses, has a tumor suppressive effect) were found between dMMR and pMMR carcinomas [11, 12].

It may be assumed that the dMMR status of the tumor and associated morphological parameters, such as low frequency of perineural invasion and predominance of the Bd1 tumor budding, are interrelated and explain lower metastatic potential. It should be noted that the absence in the tumor tissue of signs of perineural invasion, manifestations of Bd3 tumor budding at the invasive margin, and signs of hematogenous metastasis can be considered as indirect morphological parameters that may indicate the dMMR status of a colonic tumor.

CONCLUSION

Our data demonstrate the presence of morphological differences in the primary tumor tissue in colonic tumors depending on the dMMR / pMMR status of the tumor. Based on the obtained results, it can be assumed that certain histologic parameters, such as the presence of perineural invasion and the predominance of various tumor budding grades at the invasive tumor margin, can be associated with the frequency of hematogenous metastasis and, therefore, can serve as a prognostic criterion for CRC with immunohistochemically verified dMMR or pMMR molecular status of colorectal carcinoma.

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Authors' contribution

Naumov S.S., Krakhmal N.V. – conception and design, analysis and interpretation of the data, carrying out of immunohistochemistry. Tarasov M.N., Taranenko M.I., Kolobovnikova Yu.V. – collection of the material, work with archives, literary review. Udut E.V. – critical revision of the manuscript for important intellectual content. Vtorushin S.V. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

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Immunomodulatory effect of lithium salt gamma-lactone 2,3-dehydro-L-gulonic acid *in vitro*

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ABSTRACT

The aim of this work was to study the immunomodulatory effects of lithium salt gamma-lactone of 2,3-dehydro-L-gulonic acid (LiAc) on healthy blood leukocytes and leukemia cells *in vitro*.

Materials and methods. Peripheral blood lymphocytes and neutrophils obtained from healthy donors, as well as THP-1 cells (human monocytic leukemia) were used as test systems. To assess the proliferative activity, lymphocyte blast transformation was used. The antiproliferative effect was studied by the 3H-thymidine incorporation assay. Cytotoxic effects were studied using the Alamar Blue test. The effect on the phagocytic activity was studied using the method for assessing the neutrophil function during bacterial phagocytosis.

Results. LiAc exerted a dose-dependent effect on target cells, including antiproliferative and cytotoxic effects on leukemia cells and a stimulating effect on neutrophils in phagocytosis.

Conclusion. LiAc can be considered as a promising drug with immunomodulatory effects, including a suppressive effect on the proliferative activity of leukemia cells and a stimulating effect on immune mechanisms mediated by neutrophils and macrophages.

Keywords: lithium salts, immunostimulant, neutrophils, lymphocytes, monocytic leukemia, antiproliferative effect, phagocytosis

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Иммуномодулирующее действие литиевой соли гамма-лактон 2,3-дегидро-L-гулоновой кислоты *in vitro*

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РЕЗЮМЕ

Цель – изучение иммуномодулирующих свойств литиевой соли гамма-лактон 2,3-дегидро-L-гулоновой кислоты (LiAc) на нормальные лейкоциты крови и злокачественные лейкозные клетки *in vitro*.

Материалы и методы. В качестве тест-систем использованы лимфоциты и нейтрофилы периферической крови здоровых доноров, а также злокачественные клетки линии THP-1 (моноцитарная лейкемия человека). Для оценки пролиферативной активности использовалась реакция бластной трансформации лимфоцитов. Изучение антипролиферативного действия выполнено методом включения меченого 3H-тимидина. Цитотоксические эффекты препарата исследованы с помощью аламарового теста. Изучение влияния на фагоцитарную активность выполнено с помощью метода оценки функциональной активности нейтрофилов при фагоцитозе бактерий.

Результаты. LiAc оказал дозозависимое влияние на клетки-мишени, что проявилось в антипролиферативном и цитотоксическом действии в отношении лейкозных клеток и стимулирующем действии в отношении фагоцитирующих нейтрофилов.

Заключение. LiAc может рассматриваться как перспективный препарат, обладающий иммуномодулирующими свойствами, включая супрессивное влияние на пролиферативную активность лейкозных клеток и стимулирующее действие на нейтрофильно-макрофагальное звено иммунитета.

Ключевые слова: соли лития, иммуностимулятор, нейтрофилы, лимфоциты, моноцитарный лейкоз, антипролиферативный эффект, фагоцитоз

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 immune system is a complex self-regulating mechanism, providing specific and non-specific protection from both external pathogens and internal threats, including malignant cells. At the same time, an effective immune response implies a balance of immune activation to eliminate the antigen and immunosuppression, which prevents damage to one's own healthy tissues. This balance is ensured by the interaction of macrophages, and regulatory T cells; by the induction of chemokines, cytokines, and antibodies; by the expression of the corresponding receptors and inhibition and proliferation of effector cells.

Thus, normal functioning of the immunity is supported by intrinsic immunostimulatory and immunosuppressive processes [1]. Providing an external regulatory effect on the immune system is a difficult task, which is currently achieved using immunomodulators, i.e. drugs that can stimulate, inhibit or regulate components or alter the functions of the immune system [2]. The compounds with immunostimulatory or immunosuppressive effects may belong to different chemical groups and have different biological targets. Mainly, immunomodulatory effects are realized through the influence on immunocompetent cells, the processes of maturation, migration, cooperation, as well as through the interaction of these cells and their products (cytokines) with the corresponding targets [3].

Many xenobiotics and chemical compounds have unidirectional immunotoxic effects, which is manifested by a negative impact on the functioning of both local and systemic immunity, and induced immunosuppression can lead to an increase in the incidence or severity of infectious diseases and cancer [4]. Therefore, the study of the immunomodulatory properties of promising biologically active compounds is an integral stage in the development of new drugs based on them. A substance acting on cellular immunity *in vitro* will have a complex non-specific effect on the entire immune system, due to high interconnectedness of the components.

A significant number of immunostimulants are currently available as dietary supplements. However, their effect on the actual immune status is very contradictory. Not all immunostimulants have been tested with immunocompetent cells. It is especially difficult to test various herbal stimulants due to high variability of the composition and difficulties of identifying the active agent [5].

Targeted immunostimulation is one of the promising approaches to the treatment of cancer, while non-specific immunostimulation is considered as a maintenance therapy [6]. In general, immunotherapy is the most promising approach to cancer treatment and combines well with chemotherapy and immunostimulants to improve treatment outcomes [7]. Immunomodulators are an important component in the treatment of comorbid pathologies, which improves the prognosis of the primary disease. Recently, it has been shown that immune dysregulation can contribute to tumor progression [8]. Effective immunocorrection requires drugs that have low toxicity and a complex immunomodulatory effect and combine well with known methods of treating tumors, including chemotherapy and radiation therapy.

In this context, lithium preparations stand out due to the presence of a known immunotropic effect. A good example is lithium carbonate, which is widely used in psychiatric practice and is still the mainstay for the treatment of affective disorders, while having pronounced immunostimulatory properties [9, 10]. Lithium has stimulating effects towards hematopoiesis, and, thus, it is a drug for the recovery of the body after radiation exposure [11, 12]. The main molecular target explaining the hematopoietic effect of lithium is the effect on the intracellular enzyme glycogen synthase kinase-3 (GSK-3) [13].

The role and positive effects of lithium in the treatment of cancer patients, including individuals after radiation exposure, have been studied [14]. It is important to note the ability of lithium to activate antiviral immunity [15]. During the coronavirus epidemic of 2020–2021, the effectiveness of lithium drugs in the treatment of COVID-19 was shown [16]. The combination of these data forms a solid basis for an in-depth study of the mechanisms of lithium action and the search for new niches for its use, including the creation of new lithium-containing compounds with complex properties [17].

However, it should be noted that disorders related to oxidative stress play an important role in the pathogenesis of many socially sensitive human pathologies [18–20]. Oxidative stress is a fundamental phenomenon in biology that causes a cascade of reactions [21]. The influence of oxidative stress in autoimmune, mental, cardiovascular diseases, and cancer has been established, therefore, reduction of oxidative stress by antioxidant drugs is clinically justified in many cases [20]. In this context, it is important to obtain and study the properties of new lithium complexes with antioxidant activity towards immunocompetent cells, which reveals certain

prospects for obtaining drugs with combined activity that allow to modulate immunity and reduce oxidative stress in a wide range of pathologies [22–24].

The aim of this work was to study the effect of the lithium salt gamma-lactone of 2,3-dehydro-L-gulonic acid (LiAc) on the proliferative and functional activity of healthy blood leukocytes and leukemia cells, that will provide grounds for developing a promising antioxidant drug with immunomodulatory effects.

MATERIALS AND METHODS

Gamma-lactone of 2,3-dehydro-L-gulonic acid (ascorbic acid) and lithium carbonate (ACS, Sigma-Aldrich, Germany) were used to synthesize the research object. The salt preparation reaction was carried out with stirring and heating up to 40 ºC in deionized water. The reaction product was washed and sterilized with ethanol, then dried. The identity of the compound was confirmed by atomic emission spectroscopy (AES with inductively coupled plasma iCap 6300 Duo), infrared spectroscopy (Agilent Cary), thermogravimetric analysis (thermal analyzer with mass spectroscopy SDQT 600, Thermo Electron Corp.). The elemental analysis found 33% (C), 5.33% (H), 8.1% (Li2O); theoretically calculated values were 33.03% (C), 5.05% (H), 8.21% (Li2O). The water content in the salt was 16.15% (theoretical – 16.51%). The reaction product corresponded to the general formula LiC6H7O6*2H2O. The resulting powder was packed in sealed test tubes and used in experiments.

The effect of the synthesized lithium preparation on human immune blood cells was studied *in vitro* using the lymphocyte blast transformation (LBT). The method is based on the assessment of the transformation and proliferation of lymphocytes when exposed to various antigens and the mitogen phytohemagglutinin (PHA). Lymphocytes were obtained from whole blood samples of three healthy donors. Lymphocytes were isolated by density gradient centrifugation and resuspended in a standard RPMI 1640 medium containing 20% fetal bovine serum, L-glutamine, and streptomycin. Aliquots of 0.1 ml (2×10^6 cells / ml) of the cell mixture were placed in microtest plates. The drug was added to the plate in concentrations of 0.1–0.001 mg / ml with or without PHA. The microtest plates were incubated for 72 hours at 37 °C in an atmosphere of 5% CO_2. Stimulation with PHA, which induces cell proliferation, was used as a positive control. To do this, mononuclear cells (2×10^5 cells per well) in a culture medium were introduced into the wells of a 96-well flat-bottomed plate in the presence of PHA (final concentration – 15 mcg / ml). The cells were also incubated for 72 hours at 37 °C with 5% CO_2.

In the last 24 hours of the cell culture process, 1 μCi of [3H]-thymidine was added to each well of the experimental and control groups. The cells were collected by fiberglass filters (Seaton, Ind. Sterling, VA) and the amount of included [3H]-thymidine was determined using the liquid scintillation β-counter (Delta 300, model 6891, TM Analytic Inc., Netherlands).

The phagocytic activity of neutrophils was evaluated using bacterial phagocytosis [25]. Heparinized venous blood was washed with a medium 199 by centrifugation of samples to determine the phagocytic activity of neutrophils. Gram-positive bacteria *Staphylococcus aureus* – H209 were used as a substrate for phagocytosis. Bacteria were added to the leukocyte suspension. The studied compound in various concentrations (0.1–0.001 mg / ml) was added to the microtest plates. The samples were placed in the incubator for 30 minutes and shaken every 10 minutes. After incubation, the cells were fixed in formalin. Then the samples were centrifuged to make smears with the parameter determining phagocytosis. The smears were stained according to Romanowsky – Giemsa staining. Next, the following parameters were calculated. The percentage of active neutrophils was calculated as the number of neutrophils positive for *S. aureus* in terms of 100 neutrophils. The engulfment capacity of neutrophils was characterized by the phagocytic index, which was calculated as the total number of *S. aureus* cells engulfed by 100 positive neutrophils and divided by 100, which gives the average number of microbes engulfed by one active neutrophil.

In the experiment, the THP-1 monocytic leukemia cell line was used at a concentration of 2×10^5 cells per well of a 96-well plate in a complete culture medium RPMI 1640 containing 10% fetal bovine serum, L-glutamine, and antibiotics. The cells were introduced into the wells of 96-well flat-bottomed plates in 100 mcl of medium. After that, LiAc was added in the dose range from 0.1 mg / ml to 0.001 mg / ml. Then, 1 μCi of [3H]-thymidine was added to each well. The cells were incubated for 24 hours at 37 °C with 5% CO_2. The cells were collected by fiberglass filters (Seaton, Ind. Sterling, VA) and the amount of incorporated [3H]-thymidine was determined using the liquid scintillation β-counter (Delta 300, model 6891, TM Analytic Inc., Netherlands).

THP-1 monocytic leukemia cell lines and healthy human peripheral blood mononuclear cells were used as a biological object for cell cytotoxicity tests *in vitro*. The cytotoxicity of the studied drug was evaluated on cell cultures using the Alamar Blue assay. The cells were cultured in a complete RPMI 1640 medium enriched with fetal bovine serum, L-glutamine, and antibiotics and plated into a 96-well plate with 20,000 cells per well (in 180 mcl of medium). Then the drug was added to each well in the appropriate concentration (in the dose range of 0.1–0.001 mg / ml). Additional wells were used for untreated control (negative control) and control of dead cells (positive control). Then the plate was placed in the incubator with 5% CO_2 and at a temperature of 37 °C. After 48 hours of incubation, 20 mcl of Alamar Blue reagent was added to each well. The plate was placed back in the incubator for 4 hours. Then the optical density was measured at 570 nm (with background subtraction at 620 nm). Cell viability was evaluated by the formula (sample optical density (OD) – positive control OD) / (negative control OD – positive control OD) and expressed as a percentage of the surviving cells.

RESULTS

The study of the intensity of LBT after introduction into the culture of LiAc cells made it possible to establish the presence of a suppressive effect of the drug on lymphoproliferation, depending on the concentration. Thus, a dose of 0.1 mg / ml significantly inhibited cell proliferation by more than 60% compared to the control group (Fig. 1).

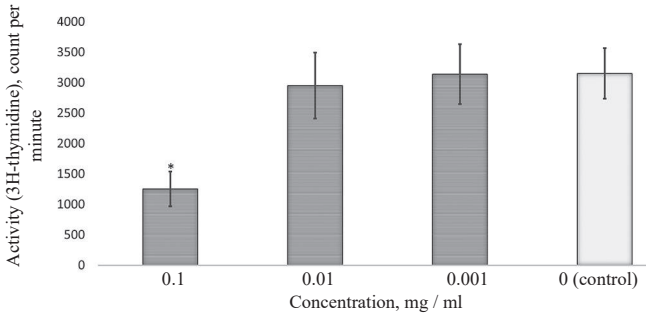

Fig. 1. The effect of LiAc on the lymphocyte PHA-stimulated proliferation (by the incorporation of 3H-thymidine (count per minute)). Here and in Fig. 2, 3, the data are presented as $M \pm SD$. * significant difference from the control, $p \leq 0.05$

The effect of the drug on the functional activity of peripheral blood neutrophilic leukocytes of donors was studied using the model of incomplete phagocytosis. The obtained results regarding the effect of the drug on phagocytic activity are presented in Table.

Table

The effect of LiAc on the phagocytic activity of neutrophils, $M \pm SD$

Parameter	Control	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
Percentage of active neutrophils, %	56 ± 3	61 ± 4	67 ± 5*	55 ± 4
Engulfment capacity of neutrophils, bact. units	4.2 ± 0.2	4.9 ± 0.2*	5.1 ± 0.5*	4.2 ± 0.3

Statistically significant differences in the experimental group vs. the control group ($p < 0.05$)

Table 1 shows that after exposure to LiAc, the percentage of active neutrophils capable of phagocytizing staphylococci and the engulfment capacity of neutrophils in the dose range of 0.1–0.01 mg / ml increase by 15–20%.

As can be seen from Table 1, LiAc does not significantly affect phagocytic activity in all dose ranges, but phagocytosis completeness at low concentrations increases slightly. The percentage of active neutrophils increases. The data obtained indicate the absence of an inhibitory effect on phagocytes in this dose range.

The cytotoxic effect of the drug on THP-1 cells was evaluated. The results are shown in Fig. 2.

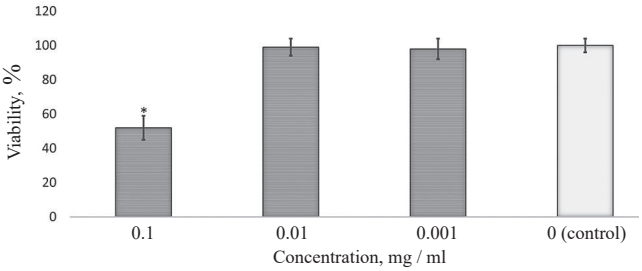

Fig. 2. Cytotoxic effect of LiAc on THP-1 cells

The antiproliferative effect of LiAc on leukemia cells was also evaluated; the results are shown in Fig. 3. Studying bioactivity on the THP-1 cell model made it possible to confirm the presence of the antiproliferative effect of the drug.

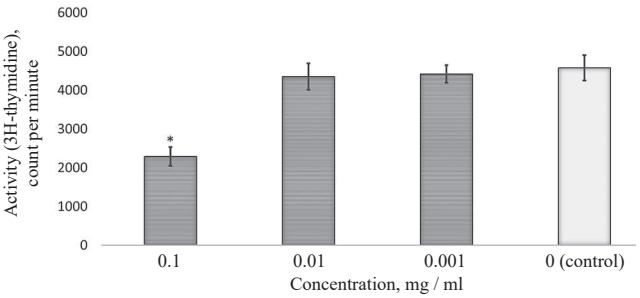

Fig. 3. The effect of LiAc on the proliferative activity of THP-1 cells, by 3H-thymidine incorporation (count per minute)

DISCUSSION

The obtained results revealed a noticeable antiproliferative effect of the drug on PHA-stimulated lymphocytes in the LBT (Fig. 1). The effect on lymphocytes observed at the dose of 0.1 mg / ml was statistically significant. In general, the antiproliferative effect on the mitogen-stimulated lymphocyte population under the influence of the studied drug correlates with the previously shown effect of ascorbic acid [26]. However, it is important to note that a stimulating effect on the activity of phagocytes was not observed. This study revealed a stimulating effect on phagocytosis parameters (Table). This phenomenon characterizes the complex properties of LiAc. At the same time, an increase in several parameters was noted, including the percentage of active neutrophils and the engulfment capacity of neutrophils in the concentration range of 0.1–0.01 mg / ml. No significant effect was recorded at lower doses. Stimulation of phagocytosis parameters may have a certain interpolation to cellular immunity in general.

Based on this, it is possible to predict the presence of a mild immunostimulating effect *in vivo*. These data generally correlate with the literature data on the stimulating effect of lithium on hematopoiesis, and in particular on granulocytopoiesis [27]. Lithium-mediated immunostimulation can be used in cases of leukopenia of various origins [28] and even immunodeficiency [29]. However, when lithium salts are used, the mechanisms of hematopoiesis induction are primarily due to the action of the cation. The anionic component of the salt provides new properties, for example, antioxidant, antihypoxant, and also participates in the regulation of hematopoiesis [30]. At the same time, there are studies showing the cytotoxic effect of antioxidants [31, 32]. *In vitro*, this effect has been proven for LiAc (Fig. 2), when a dose-dependent decrease in the viability of the leukemia cell population was shown. These data were confirmed on other types of tumor cells and via *in vivo* testing in liver cancer [33]. The study by K. Polliredddy et al. shows the inhibitory effect of ascorbate on pancreatic tumors [34].

Our study on the effect of LiAc on the THP-1 cell model with the incorporation of labeled thymidine confirmed the presence of a pronounced antiproliferative effect on actively dividing malignant cells (Fig. 3). Thus, the level of inhibition of malignant cell proliferation by the incorporation of 3H-thymidine 24 hours after exposure to the drug at a concentration of 0.1 mg / ml decreased from 4,579 ± 327 count per minute from the baseline (in the control) to 2,293 ± 241 count per minute (59% of the control values). Lower concentrations of the drug (0.001–0.01 mg / ml) did not have a suppressive effect on the cells ($p \leq 0.05$).

It is obvious that the intracellular mechanisms of the established antiproliferative and cytotoxic action are non-specific to the cell type and are generally due to the ambivalent action characteristic of ascorbates, which exhibit an antioxidant effect only in small concentrations. It should also be borne in mind that high antioxidant parameters of a substance *in vitro* do not always reflect the effects obtained *in vivo* due to the limited assessment methodology [35]. Exposure to ascorbates at high doses leads to a pro-oxidant reaction and an increase in the intracellular level of ROS, primarily hydrogen peroxide [36]. This effect can be considered as the main non-specific component responsible for the antiproliferative effect of the drug. In this case, the dualism of *in vitro* action manifests itself through a dose-dependent decrease in cell viability and proliferation.

CONCLUSION

The drug showed a significant dose-dependent suppressive effect on actively dividing cells. Suppression was noted on tumor lines and on lymphocytes stimulated to divide in LBT. A non-specific suppressive effect of the lithium complex in high concentrations was observed. At the same time, it was shown to have a stimulating effect on neutrophilic leukocytes, which play an important role in the resistance of the body to infections. The data obtained allow to consider the use of LiAc for developing drugs with immunomodulatory effect.

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Authors' contribution

Plotnikov E.V., Tretyakova M.S. – conception and design, carrying out of experiments. Krivoshchekov S.V. – obtaining, purification and quality assessment of LiAc samples, discussion of the results. Belousov M.V., Plotnikov E.V. – analysis of the results, interpretation of the data, drafting of the manuscript and critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

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Levels of metalloproteinases and adipose tissue hormones in men with coronary atherosclerosis

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ABSTRACT

Aim. To study the effect of adipose tissue hormones on the level of metalloproteinases in men with verified coronary atherosclerosis and to assess associations between the studied biomarkers and abdominal obesity.

Materials and methods. The study included 96 men aged 58.9 ± 5.1 years: 80 men with angiographically verified atherosclerosis and class II–III angina pectoris and 16 men without atherosclerosis. Anthropometric parameters were measured in all patients, and their blood was taken on an empty stomach. The blood levels of adiponectin, leptin, resistin, adipsin, amylin, and metalloproteinases (MMPs) -1, -2, -3, -7, -9, -10, -12, -13 were determined by the multiplex analysis. Statistical processing of the results was carried out using the SPSS 13.0 software.

Results. In patients with severe atherosclerosis, lipocalin, MMP-1, MMP-7, and MMP-12 levels were higher than in the control group. The blood concentration of adiponectin in patients with atherosclerosis was reduced. Inverse correlations were revealed between waist circumference and concentrations of MMP-1 and MMP-12, as well as between body mass index and MMP-1. A moderate direct relationship was revealed between resistin and MMP-2 and MMP-3; between amylin and MMP-9; between adiponectin and MMP-12; between leptin and MMP-7.

Conclusion. The results obtained suggest a relationship between the level of damage markers and adipose tissue hormones, which lead to complications of cardiovascular diseases and explain the effect of obesity on atherosclerotic plaque destabilization.

Keywords: metalloproteinases, atherosclerosis, adipose tissue hormones

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

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Conformity with the principles of ethics. All patients signed an informed consent to examination and personal data processing. The study was approved by the Ethics Committee at E.N.Meshalkin National Medical Research Center (Protocol No. 6 of 05.07.2017).

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Уровни металлопротеиназ и гормонов жировой ткани у мужчин с коронарным атеросклерозом

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РЕЗЮМЕ

Цель: изучить уровни гормонов жировой ткани и металлопротеиназ у пациентов с верифицированным коронарным атеросклерозом и оценить ассоциации изучаемых биомаркеров с абдоминальным ожирением.

Материалы и методы. В исследование включили 96 мужчин в возрасте 58,9 ± 5,1 лет: 80 мужчин с коронароангиографически верифицированным атеросклерозом и стабильной стенокардией напряжения II–III функционального класса и 16 мужчин без атеросклероза. У всех исследуемых проводилось измерение антропометрических показателей и забор крови натощак. Методом мультиплексного анализа в крови определяли уровень адипонектина, лептина, резистина, адипсина, амилина, металлопротеиназ (ММП) 1, -2, -3, -7, -9, -10, -12, -13. Статистическая обработка результатов проводилась в программе SPSS 13.0.

Результаты. У пациентов с выраженным атеросклерозом по сравнению с контрольной группой были выше уровни липокалина, ММП-1, ММП-7 и ММП-12. Концентрация в крови адипонектина у пациентов с атеросклерозом была снижена. Выявлены обратные ассоциации между окружностью талии и концентрациями ММП-1 и ММП-12, индекса массы тела с ММП-1; обнаружена прямая связь средней силы между уровнями резистина и ММП-2 и ММП-3; амилина с ММП-9; адипонектина с ММП-12; лептина с ММП-7.

Заключение. Полученные результаты позволяют говорить о связи между уровнем маркеров деструкции и гормонами жировой ткани, которые ведут к развитию осложнений сердечно-сосудистых заболеваний и объясняют влияние ожирения на дестабилизацию атеросклеротической бляшки.

Ключевые слова: металлопротеиназы, атеросклероз, гормоны жировой ткани

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

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INTRODUCTION

Recently, more and more studies have been aimed at investigating the effect of obesity on atherosclerosis and cardiovascular diseases (CVD). First of all, the presence of abdominal obesity is associated with atherosclerosis and an increased risk of adverse cardiovascular events. The data indicate that adipose tissue as an endocrine organ produces a large number of adipokines, such as leptin, resistin, adiponectin, and inflammatory cytokines that have both proatherogenic and antiatherogenic effects. Excessive accumulation of lipids in adipose tissue causes adipocyte dysfunction, as a result of which the secretion of proatherogenic

adipokines and inflammatory cytokines into the bloodstream increases. It leads to endothelial damage and formation of atherosclerotic plaques, which in the future, with an imbalance of metalloproteinases (MMP), can become unstable.

The pathophysiological processes underlying the effect of adipose tissue hormones on the level of MMPs, which may contribute to the pathogenesis of atherosclerosis, have not been sufficiently studied. Therefore, the aim of the study was to investigate the levels of adipose tissue hormones and metalloproteinases in the serum of patients with coronary atherosclerosis and to evaluate the association of the studied biomarkers with abdominal obesity.

MATERIALS AND METHODS

The collection of material for research was carried out in cooperation with E. Meshalkin National Medical Research Center. The ethical committees of the institutions approved the study, which included 96 men aged 58.9 ± 5.1 years. The main group encompassed 80 men who were hospitalized for coronary artery bypass grafting with coronary atherosclerosis verified by coronary angiography and class II–III angina pectoris. The control group included 16 men of the same age and with the same body mass index (BMI) without atherosclerosis recruited from a sample of Novosibirsk, the Research Institute of Internal and Preventive Medicine, a branch of the Institute of Cytology and Genetics SB RAS.

All patients signed an informed consent to participate in the study. Anthropometric data (height, weight, waist and hip circumference) were measured in all the subjects, and blood was taken on an empty stomach, 12 hours after the last meal. The group of patients with atherosclerosis was divided into subgroups depending on the presence / absence of abdominal obesity. The subgroup with abdominal obesity included men with a waist circumference (WC) ≥ 94 cm [1]. The blood levels of adiponectin, leptin, resistin, adipsin, amylin, and metalloproteinases (MMPs)- 1, -2, -3, -7, -9, -10, -12, -13 were determined by the multiplex assay on the Luminex MAGPIX flow fluorimetry system.

Statistical processing of the results was carried out using the SPSS 13.0 program. Biomarkers were tested for normality of distribution using the Kolmogorov – Smirnov test. Since most of the variables did not follow a normal distribution, the values in the tables were presented as the median and the interquartile range, *Me* [Q_{25}; Q_{75}]. The differences were evaluated using the Mann – Whitney test. Correlations were evaluated using the nonparametric Spearman's rank correlation coefficient. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The results obtained during the study are presented in Table 1. In patients with atherosclerosis, the level of adiponectin was significantly lower (by 20%) compared to the control group. Significant differences between the main and control groups were also obtained for MMP-1, MMP-7, and MMP-12. The level of MMP-1 was higher in the main group by 67.9%; MMP-7 – by 30.6%, and MMP-12 – by 107.1% compared to the control group. No significant differences were obtained for the other parameters (Table 1).

The results obtained in patients with atherosclerosis, depending on the absence / presence of abdominal obesity, are presented in Table 2. The levels of MMP-1 and MMP-12 were 1.7 (p = 0.001) and 1.38 (p = 0.037) times higher in patients without abdominal obesity, respectively. For the other parameters, the level of statistical significance of the differences was greater than 0.05.

Table 1

The levels of the studied biomarkers in the control group and in patients with coronary artery atherosclerosis, *Me* [Q_{25}; Q_{75}]

Parameter	Control group	Main group	*p*
Adiponectin, µg / ml	34.3 [16.4; 53.1]	24.4 [13.9; 38.5]	0.022
Adipsin, µg / ml	10.4 [7.0; 14.7]	9.6 [6.1; 13.1]	0.263
Resistin, ng / ml	21.7 [11.9; 49.1]	29.9 [11.4; 45.9]	0.716
Leptin, ng / ml	3.9 [1.2; 7.3]	4.6 [1.4; 8.2]	0.660
Amylin, pg / ml	6.2 [0.7; 16.2]	4.5 [1.6; 11.5]	0.790
MMP-1, ng / ml	5.3 [2.4; 8.9]	8.9 [5.7; 15.6]	0.002
MMP-2, ng / ml	111.7 [89.1; 126.9]	103.6 [78.3; 119.4]	0.253
MMP-3, ng / ml	47.1 [28.4; 66.5]	33.6 [21.3; 65.4]	0.202
MMP-7, ng / ml	8.5 [7.6; 11.9]	11.1 [9.4; 15.5]	0.012
MMP-9, ng / ml	236.0 [151.3; 307.6]	229.5 [131.0; 353.8]	0.611
MMP-10, ng / ml	0.62 [0.52; 0.82]	0.68 [0.52; 0.81]	0.722
MMP-12, ng / ml	0.14 [0.13; 0.17]	0.29 [0.18; 0.43]	0.000
MMP-13, pg / ml	30.4 [17.6; 71.9]	36.5 [18.5; 58.2]	0.701

Table 2

The level of adipose tissue hormones and metalloproteinases in patients with atherosclerosis, depending on the presence or absence of abdominal obesity, *Me* [Q_{25}; Q_{75}]

Parameter	Without AO	With AO	*p*
Adiponectin, µg / ml	28.3 [15.4; 41.9]	18.9 [11.7; 36.1]	0.188
Adipsin, µg / ml	10.9 [8.1; 13.6]	9.2 [5.5; 11.5]	0.07

Table 2 (continued)

Parameter	Without AO	With AO	*p*
Resistin, ng / ml	25.9 [8.2; 44.2]	16.6 [8.7; 34.7]	0.748
Leptin, ng / ml	4.0 [1.1; 7.9]	5.2 [2.1; 9.7]	0.347
Amylin, pg / ml	2.6 [1.6; 8.8]	4.5 [1.6; 10.5]	0.480
MMP-1, ng / ml	10.34 [7.1; 19.5]	6.1 [3.5; 10.0]	0.001
MMP-2, ng / ml	104.9 [82.3; 131.0]	93.7 [78.3; 112.9]	0.163
MMP-3, ng / ml	33.6 [23.6; 63.5]	35.9 [16.8; 65.8]	0.71
MMP-7, ng / ml	11.9 [9.4; 15.5]	10.2 [8.5; 14.3]	0.362
MMP-9, ng / ml	226.5 [122.1; 343.1]	205.5 [132.7; 325.9]	0.812
MMP-10, ng / ml	0.69 [0.55; 0.82]	0.68 [0.43; 0.82]	0.57
MMP-12, ng / ml	0.29 [0.17; 0.5]	0.21 [0.14; 0.33]	0.037
MMP-13, pg / ml	30.2 [17.8; 62.6]	43.15 [24.5; 58.4]	0.518

Note: AO – abdominal obesity.

In order to assess the effect of obesity on the level of metalloproteinases, we conducted a correlation analysis (using the Spearman's rank correlation coefficient). The relationship between the levels of MMP-1 (r = –0.531; p = 0.0001) and MMP-12 (r = –0.248; p = 0.032) with WC was revealed. The relationship with BMI was revealed only for MMP-1 (r = –0.264; p = 0.012). The association of MMP-2, MMP-3, MMP-7, MMP-9, and MMP-12 with adipose tissue hormones was revealed. Resistin was associated with concentrations of MMP-2 and MMP-3 (r = 0.321; p = 0.041 and r = 0.319; p = 0.002, respectively); leptin – with MMP-7 (r = 0.23; p = 0.035); adiponectin – with MMP-12 (r = 0.329; p = 0.008). The level of MMP-9 was associated with amylin (r = 0.568; p = 0.027).

DISCUSSION

The differences in the levels of biomarkers between the control group and patients with atherosclerosis were obtained for adiponectin, lipocalin-2, MMP-1, MMP-7, and MMP-12.

Adiponectin can inhibit the formation of reactive oxygen species, the release of proinflammatory cytokines, the expression of adhesion molecules, and apoptosis of smooth muscle cells and promote the outflow of cholesterol from macrophages [2]. At the same time, data on the effect of adiponectin in coronary heart disease (CHD) are contradictory. According to the meta-analysis by L. Yang et al., elevated adiponectin levels were independently associated with a higher risk of cardiovascular diseases and all-cause mortality in patients with CHD [3]. Q. Li et al. showed that, on the contrary, the frequency of adverse cardiovascular events was higher in the group of patients with low adiponectin levels [4]. In our study, we found that the level of adiponectin was reduced in patients with atherosclerosis compared to the control group, which indicates the anti-atherogenic function of adiponectin.

The association of resistin with MMP-2 and MMP-3 shows that it may enhance endothelial dysfunction, induce proliferation of smooth muscle cells, and promote the entry of mononuclear leukocytes into the intima through exposure to MMP. The study by S. Niaz et al. [5] showed a progressive increase in serum resistin levels in patients with arterial hypertension and CHD compared to healthy subjects. In our study, the level of resistin was higher in patients with atherosclerosis, but the significance level was greater than 0.05, which may be due to the small sample size and requires further study.

MMP-1 is considered to be the main enzyme responsible for collagen degradation. In addition, MMP-1 can lead to platelet activation and promote plaque expansion, rupture, and hemorrhage through degradation of collagen inside the plaque. High levels of MMP-1 are associated with an increased risk of all-cause mortality in patients with verified or possible ischemic disease [6]. We obtained higher levels of MMP-1 in patients with atherosclerosis. We also obtained data on an inverse correlation between the level of MMP-1, BMI, and WC, which differs from the data of S. Boumiza et al. [7], who found a direct correlation. It may be due to the fact that only men participated in our study.

MMP-7 plays an important role in atherosclerotic plaque destabilization, as it is associated with apoptosis of vascular smooth muscle cells and with macrophages in the necrotic nucleus. An association was established between plasma levels of MMP-7 and all-cause mortality in patients with CHD. Our data on the elevated level of MMP-7 in patients with atherosclerosis are in line with the data of other researchers [8]. C. Chavey et al. [9] showed that MMP-7 significantly decreases with obesity. Our study revealed a decrease in its level, but the differences were not statistically significant.

It is known that the expression of MMP-12 is increased in obesity. It is produced by macrophages and is associated with the progression and instability of

plaques [10, 11]. An increase in the level of circulating MMP-12 has been reported in asymptomatic patients with high cardiovascular risk associated with carotid intima–media thickness and cerebrovascular events during follow-up [12], as well as with the presence of CHD [13]. In our study, MMP-12 was elevated in patients with atherosclerosis, which is in line with the results obtained by M. Marcos-Jubilar et al. [14].

CONCLUSION

The data obtained indicate a relationship between the level of damage markers and adipose tissue hormones, which lead to the development of complications of cardiovascular diseases and explain the effect of obesity on atherosclerotic plaque destabilization.

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Authors' contribution

Polonskaya Ya.V. – conception and design, analysis and interpretation of the data, drafting of the manuscript. Kashtanova E.V. – conception and design, justification of the manuscript. Stakhneva E.M., Shramko V.S., Sadovsky E.V., Ledovskikh S.R. – analysis and interpretation of the data. Garbuzova E.V. – analysis and interpretation of the data, drafting of the manuscript in English. Kurguzov A.V., Murashov I.S. – obtaining biomaterial, analysis and interpretation of the data. Ragino Yu.I. – final approval of the manuscript for publication.

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Effect of long-term constant irradiation on retinal glia

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ABSTRACT

Aim. To study the response of retinal glial cells to constant irradiation of various intensity and to develop a mathematical model allowing to evaluate the dynamics of damage to radial glial cells and predict their photodamage depending on the duration and intensity of irradiation.

Materials and methods. Outbred sexually mature white rats ($n = 50$) weighing 180–200 g were exposed to constant round-the-clock light (200, 3,500 lux, days 1, 2, 7, 14, 30). The control group consisted of 25 non-irradiated animals. Using semi-thin sections stained with toluidine blue, we counted the number of pycnomorphic cells in the radial glial cells. Ultrastructural changes in the glial cells were studied using the JEM-100 CX-II electron microscope.

Results. The study showed that after photodamage, oligodendrocytes and astrocytes were mainly characterized by mitochondrial swelling and expansion of endoplasmic reticulum cisterns. Microglial cells at the late stage of the experiment (day 30) were localized in the inner layers of the retina; their density depended on the intensity of irradiation. The earliest (days 1, 2) changes in the radial glial cells were noted in the subretinal space and were manifested by proliferation of scleral processes and phagocytosis of dead sensorineural cell fragments. The intensification of destructive changes in the radial glial cells led to disturbances in neuron – glia interactions in the retina and a decrease in regeneration of retinal neurons (day 7–14). The developed mathematical model allowed to assess the dynamics of damage to the radial glial cells in the retina and to predict photodamage depending on the duration and intensity of irradiation.

Conclusion. Glial responses in the retina after photodamage depend on the intensity and duration of light exposure. As the duration of irradiation increases, degenerative changes in glial cells intensify and are more pronounced after high (3,500 lux) irradiation intensity.

Keywords: radial glial cells, microglial cells, astrocytes, oligodendrocytes, light, photodamage

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Влияние продолжительного постоянного освещения на глию сетчатки

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РЕЗЮМЕ

Цель. Изучить реакцию глиальной популяции сетчатой оболочки глаза при непрерывном световом облучении различной интенсивности и разработать математическую модель, позволяющую оценить динамику поражения радиальных глиоцитов и прогнозировать их световые поражения во временной и дозовой зависимости.

Материалы и методы. Беспородных половозрелых белых крыс (n = 50) массой 180–200 г подвергали постоянному круглосуточному освещению (200, 3 500 лк, 1-, 2-, 7-, 14-, 30-е сут). В качестве контроля использовали 25 необлученных животных. На полутонких срезах, окрашенных толуидиновым синим, проводили подсчет числа пикноморфных клеток радиальной глии. Ультраструктурные изменения глиоцитов изучали в электронном микроскопе JEM-100 CX-II.

Результаты. Исследование показало, что олигодендроглиоциты и астроциты после фотоповреждения в основном характеризуются набуханием митохондрий, расширением цистерн эндоплазматического ретикулума. Клетки микроглии в поздние сроки эксперимента (30 сут) локализуются во внутренних слоях сетчатки, их плотность зависит от интенсивности облучения. Наиболее ранние (1–2-е сут) изменения радиальных глиоцитов наблюдаются в субретинальном пространстве, выражаясь пролиферацией склеральных отростков и фагоцитозом фрагментов погибших нейросенсорных клеток. Усиление деструктивных изменений клеток радиальной глии приводит к нарушению глионейрональных взаимоотношений в сетчатке и снижению репаративных процессов со стороны нейронов сетчатки (7–14-е сут). Разработанная математическая модель позволяет оценить динамику поражения радиальных глиоцитов сетчатки и прогнозировать световые поражения во временной и дозовой зависимости.

Заключение. Глиальные реакции сетчатой оболочки глаза после фотоповреждения зависят от интенсивности и длительности облучения. По мере увеличения срока облучения в глиоцитах усиливаются дегенеративные изменения, более выражены после высокоинтенсивного (3 500 лк) светового облучения.

Ключевые слова: радиальная глия, микроглиоциты, астроциты, олигодендроглиоциты, свет, фотоповреждение

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

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INTRODUCTION

In the age of scientific and technological progress, people are constantly subjected to long-term exposure to numerous lighting devices, such as LED lamps, TV screens, computer displays, digital tablets, and smartphones [1–4]. Fluorescent lamps are widely used in industries and public institutions [5–7]. Replacing incandescent lamps with more economical energy-saving ones raises concerns about their safety for

human health and potential risks to the retina due to their specific spectral and energy characteristics [8–10]. LED lamps are generally characterized by intense blue light emission, which can damage structural components of the retina under both low-intensity lighting and extreme experimental conditions [1–3, 9–14].

The glial population of the retina is rather diverse. Astrocytes are located along blood vessels, oligodendrocytes are between the nerve fibers, and microglia are in the inner plexiform layer and ganglionic layer. Despite the fact that these cells do not participate directly in the process of light perception, they participate in metabolism and phagocytosis [15–17]. Radial glial cells play an important role in maintaining homeostasis and ensure protection of sensorineural cells. In case of their massive death, hypertrophy of glial cells occurs; they proliferate, migrate to the center of damage, and fill the site of injury with their processes, forming glial scars, which leads to reconstruction of the retina at a later stage of degeneration [18–22]. The available literature provides very little information on the quantitative assessment of changes in retinal glial cells in response to photodamage.

The aim of the study was to investigate the response of retinal glial cells to constant irradiation of various intensity and to develop a mathematical model allowing to evaluate the dynamics of damage to radial glial cells and predict their photodamage depending on the duration and intensity of irradiation.

MATERIALS AND METHODS

The study was performed on 75 outbred sexually mature white rats weighing 180–200 g, obtained from vivariums of Siberian State Medical University and the Goldberg Research Institute of Pharmacology and Regenerative Medicine of Tomsk NRMC. The animals were kept in a laboratory vivarium under fixed artificial light conditions (25 lux, 12:12 light – dark cycle) in accordance with the requirements of the Russian Construction Rules and Regulations II-A.9-71 to the standards of artificial lighting of rooms. In the first and second series of experiments, the animals (n = 50) were exposed to constant round-the-clock illumination (200, 3,500 lux; day 1, 2, 7, 14, 30). The control group consisted of 25 rats that were not exposed to constant round-the-clock illumination and were kept under similar conditions as experimental ones. Each experimental group contained five animals. The illumination equipment consisted of rectangular reflectors with embedded LB-40 cool white fluorescent lamps providing even illumination from five sides. The experiments were carried out in compliance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the rules of good laboratory practice (Order of the Ministry of Healthcare of the Russian Federation No. 267 of 19.06.2003).

The animals were decapitated immediately after the end of exposure to light. The central sections of the posterior segment of the eye and the optic nerves were fixed in 2.5% glutaraldehyde in the cacodylate buffer (pH 7.4) and postfixed in a 1% osmium tetroxide solution and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue. After that, the number of pycnomorphic radial glial cells of the retina was counted (200 cells per 15 fields of vision from 5 sections in each animal) at 10 × 90 magnification. Ultrastructural changes in the glial cells of the retina were studied using the JEM-100 CX-II electron microscope using ultra-thin sections contrasted with uranyl acetate and lead citrate.

Statistical data was processed using the nonparametric Mann – Whitney test in the Statistica 6.0 software program. The results of the study were presented as $M \pm m$, where M is the mean, and m is the standard error of the mean. The differences were considered significant at $p < 0.05$. Based on the results obtained in the experiment, using the methods implemented in the mathCAD software environment (interpolation, regression, approximation), a mathematical model was built that makes it possible to estimate changes in the light-induced damage to radial glial cells in the retina.

RESULTS

In the astrocytes accompanying blood vessels, the structure of the glial and fibrillary apparatus is preserved in the early stages (day 1–2) after light exposure (200, 3,500 lux), but the swelling of cisterns in cytoplasmic reticulum and mitochondria is observed. With prolonged light exposure (7–30 days, 200, 3,500 lux), most retinal astrocytes do not differ in the structure from the control ones, but the nuclei in some of them are characterized by pyknotic changes. Oligodendrocytes are located between the nerve fibers of the optic nerve, forming myelin sheaths to the axial cylinders. After exposure to low-intensity light (200, 1–30 days), focal demyelination followed by myelin phagocytosis by glial elements is observed. After

1–2 days of exposure to high-intensity light (3,500 lux), some of the organelles swell in oligodendrocytes. With prolonged (7–30 days) light exposure (200, 3,500 lux), the myelin sheath stratifies in some areas or around the entire perimeter of the nerve fiber. The processes of oligodendrocytes lying near the foci of demyelination are characterized by a decrease in the electron density and an increase in the number of fragments of phagocytized membrane myelin sheaths of various sizes and shapes.

After 30 days of light exposure (200, 3,500 lux), in the inner nuclear layer, we observed cells with an irregular (scalloped) shape of the nucleus, with high electron density of the karyoplasm and uniform distribution of chromatin. Their cytoplasm contained numerous vacuoles and lysosomes of different sizes. Based on the structure, we attributed these cells to microglia. It should be noted that their number was greater under high-intensity light exposure.

The nucleated parts of radial glial cells are located in the inner nuclear layer, and their processes penetrate the layers of the retina in different directions and form the outer and inner boundary membranes. After 1–2 days of low-intensity light exposure, numerous vacuoles and swollen mitochondria are observed in the cytoplasm of the apical regions of radial glial cells. The processes of glial cells grow through the outer boundary membrane into the subretinal space; these glial processes contain parts of the outer and inner segments of the sensorineural cells (Fig. 1). The bodies of radial glial cells containing nuclei with condensed chromatin and numerous free ribosomes in the cytoplasm are between the neurons of the inner nuclear layer, at the border with the plexiform layers (Fig. 2).

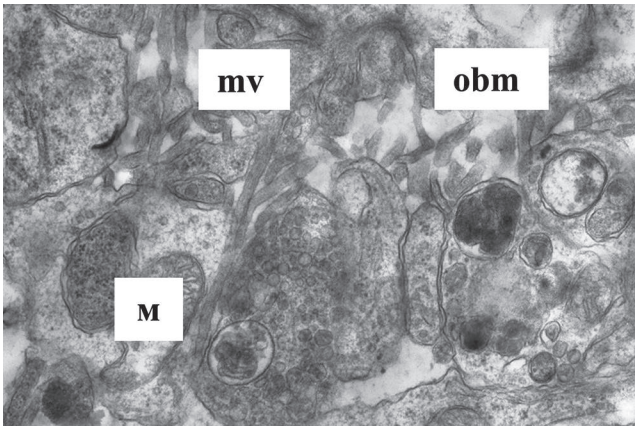

Fig. 1. Ingrowth of glial microvilli in the subretinal space and destruction of mitochondria in the inner segments of retinal sensorineural cells after light irradiation (200 lux, day 1). ×14,000. OBM – outer boundary membrane, MV – microvilli, M – mitochondria

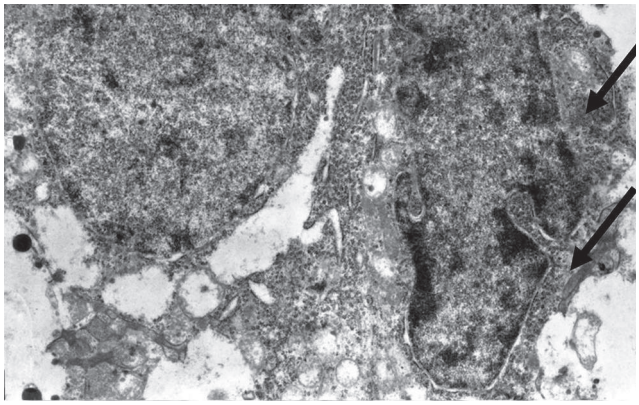

Fig. 2. An increase in the degree of chromatin condensation in the nucleus and an increase in the number of free ribosomes in the cytoplasm of a radial glial cell (arrows) after day 1 of exposure to low-intensity (200 lux, 1 day) light. ×5,800

After 1–2 days of exposure to high-intensity light, the number of radial glial cells with degenerative changes increases (Fig. 3). These cells show a decrease in the electron density and vacuolization of the scleral process cytoplasm and pyknosis of the nucleus. It is worth noting that these cells often lie on the border of the inner nuclear and plexiform layers. The structure of vitreal processes is without any changes.

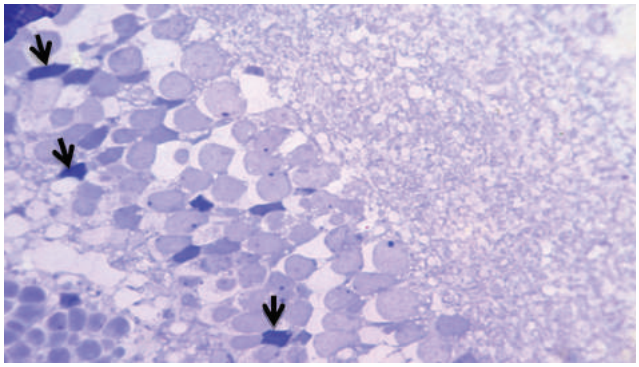

Fig. 3. Pyknosis of radial glial cells (arrows) after light exposure (3,500 lux, 2 days). ×900

After 7–14 days of low-intensity light exposure, sensorineural cells with degenerative changes appear, with fragmentation of most external and internal segments and pyknosis of the nucleus. The apical processes of glial cells surrounding the altered cells are dramatically hypertrophied and contain an increased number of membrane complexes and myelin-like bodies, as well as swollen mitochondria lacking cristae and containing fine granular material.

After 7 days of exposure to high-intensity light, multilayer glial plates appear in place of dead

sensorineural cells, surrounding their preserved nuclei and being in contact with bipolar neurons (Fig. 4). There are glial cells with hypertrophy of the vitreal processes containing swollen mitochondria, expanded cisterns of the endoplasmic reticulum, and numerous osmiophilic granules. After 14 days of exposure to high-intensity light, in the foci of massive death of sensorineural cells in the cytoplasm of the scleral processes in most glial cells, we observed a decrease in electron density, membrane complexes, and an increase in the number of vacuoles with osmiophilic material. Most of the nucleated parts of glial cells in the inner nuclear layer have normal structure.

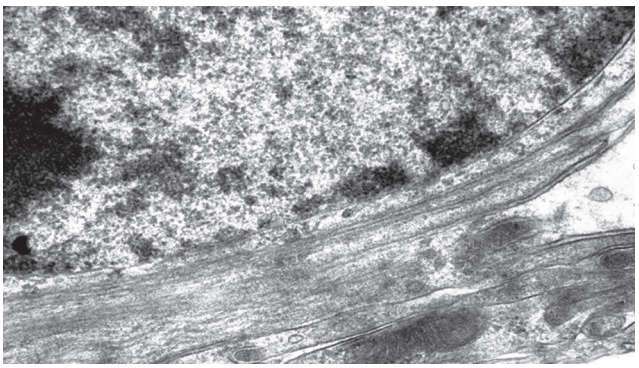

Fig. 4. Multilayer glial plates surrounding the nucleus of a sensorineural cell after 7 days of exposure to light (3,500 lux). ×15,000

After 30 days of light exposure (200 lux), proliferation of the radial glial scleral processes and vacuolization of glioplasm are observed in the outer nuclear layer, with phagocytosis of sensorineural cell nuclei with degenerative changes. After the same duration of high-intensity light exposure, severely hypertrophic processes of radial glial cells containing numerous large vacuoles and membrane complexes are observed in place of the practically absent outer nuclear layer.

In medicine, when the number of parameters of the studied trait is small, mathematical models are created for more accurate identification of various algorithms through a system of equations describing the properties of the modeling object under various experimental conditions. In our study, it was necessary to estimate the number of pycnomorphic radial glial cells in the retina taking into account the changes after exposure to light (200, 3,500 lux). Experimental data were taken to solve the problem. As a result of the data analysis, it was found that the number of pycnomorphic radial glial cells in the retina after photodamage is described by the following equation (Fig. 5, Table 1):

$$f(t) = a_0 t^{\frac{100}{J}} + a_1 t^{\frac{200}{J}} + a_2 t^{\frac{300}{J}} + a_3 e^{-t}$$

Fig. 5. The equation describing the number of pycnomorphic radial glial cells of the retina after photodamage: $f(t)$ – number of glial cells with pyknosis; J – surface illumination; a_i – constants obtained in the analysis of experimental data (Table 1); t – experiment duration

Table 1

Constants obtained in the mathematical model			
α_0	α_1	α2	α_3
Light 200 lux			
8.42	2.235	0.146	2.959
Light 3,500 lux			
2.087	0.014	0.034	3.411

Analysis of the data obtained during the modeling shows that under low-intensity (200 lux) light exposure, changes in radial glial cells were characterized by an increase in the number of pycnomorphic glial cells in the early stages of the experiment and a decrease in their number when the duration of light exposure increased. Exposure to high-intensity (3500 lux) light caused similar structural changes in radial glial cells, which were characterized by an increase in the number of dead glial cells when the duration of exposure increased. It should be noted that the obtained mathematical models confirm the experimental data and successfully describe smooth changes in the process.

The analysis of the change pattern regarding the content of pycnomorphic radial glial cells suggests that on day 7 of exposure to low-intensity light (200 lux), this parameter was 1.6 times higher than the control values (3.26 ± 0.23%) (Table 2).

Table 2

The number (%) of radial glial cells in the retina with symptoms of karyopyknosis after exposure to light of varying intensity, %	
Control	3.26 ± 0.23
day 1	
200 lux	3.72 ± 0.54
3,500 lux	2.91 ± 0.72
day 7	
200 lux	5.21 ± 1.08*
3,500 lux	8.15 ± 1.82*
day 14	
200 lux	6.73 ± 1.69*
3,500 lux	6.92 ± 1.28*
day 30	
200 lux	3.41 ± 1.32
3,500 lux	6.03 ± 1.27*

* significant differences compared to the control group, $p < 0.05$

An increase in the exposure period (14, 30 days) led to a decrease in the studied parameter, and on day 30 of the experiment, it did not significantly differ from the control values. After 7 days of exposure to high-intensity light, the content of pycnomorphic radial glial cells was 2.5 times higher than the control values and did not significantly change until day 30 of light exposure. An increase in the studied parameter points out a disruption of the adaptive and compensatory mechanisms and an increase in the destruction processes in the inner layers of the retina.

DISCUSSION

Thus, glial responses in the retina after photodamage depend on the duration and intensity of irradiation and contribute significantly to the damage. The earliest changes are observed in the subretinal space, manifested by proliferation of radial glial cell scleral processes and phagocytosis of fragments of sensorineural cells with degenerative changes. With the destruction of layers formed by sensorineural cells, we observed impaired glutamate metabolism, which regulates synaptic activity in retinal glial cells [23–25].

The intensification of damage in the studied cells leads to disruption of the glioneuronal interactions in the retina and a decrease in reparative processes in retinal neurons [26–29]. In the outer nuclear layer, foci of glial proliferation are detected and multilayer glial membranes surrounding the nuclei of sensorineural cells appear. We did not observe significant ultrastructural changes in the vitreal processes of radial glial cells.

CONCLUSION

The responses of retinal glial cells after photodamage depend on the intensity and duration of irradiation. As the irradiation period increases, degenerative changes in glial cells increase and are more pronounced after exposure to high-intensity (3500 lux) light. The developed mathematical model makes it possible to analyze the dynamics of destructive changes in retinal glial cells and to predict the results of photodamage depending on the duration and intensity of irradiation.

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Authors' contribution

Potapov A.V., Logvinov S.V., Varakuta E.Yu. – conception and design. Zhdankina A.A., Gerasimov A.V., Gereng E.A. – collection and processing of the material. Svetlik M.V., Petrov I.A. – selection of statistical analysis methods and interpretation of the data. Potapov A.V. – drafting of the manuscript. Potapov A.V., Logvinov S.V., Solonsky A.V. – editing of the manuscript.

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Studying the role of *GCLC* gene polymorphisms in predicting the clinical course of acute alcoholic pancreatitis

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ABSTRACT

The aim of the study was to evaluate the role of polymorphic loci rs12524494, rs17883901, rs606548, rs636933, rs648595, and rs761142 in the *GCLC* gene in predicting the clinical course of acute alcoholic pancreatitis (AAP).

Materials and methods. The material of the study was blood DNA samples obtained from 547 patients with AAP and 573 healthy individuals. The average age of patients was 48.9 ± 13.1 years, the average age of healthy individuals was 47.8 ± 12.1 years. Genotyping was performed using the MassARRAY 4 Analyzer. Plasma levels of total glutathione were determined using the OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit STA-312. The level of reactive oxygen species (ROS) was determined using the OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence) STA-347 (Cell Biolabs Inc., USA). The kinetic colorimetric assay was used to determine the level of amylase in the blood serum. Statistical data processing was performed using the Statistica 10.0 and SNPStats software.

Results. It was found that the polymorphic loci rs606548 (genotype C/C, odds ratio (OR) = 3.34, 95% confidence interval (CI) 1.29–8.66, $p = 0.007$), rs648595 (genotype G/T, OR = 1.56, 95% CI 1.04–2.36, $p = 0.029$), and rs12524494 (genotype A/G, $p = 0.021$) in the *GCLC* gene were predictors of an increased risk of necrotizing pancreatitis. For the genotype T/T of rs648595 (recessive model) in the *GCLC* gene, the lowest values of oxidized glutathione were found, whereas rs17883901 – G/A in the *GCLC* gene was associated with the highest ROS values in the blood. The rs761142 A/A genotype in the *GCLC* gene (OR = 1.70, 95% CI 1.12–2.59; $p = 0.010$) showed predisposition to acute peripancreatic fluid collection, and the rs648595 G allele (OR = 1.47, 95% CI 1.01–2.13; $p = 0.042$) in the *GCLC* gene exhibited predisposition to the formation of acute pancreatic pseudocysts. Predisposition to massive bleeding was associated with rs17883901 (G/A genotype, OR = 6.20, 95%CI 1.3–28.81; $p = 0.031$) in the *GCLC* gene.

Conclusion. The established genotype – phenotype associations will make it possible to predict the clinical course of AAP in a particular patient, taking into account their genetic makeup, as well as to determine the treatment strategy in a timely manner.

Keywords: acute pancreatitis, predicting, rs12524494, rs17883901, rs606548, rs636933, rs648595, rs761142, *GCLC* gene

Conflict of interest. The author declares the absence of obvious and potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the regional Ethics Committee at Kursk State Medical University (Protocol No. 3 of 11.03.2013).

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Изучение роли полиморфизмов гена *GCLC* в прогнозировании клинического течения острого алкогольно-алиментарного панкреатита

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РЕЗЮМЕ

Цель исследования: оценить роль полиморфных локусов rs12524494, rs17883901, rs606548, rs636933, rs648595, rs761142 гена *GCLC* в прогнозировании клинического течения острого алкогольно-алиментарного панкреатита (ОААП).

Материалы и методы. Материалом исследования послужили образцы ДНК крови, полученные от 547 пациентов с ОААП и 573 здоровых индивидов. Средний возраст больных составил 48,9 ± 13,1 года, здоровых лиц – 47,8 ± 12,1 года. Генотипирование проводилось на анализаторе MALDI-TOF MassARRAY-4. Определение в плазме крови уровня общего глутатиона проводилось с помощью набора Oxi Select™ Total Glutathione (GSSG/GSH) Assay Kit STA-312, уровня активных форм кислорода – с помощью набора OxiSelect™ InVitro ROS/RNS AssayKit (Green Fluorescence) STA-347 (Cell Biolabs Inc., США). Для определения уровня амилазы сыворотки крови применяли кинетический колориметрический метод. Статистическая обработка данных проводилась с использованием программы Statistica 10.0 и SNPStats.

Результаты. Установлено, что полиморфные варианты rs606548 (генотип C/C; отношение шансов (OR) 3,34; 95-й доверительный интервал (95% CI) 1,29–8,66; $p = 0,007$), rs648595 (генотип G/T; OR = 1,56; 95% CI 1,04–2,36; $p = 0,029$) и rs12524494 (генотип A/G; $p = 0,021$) гена *GCLC* являются предикторами повышенного риска развития панкреонекроза. Для генотипа T/T rs648595 гена *GCLC* (рецессивная модель) установлены наиболее низкие значения окисленного глутатиона, а генотип G/A rs17883901 *GCLC* ассоциировался с наиболее высокими значениями активных форм кислорода в крови. Предрасположенность к формированию перипанкреатического инфильтрата показал генотип A/A rs761142 *GCLC* (OR = 1,70; 95% CI 1,12–2,59; $p = 0,010$), а псевдокисты – аллель G rs648595 (OR = 1,47; 95% CI 1,01–2,13; $p = 0,042$) гена *GCLC*. Предрасположенность к летальному исходу вследствие развития аррозивного кровотечения ассоциирована с rs17883901 (генотип G/A; OR = 6,20; 95% CI 1,3–28,81; $p = 0,031$) гена *GCLC*.

Заключение. Установленные нами генно-фенотипические ассоциации позволят прогнозировать клиническое течение ОААП у конкретного больного с учетом его генетического статуса и своевременно определять лечебную тактику.

Ключевые слова: острый алкогольно-алиментарный панкреатит, прогнозирование, rs12524494, rs17883901, rs606548, rs636933, rs648595, rs761142, ген *GCLC*

Конфликт интересов. Автор декларирует отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Автор заявляет об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все лица, участвующие в исследовании, подписали добровольное информированное согласие. Исследование одобрено региональным этическим комитетом при Курском государственном медицинском университете (протокол № 3 от 11.03.2013).

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INTRODUCTION

The crucial role of oxidative stress in the pathogenesis of acute alcoholic pancreatitis (AAP) is beyond doubt [1, 2].

Glutathione is a powerful antioxidant for cell detoxification and cellular regeneration [3], which is synthesized via two ATP-dependent stages from the amino acids L-cysteine, L-glutamic acid, and glycine. At the first stage, γ-glutamylcysteine is synthesized by the enzyme γ-glutamylcysteine synthetase (or glutamate – cysteine ligase). At the second stage, the enzyme glutathione synthetase adds a glycine residue to the C-terminal group of γ-glutamylcysteine

in lymphocytes [4]. Glutathione depletion seen in acute pancreatitis is cause by deficient glutathione synthesis [4]. The study of genes encoding enzymes of glutathione metabolism is reasonable and relevant.

Glutamate – cysteine ligase (GCL) is the main glutathione-metabolizing enzyme. The GCL heterodimer consists of two subunits: catalytic (GCLC), which provides the catalytic activity of the enzyme, and modifier (GCLM), which increases the catalytic efficiency [3, 4].

Establishing the role of individual polymorphic loci in genes encoding enzymes of glutathione metabolism in the development and outcome of AAP will make it possible to predict the clinical course of the disease and determine the patient management strategy using their genetic status.

The aim of the study was to evaluate the role of polymorphic loci rs12524494, rs17883901, rs606548, rs636933, rs648595, and rs761142 in the *GCLC* gene in predicting the clinical course of AAP.

MATERIALS AND METHODS

We examined and treated 547 Russian individuals (ethnic self-identification) with AAP (154 women and 393 men), who received treatment at surgery units of Kursk hospitals – clinical sites of the Department of Surgical Diseases No. 2 in 2015–2021.

The material for the study was blood DNA samples obtained from 547 patients with AAP and 573 healthy individuals (161 women and 412 men). The average age of patients was 48.9 ± 13.1 years, the average age of healthy individuals was 47.8 ± 12.1 years. The diagnosis and severity of AAP were verified according to clinical guidelines elaborated by a working group of the Russian Society of Surgeons.

The study was performed in compliance with the ethical principles set out in the WMA Declaration of Helsinki "Ethical principles for medical research involving human subjects" (2000 revision) and Rules of Good Clinical Practice in the Russian Federation approved by the Order of the Ministry of Health of Russia No. 266 of 19.06.2003. All patients signed an informed consent to participate in the study.

The level of total glutathione was determined by the colorimetric method using the OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit (Cell Biolabs, USA). The level of reactive oxygen species (ROS) was quantified by the OxiSelect™ In Vitro ROS / RNS Assay Kit (Cell Biolabs, USA). The level of amylase in the blood serum was determined by the kinetic colorimetric assay.

Genomic DNA was extracted by the phenol – chloroform method. Genotyping of the polymorphisms was performed by the MALDI-TOF iPLEX technology using the MassArray System (Agena Bioscience Inc, USA).

To compare categorical variables between the groups, the chi-square test was used. To compare quantitative variables, the Student's *t*-test (for normally distributed variables) and the Mann – Whitney test were used. A pre-test for normal distribution was performed using the Kolmogorov – Smirnov test. The data were presented as absolute and relative values *n* (%) and as the median and the interquartile range *Me* (Q_1– Q_3).

The association of alleles and genotypes with the risk of AAP was evaluated by the value of odds ratio (OR), showing by how many times the odds of getting in the case group (disease) with exposure differs from the odds of getting in the control group (healthy individuals) without exposure. OR and 95% confidence interval (CI) were calculated by the multiple logistic regression analysis with adjustments for age and sex using the SNPStats software (http://bioinfo.iconcologia.net/snpstats/start.htm). The logistic regression analysis was also used to assess associations of DNA markers with clinical characteristics (clinical forms of the disease, symptoms, disease severity, treatment efficacy). Correction for multiplicity was carried out by permutation testing (P_{perm}) using PLINK.

RESULTS

Table 1 presents the characteristics of clinical forms and complications of AAP in the group of patients.

Table 1

Characteristics of clinical forms and complications of AAP in the main group

Parameter	Number of patients, *n* (%)
Clinical forms of AAP	
Interstitial edematous pancreatitis	281 (51.3)
Sterile pancreatic necrosis	143 (26.1)
Infectious pancreatic necrosis	123 (22.4)
Total	547 (100)
Complicated AAP	
Peritonitis	120 (21.9)
Acute peripancreatic fluid collection	154 (28.4)
Pancreatic pseudocyst	101 (18.5)
Pancreatic abscess and purulent necrotizing pancreatitis	111 (20.3)

Fifteen patients died, the causes of death were multiple organ failure, purulent septic complications (8), and life-threatening bleeding (7). The analysis of associations of the single-nucleotide polymorphisms

(SNPs) in the *GCLC* gene with the risk of AAP was carried out (Table 2).

The identification of genetic markers of an increased risk of pancreatic necrosis in patients with AAP at the onset of the disease seemed to be the most important. We analyzed the associations of the SNPs with the risk of pancreatic necrosis using data from patients with AAP only. As a result, it was found that polymorphic variants rs606548 (genotype C/C, OR = 3.34 95% CI 1.29–8.66, p = 0.007), rs648595 (genotype G/T, OR = 1.56, 95 %CI 1.04–2.36; p = 0.029), and rs12524494 (genotype A/G, p = 0.021) in the *GCLC* gene are predictors of an increased risk of pancreatic necrosis. Moreover, all the identified associations of DNA markers did not depend on the sex and age of the patients.

Table 2

Analysis of associations of the studied polymorphic variants in the *GCLC* gene with the risk of developing acute alcoholic pancreatitis

SNP ID	Genotype, allele	Number of individuals, *n* (%)		P_{perm}	p^1	$_{cor}$OR (95% CI)
		Healthy inividuals, *n* = 573	Patients with AAP, *n* = 547			
GCLC A>G (rs12524494)	A/A	397 (97.3)	420 (95)	0.01^R	0.014	1.00
	A/G	7 (1.7)	21 (4.8)			2.84 (1.19–6.74)
	G/G	4 (1)	1 (0.2)			0.24 (0.03–2.12)
	G	0.02	0.03		0.292	1.43 (0.74–2.75)
GCLC G>A (rs17883901)	G/G	472 (86.9)	469 (88)	0.5^D	0.422	1.00
	G/A	68 (12.5)	58 (10.9)			0.86 (0.59–1.25)
	A/A	3 (0.6)	6 (1.1)			2.01 (0.50–8.09)
	A	0.066	0.068		0.810	0.96 (0.69–1.35)
GCLC C>T (rs606548)	C/C	471 (95.7)	438 (93.4)	0.05^{AD}	0.160	1.00
	C/T	21 (4.3)	30 (6.4)			1.54 (0.87–2.72)
	T/T	0 (0)	1 (0.2)			–
	T	0.02	0.03		0.089	1.62 (0.93–2.83)
GCLC G>A (rs636933)	G/G	290 (60.8)	288 (61.8)	0.41^R	0.463	1.00
	G/A	172 (36.1)	157 (33.7)			0.92 (0.70–1.21)
	A/A	15 (3.1)	21 (4.5)			1.41 (0.71–2.79)
	A	0.21	0.21		0.912	1.01 (0.81–1.26)
GCLC G>T (rs648595)	T/T	153 (30.5)	126 (26.6)	0.17^D	0.342	1.00
	G/T	255 (50.9)	261 (55.1)			1.24 (0.93–1.66)
	G/G	93 (18.6)	87 (18.4)			1.14 (0.78–1.65)
	T	0.56	0.54		0.431	0.93 (0.78–1.11)
GCLC C>A (rs761142)	A/A	271 (53.8)	266 (54.9)	0.72^R	0.741	1.00
	C/A	212 (42.1)	195 (40.2)			0.94 (0.72-1.21)
	C/C	21 (4.2)	24 (5)			1.16 (0.63-2.14)
	A	0.75	0.75		0.913	1.01 (0.82-1.23)

[1] the level of significance of the association (codominant model) with the risk of developing AAP with adjustments for sex and age

Table 3

Established relationships of the studied polymorphic gene variants with quantitative parameters of the blood in patients with AAP

SNP ID	Genotypes	Oxidized glutathione, μmol / l			Reactive oxygen species, nM			Leukocytes, $\times 10^{-9}$			Amylase, U / l		
		Me	*Q1–Q3*	*p*	*Me*	*Q1–Q3*	*p*	*Me*	*Q1–Q3*	*p*	*Me*	*Q1–Q3*	*p*
1	2	5	6	7	8	9	10	11	12	13	14	15	16
GCLC (rs648595)	G/G	10.51	3.83–12.41	0.041^R	3.59	2.26–4.22	0.471	7.95	6.60–12.10	0.921	164.0	92.0–244.0	0.812
	G/T	4.59	3.16–8.51		2.32	1.60–3.39		8.20	6.60–12.90		168.0	87.0–300.0	
	T/T	4.61	2.03–8.08		2.73	2.18–3.65		7.90	6.60–12.60		148.0	84.0–280.0	
GCLC (rs17883901)	G/G	5.93	3.11–10.41	0.752	2.52	1.98–3.69	0.040^D	8.80	6.70–12.80	0.823	164.0	85.0–272.0	0.840
	G/A	5.74	2.89–8.50		5.00	1.19–5.25		7.80	6.40–12.35		96.0	68.0–195.0	
	A/A	3.29	3.29–3.29		2.18	2.18–2.18		8.30	6.70–9.90		–	–	

Note: R – recessive model, D – dominant model

However, we did not find an association of the SNPs in the *GCLC* gene with the severity of AAP (mild, moderate, and severe). We also studied the relationship of polymorphic loci in the *GCLC* gene with intermediate phenotypes of AAP in the main group: the level of oxidized glutathione (GSSG), the level of ROS, the level of amylase, and leukocyte count in the blood serum.

The influence of polymorphic variants of genes encoding the catalytic and modifier subunits of GCL on the level of oxidized glutathione in the blood serum was established. The lowest GSSG values were found for rs648595 T/T genotype in the *GCLC* gene (recessive model). The rs17883901 G/A genotypes in *GCLC* were associated with the highest levels of ROS in the blood serum. No significant effects of genotypes were established for other polymorphic loci.

We analyzed the associations of the SNPs in the *GCLC* gene with the risk of AAP complications: rs761142 A/A genotype in the *GCLC* gene (OR = 1.70, 95%CI 1.12–2.59; $p = 0.010$) showed predisposition to acute peripancreatic fluid collection, and G allele of rs648595 (OR = 1.47, 95%CI 1.01–2.13; $p = 0.042$) in the *GCLC* gene – to the formation of a pancreatic pseudocyst.

Predisposition to life-threatening bleeding and death was associated with rs17883901 (G/A genotype, OR = 6.20, 95%CI 1.3–28.81; $p = 0.031$) in the *GCLC* gene.

DISCUSSION

The depletion of reduced glutathione in the pancreas, observed in acute pancreatitis, is caused by its cleavage [5], which indicates insufficiently effective activation of the GSH synthesis in cells and contributes to destructive pancreatitis. However, within 12–24 hours, GSH is redistributed towards the inflammatory focus due to endogenous reserves [6], since beta cells demonstrate low expression of antioxidant enzymes [7].

J. Pereda et al. found that the GSH level remained low in pancreatic necrosis for several hours without an increase in the level of the enzyme or GCL subunit mRNA, despite the binding of RNA polymerase II to their promoters and coding regions. On the contrary, in edematous pancreatitis, the GSH level quickly recovered, and the expression of the protein increased significantly due to increased transcription mediated by the effect of c-MYC, NF-kB and SP-1 on promoters. At the same time, an increase in the activity of cytosolic ribonuclease was not found in this case [8].

As a result of the study, we found that the polymorphic variants rs606548 (genotype C/C, OR = 3.34 95%CI 1.29–8.66, $p = 0.007$), rs648595 (genotype G/T, OR = 1.56, 95%CI 1.04–2.36; $p = 0.029$), and rs12524494 (genotype A/G, $p = 0.021$) in the *GCLC* gene are predictors of an increased risk of pancreatic necrosis. For the rs648595 T/T genotype in the *GCLC* gene (recessive model), the lowest values of oxidized glutathione were found, and the rs17883901 G/A *GCLC* genotype was associated with the highest values of ROS in the blood serum. The rs761142 A/A *GCLC* genotype (OR = 1.70, 95%CI 1.12–2.59; $p = 0.010$) showed predisposition to acute peripancreatic fluid collection, and G allele of rs648595 (OR = 1.47, 95%CI 1.01–2.13; $p = 0.042$) in the *GCLC* gene – to the formation of a pancreatic pseudocyst. Predisposition to fatal bleeding was associated with rs17883901 (G/A genotype, OR = 6.20, 95%CI 1.3–28.81; $p = 0.031$) in the *GCLC* gene.

The effect of the polymorphic variants in the *GCLC* gene in AAP has not been studied before. However, the study on type 2 diabetes mellitus revealed that SNPs rs17883901, rs636933, and rs648595 in the *GCLC* gene have a protective effect on disease progression, and their effects are mediated by an increased level of GSH [9].

To analyze the effect of the SNPs on gene expression in the pancreas, liver, and blood, we used the bioinformatic resources of the GTEx database. The polymorphic variants rs636933, rs648595, and rs761142 were associated with increased expression of the *GCLC* gene in the pancreas ($p \leq 0.0002$) and liver, with the exception of rs636933 ($p \leq 0.02$).

CONCLUSION

The established genotype – phenotype associations will make it possible to predict the clinical course of AAP in a particular patient, using their genetic status, and to determine the treatment strategy in a timely manner.

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ORIGINAL ARTICLES

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Notch signaling pathway in the development of imbalanced immune responses in patients with disseminated pulmonary tuberculosis

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ABSTRACT

Aim. To determine the role of the Notch signaling pathway in the regulation of Th1 / Th2 lymphocyte balance in patients with disseminated drug-sensitive (DS) and drug-resistant (DR) pulmonary tuberculosis (PT).

Materials and methods. Mononuclear leukocytes were isolated from the venous blood of 13 patients with disseminated PT by density gradient centrifugation. The cells were cultured for 72 h in the complete cell culture medium at 5% CO_2 and 37 °C. Preliminarily, CFP10 and ESAT6 mycobacterial antigens or γ-secretase inhibitor DAPT (5 μM / l; 10 μM / l) together with CFP10 and ESAT6 antigens were added to the culture medium. Immunophenotyping of Th1 and Th2 lymphocytes was performed by multicolor flow cytometry by determining the expression of CD4 receptor and intracellular transcription factors T-bet and GATA-3.

Results. In patients with disseminated DS and DR PT, an increase in the number of Th1 and Th2 lymphocytes was found in intact cultures. Stimulation of cells with mycobacterial antigens CFP10 and ESAT6 resulted in an increase in the number of $CD4^+T\text{-}bet^+$ and $CD4^+GATA\text{-}3^+$ cells in all comparison groups. Addition of CFP10 and ESAT6 antigens and DAPT (10 μM / l) to the incubation medium was accompanied by a decrease in the number of Th2 lymphocytes in PT patients in both groups. A rise in the number of Th1 cells was registered only in patients with DS PT. Suppression of the Notch signaling pathway with the γ-secretase inhibitor DAPT (10 μM / l) resulted in an increase in the Th1 / Th2 lymphocyte balance in both DS and DR variants of the disease.

Conclusion. The Notch signaling pathway has a modulating effect on the differentiation of the key lymphocyte populations that determine the balance between cell-mediated and humoral immune responses to PT. Suppression of the Notch signaling cascade by the γ-secretase inhibitor DAPT (10 μM / l) *in vitro* promotes an increase in the Th1 / Th2 ratio in patients with disseminated DS and DR PT. The positive regulatory effect on the Th1 / Th2 lymphocyte balance allows to consider the Notch signaling pathway as a promising potential target in the development of new approaches to the pathogen-specific therapy for PT.

Keywords: Notch signaling pathway, pulmonary tuberculosis, lymphocytes, γ-secretase, helper T cells

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No 7924 of 14.10.2019).

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Сигнальный путь Notch в развитии дисбаланса иммунных реакций у больных диссеминированным туберкулезом легких

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РЕЗЮМЕ

Цель исследования. Определить роль сигнального пути Notch в регуляции баланса Th1/Th2-лимфоцитов у больных диссеминированным лекарственно-чувствительным (ЛЧ) и лекарственно-устойчивым (ЛУ) туберкулезом легких (ТЛ).

Материалы и методы. Из венозной крови 13 пациентов с диссеминированным ТЛ мононуклеарные лейкоциты выделяли методом градиентного центрифугирования. Клетки культивировали в течение 72 ч в полной питательной среде при 5%-м CO_2 и температуре 37 °С, предварительно добавляя в инкубационную среду антигены микобактерий туберкулеза CFP10-ESAT6 или ингибитор γ-секретазы DAPT (5 мкМ/л; 10 мкМ/л) вместе с антигенами CFP10-ESAT6. Иммунофенотипирование Th1- и Th2-лимфоцитов проводили методом проточной лазерной многоцветной цитофлуориметрии посредством определения экспрессии рецептора CD4 и внутриклеточных транскрипционных факторов T-bet и GATA-3.

Результаты. У больных диссеминированным ЛЧ и ЛУ ТЛ установлено увеличение количества Th1- и Th2-лимфоцитов в интактных культурах. Стимуляции клеток антигенами микобактерий CFP10-ESAT6 способствовала повышению числа $CD4^+T\text{-}bet^+$ и $CD4^+GATA\text{-}3^+$ клеток во всех группах сравнения. Добавление в инкубационную среду антигенов CFP10-ESAT6 и DAPT (10 мкМ/л) сопровождалось уменьшением количества Th2-лимфоцитов у больных ТЛ обеих групп. Повышение числа Th1-клеток регистрировалось только у пациентов с ЛЧ ТЛ. Подавление сигнального пути Notch с помощью ингибитора γ-секретазы – DAPT (10 мкМ/л) приводило к повышению коэффициента соотношения Th1/Th2-лимфоцитов как при ЛЧ, так и при ЛУ вариантах заболевания.

Заключение. Сигнальный путь Notch оказывает модулирующее действие на дифференцировку ключевых популяций лимфоцитов, определяющих динамический баланс клеточно-опосредованных и гуморальных реакций противотуберкулезного иммунитета. Угнетение молекулярного каскада Notch ингибитором γ-секретазы DAPT (10 мкМ/л) в условиях *in vitro* способствует увеличению коэффициента соотношения Th1/Th2 у больных диссеминированным ЛЧ и ЛУ ТЛ. Положительное регулирующее действие на баланс Th1/Th2-клеток позволяет рассматривать сигнальный путь Notch в качестве перспективной потенциальной мишени в разработке новых подходов к патогенетической терапии туберкулеза.

Ключевые слова: Notch-сигнальный путь, туберкулез легких, лимфоциты, γ-секретаза, Т-хелперы

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

Источник финансирования. Работа выполнена в рамках плановой темы НИР Сибирского государственного медицинского университета по программе САЕ «Молекулярная медицина» (Молекулярно-клеточные основы воспаления при социально значимой патологии).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом СибГМУ (протокол № 7924 от 14.10.2019).

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INTRODUCTION

A progressive course of tuberculosis is based on hyperergic inflammation involving a wide range of immunocompetent cells [1; 2]. Th2 lymphocytes, which regulate the development of antibody-mediated immune responses, together with Th1 cells participate in the formation of protective responses [3; 4] and, conversely, can contribute to immune-mediated tissue damage and persistence of mycobacteria [5; 6]. It has been established that a more severe course of pulmonary tuberculosis (PT) is associated with an increase in the IL-4 mRNA concentration in the blood [6; 7], high titers of antigen-specific IgG, and increased expression of suppressor of cytokine signaling SOCS3 mRNA [7].

Multiple interactions between cells of the immune system are determined not only by cytokines and chemokines (IFNγ, TNFα, IL-4, IL-12, IL-27, etc.), but also by receptor – ligand interactions [8; 9]. Notch ligands and receptors associated with the intracellular signaling cascade regulating cell differentiation occupy a prominent place among receptor cooperation mechanisms [10]. Dysfunction of molecular mechanisms in the Notch signaling pathway at any stage of implementation (expression of receptors, ligands, enzyme activity, etc.) can contribute to disruption of an effective immune response against Mycobacterium tuberculosis and progression of the disease. Thus, in experiments on murine macrophages, it was established that a BCG-induced increase in the expression of Notch1 receptor protein and subsequent activation of its signaling pathway led to a rise in the expression of SOCS3 protein that provides negative regulation of cytokine signaling [11]. The demonstrated increase in leukocyte expression of molecules initiating the Notch signaling cascade (Notch1/2 and DLL4 mRNA) in patients with PT without changes in the expression of their target genes (Hes1, Hey1) [12] leaves the question about the role of the Notch signaling pathway in the immunopathogenesis of PT open.

One of the molecular approaches to evaluate the role of the Notch signaling cascade in the pathogenesis of various immune-mediated diseases is inhibition of the activity of γ-secretase, a key proteolytic enzyme that initiates the release of the Notch intracellular domain [13]. The properties of the γ-secretase inhibitor DAPT (N-[N-(3,5-difluorophenacetyl)-1-alanyl]-s-phenylglycine t-butyl ester) are being actively studied as a potential drug for treatment of cancer, as well as neurological, cardiovascular, and cerebrovascular diseases [14].

The aim of the study was to determine the role of the Notch signaling pathway in the regulation of Th1 / Th2 lymphocyte balance in patients with disseminated drug-sensitive (DS) and drug-resistant (DR) PT.

MATERIALS AND METHODS

The study involved 13 patients with newly diagnosed disseminated PT (mean age 47.3 ± 5.21 years), who were hospitalized in Tomsk Phthisiopulmonology Medical Center.

The patients were divided into two groups depending on the sensitivity of Mycobacterium tuberculosis to anti-TB drugs: group 1 encompassed 7 patients with bacteriologically confirmed Mycobacterium tuberculosis resistant to one or more anti-TB drugs (isoniazid and rifampicin); group 2 included 6 patients excreting drug-sensitive Mycobacterium tuberculosis. The control group consisted of 10 healthy volunteers with comparable age and gender characteristics.

The study material was whole venous blood taken before treatment with anti-TB drugs. Mononuclear leukocytes were isolated from the blood by density gradient centrifugation (ρ = 1.077 g / ml). Mycobacterium tuberculosis antigens (AG) CFP10 and ESAT6 (Diaskintest, Generium, Russia) at a dose of 10 μg / ml or the γ-secretase inhibitor (DAPT, Tocris Bioscience, UK) at a dose of 5 or 10 μM / l, pre-dissolved in 0.1% dimethyl sulfoxide (DMSO) solution (Sigma-Aldrich, USA), together with CFP10 and ESAT6 were added to the incubation medium. The indicated dose was determined by evaluating the cytotoxicity of the tuberculosis recombinant allergen containing CFP10/ESAT-6 protein by the MTT assay. DMSO and the γ-secretase inhibitor at the indicated concentrations did not cause cell death *in vitro*. Cells were cultured in the RPMI-1640 medium with L-glutamine (Biolot LLC, Russia). The cells were incubated in 5% CO_2 at 37 °C for 72 h. Immunophenotyping of Th1 and Th2 lymphocytes was performed by multicolor flow cytometry by determining the expression of surface CD4 receptor (FITC, BD Biosciences, USA) and intracellular transcription factors – T-bet (Alexa Fluor 405, R&D Systems Inc., USA) and GATA-3 (PerCP-eFluor 710, BD Biosciences, USA).

The statistical data were processed using IBM SPSS statistics 25 (Statistical Package for the Social Sciences, USA) and Microsoft Office 2013. The Shapiro – Wilk test was used to check the data for normality of distribution. The data were presented as the median (*Me*) and the 25th and 75th percentiles (Q_1 and Q_3), as quantitative variables in the study groups

did not follow normal distribution. The nonparametric Mann – Whitney *U* test was used to estimate the level of significance of differences in quantitative variables between the study samples. The Wilcoxon test was used to assess the significance of differences in dependent data within the group. The results of the statistical analysis were considered significant at $p < 0.05$.

RESULTS

The study showed that the development of disseminated PT was accompanied by an increase in the number of $CD4^+T\text{-}bet^+$ cells. Thus, in patients with DS PT, the number of Th1 lymphocytes exceeded similar parameters in healthy donors by 2 times ($p < 0.01$) and in patients with DR PT –by 1.7 times ($p < 0.01$). The Th2 lymphocyte count in intact cultures in PT patients with different types of mycobacterial resistance was 2.5 times higher ($p < 0.001$) than in healthy individuals (Table 1).

After stimulation of the cells with mycobacterial antigens CFP10 and ESAT6, an increase in the number of Th1 and Th2 cells was registered in all groups under study.

Table 1

Relative content of Th1 and Th2 lymphocytes in peripheral blood (% of the total lymphocyte count) in patients with disseminated pulmonary tuberculosis, *Me* (Q_1–Q_3)

Parameters	Healthy donors	Patients with pulmonary tuberculosis	
		Drug-sensitive	Drug-resistant
Th1 lymphocytes ($CD4^+T\text{-}bet^+$)			
Intact culture	1.25 (1.12–1.37)	2.51 (2.48–2.59) $p_1 < 0.001$	2.19 (2.17–2.22) $p_1 < 0.001$ $p_4 = 0.008$
With added AG (CFP10 and ESAT6)	1.30 (1.18–1.42) $p_2 = 0.012$	2.54 (2.51–2.66) $p_1 < 0.001$ $p_2 = 0.041$	2.34 (2.31–2.34) $p_1 < 0.001$ $p_2 = 0.043$ $p_4 = 0.008$
With added AG and DAPT (5 μM / l)	1.37 (1.21–1.44) $p_2 = 0.012$ $p_3 = 0.012$	2.56 (2.52–2.64) $p_1 < 0.001$	2.30 (2.27–2.31) $p_1 < 0.001$ $p_2 = 0.042$ $p_4 = 0.008$
With added AG and DAPT (10 μM / l)	1.95 (1.7–2.04) $p_2 = 0.012$ $p_3 = 0.012$	2.68 (2.63–2.73) $p_1 < 0.001$ $p_2 = 0.043$ $p_3 = 0.043$	2.34 (2.29–2.37) $p_1 < 0.001$ $p_4 = 0.008$
Th2 lymphocytes ($CD4^+GATA\text{-}3^+$)			
Intact culture	1.04 (0.99–1.01)	2.57 (2.49–2.63) $p_1 < 0.001$	2.67 (2.65–2.69) $p_1 < 0.001$
With added AG (CFP10 and ESAT6)	1.12 (1.08–1.14) $p_2 = 0.012$	2.73 (2.64–2.73) $p_1 < 0.001$ $p_2 = 0.043$	2.70 (2.68–2.71) $p_1 < 0.001$ $p_2 = 0.043$
With added AG and DAPT (5 μM / l)	0.91 (0.82–0.98) $p_3 = 0.012$	2.65 (2.61–2.65) $p_1 < 0.001$	2.68 (2.62–2.69) $p_1 < 0.001$
With added AG and DAPT (10 μM / l)	0.68 (0.63–0.72) $p_2 = 0.012$ $p_3 = 0.012$	2.19 (2.18–2.21) $p_1 < 0.001$ $p_2 = 0.043$ $p_3 = 0.043$	2.18 (2.13–2.19) $p_1 < 0.001$ $p_2 = 0.043$ $p_3 = 0.043$

Note (here and Table 2): p_1 – the level of statistical significance of differences compared to similar parameters in healthy donors; p_2 – in the intact culture; p_3 – during antigen stimulation (AG); p_4 – in patients with DS PT; DAPT – N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-s-phenylglycine t-butyl ester.

The analysis of the population composition of lymphocytes in patients with DS and DR PT after sequential addition of the γ-secretase inhibitor (DAPT) at a concentration of 5 μM / l to the intact cultures in combination with CFP10 and ESAT6 antigens did not reveal any significant differences from the corresponding data obtained when stimulating the cells with antigens alone. A significant ($p = 0.012$)

decrease in $CD4^+GATA\text{-}3^+$ cells and an increase in $CD4^+T\text{-}bet^+$ lymphocytes was registered in healthy donors (Table 1).

Increasing the DAPT concentration to 10 μM / l led to a decrease in the proportion of Th2 cells in the cell cultures in PT patients in both groups ($p = 0.043$) and healthy donors ($p = 0.012$). Changes in the number of $CD4^+T\text{-}bet^+$ lymphocytes were not unequivocal. Thus, an increase in the number of Th1 cells was registered only in patients with DS PT ($p = 0.043$) and in the control group ($p = 0.012$). The number of $CD4^+T\text{-}bet^+$ lymphocytes in DR PT patients was comparable with the same indices obtained during incubation of the cells with CFP10 and ESAT6 antigens.

The comparison of the results obtained between the groups of PT patients showed that under all applied cell culture conditions, the number of Th1 lymphocytes in patients with DR PT was smaller ($p = 0.008$) than in patients with DS PT.

The calculation of the Th1 / Th2 lymphocyte ratio revealed significant differences for the indices obtained during incubation of mononuclear leukocytes with CFP10 and ESAT6 and DAPT (10 μM / l). Inhibition of the Notch signaling pathway resulted in the increased Th1 / Th2 cell ratio relative to the intact and CFP10 – ESAT6-stimulated cultures in PT patients ($p_3 = 0.043$) and healthy donors ($p_3 = 0.012$). There were no significant differences between the groups of patients with different mycobacterial sensitivity (Table 2).

Table 2

Th1 / Th2 cell ratio in patients with disseminated pulmonary tuberculosis, %, *Me* (Q_1–Q_3)

Th1/Th2 ratio	Healthy donors	Patients with pulmonary tuberculosis	
		Drug-sensitive	Drug-resistant
Baseline	1.2 (1.13–1.25)	0.96 (0.94–0.97)	0.93 (0.88–0.95)
With added AG (CFP10 and ESAT6)	1.16 (1.09–1.24)	0.96 (0.95–0.99)	0.95 (0.94–0.95)
With added AG and DAPT (10 μM / l)	2.88 (2.68–2.82) $p_2 = 0.012$ $p_3 = 0.012$	1.27 (1.21–1.28) $p_1 = 0.003$ $p_2 = 0.043$ $p_3 = 0.043$	1.25 (1.24–1.25) $p_1 = 0.003$ $p_2 = 0.043$ $p_3 = 0.043$

DISCUSSION

Protective control over the infectious process caused by Mycobacterium tuberculosis is ensured by cooperative interaction of a variety of immunocompetent cells, realized via juxtacrine and paracrine signaling mechanisms. The role of the main population ensuring the development of the adaptive immune response belongs to the pool of antigen-specific $CD4^+$ T lymphocytes [15]. Their crucial role in the pathogenesis of PT is determined by their ability to increase phagocytic activity of macrophages [16], induce chemokine-mediated migration of $CD8^+$ T cells, their cytolytic activity, and their secretion of cytokines (IFNγ, TNFα) and granzymes [15], and is also confirmed by a high risk of disease development in HIV patients [15; 17; 18].

The increased proportion of Th1 and Th2 lymphocytes in the intact cultures of patients with PT indicates complex involvement of cell-mediated and humoral immune responses in the development of a protective response against Mycobacterium tuberculosis. A disseminated course of PT indicates the failure of the responses, aimed at restraining primary mycobacterial infection. An increase in the number of Treg and Th2 cells can be considered as one of the factors contributing to the progression of the pathological process.

Highly specific molecules ESAT6 and CFP10, secreted only by dividing Mycobacterium tuberculosis, play a key role in the development of tuberculosis infection [19]. The ESAT-6 protein has lytic activity, promotes pathogen entry into the cell, and destabilizes phagosomes, allowing mycobacteria to enter the macrophage cytosol and avoid lysis. The CFP-10 antigen forms a complex with ESAT-6 and ensures its delivery to the site of action [20]. The single recombinant ESAT-6 –CFP10 protein is designed to evaluate the cell-mediated immune response to Mycobacterium tuberculosis. The increased number of Th1 and Th2 lymphocytes recorded in all the studied samples in response to stimulation of the cell cultures with mycobacterial antigens CFP10 and ESAT6 reflects the physiological cellular response and may indicate a preserved antigen recognition function and relatively effective intercellular cooperation.

The question on the functional significance of Th2 lymphocytes in the pathogenesis of the progressive course of tuberculosis remains open. Classical antibody-mediated immune responses (opsonization,

complement activation, phagocytosis, lysosomal degradation) can potentially be effective against Mycobacterium tuberculosis [3]. The leading cytokine of humoral immune response is IL-4, produced by Th2 lymphocytes. Molecular mechanisms of IL-4 involvement in immune responses are associated with suppression of TNFα-induced apoptosis in infected cells, reduction of iNOS activity, and enhancement of proliferation of antigen-specific regulatory T lymphocytes [5]. The works by domestic [21; 22] and foreign authors [23–25] established that the polarization of the immune response toward Treg- and Th2-dependent responses is a key element of the immune imbalance in tuberculosis infection. The intensity of the functional activity of Th2 lymphocytes, as well as the titer and spectrum of antibodies formed by plasma cells may be crucial. In view of this, the established decrease in the proportion of Th2 lymphocytes in the cell cultures of PT patients in both groups under the effect of DAPT (10 μM / l) with a parallel increase in the number of Th1 cells in DS PT can be considered as a possible mechanism contributing to the restoration of an effective dynamic balance between the main populations regulating the intensity of destructive processes and providing protective control over the spread of the infection.

A recorded and maintained under various experimental conditions smaller (than in DS PT) number of Th1 lymphocytes in patients with DR PT proves significant disruptions of intercellular cooperation mechanisms, induced by resistant strains of mycobacteria among others. It was established that the development of DR PT is associated with a significant increase in the number and activity of immunosuppressive regulatory T cells (Treg), their production of IL-10, deficiency of NK cells, IFNγ, and IL-2 (the main factor ensuring proliferation of antigen-specific lymphocytes) [24; 26]. Therefore, a possible reason for the initially smaller number of Th1 lymphocytes in DS PT patients in the intact culture and the preservation of this trend when the cells were stimulated with antigens could be the mechanisms induced by drug-resistant mycobacteria to disrupt cell-mediated immune responses that contribute to the persistence of the pathogen and progression of the disease.

The Th1 / Th2 ratio to a certain extent reflects the immune pattern of T lymphocytes [4; 27]. With respect to mycobacterial infection, the predominance of Th1 cells and cell-mediated immune responses provides effective protection against the pathogen, while the predominance of Th2 lymphocytes and humoral immune responses facilitates the development of hyperergic inflammation with immune-mediated tissue damage. The increase in the Th1 / Th2 ratio under the effect of DAPT (10 μM / l) in PT patients compared to the intact and antigen-stimulated cultures (as well as their compliance with baseline values in healthy donors) can be considered as a possible mechanism regulating the restoration of a balance between lymphocyte populations through inhibition of the Notch signaling pathway, which may contribute to more effective development of the immune response and slow down destructive processes.

CONCLUSION

The Notch signaling pathway has a modulating effect on the differentiation of key lymphocyte populations that determine the dynamic balance between cell-mediated and humoral immune responses. Inhibition of the Notch signaling cascade by the γ-secretase inhibitor DAPT (at a dose of 10 μM / l) under *in vitro* conditions promotes an increase in the Th1 / Th2 ratio in patients with disseminated DS and DR PT. The positive regulatory effect on the Th1 / Th2 balance allows the Notch signaling pathway to be considered as a promising potential target in pathogen-specific therapy for PT. The contribution of the Notch signaling pathway to the regulation of differentiation of other T lymphocyte populations (Th17, Treg) involved in the immunopathogenesis of PT requires further research.

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Association of *CDKN2A/B* deletions with survival of patients with diffuse large B-cell lymphoma

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ABSTRACT

Aim. To define the association of *CDKN2A/B* deletions in the 9p21 locus with survival of patients with diffuse large B-cell lymphoma.

Materials and methods. The study included 105 patients with diffuse large B-cell lymphoma who received first-line therapy with R-CHOP. A deletion of 9p21 was detected by fluorescent in situ hybridization of tumor tissue biopsy samples. Deletions of *CDKN2A* and *CDKN2B* were determined by real-time quantitative polymerase chain reaction. The overall survival and the progression-free survival were calculated by the Kaplan – Meier method with plotting of survival curves (the log-rank test). The risk of event occurrence was determined by the Cox regression analysis with the calculation of the risk ratio (RR) and 95% confidence interval (CI). The differences between the variables were considered statistically significant at $p < 0.05$.

Results. The deletion of the chromosomal region 9p21 was detected in the biopsy samples in 16.2% of patients. The *CDKN2A* deletions were detected in 23.8% of patients and *CDKN2B* loss – in 28.6% of patients. The progression-free survival was significantly lower in patients with the 9p21 deletion than in those without this aberration: 29.4% vs. 62.5%, respectively ($p = 0.012$; RR = 2.26; 95% CI = 1.17–4.38). The risk of disease progression at low and low-intermediate values of the International Prognostic Index was 5.9 times higher in patients with the *CDKN2B* deletion than in patients without this abnormality.

Conclusion. Deletion of the chromosomal region 9p21 is associated with low progression-free survival in patients with diffuse large B-cell lymphoma. Loss of *CDKN2B* is associated with a high risk of disease progression in patients with low and low-intermediate risk according to the International Prognostic Index.

Keywords: deletion of the 9p21 locus, diffuse large B-cell lymphoma, *CDKN2A/B*

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

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Взаимосвязь делеций генов *CDKN2A* и *CDKN2B* с выживаемостью больных диффузной В-крупноклеточной лимфомой

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РЕЗЮМЕ

Цель. Определить взаимосвязь делеций генов *CDKN2A* и *CDKN2B* в локусе 9p21 с выживаемостью больных диффузной В-крупноклеточной лимфомой.

Материалы и методы. В исследование включены 105 пациентов с диффузной В-крупноклеточной лимфомой, получавших терапию первой линии по схеме R-CHOP. Делецию 9p21 выявляли с помощью флуоресцентной гибридизации *in situ* биопсийных образцов опухолевой ткани. Делеции в генах *CDKN2A* и *CDKN2B* устанавливали количественной полимеразной цепной реакцией в реальном времени. Общую и беспрогрессивную выживаемость рассчитывали по методу Каплана – Мейера с графическим построением кривых (log-rank тест). Риск наступления события вычисляли методом регрессионного анализа Кокса с расчетом отношения рисков (ОР) и 95%-го доверительного интервала (95%-й ДИ). Различия между показателями считали статистически значимыми при $p < 0,05$.

Результаты. Делеция хромосомного региона 9p21 обнаружена в биопсийных образцах 16,2% больных. Поломки в гене *CDKN2A* выявлены у 23,8% пациентов, утрата *CDKN2B* –у 28,6%. Беспрогрессивная выживаемость значимо ниже у обследованных с делецией 9p21, чем у лиц без данной аберрации: 29,4% против 62,5% соответственно ($p = 0,012$; ОР = 2,26; 95%-й ДИ = 1,17–4,38). Риск прогрессии заболевания при низком и низком промежуточном показателе международного прогностического индекса в 5,9 раза выше у пациентов с делецией гена *CDKN2B*, чем у больных без указанной аномалии.

Заключение. Делеция хромосомного региона 9p21 связана с низкой беспрогрессивной выживаемостью больных диффузной В-крупноклеточной лимфомой. Утрата гена *CDKN2B* ассоциирована с высоким риском прогрессии заболевания у пациентов низкого и низкого промежуточного риска согласно международному прогностическому индексу.

Ключевые слова: делеция локуса 9p21, диффузная В-крупноклеточная лимфома, *CDKN2A/B*

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. Исследование одобрено локальным этическим комитетом при КНИИГиПК ФМБА России (протокол № 34 от 09.12.2022).

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a group of heterogeneous tumors with different clinical manifestations, morphological characteristics, genetic aberrations, and different responses to therapy and prognosis [1]. More than half of patients with DLBCL respond well to the standard R-CHOP chemotherapy regimen (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone). However, relapses or refractory forms of the disease leading in most cases to death develop in 30–40% of cases [2]. Currently,

the international prognostic index (IPI) and its modifications are considered to be a simple and reproducible tool for estimating the individual risk of early disease progression. However, according to some authors, its practical application does not always accurately estimate the individual risk of therapy failure, since IPI is mainly based on clinical characteristics, so it is necessary to search for new markers associated with an unfavorable course of the disease [3].

Some of the reasons for the heterogeneity of clinical manifestations in DLBCL are molecular biological features of tumor cells [4, 5]. Extensive data on genetic disorders associated with the development of the disease and (or) neoplastic progression have been obtained using the next-generation sequencing methods [6, 7].

Some of the most frequently mutated genes in various malignant neoplasms are genes encoding inhibitors of cyclin-dependent kinases 2A/B (*CDKN2A* and *CDKN2B*), localized in the chromosomal region 9p21. They belong to the tumor suppressor family. *CDKN2A* and *CDKN2B* encode the corresponding proteins p16INK4a and p15INK4B, which are almost identical in structure and biochemical properties. Both proteins play an important role in the cell cycle control, blocking it during the G1 to S-phase transition by binding cyclin-dependent kinases 4 and 6 (CDK4/6). According to the literature, G1/S checkpoint dysfunction leads to uncontrolled proliferation of tumor cells [8].

Deletions of *CDKN2A/B* (9p21) occur in 20–30% of DLBCL cases and, according to some foreign authors, are associated with an unfavorable course of the disease [9]. No information was found on the influence of genetic aberrations in the chromosomal region 9p21 on the prognosis of the disease in the domestic literature. Therefore, the study on the prognostic value of aberrations in the 9p21 locus in patients with DLBCL is relevant.

The aim of the study was to determine the relationship of *CDKN2A/B* deletions at the 9p21 locus with survival of patients with DLBCL.

MATERIALS AND METHODS

The retrospective study included 105 patients with newly diagnosed DLBCL who received treatment at the clinic of KRIHBT in 201–2019. The average age was 59 (49–67) years. Among them, 50.5% (53/105) were men, 49.5% (52/105) were women. Stages 1 and 2 of the disease were determined in 40% (42/105) of the examined patients (according to Ann Arbor staging classification), stages 3 and 4 were determined in 60% (63/105) of patients. Half of the patients (52/105) belonged to high and high-intermediate risk groups according to IPI. All patients received standard first-line R-CHOP induction immunochemotherapy. The immunohistochemical (IHC) subtype of the tumor was determined based on the IHC algorithm proposed by C.P. Hans [10]: GCB subtype was found in 27.6% (29/105) of cases, non-GCB subtype was detected in 72.4% (76/105) of cases. A complete response to R-CHOP therapy was achieved in 64.8% (68/105) of those examined, a partial response was noted in 18.1% (19/105) of cases. Stabilization of the process and refractoriness to treatment were noted in 17.1% (18/105) of patients. Five-year overall survival (OS) was 64.8%, five-year progression-free survival (PFS) was 57.1%. The median follow-up of patients was 48 (20–60) months.

The deletion of the chromosomal region 9p21 was determined using fluorescent *in situ* hybridization (FISH) of biopsy samples of tumor tissue using the Kreatech CDKN2A (9p21) / 9q21 FISH probe according to the standard method in accordance with the manufacturer's protocol. The deletions of exons 1α, 2 in the *CDKN2A* gene and exon 1 in the *CDKN2B* gene were determined by quantitative real-time polymerase chain reaction (PCR) [11].

Statistical data processing was performed using the STADIA software. The frequency of occurrence of nominal independent variables in the groups, divided according to the characteristics under study, was compared using the Pearson's chi-square test (χ^2). The five-year OS and PFS were calculated by plotting the Kaplan – Meier survival curves. The differences between survival rates in the groups of patients were determined using the log-rank test. The risk of disease progression was calculated using the Cox regression analysis with the calculation of the hazard ratio (HR) and 95% confidence interval (CI). The selection of variables was performed by backward elimination (Wald's test). The differences between the parameters were considered statistically significant at $p < 0.05$.

RESULTS

A deletion of the 9p21 chromosomal region was found in 16.2% (17/105) of the examined patients. All the results of the FISH analysis were confirmed by PCR, deletions were detected in 31.4% (33/105) of patients with DLBCL. On the one hand, the obtained data are due to higher sensitivity of PCR compared

to FISH in assessing copy number aberrations in histologic specimens. On the other hand, in some cases, a significantly smaller DNA area is deleted compared to the area covered by a commercial DNA probe. It does not lead to attenuation of the fluorescent signal. The deletions of exons 1α and (or) 2 in the *CDKN2A* gene were found in the formalin-fixed paraffin-embedded (FFPE) samples in 23.8% (25/105) of patients, the deletion of exon 1 in *CDKN2B* was found in 28.6% (30/105) of cases. All patients were divided into groups depending on the presence or absence of deletions of the chromosomal region 9p21 in the *CDKN2A* and *CDKN2B* genes.

There were no significant associations of the 9p21 deletions with clinical and laboratory characteristics of patients (age, disease stage, IHC subtype, IPI risk group) (data not shown).

The relationship between deletions of *CDKN2A/B* (9p21) and OS of patients has not been established (data not shown). The five-year PFS of patients with del9p21 was significantly lower compared to that of patients without this aberration: 29.4% (*Me* = 19 months) versus 62.5% (*Me* was not reached), respectively ($p = 0.012$; Fig. 1, *a*). The risk of disease progression in the patients with the 9p21 deletion in the FFPE samples was 2.26 times higher than in patients without genetic damage (HR = 2.26; 95% CI = 1.17–4.38). Associations between the presence or absence of *CDKN2A* deletions and PFS were not found (data not shown).

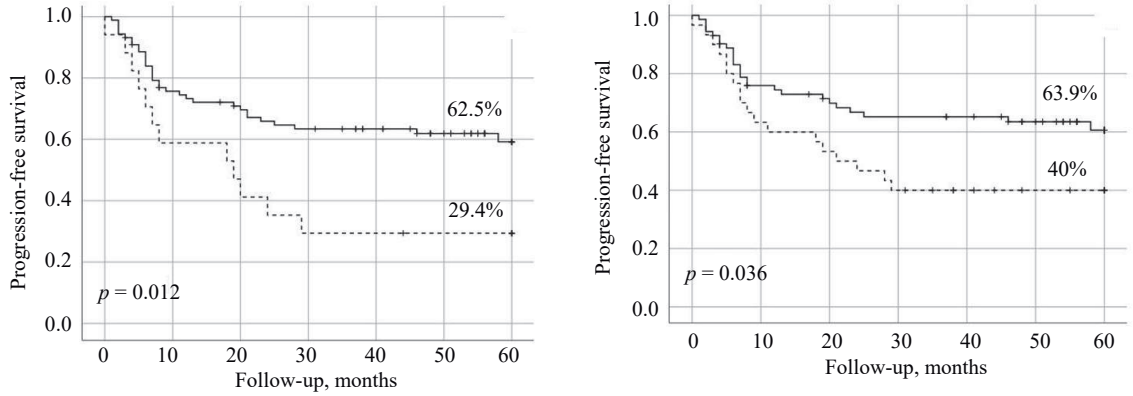

Fig. 1: Progression-free survival of patients: *a* – with a deletion of the chromosomal region 9p21 ($n = 17$, dotted line) and without aberrations ($n = 88$, solid line); *b* – with *CDKN2B* deletion ($n = 30$, dotted line) and without aberrations ($n = 75$, solid line)

The five-year PFS in patients with a *CDKN2B* deletion was lower than in patients without this anomaly: 40% (*Me* = 21 months) versus 63.9% (*Me* was not reached), respectively ($p = 0.036$; Fig.1, *b*). The risk of disease progression in patients with del*CDKN2B* was 1.9 times higher than in patients without the deletion (HR = 1.87; 95% CI = 1.03–3.42).

According to the results of the univariate Cox regression analysis, predictors of low PFS in patients with DLBCL were IPI > 2 ($p < 0.001$; HR = 6.22; 95% CI = 3.05–12.68), non-GCB subtype ($p = 0.058$; HR = 2.10; 95% CI = 0.98–4.51), deletion of the chromosomal region 9p21 ($p = 0.016$; HR = 2.26; 95% CI = 1.17–4.38) or *CDKN2B* deletion ($p = 0.041$; HR = 1.87; 95% CI = 1.03–3.42).

The multivariate Cox proportional hazard model (Table 1) includes parameters that have passed selection by the significance level (IPI > 2, non-GCB subtype, del9p21). A loss of the chromosomal region 9p21 was identified as an independent predictor of low PFS along with IPI > 2. The risk of disease progression was 1.95 times higher in patients with a 9p21 deletion than in those without aberrations at the study locus ($p = 0.031$; HR = 1.95; 95% CI = 1.07–3.56).

Table 1

Multivariate Cox regression analysis of predictors of progression-free survival in patients with diffuse large B-cell lymphoma, *n* = 105

Parameter	HR	95% CI	*p*
IPI >2	5.82	2.85–11.91	<0.001
del 9p21	1.95	1.07–3.56	0.031

The relationship between the presence of genetic aberrations at 9p21 and the survival rates of patients with low or low-intermediate risk (according to IPI) was studied. In patients with *CDKN2A* or *CDKN2B*

deletions, the PFS was lower than in those with an intact locus: 66.7 vs. 86.1% (p = 0.109; *Me* was not reached; Fig. 2, *a*) and 60 vs. 88.9%, respectively (p = 0.009; *Me* was not reached; Fig. 2, *b*).

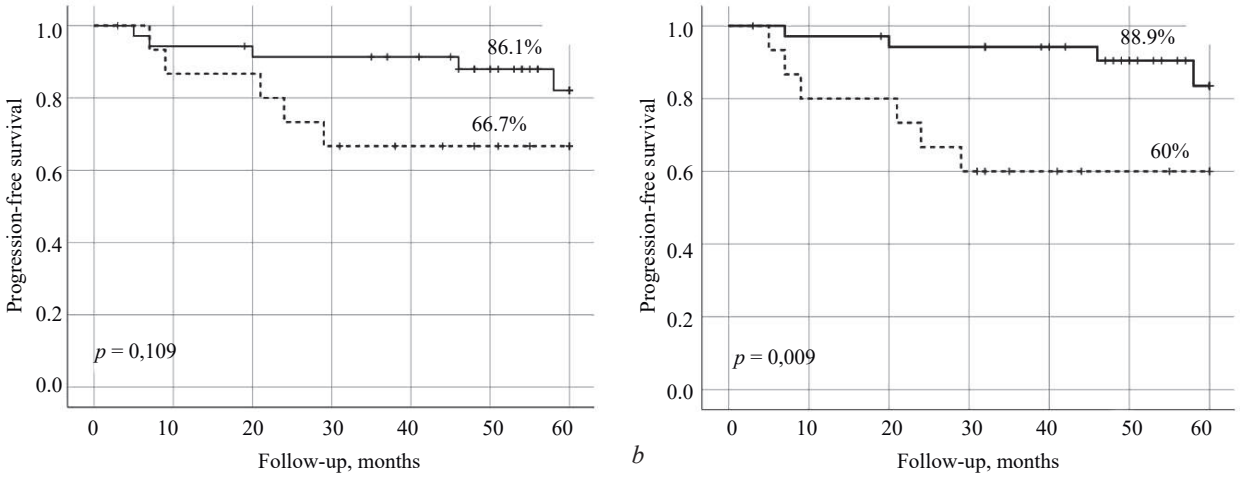

Fig. 2. The progression-free survival in patients with low or low-intermediate risk in the presence of a deletion: *a* – in the CDKN2A gene (n = 15, dotted line), *b* – CDKN2B (n = 15, dotted line) and without aberrations (n = 38, solid line)

Only *CDKN2B* deletion showed statistical significance in the univariate Cox regression analysis for PFS (p = 0.018; HR = 4.67; 95% CI = 1.30–16.81). Assessing the effect of several predictors on disease progression, such as age ≥ 60 years (p = 0.140; HR = 2.6; 95% CI = 0.73–9.21), del *CDKN2A* (p = 0.124; HR = 2. 65; 95% CI = 0.76–9.22), del *CDKN2B* (p = 0.018; HR = 4.67; 95% CI = 1.30–16.81), it was found (Table 2) that patients with del *CDKN2B* had a higher risk of disease progression (by 5.9 times) than those without the gene loss (p = 0.010; HR = 5.9; 95% CI = 1.54–22.61).

Table 2

Multivariate Cox regression analysis of predictors of progression-free survival in patients with low and low-intermediate risk, n = 53

Parameter	HR	95% CI	p
del *CDKN2B*	5.90	1.54–22.61	0.010
Age ≥60 years	3.25	0.87–12.08	0.079

DISCUSSION

DLBCL is a heterogeneous lymphoid neoplasm with variable gene expression patterns and genetic abnormalities that contribute to different clinical courses of the disease and responses to therapy. *CDKN2A/B* aberrations can disrupt various biological programs, in particular DNA damage response (via the p14-ARF/p53 pathway) and cell cycle regulation (via the RB/p16 tumor-suppressive pathway). When the latter is impaired, neoplastic cells accumulate additional mutations, contributing to clonal tumor evolution, genome instability, and, as a result, drug resistance and disease progression [8]. So, B. Chapuy et al. identified a subset of DLBCL variants with biallelic inactivation of *TP53* and a loss of *CDKN2A*, characterized by genomic instability and low survival rates, regardless of the gene expression profile [7].

According to the obtained data, the deletion of the chromosomal region 9p21, established by the FISH method, was found in 16.2% of patients. The results of molecular cytogenetic studies were confirmed by PCR. The loss of *CDKN2A* was found in 23.8% of patients, the loss of *CDKN2B* – in 28.6% of individuals. The obtained data generally correspond to the data mentioned in the literature [9]. The high frequency of genetic disorders determined by PCR is probably due to the high sensitivity of the applied analysis, in contrast to FISH. Although FISH does not detect deletions of smaller regions (microdeletions) and small subclones, the method is considered as specific and indicative. The simultaneous use of technologies is complementary and minimizes errors.

Several studies have shown the association of del9p21 with the prognostically unfavorable ABC subtype of DLBCL [12, 13]. In our study, no similar pattern was found. Perhaps the differences are due to the methods of determining the subtype: the analysis based on the gene expression profile and immunohistochemical methods.

It was found that the deletion of the chromosomal region 9p21, along with IPI > 2, was an independent

predictor of low PFS in patients with DLBCL. The risk of disease progression was two times higher in patients with the 9p21 deletion than in patients without aberrations at the locus under study. It was determined that the PFS of patients belonging to low or low-intermediate risk groups with a *CDKN2B* deletion was significantly shorter than in the individuals without the genetic damage. The risk of progression was more than five times higher in patients with IPI ≤ 2 with a deletion of *CDKN2B* compared to the same parameter in patients without the gene loss. The results are partially in line with the data obtained by F. Jardin et al., who found that *CDKN2A* and (or) *CDKN2B* deletions were associated with shorter overall and disease-free survival of patients [9]. At the same time, we did not reveal any differences in the OS of patients depending on the presence or absence of deletions of the chromosomal region 9p21 and (or) *CDKN2B*.

Associations between the presence of del *CDKN2A* and survival rates of patients with DLBCL were not found. The results are similar to those reported by C.R. Bolen et al. [12] and K. Karube et al. [13]. At the same time, researchers have shown that complex changes in the TP53/CDKN2A biological pathway are associated with low survival rates (OS and PFS), regardless of IPI and the molecular subtype of the disease [13].

CONCLUSION

The deletion of the chromosomal region 9p21 is associated with low PFS rate in patients with DLBCL. The deletion of the *CDKN2B* gene is associated with a high risk of disease progression in low- and low-intermediate risk patients (IPI). Aberrations in the chromosomal region 9p21 (del *CDKN2A/B*) are determined using both PCR and FISH. The obtained results can be used as additional molecular genetic criteria for assessing the unfavorable course of DLBCL.

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Authors' contribution

Sarpova M.V. – conception and design, analysis and interpretation of the data. Tregubova E.V. – analysis and interpretation of the data. Diakonov D.A. – conception and design, justification of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Vaneeva E.V. – analysis and interpretation of the data. Posin V.A. – justification of the manuscript and critical revision of the manuscript for important intellectual content. Samarina S.V. – collection of clinical data about patients. Nazarova E.L. – justification of the manuscript and critical revision of the manuscript for important intellectual content.

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Analysis of in vitro fertilization programs in patients with functional ovarian cysts and anovulatory infertility

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ABSTRACT

Background. There are different opinions about the effect of functional ovarian cysts on the duration of controlled ovarian hyperstimulation, the dose of gonadotropins, the number and quality of collected oocytes and produced embryos, and the frequency of pregnancy.

Aim. To analyze in vitro fertilization (IVF) programs in women with anovulatory infertility and ovarian retention.

Materials and methods. A prospective study included 71 women aged 18–44 years. The main group (I) included patients (n = 38) with anovulatory infertility and functional ovarian cysts (FOC) diagnosed by ultrasound before enrollment in the IFV program. Patients of this group underwent ultrasound-guided transvaginal puncture of ovarian cyst followed by cytology. The comparison group (II) (n = 33) encompassed patients with anovulatory infertility without FOC, who went through the IVF program. The control group (III) included apparently healthy individuals (n = 15).

The study algorithm included collection of clinical and anamnestic data of the patients, data of laboratory and instrumental studies, parameters of a stimulated IVF cycle, characteristics of oogenesis and early embryogenesis, and assessment of IVF program effectiveness.

Conclusion. It was established that in FOC and anovulatory infertility, the number of collected oocytes was smaller; however, the number of the best quality embryos and the frequency of pregnancy did not differ.

Keywords: functional ovarian cysts, assisted reproductive technology, in vitro fertilization, ovulation induction

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Siberian State Medical University (Protocol No. 9455 of 27.04.2023).

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Анализ программ экстракорпорального оплодотворения при ановуляторном бесплодии у пациенток с ретенционными образованиями яичников

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РЕЗЮМЕ

Введение. Существуют различные мнения о влиянии функциональных кист яичников на длительность стимуляции суперовуляции, дозу гонадотропинов, количество и качество полученных ооцитов и эмбрионов, частоту наступления беременности.

Цель исследования – провести анализ программ экстракорпорального оплодотворения (ЭКО) при ановуляторном бесплодии у пациенток с ретенционными образованиями яичников.

Материалы и методы. Проведено проспективное исследование 71 женщины в возрасте 18–44 года. Основную группу (I) составили пациенты (n = 38) с ановуляторным бесплодием и функциональными кистами яичников (ФКЯ), диагностированными при ультразвуковом исследовании непосредственно перед вступлением в программу ЭКО. Пациентам данной группы проводилась трансвагинальная пункция кисты под ультразвуковым контролем с последующим цитологическим исследованием. В группу сравнения (II) (n = 33) включались пациенты с ановуляторным бесплодием без ФКЯ, которым проведена программа ЭКО. В группу контроля (III) вошли условно здоровые пациенты (n = 15).

Алгоритм исследования включал анализ клинико-анамнестических характеристик пациентов, данных лабораторного и инструментального исследования, показателей стимулированного цикла, характеристику показателей оогенеза и раннего эмбриогенеза, а также оценку эффективности программы ЭКО.

Заключение. Установлено, что при функциональных кистах яичника и бесплодии получено более низкое количество ооцитов при стимуляции, однако количество топовых эмбрионов, частота наступления беременности и живорождения не отличались.

Ключевые слова: функциональные кисты яичников, лечение функциональных кист, вспомогательные репродуктивные технологии, экстракорпоральное оплодотворение, стимуляция овуляции

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтовинтересов, 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 СибГМУ (протокол № 9455 от 27.04.2023).

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INTRODUCTION

The problems of diagnosis and treatment of infertility are some of the main clinical and socially sensitive problems in modern medicine, which is due not only to the significant prevalence of various infertility factors, but also to their tendency to increase [1]. Assisted reproductive technologies (ART), including in vitro fertilization (IVF), are some of the effective and popular methods to

overcome infertility [2]. According to the register of the Russian Association of Human Reproduction, more than 36,000 children were born following IVF in Russia in 2019 and more than 34,000 children were born in 2020 [3]. The indication for IVF is the ineffectiveness of infertility treatment by other methods for 12 months for women younger than 35 years and for 6 months for women older than 35 years [4].

According to the literature, every fourth patient with infertility has ovarian masses of various genesis and 80% of them are follicular ovarian cysts. The majority of retention masses are functional ovarian cysts (FOC): follicular cysts and corpus luteum cysts [5–9]. Over the last decade, the prevalence of FOC has increased from 6–12 to 25% [9–13]. In 60% of cases, they occur in patients of the reproductive age [14, 15]. Infertility in patients with FOC reaches 41% [16, 17].

The pelvic ultrasound examination is the starting point for planning ART and its purpose is to assess the endometrium, detect uterine cavity anomalies, count the number of antral follicles, and detect ovarian pathology, in particular ovarian cysts [4]. Management strategy for patients with FOC in IVF programs remains controversial. B. Kumbak et al. (2009), E.B. Rudakova et al. (2014) believe that FOC do not affect the effectiveness of IVF programs [18, 19]. H.S. Qublan et al. (2006), R.D. Firouzabadi et al. (2010), and R. Levi et al. (2003) suggest that FOCs detected before the start of stimulation in IVF programs adversely affect its outcome as they require higher doses of gonadotropins, are associated with a lower ovarian response, and reduce the pregnancy rate [20–22].

There are different approaches to the management of patients with FOC in ART programs: surgical (transvaginal puncture of the cyst with a subsequent cytological examination) and conservative (administration of gonadotropin-releasing hormone (GnRH) agonists and antagonists for 3–7 days) methods, as well as a wait-and-see approach [18–27]. Despite a significant number of studies, the impact of FOC on the course and outcome of IVF protocols, namely on the features of controlled ovarian superovulation, the number and quality of oocytes, as well as the parameters of oogenesis and early embryogenesis, requires further research.

The aim of the study was to analyze IVF programs in patients with anovulatory infertility and FOCs detected in the IVF program.

MATERIALS AND METHODS

We conducted a prospective study that included 71 women of reproductive age who applied to the Center for Assisted Reproductive Technologies of Siberian State Medical University. The main group (I) consisted of (n = 38) patients with anovulatory infertility and FOCs diagnosed by ultrasound immediately before enrollment in the IVF program. Patients in this group underwent ultrasound-guided transvaginal cyst puncture followed by the cytological examination. The comparison group (II) (n = 33) included patients with anovulatory infertility without FOC who went through IVF. The control group (III) included healthy patients (n = 15).

Inclusion criteria:

1) age 18–44 years;

2) anti-Müllerian hormone (AMH) level in the blood of women 1.2–3.5 ng / ml;

3) informed consent to participate in this study;

4) body mass index (BMI) 18.5–30.0.

Exclusion criteria:

1) the age under 18 and over 45 years;

2) hyperprolactinemia;

3) moderate and severe forms of genital endometriosis (ASRM ≥ III, 1996);

4) hypothyroidism;

5) uterine myoma requiring surgical intervention;

6) true ovarian tumors;

7) contraindications to IVF according to the Order of the Ministry of Healthcare of the Russian Federation No. 803n of 31.07.2020 “On the Procedure for the Use of Assisted Reproductive Technologies, Contraindications, and Restrictions on Their Use” (hereinafter – Order No. 803n);

8) the woman’s refusal to participate in the study.

The main group included patients with unilateral FOC persisting for no more than 3 months and being 25–60 mm in diameter according to the ultrasound examination. In case of FOC detection, patients were tested for CA-125 and HE-4. If the concentration of these markers increased above the reference values, the patient was excluded from the study.

The research algorithm included an analysis of clinical characteristics and medical history of the patients, laboratory tests and clinical investigation data, stimulated cycle parameters, oogenesis and early embryogenesis parameters, as well as an assessment of the effectiveness of the IVF treatment. All patients were examined in accordance with the Order No. 803n. All patients underwent ovulation stimulation

according to the protocol with GnRH antagonists. Statistical data was processed using the SPSS 23 program. The Levene's test was used to determine the homogeneity of dispersions. The Kolmogorov – Smirnov test with the Lilliefors correction, the Shapiro – Wilk test, and visual assessment of histograms were used to assess the normal distribution of variables. The Mann – Whitney test was used to compare quantitative data from two independent groups, and the Kruskal – Wallis test was used to compare data from more than two independent groups, followed by an analysis of a posteriori comparisons by the Dunn's test (Dunn, 1964) and the Conover–Iman test (Conover, Iman, 1979). When comparing qualitative variables, contingency tables with the Pearson's $\chi 2$ test were used. The data were presented as the median and the interquartile range (*Me* $(Q_{25}–Q_{75})$), or the mean and the standard error ($M \pm SD$). The critical significance level p for all statistical analysis procedures was 0.05.

RESULTS

The mean age of all the examined individuals (n = 71) was 34.0 (30.0–39.0) years. The statistical analysis showed age homogeneity of the main group and the comparison group (p = 0.746). In the vast majority of cases, menarche in women of all groups occurred at the age of 12–15 years. The mean age of menarche in all the subjects (n = 71) was 13.5 (12.0–14.0) years. The mean duration of the menstrual cycle in all the subjects (n = 71) was 30.0 (27.0–37.0) days. Menstrual disorders were established in the medical history of 51 (71.8 %) patients. Oligomenorrhea occurred in 12 (16.9%) patients, while abnormal uterine bleeding (AUB) in the absence of chronic endometrial pathology was observed in 20 (28.1%) women. Dysmenorrhea was observed in 21 (29.5%) patients. The statistical analysis of groups I and II showed their homogeneity in all the studied menstrual cycle parameters.

The mean duration of infertility was 6.0 ± 0.3 years (p = 0.929). Combined infertility (combination of both male and female factors) occurred in 19 (26.7 %) patients. Combined female infertility was diagnosed in 25 (35.2 %) patients. Tubal factor and endometriosis were detected in 18 (25.4 %) and 12 (12.1 %) patients, respectively. The statistical analysis of homogeneity in groups I and II did not reveal any differences.

The health status of all patients was assessed as satisfactory. At the time of enrollment in the ART program, 50.5% of women had extragenital pathology that was in a stable remission or compensated. Compensated pathology of the thyroid gland in 35.2% of the patients and pathology of the gastrointestinal tract in remission in 16.9% of patients of all groups were the most common.

Hormonal profile (AMH, follicle stimulating hormone (FSH), luteinizing hormone (LH)) and ultrasound data on the antral follicle count (AFC) were used to assess the ovarian reserve of patients in all groups (Table 1).

Table 1

Analysis of ovarian reserve parameters, *Me* ($Q_{25}–Q_{75}$)

Parameter	Group I, n = 38	Group II, n = 33	Group III (control), n = 15	p (the Kruskal – Wallis test)
FSH, mU / ml	5.60 (5.1–8.4)	5.3 (3.8–8.5)	4.6 (3.1–5.3)	0.608
LH, mU / ml	6.9 (3.2–7.4)	6.5 (3.0–6.9)	3.9 (2.8–4.8)	0.043*
AMH, ng / ml	2.51 (1.4–3.4)	2.4 (1.8–3.1)	3.3 (3.0–3.8)	0.841
Estradiol, pg / ml	42.9 (30.0–64.0)	42.0 (30.3–69.0)	41.6 (30.5–65.0)	0.906
AFC	10.0 (5.0–13.0)	11.0 (8.5–13.0)	13.6 (11.0–14.0)	0.449

Note: pairwise comparison of groups I and II by the Mann – Whitney test did not reveal statistically significant differences (p = 0.442), while comparison of groups I–III and II–III revealed significant differences, p = 0.032 and p = 0.028, respectively.

Thus, the analysis of ovarian reserve data shows the homogeneity of groups I and II. The homogeneity of the groups was achieved by fulfilling the inclusion criteria, namely limiting the AMH level to 1.2–3.5 ng / ml and BMI for participation in the study. Thus, all the studied groups of patients had the expected normal response to ovulation stimulation, which makes it possible to obtain true data on the influence of FOC on the effectiveness of ovarian stimulation. Ovulation stimulation in all patients who participated in the study was performed according to a protocol using GnRH antagonists.

GnRH antagonists were added on day 6 of gonadotropin stimulation. The study of induced cycle parameters in the main group and the comparison group did not reveal any statistically significant differences in either the starting dose or the duration of stimulation (Table 2).

Table 2

Stimulated cycle data			
Parameter	Group I, *n* = 38	Group II, *n* = 33	*p*
Starting dose of gonadotropins, IU, *M* ± *SD*, *Me* (Q_{25}–Q_{75})	194.7 ± 10.4 150 (150–225)	200 ± 7.3 225 (150–225)	0.932
Total dose of gonadotropins, IU, *M* ± *SD*, *Me* (Q_{25}–Q_{75})	2,373.7 ± 220.9 1,950 (1,350–2,850)	1,981.8 ± 82.9 2,175.0 (1,750.0–2,250.0)	0.875
Duration of stimulation, days, *M* ± *SD*, *Me* (Q_{25}–Q_{75})	10.8 ± 0.9 11.0 (9.0–14.0)	10.5 ± 0.3 (11.0; (9.0–11.0)	0.910
Trigger, *n* (%): – triptorelin – α-subunit of hCG	34 (89.4%) 4 (10.5%)	30 (90.9%) 3 (9.1%)	1.000

Note: hCG is human chorionic gonadotropin.

The effectiveness of the IVF program is expressed not only by the response to ovulation stimulation, but also by the quantity and quality of oocytes and embryos obtained. Based on the data obtained, we analyzed the parameters of oogenesis and early embryogenesis. The average number of punctured follicles in group I and II (n = 38 and n = 33, respectively) was 10.2 (from 7 to 13). The number of punctured follicles was significantly higher in the comparison group with anovulatory infertility and absence of FOC than in the patients of the main group (p = 0.006). A significant increase in the number of obtained oocytes in the group of patients without FOC was also observed (p = 0.002). However, the proportion of mature oocytes was higher in the group with FOC (p = 0.014), which ultimately resulted in an equal number of best quality embryos in both groups (p = 0.097) (Table 3).

Table 3

Characteristics of oogenesis and early embryogenesis			
Parameter	Group I, *n* = 38	Group II, *n* = 33	*p*
Number of punctured follicles, *M* ± *SD*, *Me* (Q_{25}–Q_{75})	7.9 ± 0.5 8.0 (5.0–11.0)	10.2 ± 0.5 11.0 (7.0–13.0)	0.013
Number of oocytes, *M* ± *SD*, *Me* (Q_{25}–Q_{75})	7.24 ± 0.5 7.0 (5.0–11.0)	9.45 ± 0.6 9.0 (6.0–13.0)	0.022
Proportion of mature oocytes (MII), *n* (%)	243/312 (77.9%)	198/283 (69.9%)	0.007
Number of fertilized oocytes, *M* ± *SD*, *Me* (Q_{25}–Q_{75})	4.9 ± 0.5 4.0 (3.0–6.0)	6.58 ± 0.6 6.0 (3.5–8.5)	0.075
Number of best quality embryos on day 5, *M* ± *SD*, *Me* (Q_{25}–Q_{75})	2.34 ± 0.3 2.0 (1.0–3.25)	3.3 ± 0.5 2.0 (1.5–3.5)	0.097

The effectiveness of IVF was analyzed (Table 4). The pregnancy rate per cycle initiated and per embryo transfer was 21/71 (29.5 %) and 31/71 (43.7 %) in group I and II, respectively. The Take baby home parameter, i.e. the delivery of a baby, was 24/71 (33.9 %). The Pearson's $\chi 2$ test and contingency tables showed that implantation, pregnancy per cycle initiated (transfer), and live birth rates were similar regardless of the presence (absence) of FOC before entering the IVF treatment.

Table 4

Clinical effectiveness of IVF programs			
Parameter	Group I, *n* = 38	Group II, *n* = 33	*p*
Pregnancy rate per cycle initiated, *n* (%)	11 (28.9%)	10 (30.3%)	0.627
Pregnancy rate per embryo transfer, *n* (%)	16 (42.1%)	15 (45.5%)	0.886
Pregnancy termination, *n* (%)	2 (5.3%)	3 (9.1%)	0.579
Ectopic pregnancy, *n* (%)	1 (1.5%)	0 (0%)	1.000
Take baby home, *n* (95% CI)	12 (31.6%)	12 (36.4%)	1.000

CONCLUSION

FOC may have a negative effect on the effectiveness of ovarian stimulation, which can manifest itself by a lower follicle count (p = 0.006) and a smaller number of oocytes produced (p = 0.002) in anovulatory infertility. This effect is probably mediated both by the mechanical effect of FOC on the ovary, which prevents follicle growth, and by an altered blood supply which prevents follicle development, resulting in a reduced response to stimulation and a smaller number of oocytes produced in the anovulatory infertility group with FOC compared to the group without FOC.

Presumably, FOC may have an active paracrine or endocrine effect resulting from the release of estradiol. Cysts associated with increased estradiol levels may cause a premature increase in LH and progesterone levels, which leads to a decrease in the oocyte quality and adversely affects the endometrium. The presumed mechanism of the negative effect of FOC on the number of oocytes during ovarian stimulation is consistent with the literature data [20, 23, 24]. However, the proportion of mature oocytes (MII) was higher in the group of patients with FOC compared to the group of patients with anovulatory infertility without FOC, which proves that there is no pronounced negative effect of follicular cysts and corpus luteum cysts on the quality and competence of oocytes.

The number of best quality embryos (category A and B blastocysts according to the Gardner blastocyst

grading system aimed at assessing human embryos at the blastocyst stage proposed by Gardner, Schoolcraft (1999), based on the analysis of trophectoderms, inner cell mass, and blastocyst cavity size) also did not differ in the two groups ($p = 0.097$), which confirms the literature data on the absence of a possible negative effect of FOC on the effectiveness of the IVF program [18, 19]. Program effectiveness estimated as the number of pregnancies per cycle initiated and the number of pregnancies per transfer was also similar in both groups ($p = 0.89$ and $p = 0.89$, respectively). Thus, FOCs detected before patient entering the protocol do not have a pronounced adverse effect on the outcome of ART programs.

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Authors' contribution

Timofeeva O.S., Petrov I.A., Tikhonovskaya O.A., Logvinov S.V. – conception and design. Timofeeva O.S., Gaifulina J.F., Samoilova Yu.G., Petrova M.S., Yuriev S.Yu., Dmitrieva M.L., Zhdankina A.A., Gerasimov A.V., Mikheenko G.A. – data search and analysis. Timofeeva O.S. – drafting of the manuscript. Petrov I.A., Tikhonovskaya O.A., Logvinov S.V. – final approval of the manuscript for publication.

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Hydration status in patients hospitalized with acute decompensated heart failure depending on the severity of glucose metabolism disorder

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ABSTRACT

Aim. To study the hydration status according to clinical parameters and laboratory and instrumental research findings at admission and discharge in patients hospitalized with acute decompensated heart failure (ADHF), depending on the severity of glucose metabolism disorder.

Materials and methods. The study included 280 patients (53% men, average age 70.1 ± 10.8 years) with ADHF. 72.5% of patients had arterial hypertension in the medical history, 60% of patients had coronary artery disease. In all patients, the level of glycated hemoglobin (HbA1c) was determined to assess the glucose metabolism status. 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 of 5.7–6.4% – in the prediabetes group, at HbA1c ≥ 6.5% – in the type 2 diabetes group. The patients underwent a standard physical examination at admission and at discharge, as well as a clinical and comprehensive assessment of congestion (determination of N-terminal pro B-type natriuretic peptide (NT-proBNP), lung ultrasound, liver Fibroscan testing, including calculation of a controlled attenuation parameter, bioimpedance analysis of the body).

Results. The frequency of glucose metabolism disorders in patients hospitalized with ADHF was 57.5% (n = 161), while prediabetes was detected in 17.1% of patients (n = 48) and type 2 diabetes – in 40.4% (n = 113) of cases. Congestion at admission was detected in all patients. A significantly higher frequency of residual (61%) and a lower frequency of subclinical congestion (10%) were revealed in patients with ADHF and type 2 diabetes, compared to patients without glucose metabolism disorders (39% for residual congestion, 27% for subclinical congestion) and prediabetes (40% for residual congestion, 25% for subclinical congestion), respectively. There were no significant differences in the frequency of euvolemia at discharge, depending on the glucose metabolism disorder.

Conclusion. To assess congestion phenomena at discharge, it is necessary to use clinical, laboratory, and instrumental assessments for patients with ADHF and glucose metabolism disorders. However, in patients with ADHF and prediabetes, it is preferable to focus on the laboratory and instrumental assessment of congestion, while in patients with ADHF and type 2 diabetes, both clinical and laboratory and instrumental assessment of congestion should be performed.

Keywords: heart failure, congestion assessment, NT-proBNP, lung ultrasound, liver Fibroscan testing, glucose metabolism disorder

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

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Статус гидратации у пациентов, госпитализированных с декомпенсацией острой сердечной недостаточности в зависимости от степени нарушения углеводного обмена

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РЕЗЮМЕ

Цель: изучить статус гидратации по клиническим и лабораторно-инструментальным параметрам при поступлении и выписке у пациентов, госпитализированных с острой декомпенсацией хронической сердечной недостаточности (ОДХСН), в зависимости от степени нарушения углеводного обмена (НУО).

Материалы 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 проводили исследование уровня гликозилированного гемоглобина (HbA1c). Пациенты были разделены на группы в зависимости от полученных результатов: при значениях HbA1c < 5,7% включали в группу без НУО, 5,7–6,4% – в группу предиабета, ≥6,5% – в группу с сахарным диабетом 2-го типа (СД2). Пациентам проводили стандартное физическое обследование при поступлении и при выписке, а также делали клиническую и комплексную оценку застоя (определение концентрации мозгового натрийуретического пептида (NT-proBNP), ультразвуковое исследование (УЗИ) легких, фибросканирование печени, включая расчет контролируемого параметра затухания ультразвука, биоимпедансный анализ состава тела).

Результаты. Частота НУО у пациентов, госпитализированных с декомпенсацией хронической сердечной недостаточности (ХСН), составляет 57,5% (*n* = 161), при этом предиабет был выявлен в 17,1% (*n* = 48), СД2 – в 40,4% (*n* =113) случаев. Застойные явления при поступлении отмечены у всех пациентов. Выявлены достоверно более высокая частота остаточного (61%) и более низкая частота субклинического застоя (10%) у пациентов с ХСН и СД2 в сравнении с пациентами без НУО (39% остаточный, 27% субклинический застой) и предиабетом (40% остаточный, 25% субклинический застой) соответственно. Не показано достоверных различий по частоте эуволемии при выписке в зависимости от НУО.

Заключение. Пациентам с ОДХСН и НУО для оценки застойных явлений при выписке необходимо использовать клиническую и лабораторно-инструментальную оценку застоя. Однако у пациентов с ОДХСН и предиабетом предпочтительно сделать акцент на лабораторно-инструментальной оценке застоя, а пациентам с ОДХСН и СД2 – на клинической и лабораторно-инструментальной оценке застоя.

Ключевые слова: сердечная недостаточность, оценка застоя, NT-proBNP, УЗИ легких, фибросканирование печени, 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

Type 2 diabetes mellitus (T2DM) and heart failure (HF) are common comorbid conditions. In addition, new-onset T2DM and prediabetes are often found in patients hospitalized with acute decompensated heart failure (ADHF) and are independently associated with an increased risk of both all-cause and cardiovascular mortality [1].

The leading pathophysiological mechanism in ADHF and the reason for hospitalization is systemic congestion, which is associated with an unfavorable disease prognosis [2]. Systemic congestion leads to dysfunction of target organs, which has an important clinical and prognostic value. Quite often, congestion phenomena can go unnoticed, since in some cases, they do not manifest clinically [3], but can only be detected by laboratory and (or) instrumental methods. The instrumental methods for assessing congestion, which have a prognostic value according to literature data, include determining the level of brain-natriuretic peptide (NT-proBNP), assessing the number of B-lines according to lung ultrasound, estimating liver density by transient elastography, and assessing the hydration status by the bioelectrical impedance vector analysis (BIVA). Patients with T2DM with both heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) are characterized by a more advanced NYHA functional class of the disease and have more symptoms and signs associated with HF than patients without T2DM [4, 5]. In the CHARM, DIG, and I-PRESERVE trials, high frequency of symptoms and signs of congestion was found in patients with T2DM. Similar data in patients with prediabetes have not been presented in the literature.

Thus, the aim of this work was to study the hydration status according to clinical parameters and laboratory and instrumental research findings at admission and discharge in patients hospitalized with ADHF, depending on the severity of glucose metabolism disorder (prediabetes, T2DM) and without metabolism disorders.

MATERIALS AND METHODS

A prospective, observational study on investigating the features of chronic heart failure (CHF) in patients with glucose metabolism disorder included 280 people hospitalized with ADHF.

ADHF was diagnosed on the basis of current clinical guidelines: rapid aggravation of symptoms and signs of HF requiring emergency hospitalization of the patient and intensive therapy in combination with objective signs of heart failure (systolic and (or) diastolic dysfunction, left ventricular hypertrophy (LVH), left atrial enlargement according to echocardiography, and an increase in the NT-proBNP level).

Patients with acute coronary syndrome, terminal hepatorenal syndrome, non-cardiogenic edematous disorder, active cancer, exacerbation of chronic obstructive pulmonary disease (COPD), bronchial asthma (BA), pneumonia, type 1 diabetes mellitus, severe cognitive impairments, suspected or conformed COVID-19, verified hepatitis (cirrhosis of the liver), immobilized patients, and those who could not undergo BIVA (due to amputated limbs, ulcers or pronounced trophic changes in the skin of the limbs, the presence of metal implants) were not included in the study.

Glycated hemoglobin (HbA1c) was determined 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%, the patients were included in the group without glucose metabolism disorders, at HbA1c of 5.7–6.4% – in the prediabetes group, at HbA1c ≥ 6.5% – in the T2DM group.

In the first 24 hours from the moment of hospitalization and at discharge, all patients included in the study underwent standard physical, laboratory and instrumental examinations, including lung ultrasound, determination of the NT-proBNP level, liver Fibroscan testing with the calculation of a controlled attenuation parameter (CAP), and BIVA of body composition (Fig. 1).

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

Therapy of patients in the outpatient setting included loop diuretics 72.8%, mineralocorticoid receptor antagonists (MRA) – 55%, ACEi /ARB / ARNI – 77.1%, beta-blockers – 70.0%, cardiac glycosides – 18.5%, and oral anticoagulants – 55%. In the in-patient setting, all patients received loop diuretics, MRA – 74.2%, ACEi / ARB / ARNI – 94.2%, beta blockers – 95.3%, cardiac glycosides – 18.5%, and oral anticoagulants – 66%.

To assess clinical congestion, the Composite Congestion Score (CCS) was used. 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. When summing up the scores, the score ≥ 1 was considered as clinical congestion at admission and as residual congestion with clinical manifestations at discharge.

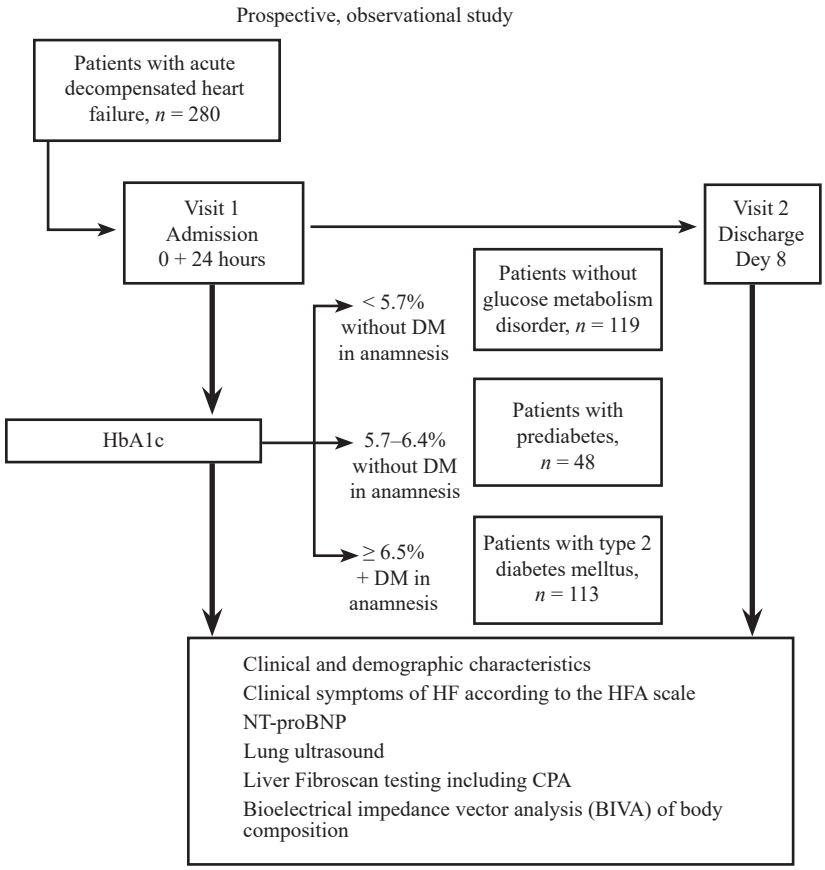

Fig. 1. Design of the study

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
BMI, kg/m², *M*±*SD*	32.1 ±5.7
NYHA functional class of HF, *n* (%) II III IV	 90 (32%) 123 (44%) 67 (24%)
LVEF, %, *n* (%)	45.1 ±11.9
LVEF: <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%)
COPD / BA, *n* (%)	47 (17%)

Note: BMI – body mass index, LVEF – left ventricular ejection fraction.

The concentration of NT-proBNP was determined by the enzyme-linked immunosorbent assay (ELISA) using the NT-proBNP-ELISA-BEST test systems, the A-9102 reagent kit (Vector-Best, Russia). Lung ultrasound (VIVID iq, GE) with the calculation of the sum of B-lines was performed in 8 zones. Transient elastography (TE) was performed using the FibroScan ® 502 Touch device (Echosens, France) according to the standard procedure. BIVA was performed using the ABC-01 analyzer (Medass, Russia).

Liver ultrasound was regarded as a method for assessing congestion in the pulmonary circulation, transient elastography – as a method for assessing congestion in the systemic circulation, BIVA and determining NT-proBNP – as methods for assessing systemic congestion.

At discharge, the following groups of patients were differentiated: patients with residual congestion (clinical and laboratory and instrumental assessments), patients with subclinical congestion, and euvolemic or well compensated patients.

Residual congestion at discharge was evidenced by clinical and instrumental and laboratory data confirming

the presence of congestion. Subclinical congestion was evidenced by the absence of clinical and the presence of instrumental research findings confirming the presence of congestion. The absence of clinical and instrumental research findings confirming congestion was regarded as a state of euvolemia or compensation.

For statistical data processing, MedCalc Software's VAT Version 19.0 and IBM SPSS Statistics (version 26.0) were used. Quantitative variables were presented as the arithmetic mean and the standard deviation ($M \pm SD$) for normal distribution and as the median and the interquartile range $Me\ (Q_1; Q_3)$ for non-normal distribution.

RESULTS

The frequency of glucose metabolism disorders in patients hospitalized with ADHF was 57.5% (n = 161), while prediabetes was detected in 17.1% of cases (n = 48), and T2DM – in 40.4% (n = 113) of cases.

The status of congestion phenomena at admission and discharge was analyzed in all patients hospitalized with ADHF. Congestion at admission was detected in all patients. Patients with glucose metabolism disorders at admission had significantly higher frequency of typical clinical symptoms and signs of CHF, such as wheezing in the lungs, orthopnea, swollen cervical veins, and edema of the lower extremities. They also had significantly higher values of liver density, CAP, the number of B-lines on lung ultrasound, and the level of NT-proBNP and significantly lower 6-min walk test values (6MWT) and active resistance and impedance according to BIVA, which indicates more pronounced manifestations of congestion compared to patients without glucose metabolism disorders (Table 2).

The clinical and laboratory and instrumental assessments of congestion in patients with ADHF, depending on the degree of glucose metabolism disorders at discharge, are presented in Table 3. At discharge, the frequency of residual congestion in patients with glucose metabolism disorders was significantly higher (55% vs. 39%, $p < 0.01$), and the frequency of subclinical congestion was significantly lower (14% vs. 27%, $p < 0.01$) than in the group of patients without glucose metabolism disorders. At the same time, the frequency of congestion in the prediabetes group was comparable with that in the group without glucose metabolism disorders. The differences in the frequency were revealed due to the group of patients with T2DM (Fig. 2). There were no significant differences in the frequency of euvolemia or compensation at discharge, depending on glucose metabolism disorders.

Table 2

Clinical and laboratory and instrumental assessment of congestion in patients with ADHF, depending on the degree of glucose metabolism disorders at admission, n = 280

Parameter	CHF without glucose metabolism disorders, n = 119	CHF with prediabetes, n = 48	CHF with type 2 diabetes, n = 113
6MWT, m	255.2 ± 111.4	211.3 ± 116.2*	227.7 ± 9.3*#
Clinical assessment of congestion			
Dyspnea, n (%)	113 (94.9)	46 (95.8)	111 (98.2)
Wheezing in the lungs, n (%)	50 (42.0)	37 (77.1)***	78 (69)*
Orthopnea, n (%)	79 (66.4)	35 (72.9)***	78 (69)
Swollen cervical veins, n (%)	46 (38.7)	23 (47.9)**	52 (46)
Edema of the lower extremities, n (%)	73 (61.3)	41 (85.4)**	105 (92.9)*
Laboratory and instrumental assessment of congestion			
NT-proBNP, pg / ml, $Me\ (Q_1; Q_3)$	1,700 (690; 2,901)	1,797 (1,040; 2,941)	2,130 (1,150; 3,201)*
Number of B-lines, $M \pm SD$	31,4 ± 17	34.9 ± 15.4	36 ± 17.9*
Liver density, кPa, $M \pm SD$	10.6 ± 8.9	14.3 ± 10.2**	14.3 ± 10.8**
CAP, dB / m, $M \pm SD$	231 ± 72.1	254.9 ± 51.4**	256.9 ± 55.3**
Active resistance, Om, $M \pm SD$	403.5 ± 76.9	382.5 ± 74.9	377.8 ± 73.48
Reactance, Om, $M \pm SD$	35.6 ± 9.3	32.5 ± 10.7	33.2 ± 9.8
Impedance Z, BIVA, $M \pm SD$	405.2 ± 77.1	383.9 ± 75.4	379.3 ± 73.9*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the group of CHF patients without glucose metabolism disorders; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared to the prediabetes group and T2DM group (here and in Table 3).

Table 3

Clinical and laboratory and instrumental assessment of congestion in patients with ADHF, depending on the degree of glucose metabolism disorders at discharge, *n* = 280

Parameter	CHF without glucose metabolism disorders, *n* = 119	CHF with prediabetes, *n* = 48	CHF with type 2 diabetes, *n* = 113
Clinical assessment of congestion			
Dyspnea, *n* (%)	55 (46.2%)	26 (54.1%)	65 (57.5%)
Wheezing in the lungs, *n* (%)	15 (12.6%)	12 (25.0%)	40 (35.3%)***
Orthopnea, *n* (%)	25 (21.0%)	11 (22.9%)	30 (26.5%)
Swollen cervical veins, *n* (%)	19 (15.9%)	9 (18.7%)	24 (21.2%)
Edema of the lower extremities, *n* (%)	24 (20.1%)	17 (35.4%)	59 (52.2%)***#
Laboratory and instrumental assessment of congestion			
NT-proBNP, pg / ml, *Me* (Q_1; Q_3)	693.5 (341; 1,501)	957 (659; 1,727)***	1,252 (904; 2,146)***
Number of B-lines, *M* ± *SD*	16.5 ± 11.9	21.9 ± 15.6**	21.8 ± 11.1**
Liver density, кPa, *M* ± *SD*	5.6 ± 2.2	7.6 ± 4.4	7.8 ± 4.6
CAP, dB / m, *M* ± *SD*	454.0 ± 74.7	417.4 ± 81.1***	415.8 ± 80.0***
Active resistance, Om, *M* ± *SD*	42.8 ± 9.2	38.4 ± 11.6**	38.4 ± 9.8**
Reactance, Om, *M* ± *SD*	456.0 ± 75.1	419.2 ± 81.7**	417.7 ± 80.5**

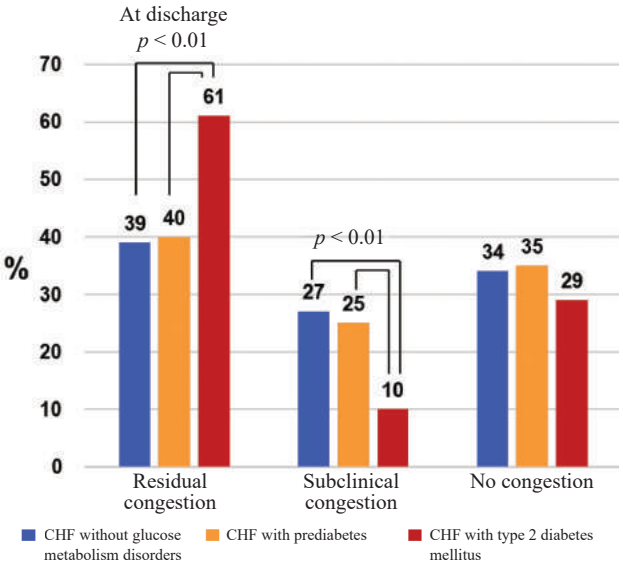

Fig. 2. Frequency of congestion at discharge in the studied groups

DISCUSSION

CHF and DM are quite common diseases. In the general population, HF is associated with higher prevalence of T2DM compared to patients without HF [4–6]. In our study, the incidence of glucose metabolism disorders in patients hospitalized with ADHF was 57.5%, while prediabetes was detected in 17.1% of cases, and T2DM – in 40.4% of cases, which is consistent with the literature data. In the registers of hospitalized patients with HF in North America and Europe, the prevalence of T2DM is about 40–45% [7]. According to a large European registry, DM is diagnosed in 36% of outpatient patients with CHF [8], while among patients hospitalized for acute HF, DM is detected in < 50% [9].

In clinical studies of patients with CHF, the prevalence of T2DM was about 30%, regardless of the HF phenotype (i.e. HFrEF and HFpEF) [3, 5, 8, 10–16]. It is important to note that in patients with HF without DM, the risk of developing DM is higher and increases with increasing severity of HF and the use of loop diuretics [17]. In addition, newly diagnosed T2DM and prediabetes are often found in patients hospitalized with ADHF. In the PARAGON-HF study, which involved 4,796 patients with HFpEF, 50% of patients had T2DM, and 18% of patients had prediabetes, that is 2/3 of the patients had glucose metabolism disorders. In the PARADIGM-FH study, it was shown that among 8,274 patients with systolic HF, 35% had a history of T2DM. The examination conducted before the start of the study revealed an additional 13% of patients with newly diagnosed T2DM (HbA1c > 6.5%) and 25% with prediabetes (HbA1c 6.0–6.4%). That is, in 38% of patients who lived to HF with LVEF ≤ 40%, clinically significant glucose metabolism disorders (prediabetes and T2DM) were not detected in time [5].

Fluid volume overload and congestion remain common causes of hospitalizations with HF. Patients with DM have increased neurohumoral activation and changes in sodium absorption, which may predispose to congestion, cardiorenal syndrome, and decreased sensitivity to diuretics. Hyperglycemia in the context of DM causes increased regulation of sodium – glucose cotransporter-2, which leads to an

increase in sodium absorption by the proximal parts of the kidneys, an increase in the fluid volume, and a decrease in sensitivity to diuretics [18].

In the CHARM, DIG, and I-PRESERVE studies, greater frequency of symptoms and signs of congestion was shown in patients with DM. Despite the fact that the status of congestion was not directly studied in the GWTG-HF study, it revealed a more frequent need for mechanical ventilation and dialysis / ultrafiltration and deterioration of kidney function in patients with T2DM, which may indicate an increase in the fluid volume load. The SOLVD-Prevention study showed that patients with asymptomatic left ventricular systolic dysfunction and T2DM had a higher probability of disease progression to symptomatic HF than those without T2DM [19].

Our study shows that not only patients with T2DM, but also patients with prediabetes were characterized by significantly higher frequency of typical clinical symptoms and signs of CHF, such as wheezing in the lungs (69 and 77.1 versus 42%), orthopnea (69 and 72.9 versus 66.4%), swollen cervical veins (46 and 47.9 versus 38.7%), and edema of the lower extremities (92.9 and 85.4 versus 61.3%) compared to patients without glucose metabolism disorders, respectively. In addition, patients with glucose metabolism disorders were characterized by significantly more pronounced laboratory and instrumental signs of congestion. Thus, in patients with T2DM and prediabetes, in contrast to patients without glucose metabolism disorders, we detected significantly higher values of liver density (14.3 ± 10.8 kP and 14.3 ± 10.2 versus 10.6 ± 8.9 kPa, $p < 0.01$), CAP (256.9 ± 55.3 and 254.9 ± 51.4 versus 231 ± 72.1 dB/m, $p < 0.01$), the number of B-lines on lung ultrasound (36 ± 17.9 and 34.9 ± 15.4 versus. 31.4 ± 17, $p < 0.05$), and NT-proBNP (2,130 and 1,797 versus 1,700 pg / ml, $p < 0.05$) and significantly lower impedance values in BIVA (379.3 ± 73.9 and 383.9 ± 75.4 versus 405.2 ± 77.1, $p < 0.05$).

Patients with CHF and T2DM at discharge were characterized by significantly higher frequency of residual congestion (61%) and lower frequency of subclinical congestion (10%), compared to patients without glucose metabolism disorders (39% for residual congestion, 27% for subclinical congestion) and prediabetes (40% for residual congestion, 25% for subclinical congestion), respectively. There were no significant differences in the frequency of achieving euvolemia at discharge, depending on glucose metabolism disorders.

CONCLUSION

To assess congestion phenomena at discharge, it is necessary to use clinical and laboratory and instrumental assessments for patients with ADHF and glucose metabolism disorders. However, in patients with ADHF and prediabetes, it is preferable to focus on the laboratory and instrumental assessment of congestion, while in patients with ADHF and T2DM, both clinical and laboratory and instrumental assessment of congestion should be performed.

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Authors' contribution

Tolkacheva V.V. – conception and design, analysis and acquisition of the data, drafting of the manuscript. Diane M.L., Khutsishvili N.I., Nazarov I.S., Smirnov I.P. – collection and processing of the materials. Cabello Montoya F.E., Galochkin S.A. – analysis and acquisition of the data, drafting of the manuscript. Kobalava Zh.D. – conception and design.

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Mathematical modeling of physiological parameters in traumatic shock caused by lower limb blast injury

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ABSTRACT

The aim of this study was to apply integrative physiological mathematical models to simulate physiological parameters in traumatic shock caused by lower limb blast injury.

Materials and methods. At the first stage of mathematical modeling, we applied lumped parameter integrative physiological models, and at the second stage we used neural networks.

Results. We developed a clinical decision support system that allows to determine the intensity of blood loss in lower limb blast injuries according to physiological monitoring data.

Conclusion. The developed approaches make it possible to partially solve the problem associated with the impossibility of accumulating a sufficient amount of medical data for a specific person to create an adequate personalized clinical decision support system.

Keywords: mathematical modeling, traumatic shock, bleeding, clinical decision support systems

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Математическое моделирование физиологических показателей при травматическом шоке, вызванном взрывной травмой нижних конечностей

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РЕЗЮМЕ

Целью настоящего исследования является применение интегративных физиологических математических моделей для моделирования физиологических показателей при травматическом шоке, вызванном взрывной травмой нижних конечностей.

Материалы и методы. На первом этапе математического моделирования использовались интегративные физиологические модели с сосредоточенными параметрами, а на втором этапе – нейронные сети.

Результаты. Разработана система поддержки принятия врачебных решений, позволяющая по данным физиологического мониторинга определять интенсивность кровопотери при минно-взрывной травме нижних конечностей.

Заключение. Разработанные подходы позволяют частично решить проблему, связанную с невозможностью накопления достаточного количества медицинских данных для конкретного человека с целью создания адекватной персонализированной модели поддержки принятия врачебных решений.

Ключевые слова: математическое моделирование, травматический шок, кровотечение, системы поддержки принятия врачебных решений

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

Assessing the severity and predicting the development of traumatic shock in mine blast injuries is a relevant task of modern military science. Blast injury is a special type of gunshot injury, characterized by a combination of injuries resulting from direct or indirect exposure to an explosion. Blast injury requires a special approach to the assessment of the severity and the condition of the wounded, which is the key to the effectiveness of medical and evacuation measures [1].

The development of traumatic shock is determined by the type of injury, the volume of mechanical damage to tissues and organs, blood loss and hypovolemia,

pain intensity and, a body reaction to aggression, and the duration of the pathological condition. From the pathogenic point of view, traumatic shock is a severe multicomponent reaction of the body to severe mechanical damage and is identified by clinicians as the first stage of the so-called traumatic disease. The main pathogenic element of shock is generalized tissue hypoperfusion, which disrupts homeostatic mechanisms and leads to irreversible cellular damage. Tissue hypoperfusion entails the development of irreversible metabolic, biochemical, and enzymatic cellular disorders, and in the absence of adequate treatment – death [2].

The concepts of "severity of injury", "severity of damage", and "severity of condition" are interrelated, but are not synonymous. The severity of the damage depends on its location, the extent of the anatomical damage, and the functional significance of the affected organ or anatomical and functional area. The severity of the condition is associated with the severity of the injury and the severity of functional disorders, time that passed since the injury, the initial condition of the person, and the amount of medical care provided. Methods for assessing the severity of injury using combined approaches, including parameters of the injury severity (morphological signs) and parameters of the condition severity (functional signs), have proven to be extremely effective [3–5]. This article focuses on the assessment of functional signs, which implies further development of the methodology with additional criteria for assessing the injury severity.

Numerous classifications of acute blood loss with the development of shock ultimately come down to a discussion of the role of two components of impaired oxygen-carrying capacity of the blood. The first component is associated with impaired myocardial contractility due to several reasons: hypoxia, myocardial ischemia, the effect of myocardial depression factors of various etiologies, concomitant pathology, intensive care strategy used, etc. The second component which is most discussed and directly caused by blood loss is associated with primary circulatory system disorders due to deficient circulating blood volume (CBV); therefore, with the development of metabolic and microcirculatory disorders, it is called hypovolemic shock. However, the cause of shock due to acute blood loss is of great practical importance only in early stages of the process, since subsequently, due to the convergence of pathophysiological parameters, it loses its specificity associated with the etiological factor [6]. Based on the above, the use of mathematical modeling may be effective for solving problems in developing a clinical decision support system (CDSS) to assess the severity and predict the development of traumatic shock when monitoring the condition of a serviceman at the frontline stages of evacuation, as well as to develop activities for simulation training.

The aim of this study was to use integrative physiological mathematical models to simulate physiological parameters in traumatic shock caused by lower limb blast injury.

MATERIALS AND METHODS

To simulate physiological parameters in traumatic shock caused by lower limb blast injury, we used the Pulse Physiology Engine [7], a multi-platform universal human physiology simulator, modified for work. The system is used to enable accurate and consistent physiology simulation in real time. The structure of the developed engine includes the main core, which is the basic software that manages the engine components using interfaces. Engine components include verified models of physiological mechanisms and pharmacokinetic (pharmacodynamic) models. These models belong to the class of lumped parameter mathematical models and are based on ordinary differential equations (ODEs) taking into account feedback mechanisms.

Unlike systems in which lumped parameter models are typically used to model individual physiological functions and behaviors, the engine is used to examine the physiological state of the body based on physiological functions in each individual subsystem.

The cardiovascular subsystem includes the heart and blood vessels of pulmonary and systemic circulation, and the respiratory subsystem models various components of the airways. These two subsystems interact through the alveolar – capillary barrier to mediate gas exchange. The simulation involves diffusion due to partial pressure between liquid (blood) and gas (air). The result of the simulation is the pressure and volume values in the capillaries and airways. Feedback mechanisms occur through baroreceptors. The baroreceptor mechanism rapidly regulates blood pressure (BP) based on negative feedback. A drop in blood pressure is detected by baroreceptors and leads to an increase in heart rate (HR) and vascular resistance. These changes are needed to maintain constant blood pressure at rest by calculating the sympathetic (1) and parasympathetic (2) responses.

$$\eta_s(P_a) = [1 + P_a / P_{a,s})^v]^{-1}, \quad (1)$$

$$\eta_p(P_a) = [1 + P_a / P_{a,s})^{-v}]^{-1}, \quad (2)$$

where v – baroreceptor parameter, P_a – mean blood pressure, $P_{a,s}$ – fixed value of P_a. These values are then used to calculate changes in heart rate (HR) (3), elasticity (E) (4), systemic vascular resistance (R) (5) and compliance (C) (6).

$$dHR / dt = -\tau_{HR}^{-1} (-HR + \alpha_{HR}\eta_s(P_a) + \beta_{HR}\eta_p(P_a) + \gamma_{HR}), \quad (3)$$

$$dE / dt = -\tau_E^{-1} (-E + \alpha_E\eta_s(P_a) + \gamma_{HR}), \quad (4)$$

$$dE / dt = -\tau_R^{-1} (-R + \alpha_R\eta_s(P_a) + \gamma_R), \quad (5)$$

$$dC / dt = -\tau_C^{-1} (-R + \alpha_C\eta_s(P_a) + \gamma_C), \quad (6)$$

Here HR, E, R, and C are relative values of heart rate, elasticity, vascular resistance and compliance, respectively; α, β, γ – model parameters, τ – time parameters of the corresponding processes. These time-dependent changes are introduced into a model of the cardiovascular system by changing components with lumped parameters, scale factors determining vascular resistance, blood volume, and heart rate are defined.

In terms of mathematical modeling, the amount of physiological data generated is limited only by the variations of independent variables. Therefore, it is fundamentally possible to generate an arbitrarily large array of data for subsequent training of the CDSS model. The approach was tested by generating an array of data containing 10,000,000 records including changes in physiological parameters over 20 minutes: diastolic blood pressure, systolic blood pressure, heart rate, respiratory rate, blood oxygen saturation (SpO_2), temperature in lower limb blast injury accompanied by acute blood loss of varying intensity (the modeling step for the rate of blood loss from the lower limb is 10 ml / min). The total volume of generated data was 16.2 GB in CSV format.

RESULTS

The developed CDSS is a cyber physical system (CPS), which implies a set of physical processes and systems, computer and other devices, Internet resources and users coordinately interacting with one another through computer implementation of algorithms (protocols) aimed at solving a wide range of multi-purpose tasks in the field of network technologies. To visualize data in real time, software generating model signals was developed in accordance with the specified initial conditions of the mathematical model (Fig. 1).

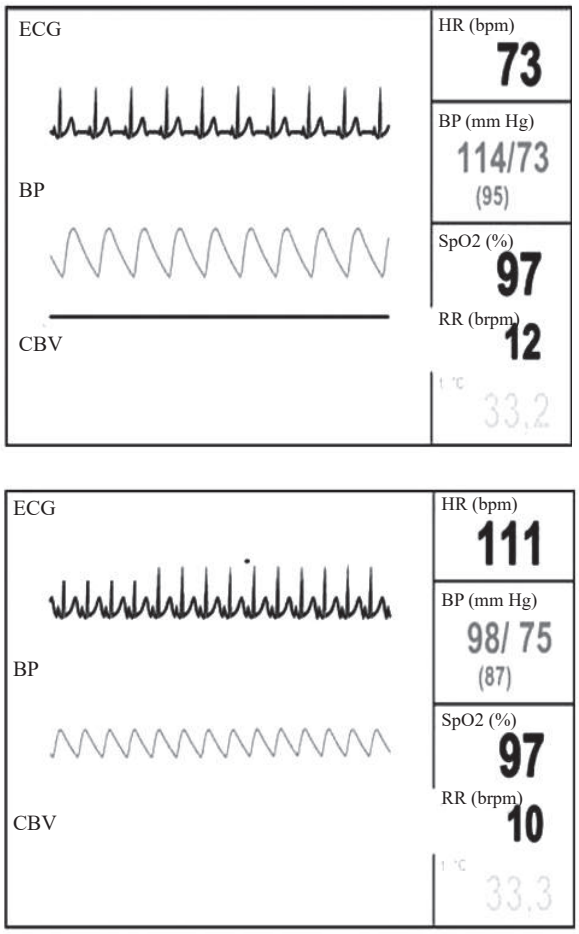

Fig. 1. An example of the results of mathematical modeling of physiological parameters in traumatic shock caused by lower limb blast injury: *a* is the initial state; *b* is the state of compensated traumatic shock

The physiological parameters obtained as a result of solving the direct problem of mathematical modeling represent an array of data in which variations of physiological parameters in dynamics are compared, allowing to identify the most likely combination of vital signs with different blood loss intensity.

The development of the final CDSS includes several stages: 1) building a personalized database (DB) of the examined persons based on the measurement of physiological parameters, modeling a number of physiological conditions in both normal and critical conditions on the basis of a computer simulator of human physiological functions used in the system; 2) training a classifier used in the system that can determine the nature of a person's pathological condition by comparing the flow of measured physiological parameters of a person with a set of records in a personalized database.

The priority task of the CDSS is to monitor data using sensors of vital physiological parameters, create the medical data flow in an established format using software, and use a software component to compare the data flow with a personalized database in order to detect a critical condition (CC). If a critical condition is detected, its type is determined (the CC is indexed), and information about the CC and its type is sent to the person responsible for making a clinical decision.

The general diagram of the developed CDSS is shown in Fig. 2, 3. In this system, module 1 is implemented on the basis of the Pulse Engine software package, which generates a personalized object database. Module 2, which classifies object states, is implemented as a set of deep neural networks trained on the object database. CDSS includes the following interconnected structural elements: array of X vectors of personalized database obtained by measuring state parameters (Fig. 2).

Module 2 monitors the functional states of an object by comparing the input stream of measured physiological parameters of the object, detecting CC and indexing it. In the system under development, module 2 is implemented in the form of neural systems. The training of neural networks, which makes it possible to determine the CC of an object, is carried out using a set of CC from a personalized object database generated by module 1.

The input array consists of X vectors of the patient's primary data. Vector X has the following structure: $X = (X_1, X_2)$. Here X_1 is a vector of anthropometric parameters, and X_2 is a vector of physiological parameters of the patient.

The components of vector X_1 include such parameters as height, weight, gender, baseline values of vital signs at rest and on exertion. If necessary, the list of input parameters can be significantly expanded. Currently, most of the input parameters (parameters of the endocrine system, hemostasis, nervous system, etc.) are recorded as average values.

The components of vector X_2 include heart rate (number of contractions / min); SpO_2, the normal level 95%; respiration rate (breaths / min); blood pressure (mm Hg).; physical activity; temperature (°C). Let us consider the structural elements of the CDSS presented in Fig. 2 in more detail.

The vector supplied to the input system (X) consists of the measured parameters of the patient's condition. The list of patient parameters can be adjusted depending on the specific conditions of applying the CDSS.

Module 1 generates a personalized patient database consisting of model vectors (Y) of the patient's condition in a given range of model parameters ($a = (a_1, \ldots, a_k)$). The Pulse Physiology Engine performs this function in the developed CDSS.

For a given set of parameters of model a and input vector X, module 1 generates a time series of vectors $Y(a, t)$ (the information flow of the object data) at the output. The time variable t with sample spacing δ is defined as the characteristic time of the modeled physiological process. For example, a step can be set to 1 minute for blood loss. You make the step δ "small" compared to its consequence, i.e. when the modeled process leads to a change in the state of an object (to a transition from a normal state to a critical one). For example, the time series appears when the process of blood loss does not immediately lead to the transition from a normal condition to a critical one (the effect is accumulated).

In Fig. 3, the "External Expert" block includes the function of configuring the CDSS, which consists in setting the vector of model parameters, $a = a_{(j)}$, at which module 1 generates a vector flow of vectors $Y_{(j)}(a_{(j)}, t)$, representing the j CC of the object . It will be designated as cr. j. Let us assume that the value $j = 1$ corresponds to the patient's CC, which occurs in blood loss at a rate of 10 ml / min.

Module 2. The neural network should perform the function of assessing the state, including the critical condition of the object according to (tested) input vector of the measured parameters of the object's state. The use of neural network machine learning algorithms makes it possible to turn from mathematically complex solutions of inverse problems for dynamical system through multiple integration to solving simple models with a known structure (weighting factors and activation functions). The input vector for the neural network is a time series of vectors $Y(a,t)$ generated by module 1. Accordingly, the output of the network will be a time series of vectors of the form $Z_{cr}(t_1) = (h_1(t_1), h_2(t_1), \ldots, h_R(t_1))$.

Here the time variable t_1 changes at a scale different from the time scale t set in module 1. Sample spacing Δ time t_1 is greater than the step δ of a physiological process, for example, blood loss, i.e. t_1 is a "slow" time compared to a "fast" time t.

The values $h_1(t_1), h_2(t_1), \ldots, h_R(t_1)$ – components of vector $Z_{cr}(t_1)$ – represent probabilities, for example, $h_1(t_1)$ – there is a probability that the patient is in CC 1 in the time interval $(t_1, t_1 + \Delta)$.

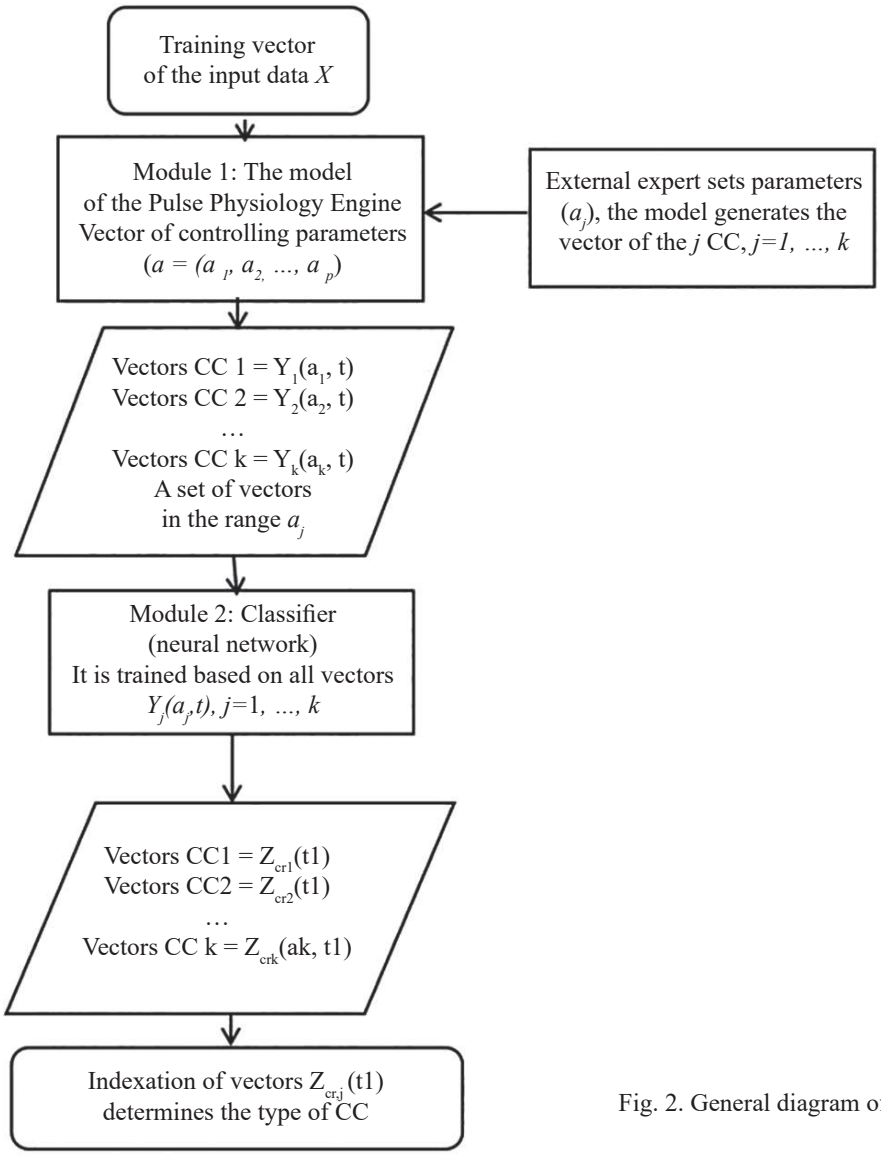

Fig. 2. General diagram of the clinical decision support system

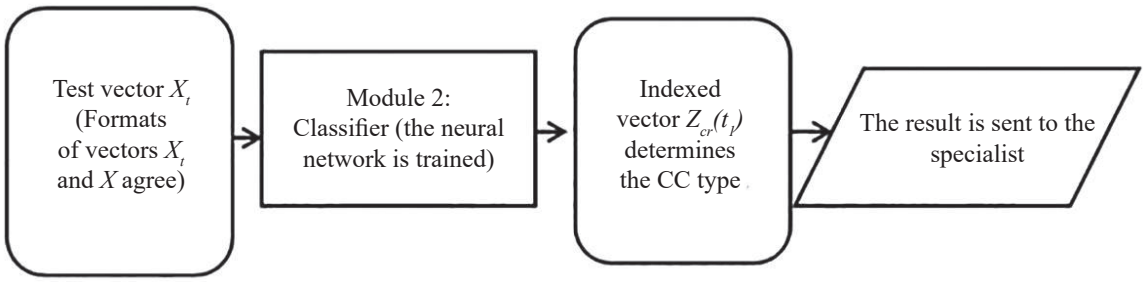

Fig. 3. Decision support system testing scheme

Neural networks are trained through operations with a personalized database object. Based on the results of the work of module 2, CDSS algorithms are obtained, which are "input – output" models. The input of the model consists of an array of data with vital parameters accumulated over a fixed period of time (60 seconds). At the output, the system forms a vector containing information about the state of the object and calculated physiological parameters that are highly informative content for medical specialists (rate of blood loss, volume of blood loss).

A software application in Python was developed to build the final model of the CDSS based on neural network algorithms. Using the annotated data set generated at the previous stage, several neural networks are built including long short-term memory (LSTM), Autoencoder, and convolutional neural network (CNN). The final CDSS algorithm performs the following functions: classification of states according to physiological monitoring (heart rate, systolic BP, diastolic BP, SpO_2, respiration rate, body temperature), restoration of the data array if some of the values are missing. If a patient is bleeding, the system will determine the rate of blood loss, the volume of blood loss and the time of blood loss onset. Several deep neural network architectures have been proposed:

1) LSTM network whose main task is to classify the physiological state. It belongs to recurrent neural networks capable of learning long-term dependencies. LSTM is specifically designed to detect events in a changing process mode.

2) Autoencoder network whose main task is to recreate a data array if there are gaps and predict changes in the trajectory of parameters.

3) CNN network whose main task is to calculate the rate of blood loss, the volume of blood loss, and the time of the bleeding onset. A convolutional neural network is a specialized artificial neural network architecture that promotes efficient image recognition. The developed algorithm makes it possible to calculate a neural network using a sample of simulated parameters in Python. When assessing the quality of the modeled structure, final accuracy was 0.992 (99.1%) and 0.997 (98.9%) according to MSE and MAE, respectively.

CONCLUSION

The developed approaches make it possible to partially solve the problem associated with the inability to accumulate a sufficient amount of medical data for a particular person to create an adequate personalized model to support clinical decision-making. In the future, the proposed algorithm will make it possible to create a hardware solution for assessing the need for medical care in case of lower limb blast injury, which is especially important at the pre-hospital phase and in emergency care during medical evacuation. Criteria for assessing the injury severity remain an important problem. The complexity of including these parameters in the mathematical model does not allow to use the developed methodology alone. In addition, to confirm the results of mathematical modeling, a set of clinical data is required to verify the model.

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Proinflammatory biomarkers and platelet aggregation activity in patients with coronary artery disease

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ABSTRACT

Aim. To determine concentrations and identify the relationship of biomarkers (endocan / cell-specific molecule-1, fatty acid binding protein 4 (FABP 4), placental growth factor (PlGF), oncostatin M), with parameters of collagen-induced platelet aggregation in patients with coronary artery disease (CAD).

Materials and methods. In patients with CAD (n = 51), serum levels of endocan, FABP 4, PlGF, oncostatin M, and platelet aggregation indices (collagen at concentrations of 2 and 10 mmol / l) were determined. Patients were divided into groups with and without high residual platelet reactivity (HRPR). Correlation coefficients between concentrations of proinflammatory biomarkers and platelet aggregation indices were determined in patients of both groups.

Results. In patients with HRPR, the concentrations of endocan and PlGF were significantly higher, and the concentrations of FABP4 and oncostatin M were lower than in the first group. In patients with HRPR, a correlation was found between the concentration of endocan and the degree of platelet aggregation in the presence of 2 mmol / l of collagen ($\rho = 0.48$; $p = 0.01$), between the concentration of PlGF and the degree of platelet aggregation in the presence of 10 mmol / l of collagen ($\rho = 0.58$; $p = 0.01$), as well as between the concentration of FABP 4 and the size of aggregates at both collagen concentrations ($\rho = 0.42$; $p = 0.03$) and ($\rho = 0.70$; $p = 0.01$) and the degree of platelet aggregation in the presence of 10 mmol / l of collagen ($\rho = 0.43$; $p = 0.01$).

Conclusion. In all examined CAD patients, regardless of the residual platelet reactivity, the levels of endocan and FABP 4 increased compared to the reference values. In patients with HRPR, the content of parameters (endocan, PlGF) contributing to plaque growth was elevated, and in patients without HRPR, the levels of platelet-activating factors (FABP 4, oncostatin M) were increased, which determines a personalized approach to prescribing therapy for these groups of patients. In patients with CAD, platelet aggregation indices were associated with concentrations of proinflammatory biomarkers (endocan, PlGF, and FABP 4), which contribute to the development of endothelial dysfunction.

Keywords: aggregation, platelet, collagen, coronary artery disease, biomarkers

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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 Cancer Research Institute of Tomsk NRMC (Protocol No. 139 of 18.11.2015).

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Провоспалительные биомаркеры и агрегационная активность тромбоцитов у пациентов с ишемической болезнью сердца

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РЕЗЮМЕ

Цель: определить концентрации и выявить связь биомаркеров (эндокана-1, белка, связывающего жирные кислоты, 4 (FABP 4), плацентарного фактора роста (PLGF), онкостатина М с показателями коллаген-индуцированной агрегации тромбоцитов у пациентов с ишемической болезнью сердца (ИБС).

Материалы и методы. У пациентов с ИБС (n = 51 человек) определяли сывороточную концентрацию эндокана-1, уровень FABP 4, PLGF, онкостатина М и показатели агрегации тромбоцитов (коллаген в концентрации и 10 мкмоль/л). Пациенты разделены на группы с высокой остаточной реактивностью тромбоцитов (ВОРТ) и без нее. Определялись коэффициенты корреляции между концентрациями провоспалительных биомаркеров и показателями агрегации тромбоцитов.

Результаты. У всех обследованных пациентов с ИБС вне зависимости от остаточной реактивности тромбоцитов повышена концентрация эндокана-1 и FABP 4 по сравнению с референсными значениями. У пациентов с ВОРТ концентрация эндокана-1 и PLGF значимо выше, а концентрации FABP 4 и онкостатина М ниже, чем в первой группе. У пациентов с ВОРТ выявлена корреляция между концентрацией эндокана-1 и степенью агрегации тромбоцитов в присутствии 2 мкмоль/л коллагена (ρ = 0,48; p = 0,01), концентрацией PLGF и степенью агрегации в присутствии 10 мкмоль/л коллагена (ρ = 0,58; p = 0,01), а также между концентрацией FABP 4 и размерами агрегатов при обеих концентрациях коллагена (ρ = 0,42; p = 0,03) и (ρ = 0,70; p = 0,01) и со степенью агрегации в присутствии 10 мкмоль/л коллагена (ρ = 0,43; p = 0,01).

Заключение. У пациентов с ВОРТ увеличено содержание факторов (эндокан-1, PLGF), способствующих росту бляшки, а у пациентов без таковой – факторов активации тромбоцитов (FABP 4, онкостатин М), что обусловливает персонифицированный подход к назначению терапии для больных этих групп. У пациентов с ИБС показатели агрегации тромбоцитов ассоциированы с концентрациями провоспалительных биомаркеров, которые способствуют развитию эндотелиальной дисфункции.

Ключевые слова: агрегация, тромбоцит, коллаген, ишемическая болезнь сердца, биомаркеры

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

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INTRODUCTION

Despite the ongoing therapeutic and preventive measures, coronary artery disease (CAD) remains the most common cardiovascular pathology in Russia. High residual platelet reactivity (HRPR) in the context of broad-spectrum antiplatelet therapy in patients is associated with the development of ischemic complications, which has been proven by numerous studies and data of the meta-analysis [1–3]. The main causes of CAD development are coronary stenosis and microvascular endothelial dysfunction [4]. It is known that endocan acts as a marker of endothelial dysfunction; its release is one of the earliest pathogenetic events observed in atherosclerosis, thrombosis, and chronic heart failure [5]. Researchers discuss the role of platelets as primary factors in the pathogenesis of cardiovascular diseases through modulation of immune responses, which are currently considered to be the driving force in atherogenesis. Proinflammatory cytokines promote the expression of enzymes that lead to atherosclerotic plaque destabilization (APD), followed by plaque rupture [6]. After the atherosclerotic plaque rupture, macrophages and smooth muscle cells produce a tissue factor that triggers a coagulation cascade leading to thrombosis. Platelets are activated due to their interaction with collagen in the extracellular matrix of the plaque. It has been shown that oncostatin M can contribute to the development of atherosclerosis and vascular destabilization [7, 8]. Quite often, thrombosis develops at the site of hemodynamically insignificant stenosis in the coronary arteries [4]. Thus, the development of arterial and venous thrombosis was associated with an increase in the content of placental growth factor (PlGF) in patients with antiphospholipid syndrome [9].

Biochemical markers are the most important tool for timely diagnosis and prediction of a risk of developing cardiovascular pathology [10]. Both clinical and experimental data have shown that fatty acid binding protein type 4 (FABP4) plays an essential role in the development of atherosclerosis and CAD, and it is directly associated with left ventricular hypertrophy and cardiac dysfunction [11, 12]. The multi-marker approach should more accurately reflect the key links in the pathogenesis and biochemical interactions compared to the use of individual markers. In this regard, there is a growing interest in the development and use of combinations of biomarkers.

In this work, concentrations of proinflammatory biomarkers (endocan, FABP4, PlGF, and oncostatin M) will be determined in patients with CAD in the presence and absence of HRPR, and their relationship with the parameters of collagen-induced platelet aggregation will be evaluated. Knowledge in this area of research is relevant for both clinical and fundamental medicine.

The aim of the study was to determine concentrations and identify the relationship of proinflammatory biomarkers (endocan / cell-specific molecule-1, FABP 4, PlGF, oncostatin M) with parameters of collagen-induced platelet aggregation in patients with CAD.

MATERIALS AND METHODS

A single-stage, simple comparative study was conducted. The study included 51 patients with CAD (75% of them were men). The recruitment of patients was performed at the Cardiology Research Institute in accordance with the principles of the Declaration of Helsinki. The study included patients aged 41–83 years. All examined patients received combined basic therapy in accordance with the guidelines for CAD treatment.

Criteria for inclusion in the study: stable CAD and continuous antiplatelet therapy for 6 months (cardiomagnil, 75mg). Exclusion criteria from the study: combined antiplatelet therapy, acute vascular complications within less than 6 months before the inclusion in the study; severe concomitant pathology; clinical and laboratory signs of acute inflammation; refusal to participate in the study.

To obtain blood serum, whole peripheral blood of patients stabilized with 3.8% sodium citrate was centrifuged at 3,000 rpm for 15 minutes at room temperature. The obtained serum samples were stored at –40 °C. Concentrations of proinflammatory biomarkers endocan, FABP 4, PlGF, and oncostatin M were determined in the samples. The study was conducted at the Medical Genomics Collective Use Center of Tomsk NRMC by the multiplex immunoassay (Luminex FLEXMAP 3D platform, USA) using the Human Cardiovascular Disease Panel 1 (Merck KGaA, Darmstadt, Germany). The parameters of collagen-induced platelet aggregation were determined by the Born's method in the modification of Z.A. Gabbasov on the two-channel laser analyzer (220 LA Research and Production Company Biola, Russia).

To isolate the platelet suspension, blood was drawn into test tubes with 3.8% sodium citrate as an anticoagulant. All samples were examined using a standard approach (hereinafter referred to as Method 1), as well as by the authors' own patented

methodology (hereinafter referred to as Method 2). In Method 1, the aggregation inductor (collagen) was introduced once at a final concentration of 2 mmol / l at 10 seconds of measurement. According to Method 2, aggregation parameters were determined 5 times when adding 2 mmol / l of collagen at 10 seconds, 1, 2, 3, and 4 minutes of the platelet aggregation evaluation with the final concentration of collagen in the sample being 10 mmol / l [3].

The degree of platelet aggregation (%) was evaluated by the maximum amount of light transmission, and the size of the aggregate was evaluated by the average aggregate size curve (rel. units). The examined patients were divided into two groups. Group 1 ($n = 27$) included patients who had no HRPR. The degree of collagen-induced aggregation measured by both methods did not exceed 60%, and the size of aggregates was less than 4 rel. units. Group 2 included patients ($n = 24$) whose degree of platelet aggregation was ≥ 60%, and the size of aggregates was ≥ 4 rel. units according to at least one of the methods. The parameters were determined by both methods. According to [3], patients of the second group corresponded to a group of individuals with HRPR.

Statistical data processing was carried out using the SPSS (version 19) and STATISTICA 10.0 software packages. To assess the distribution of quantitative variables, the Shapiro – Wilk test was used. The statistical significance of differences for two independent samples was assessed using the Mann – Whitney *U*–test. For comparison with a given value of one parameter, the Student's *t*–test was used, after applying the Box – Cox data transformation method. The Spearman's rank correlation coefficient (ρ) was used to evaluate the relationships between the variables. The data were presented as the median and the interquartile range ($Me\ (Q_1; Q_3)$). The results of the comparative and correlation analysis were considered statistically significant at $p < 0.05$.

RESULTS

Platelet aggregation indices in patients assigned to groups 1 and 2 significantly differed, according to [3]. The groups of patients were comparable by gender, age, duration of CAD, the number of prior myocardial infarctions, and concomitant pathology. Drug therapy did not differ significantly between the groups of patients (aspirin, calcium antagonists, statins, diuretics, nitrites, angiotensin II receptor blockers, angiotensin converting enzyme inhibitors).

The concentrations of endocan and FABP 4 in both groups of patients significantly exceeded the maximum reference values. Thus, the concentration of FABP 4 increased by 10 times compared to the reference values (Table). The concentrations of endocan and PlGF were significantly increased in the group of patients with HRPR compared to patients of group 1, and the levels of FABP 4 and oncostatin M, on the contrary, were reduced (Table).

Table

Concentrations of biomarkers in patients with coronary artery disease and their reference values

Parameter	Group 1, $n = 27$, Me (Q_1; Q_3)	Group 2, n = 24, $Me\ (Q_1; Q_3)$	p value between the groups	Reference values Mean (Min.; Max.)
Endocan, ng / ml	2.13 ($p = 0.02$) (1.89; 2.66)	2.61 ($p = 0.01$) (2.07; 2.96)	0.03	0.94 (0.65; 1.72)
PlGF, pg / ml	4.22 (2.86; 12.19)	9.51 (5.48; 18.73)	0.01	8.72 (0.0; 39.98)
FABP 4, ng / ml	68.74 ($p = 0.01$) (45.60; 75.44)	51.52 ($p = 0.01$) (25.59; 55.47)	0.02	5.34 (0.0; 11.83)
Oncostatin M, pg / ml	26.12 (5.02;45.68)	14.93 (2.98; 28.45)	0.04	22.73 (4.07; 53.83)

Note: p is the level of significance of differences between the groups of CAD patients and in comparison with the reference values.

The correlation analysis revealed the following. In group 1 (patients without HRPR), a positive correlation was found between the concentration of PlGF and the size of aggregates according to both aggregation methods ($r = 0.39$; $p = 0.01$) and ($r = 0.62$; $p = 0.02$). In addition, a correlation was found between the concentration of oncostatin M and the degree of aggregation determined in the presence of 10 mmol / l of collagen ($r = 0.82$; $p = 0.01$).

In group 2 (patients with HRPR), correlations were found between the concentration of endocan and the degree of aggregation determined in the presence of 2 mmol / l of collagen ($r = 0.48$; $p = 0.01$), as well as between the concentration of PlGF and the degree of aggregation measured in the presence of 10 mmol / l of collagen ($r = 0.58$; $p = 0.03$). In addition, correlations were found between the concentration of FABP 4 and the size of aggregates measured in the presence of

two concentrations of collagen (r = 0.42; p = 0.04), (r = 0.70; p = 0.01), as well as the degree of aggregation determined in the presence of 10 mmol / l of collagen (r = 0.43; p = 0.01).

DISCUSSION

The present work was an open, single-center, cross-sectional study. In the present study, we found that in patients of group 2 (with HRPR), the concentration of endocan and PlGF increased, which can contribute to the growth of atherosclerotic plaques, and in patients of group 1 (without HRPR), FABP 4 and oncostatin M, factors contributing to the activation of platelets, were elevated. In addition, we showed that in patients with CAD, platelet aggregation indices are associated with concentrations of proinflammatory biomarkers (endocan, PlGF, and FABP 4), which may contribute to the development of endothelial dysfunction.

In the present study, the levels of endocan and FABP 4 were significantly increased in patients with CAD in both groups compared to reference values, which indicates the development of endothelial dysfunction in patients with CAD [13]. As is known, endothelial dysfunction is one of the earliest pathogenetic events in the development of atherosclerosis, hypertension, and thrombosis [14]. An increase in the level of endocan, which is a potential marker of inflammation and cardiovascular diseases [6, 15], found in this study, confirms this theory. In addition, the revealed correlation between the concentration of endocan and platelet aggregation in patients with HRPR (group 2) indicates endothelial dysfunction leading to increased thrombosis. Platelet activation products promote endocan release by endothelial cell culture *in vitro*, which was pronounced in patients with transfusion complications [5].

A ten-fold increase in the concentration of FABP 4 in patients of both groups confirms the proatherogenic and prothrombotic effects of this marker. FABP 4 has been shown to mediate inhibition of the peroxisome proliferator-activated receptor (PPAR)γ [16]. The latter, in turn, inhibits the activation of platelets and the release of active mediators from them [17]. FABP 4 plays an important role in the development of atherosclerosis and CAD and is associated with left ventricular hypertrophy and cardiac dysfunction [11, 12]. The prothrombotic role of FABP 4 in this study is also confirmed by the revealed correlations between its concentration and the size of aggregates in patients with HRPR.

In the group of patients with HRPR, an elevated concentration of PlGF was found compared to group 1. PlGF promotes neoangiogenesis in CAD, which is considered as an adaptive response aimed at improving perfusion of the ischemic myocardium by increasing the number and size of collateral arteries [9, 18].

At the same time, the study established correlations of PlGF with platelet aggregation parameters in both groups. PlGF is a placental growth factor capable of stimulating angiogenesis and inducing atherosclerosis by binding and activating its membrane-bound receptor, soluble fms-like tyrosine kinase-1. Expression of PlGF in atherosclerotic lesions activates monocytes and macrophages, which subsequently produce inflammatory and angiogenic mediators, leading to an increasing risk of plaque rupture. Conversely, inhibition of PlGF reduces the size of atherosclerotic plaques [8]. The development of thrombosis is associated with an increase in the content of PlGF in patients with antiphospholipid syndrome [9]. The relationship between platelet aggregation parameters and PlGF is shown in women with preeclampsia [19], however, the molecular mechanisms that may underlie this relationship are unknown and require further study.

It is possible that the associations of platelet aggregation parameters in CAD patients of group 1 with the levels of oncostatin M and PlGF revealed in this study prove the modulating effect of platelets on the inflammatory response in the endothelium.

In this study, a correlation was established between the concentration of oncostatin M and the degree of platelet aggregation in group 1. Oncostatin M is known to contribute to the development of atherosclerosis and vascular destabilization [20, 21]. In addition, oncostatin M is considered as a megakaryocyte colony-forming factor that promotes thrombocytopoiesis [7]. There is evidence that under the influence of oncostatin M, activation of the signal transducer and activator of transcription (STAT)3 occurs, which plays an important role in collagen-mediated aggregation [8].

The role of platelets in the atherosclerosis and pathogenesis of cardiovascular diseases is very significant, since platelets, in addition to their contribution to thrombosis and hemostasis, modulate inflammatory and immune responses [22]. One of the first signals for platelet activation is collagen, the main protein of connective tissue that is exposed when a vessel is damaged. In addition to vascular damage, various proinflammatory mediators activate platelets

[11, 21]. The study revealed multiple correlations between platelet aggregation and the levels of serum biomarkers of cardiovascular diseases. In this study, it was revealed that the levels of endocan and FABP 4 were increased in patients of both groups, which points to the possible inflammatory damage of the vascular endothelium and platelet activation. At the same time, the differences in the concentration of some biomarkers were found in patients of both groups. Thus, in patients without HRPR, the concentrations of FABP 4 and oncostatin M were elevated, and in patients with HRPR, the concentrations of endocan and PlGF were increased. Based on this, it can be assumed that in group 1, the increase in thrombus formation is mainly associated with platelet activation, and in group 2, it may be associated with plaque growth. All of the above creates opportunities for a personalized approach to the prevention and treatment of patients with CAD. In particular, antiplatelet therapy requires certain adjustments in the group of patients with HRPR, namely, prescription of additional anti-atherosclerotic drugs or an increase in the dose of antiplatelet agents.

CONCLUSION

It was found that in patients with CAD, the levels of proinflammatory biomarkers (endocan 1 and FABP 4) were elevated compared to the maximum reference values. It was revealed that the concentrations of endocan 1 and PlGF were significantly increased in patients with collagen -induced HRPR. The study revealed the presence of correlations between the increased size of aggregates, the degree of platelet aggregation and the concentrations of proinflammatory biomarkers (endocan 1, PlGF, and FABP 4). At the same time, in patients with HRPR, the concentrations of endocan 1 and PlGF, contributing to plaque growth, were increased, and in patients without HRPR, platelet activation factors FABP 4 and oncostatin M were elevated, which determines various adjustments in therapy for patients of these groups.

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Authors' contribution

Petrova I.V., Kovalev I.V. – critical revision of the manuscript for important intellectual content, approval of the manuscript for publication. Trubacheva O.A., Vasiliev V.N. – conception and design, interpretation and analysis of the data, drafting of the manuscript. Yakimovich I.Yu., Kologrivova I.V. – justification of the manuscript. Trubacheva O.A., Schneider O.L – carrying out of the experiment.

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Phenotypes of the no-reflow phenomenon during percutaneous coronary interventions in myocardial infarction

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ABSTRACT

Aim. Using the cluster analysis, to determine and describe clinical and pathogenetic phenotypes of the coronary microvascular obstruction phenomenon (CMVO) that occurs during percutaneous coronary interventions (PCI) in patients with myocardial infarction (MI).

Materials and methods. The study included 190 patients with CMVO that occurred during PCI for type 1 MI: 137 (72%) men, 53 (28%) women, the median age was 64 [56; 70] years. The study was conducted in 2013–2020. CMVO criteria: blood flow < 3 points in the infarct-related artery (IRA) according to the TIMI flow grade (TFG); perfusion < 2 points according to the Myocardial Blush Grade; ST segment resolution < 70%. ST-elevation MI (STEMI) was found in 170 patients (89%). Primary PCI was noted in 127 (67%) cases. Nine patients (4.7%) died. Phenotyping was performed using the expectation – maximization (EM) algorithm.

Results. Three phenotypes were identified in a ratio of 56% (n = 106) / 27% (n = 52) / 17% (n = 32). The values of the parameters are the following, respectively: age 62 [54; 67] / 73 [67; 79] / 59 [50; 65] years; women 8 (8%) / 39 (77%) / 6 (19%); STEMI 102 (96%) / 43 (83%) / 25 (78%); thrombolysis 46 (43%) / 6 (12%) / 11 (34%); class 1 [1; 2] / 2 [1; 4] / 2 [2; 2] acute heart failure; platelet-to-lymphocyte ratio 110 [78; 153] / 106 [85; 132] / 132 [100; 182]; glucose at admission 8.0 [6.9; 9.6] / 11.1 [8.8; 15.2] / 7.5 [6.1; 8.1] mmol / l; total cholesterol 4.7 [4.2; 5.4] / 5.3 [3.7; 6.2] / 5.1 [4.5; 6.2] mmol / l; glomerular filtration rate according to CKD-EPI 77 [64; 88] / 58 [46; 74] / 81 [64; 88] ml /min / 1.73m^2; Syntax Score 15 [10; 21] / 20 [14; 26] / 8 [5; 10]; Syntax Score in the IRA 9 [8; 15] / 12 [7; 16] / 6 [3; 7]; coronary collaterals according to Rentrop: grade 0 [0; 1] / 0 [0; 1] / 0 [0; 0]; thrombosis of the IRA according to the TIMI thrombus grade 5 [5; 5] / 5 [3; 5] / 1 [0; 2]; TFG 0 [0; 0] / 0 [0; 1] / 2 [2; 3]; aspiration thrombectomy 30 (28%) / 7 (13%) / 4 (13%); IRA diameter 3.5 [3.0; 3.5] / 3.0 [2.8; 3.5] / 3.5 [3.0; 3.5] mm; balloon angioplasty 99 (93%) / 45 (87%) / 16 (50%); PCI of 2 or more arteries 0 (0%) / 4 (8%) / 3 (9%). Deaths – 2 (1.9%), 7 (13.5%), and 0 (0%) patients, respectively (p = 0.002, $\chi 2$ Pearson).

Conclusion. Three phenotypes were identified. Phenotype 1: severe IRA thrombosis, mostly men, moderate atherosclerotic lesions. Phenotype 2: mostly elderly women, high hyperglycemia, severe atherosclerotic lesions, severe AHF, impaired renal function, IRA thrombosis. Phenotype 3: mostly men, minor changes in the coronary arteries, absence of significant thrombosis and preserved blood flow in the IRA before PCI, elevated levels of inflammatory markers and total cholesterol.

Keywords: no-reflow phenomenon, myocardial infarction, percutaneous coronary intervention, cluster analysis, classification

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Privolzhsky Research Medical University (Protocol No. 5 of 08.04.2022).

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Фенотипы синдрома коронарной микрососудистой обструкции (no-reflow), развивающегося в ходе выполнения чрескожных коронарных вмешательств при инфаркте миокарда

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РЕЗЮМЕ

Цель: определить и охарактеризовать клинико-патогенетические фенотипы феномена коронарной микрососудистой обструкции (КМСО), возникающего при выполнении чрескожных коронарных вмешательств (ЧКВ) у пациентов с инфарктом миокарда (ИМ), используя метод кластеризации.

Материалы и методы. В исследование включены 190 больных с КМСО в ходе ЧКВ при ИМ I типа, в том числе 137 (72%) мужчин, 53 (28%) женщины. Медиана возраста – 64 [56; 70] года. Исследование проведено в 2013–2020 гг. Критерии КМСО: кровоток < 3 баллов в инфаркт-ответственной артерии (ИОА) по TIMI flowgrade (TFG); перфузия < 2 баллов по Myocardial blush grade; резолюция сегмента ST < 70%. ИМ с подъемом ST (ИМпST) у 170 больных (89%). Первичное ЧКВ наблюдалось в 127 (67%) случаях. Скончались 9 пациентов (4,7%). Фенотипирование осуществлялось с помощью алгоритма кластеризации (expectation-maximization – EM).

Результаты. Выявлены три кластера в соотношении 56% (n = 106) / 27% (n = 52) / 17% (n = 32). Значение параметров, соответственно: возраст 62 [54; 67] / 73 [67; 79] / 59 [50; 65] года; женщины 8 (8%) / 39 (77%) / 6 (19%); ИМпST 102 (96%) / 43 (83%) / 25 (78%); тромболитическая терапия 46 (43%) / 6 (12%) / 11 (34%); острая сердечная недостаточность 1 [1; 2] / 2 [1; 4] / 2 [2; 2] класса; отношение тромбоцитов к лимфоцитам 110 [78; 153] / 106 [85; 132] / 132 [100; 182]; глюкоза при поступлении 8,0 [6,9; 9,6] / 11,1 [8,8; 15,2] / 7,5 [6,1; 8,1] ммоль/л; общий холестерин 4,7 [4,2; 5,4] / 5,3 [3,7; 6,2] / 5,1 [4,5; 6,2] ммоль/л; скорость клубочковой фильтрации по CKD-EPI77 [64; 88] / 58 [46; 74] / 81 [64; 88] мл/мин/1,73 м^2; SyntaxScore 15 [10; 21] / 20 [14; 26] / 8 [5; 10] баллов; Syntax Score в ИОА 9 [8; 15] / 12 [7; 16] / 6 [3; 7] баллов; коллатерали по Rentrop 0 [0; 1] / 0 [0; 1] / 0 [0; 0] степени; тромбоз ИОА по TIMI thrombus grade 5 [5; 5] / 5 [3; 5] / 1 [0; 2] степени; TFG 0 [0; 0] / 0 [0; 1] / 2 [2; 3] степени; аспирационная тромбэктомия 30 (28%) / 7 (13%) / 4 (13%); баллонная ангиопластика 99 (93%) / 45 (87%) / 16 (50%); диаметр ИОА 3,5 [3,0; 3,5] / 3,0 [2,8; 3,5] / 3,5 [3,0; 3,5] мм; ЧКВ двух и более артерий 0 (0%) / 4 (8%) / 3 (9). Смертельные исходы – 2 (1,9%), 7 (13,5%) и 0 (0%) пациентов соответственно (p = 0,002; χ2-Пирсона).

Заключение. Определены три фенотипа. Фенотип 1: выраженный тромбоз ИОА, преимущественно мужчины, умеренное атеросклеротическое поражение. Фенотип 2: преимущественно женщины старческого возраста, высокая гипергликемия, выраженное атеросклеротическое поражение, тяжелая сердечная недостаточность, нарушенная функция почек, тромбоз ИОА. Фенотип 3: преимущественно мужчины, незначительные изменения коронарных артерий, отсутствие значимого тромбоза и сохраненный кровоток в ИОА до ЧКВ, повышенные уровни маркеров воспаления и общего холестерина.

Ключевые слова: инфаркт миокарда, коронарная микрососудистая обструкция, no-reflow, чрескожное коронарное вмешательство, кластеризация, фенотипирование, клинико-патогенетические фенотипы

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

Источник финансирования: работа выполнена в рамках программы «Приоритет 2030».

Соответствие принципам этики. Все пациенты подписали информированное согласие на проведение исследования. Исследование одобрено локальным этическим комитетом ФГБОУ ВО «ПИМУ» Минздрава России (протокол № 5 от 08.04.2022).

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INTRODUCTION

Percutaneous coronary intervention (PCI) is the basic procedure for reperfusion in myocardial infarction (MI). In 5–10 % of cases, blood flow restoration in the infarct-related artery (IRA) does not lead to sufficient perfusion of the myocardium due to coronary microvascular obstruction (CMVO, no-reflow phenomenon) [1, 2]. CMVO is associated with increased in-hospital mortality and worse short-term and long-term survival rates [2].

It is worth noting that the data accumulated for the last years concerning risk factors and prognosis of CMVO evidence of advances in the medical science; however, the issue of developing new effective approaches to CMVO treatment is still unresolved. It is likely due to the multifactorial nature of the pathogenesis of this condition and clinical diversity of patient groups having this complication [3]. There are several mechanisms leading to CMVO; they can occur simultaneously and vary in different patients [4]. The existing data suggest that the risk of CMVO development is associated with the severity of IRA thrombosis, plaque features, severity and intensity of MI, systemic inflammations, carbohydrate metabolism disorders, and reperfusion features [5].

A rational approach to effective treatment of CMVO could be division of all patients with this complication into groups (clusters) with further identification of the leading pathogenetic mechanism in each group and determination of an appropriate therapy target. Currently only one pathogenetic classification of CMVO is known that distinguishes the following mechanisms: 1) microthromboembolism; 2) ischemic injury; 3) reperfusion injury; 4) individual susceptibility [3]. It should be noted that this classification was made up empirically and for the following 13 years, no effective therapeutic algorithm has been developed on its basis. The reason for such an ineffective empirical approach may be an impossibility to correlate scattered theoretical data concerning the pathogenesis of CMVO with clinically available markers.

To create an objective and practically-oriented classification, mathematical methods, including different clustering techniques, can be used. To date such approaches to resolving the issue of CMVO have not been used.

The aim of the study: using the cluster analysis, to determine and describe clinical and pathogenetic phenotypes of CMVO during PCIs for MI.

MATERIALS AND METHODS

A retrospective study was conducted. Out of 18,079 patients admitted to Regional City Clinical Hospital No. 13 of the Nizhny Novgorod Avtozavodskoy District with the diagnosis of acute coronary syndrome in 2013–2020, 7,456 patients with type 1 MI were selected; they underwent emergency PCI. Among patients with MI and PCI, 232 (3.1%) patients were identified who developed CMVO during the surgery. The patients with restricted coronary blood flow and myocardial perfusion due to other causes (initial cardiogenic shock, spasm or dissection of the coronary artery, etc.) were excluded from the study. The mortality rate in this group was 13.8% (32 in-hospital deaths). Since the absence of missing data in the analyzed dataset is a necessary condition for a cluster analysis, the inability to obtain necessary data for organizational reasons and due to the retrospective nature of the study (mostly due to the lack of certain laboratory tests) became an additional exclusion criterion. Thus, 190 patients with MI who developed CMVO during PCI and had a complete dataset for the analysis were selected for the study. The study was approved by the local Ethics Committee.

The diagnosis of type 1 MI was made based on clinical, electrocardiographic, and biochemical criteria according to the third and fourth universal definitions [6]. The severity of acute heart failure (AHF) was assessed using the Killip classification [1].

The term PCI was used to refer to stent implantation in the IRA, resulting in epicardial coronary artery patency restoration with residual stenosis of less than 50% and exclusion of complications, such as dissection, perforation, persistent spasm or severe thromboembolism of the coronary artery (CA). The following scales were used to describe the CA anatomy and characterize the results of PCI: 1) Thrombolysis in Myocardial Infarction (TIMI) flow grade (TFG) [3] for assessing coronary blood flow in the IRA before and after PCI; 2) TIMI thrombus grade (TTG) [1] for assessing the severity of thrombus burden in the IRA after PCI; 3) Myocardial Blush Grade (MBG) [3] for assessing myocardial perfusion after PCI; 4) Rentrop [5] for grading collaterals to the IRA; 5) Syntax Score (SS) [1] – for quantitative description of the severity of atherosclerotic lesion in the CA (evaluated on the whole and in the IRA).

The CMVO phenomenon was diagnosed according to the guidelines of the European Society of Cardiology [7]: 1) TFG score of less than 3; 2) MBG score of less than 2; 3) less than 70% resolution of ST-segment changes on the electrocardiogram (ECG) within 60–90 minutes after PCI.

The median age was 64 [56; 70] years. The study included 137 (72%) men and 53 (28%) women. Of the 190 patients included in the study, 57 (30%) people had a history of coronary artery disease (CAD), and 52 (27%) individuals had a history of diabetes mellitus; 170 (89%) patients were admitted with ST-segment elevation myocardial infarction (STEMI). Upon admission, 4 (2%) patients had class III AHF; cardiogenic shock was diagnosed in 17 (9%) patients.

The median total SS was 15 [9; 21] and SS in the IRA was 9 [7; 15]. The left main coronary artery or the left anterior descending artery were defined as the IRA in 81 (43%) patients. Initial severe thrombosis of the IRA (TTG score 4–5) was detected in 146 (77%) patients, CA occlusion was found in 150 (79%) patients, no visible collaterals to the IRA (Rentrop grade 0–1) were noted in 170 (90%) patients. CA ectasia, according to the definition by P.S. Swaye [8], was diagnosed in 13 (7%) patients.

Of the 190 patients included in the study, primary PCI was performed in 127 (67%) patients, and a pharmacoinvasive strategy (systemic thrombolytic therapy preceding PCI) was applied in 63 (33%) patients. The symptom-to-balloon time (from the onset of the status anginosus to the blood flow restoration by PCI) was 9.7 [4.8; 16.0] hours. Stenting was combined with balloon angioplasty in 160 (84%) cases. Vacuum aspiration thrombectomy was performed in 41 (22%) patients, concurrent PCI on multiple CAs was performed in 7 (4%) patients. The median number of stents implanted was 1 [1; 2], the median length of the implanted stents was 30 [26; 51] mm, and the median stent inflation pressure was 14 [12; 15] atm. The median diameter of the IRA was 3.5 [3.0; 3.5] mm.

The following strategies were used in the operating room to treat CMVO: intracoronary administration of isosorbide dinitrate in 80 (42%) patients; intracoronary administration of verapamil in 43 (23%) patients; intra-aortic balloon counterpulsation in 10 (5%) patients; and glycoprotein IIb / IIIa inhibitors in 6 (3%) patients.

The median values of the key laboratory parameters on admission were as follows: glucose – 8.3 [7.0; 10.5] mmol / l, platelet-to-lymphocyte ratio (PLR) – 111 [83; 149], total cholesterol (TC) – 4.9 [4.1; 5.7] mmol / l, glomerular filtration rate (GFR) according to the CKD-EPI equation 76 [57; 86] ml / min / 1.73m^2, leukocytes – 11.3 [8.7; 14.2] × 10^9 / l, neutrophils – 5.1 [4.0; 6.6] × 10^9 / l, troponin I – 0.76 [0.10; 6.35] ng / ml.

After PCI, the resolution of ST-segment changes on ECG was observed in 110 (58%) patients, Q-wave myocardial infarction developed in 172 (91%) patients. The median left ventricular ejection fraction (LVEF) calculated by the Simpson's method was 46 [41; 50] % on discharge. Nine patients (4.7%) died in the hospital. The causes of death were: cardiogenic shock in 5 (56%) cases, mechanical complications of MI in 2 (22%) cases, pulmonary edema in 1 (11%) patient, and thromboembolic complications in 1 (11%) patient.

To conduct clustering, we selected certain parameters that were predictors of CMVO development according to the current literature on the topic [5]. From the anamnestic and clinical parameters, the following were assessed: age, gender, medical history of CAD, admission with STEMI, AHF class, hemodynamic status, and systemic thrombolytic therapy. Out of the parameters characterizing CAs and performed PCI, the following were noted: the symptom-to-balloon time, total SS, SS in the IRA, presence of CA ectasia, lesion in the stem of the left coronary artery or left anterior descending artery, lesion in the right coronary

artery, collateral grading according to Rentrop, IRA diameter, TTG, TFG, size of the CA atherosclerotic lesion, balloon angioplasty (pre- and post-dilation) or vacuum aspiration thrombectomy, number, length, and implantation pressure of stents, concurrent stenting of multiple CAs. Laboratory tests performed on admission were the following: blood glucose level, leukocytes, lymphocytes, neutrophils, TC, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), cardiac troponin I, mean platelet volume, PLR, neutrophil-to-lymphocyte ratio (NLR), and estimated glomerular filtration rate (GFR). To evaluate the outcomes and severity of MI, in-hospital mortality, development of Q-wave MI, and LVEF were assessed.

Before conducting clustering, all quantitative variables were standardized to the mean and the standard deviation (*Mean* ± *SD*) [9]. Clustering was performed using the expectation – maximization (EM) algorithm, considering the type of distribution of the quantitative variables. The number of clusters was chosen using V-fold cross-validation [10].

In the statistical analysis, the Kolmogorov – Smirnov test was used to determine the nature of the distribution. Depending on the distribution, the Mann – Whitney and Kruskal – Wallis tests were used to evaluate the statistical significance of differences in the quantitative variables. The $\chi 2$ Pearson's test (including the Yates' correction) and the Fisher's exact test were used to evaluate the significance of differences in the categorical variables. The differences were considered statistically significant at $p < 0.05$. The Bonferroni correction was used for multiple comparisons (maximum number of compared groups – 3, p-value after correction < 0.018). The quantitative data were presented as the median and the interquartile range (*Me [Q_1; Q_3]*). The clustering and statistical analysis were performed using the Statistica 12.0 (StatSoft, USA) and MedCalc 11.5 (MedCalc Software, Belgium) software.

RESULTS

During clustering, a model consisting of three clusters was obtained based on 18 variables ($p <$ 0.05 for each parameter). The model included: age, gender, type of MI, AHF class, total SS, SS in the IRA, coronary collaterals by Rentrop, IRA diameter, TTG, TFG, PLR, GFR, TC, blood glucose level on admission, systemic thrombolytic therapy, vacuum aspiration thrombectomy, balloon angioplasty, and PCI in 2 or more CAs. The patients were distributed into the clusters as follows: cluster 1 included 106 (56%) patients, cluster 2 included 52 (27%) patients, cluster 3 encompassed 32 (17%) patients. The standardized mean values of the quantitative variables in the identified clusters are presented in Fig. 1, and the percentage of qualitative variables – in Fig. 2. For display convenience, ordinal parameters were transformed into binary ones (the threshold values were chosen based on clinical significance and are generally accepted) [5].

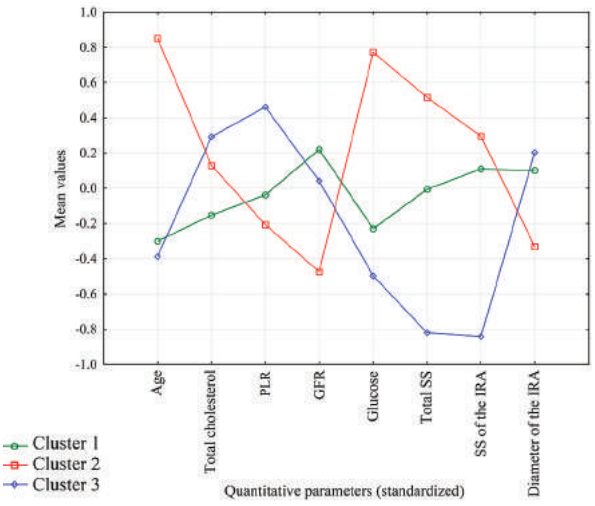

Fig. 1. Standardized mean values of quantitative variables in the clusters: IRA – infarct-related artery, GFR – glomerular filtration rate, PLR – platelet-to-lymphocyte ratio, SS – Syntax Score

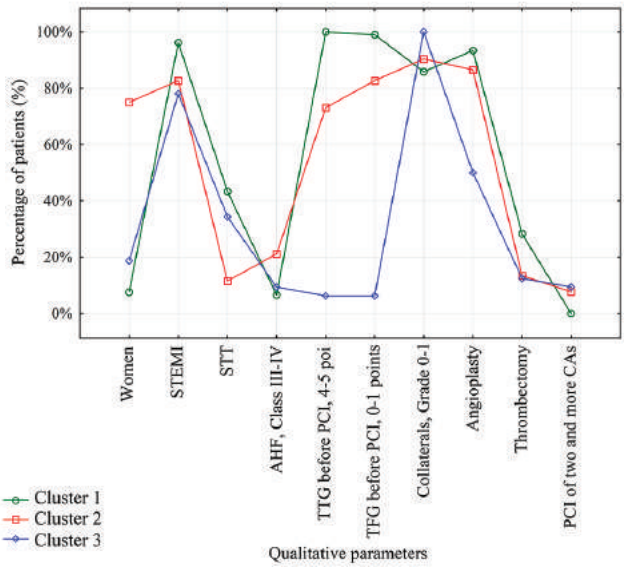

Fig. 2. Percentage of qualitative variables in the clusters: STEMI – ST-segment elevation myocardial infarction, CA – coronary artery, AHF – acute heart failure, PCI – percutaneous coronary intervention, TIMI – Thrombolysis in Myocardial Infarction, TFG – TIMI flow grade, TTG – TIMI thrombus grade

Table compares the groups by the parameters included in the clustering model (multiple and pairwise comparisons of the clusters adjusted to the number of tests). The clusters differed significantly in hospital outcomes "death" and "left ventricular ejection fraction" (Fig. 3). No differences in the frequency of Q-wave MI were found: cluster 1 – 99 (93%) patients, cluster 2 – 47 (90%) patients, cluster 3 – 26 (81%) patients, $p = 0.12$.

Table

Comparison of the clusters by the parameters included in the clustering model

Parameter	Cluster 1, n = 106	Cluster 2, n = 52	Cluster 3, n = 32	p-value
Age, years, *Me* [Q_1; Q_3]	62 [54; 67][2]	73 [67; 79][1, 3]	59 [50; 65][2]	<0.001
Female / male, n (%)	8 (8) / 98 (92)[2]	39 (75) / 13 (25)[1, 3]	6 (19) / 26 (81)[2]	<0.001
Admitted with STEMI, n (%)	102 (96)[2, 3]	43 (83)[1]	25 (78)[1]	0.002
Systemic thrombolytic therapy, n (%)	46 (43)[2]	6 (12)[1, 3]	11 (34)[2]	<0.001
AHF, class, *Me* [Q_1; Q_3]	1 [1; 2][2]	2 [1; 4][1]	2 [1; 2]	<0.001
Blood glucose level, mmol / l, *Me* [Q_1; Q_3]	8.0 [6.9; 9.6][2]	11.1 [8.8; 15.2] [1, 3]	7.5 [6.1; 8.1] [2]	<0.001
PLR, *Me* [Q_1; Q_3]	110 [78; 153] [3]	106 [85; 132][3]	132 [100; 182][1, 2]	0.04
Total cholesterol, mmol / l, *Me* [Q_1; Q_3]	4.7 [4.2; 5.4][2, 3]	5.3 [3.7; 6.2][1]	5.1 [4.5; 6.2][1]	0.047
GFR, ml / min / 1.73 m^2, *Me* [Q_1; Q_3]	77 [64; 88][2]	58 [46; 74] [1, 3]	81 [64; 88] [2]	<0.001
Total Syntax Score, points, *Me* [Q_1; Q_3]	15 [10; 21][2, 3]	20 [14; 26][1, 3]	8 [5; 10] [1, 2]	<0.001
Syntax Score in the IRA, points, *Me* [Q_1; Q_3]	9 [8; 15] [3]	12 [7; 16] [3]	6 [3; 7] [1, 2]	<0.001
Collateral grading, grade, *Me* [Q_1; Q_3]	0 [0; 1] [3]	0 [0; 1] [3]	0 [0; 0] [1, 2]	0.01
TIMI thrombus grade, grade, *Me* [Q_1; Q_3]	5 [5; 5][2, 3]	5 [3; 5][1, 3]	1 [0; 2] [1, 2]	<0.001
TIMI flow grade, grade, *Me* [Q_1; Q_3]	0 [0; 0][2, 3]	0 [0; 1][1, 3]	2 [2; 3] [1, 2]	<0.001
Vacuum aspiration thrombectomy, n (%)	30 (28)[2, 3]	7 (13)[1]	4 (13)[1]	0.04
Balloon angioplasty, n (%)	99 (93)[3]	45 (87)[3]	16 (50)[1, 2]	<0.001
IRA diameter, mm, *Me* [Q_1; Q_3]	3.5 [3.0; 3.5][2]	3.0 [2.8; 3.5] [1, 3]	3.5 [3.0; 3.5] [2]	0.02
PCI in 2 or more CAs, n (%)	0 (0)[2, 3]	4 (8)[1]	3 (9)[1]	0.009

[1], [2], [3] the value of the parameter is statistically significant ($p < 0.018$, with the Bonferroni correction) and differs from the identical parameter in cluster 1, 2 or 3, respectively.

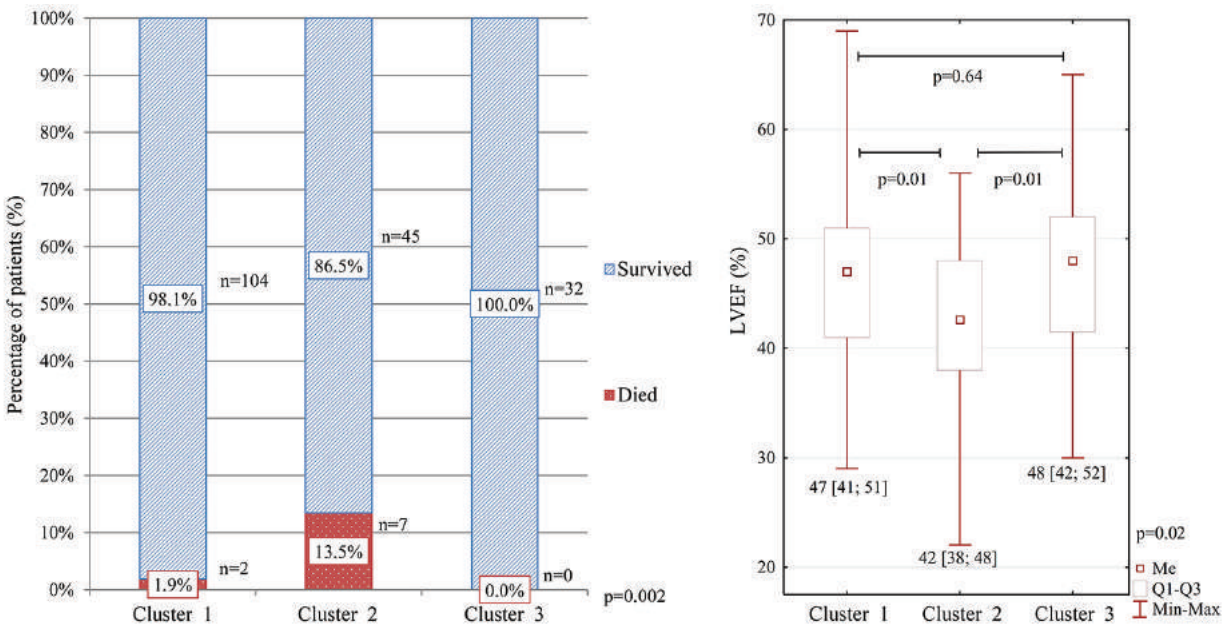

Fig. 3. Comparison of the clusters by the outcomes "death" and "LVEF": Min – Max – minimum and maximum values

DISCUSSION

The goal of the cluster analysis is to divide a sample into groups (clusters) in such a way that each cluster consists of similar objects, while objects from different clusters differ significantly from one other. The researcher's task in the cluster analysis is to knowledgeably select input parameters based on the relevant data on the research topic. Since clustering is a type of unsupervised machine learning (the "correct" division is not known in advance), the resulting classification is generally objective and is more a product of mathematical analysis rather than personal empirical choice [9, 10]. The researcher's ultimate task in this case is to analyze and explain the obtained results.

It should be noted that in some situations, the values of the parameters from different clusters may overlap, which is considered to be acceptable, since any parameter used for the classification is characterized by a certain proportion of false positive and false negative results. The evidence of statistically significant differences in the parameters between the clusters and, accordingly, the correctness of the obtained model is a p value of less than 0.05 for all parameters. It should be noted that in the cluster analysis, the original sample is divided by a combination of many features, even though individual parameters in the clusters may match [9, 10].

The analysis revealed significant heterogeneity in the group of patients with CMVO, resulting in the formation of 3 clusters with statistically significant differences (Table). The first cluster was the largest: 106 (56%) patients out of 190. It mainly included relatively young (62 [54; 67] years old) men (92%) with moderate atherosclerotic lesions of the CA (SS 15 [10; 21]). The prevailing predictors of the CMVO development in this cluster included high thrombus burden of the IRA (TTG 5 [5; 5]) and the associated sharp decrease in the baseline coronary blood flow (TFG 0 [0; 0]).

The association of CMVO in the group with intracoronary thrombosis is confirmed by the highest proportion of patients with STEMI – 95%, p = 0.02 (the development of STEMI is usually due to thrombotic occlusion of the IRA) [7, 11] and the most frequent use of balloon angioplasty (93%, $p < 0.001$) and vacuum aspiration thrombectomy (28%, $p = 0.04$) (also explained by the initially high thrombus burden of the IRA) [7, 11]. Thus, it would be reasonable to define the first cluster as a "microthromboembolic" phenotype, thereby underlining the most likely part of the pathogenesis – distal microembolism with thrombus debris fragmentation by PCI [2, 5, 11].

The second cluster (27% of the included patients) was characterized by the predominance of elderly women (75%) (73 [67; 79] years old) with high hyperglycemia (glucose 11.1 [8.8; 15.2] mmol / l, diabetes mellitus in 60% of the patients), severe atherosclerotic lesions of the CAs (SS 20 [14; 26]), the smallest diameter of the IRA (3.0 [2.8; 3.5] mm), reduced kidney function (GFR 58 [46; 74] ml / min / 1.73m^2), and hospitalization with severe AHF (class 2 [1; 4]). It should be noted that patients in this group also had high thrombus burden (TTG 5 [3; 5]) and reduced coronary blood flow (TFG 0 [0; 1]).

On the one hand, it is obvious that the trigger for the CMVO development in this group was also distal peripheral microembolism caused by intracoronary thrombus debris during PCI. On the other hand, it is known that the presence of severe persistent endothelial dysfunction, which is a predictor of the CMVO development [1, 5, 11], is characteristic of patients with the aforementioned clinical profile (especially those with diabetes). Apparently, the clustering algorithm correctly identified the most vulnerable and severe group of elderly patients. It is the age that was the factor that largely determined the clinical profile in this group: severe ischemic heart disease, severe atherosclerosis of the CAs, and comorbidity (diabetes, renal failure) [1, 5, 11]. Therefore, this cluster should be referred to as an "age-associated" phenotype. It should be noted that the rare use of thrombolytic therapy among patients in this cluster (12%, $p < 0.001$) was also likely due to their advanced age and associated hemorrhagic risk.

The third cluster (17% of the included patients) was mainly represented by relatively young (59 [50; 65] years old) men (81%) with minor atherosclerotic lesions of the CAs (SS 8 [5; 10]), almost no intracoronary thrombosis (TTG 1 [0; 2]), preserved blood flow (TFG 2 [2; 3]) in the IRA, and no visible coronary collaterals (Grade 0 [0; 0]). At the same time, this group had a relatively high level of TC (5.1 [4.5; 6.2] mmol / l) and the highest value of PLR (132 [100; 182]) – a parameter reflecting the severity of inflammation and a reliable predictor of the CMVO development [12].

The absence of severe IRA thrombosis in combination with dyslipidemia and severe inflammation suggests that the CMVO development in the patients of this group was caused by the rupture of a large, lipid-rich

atherosclerotic plaque with subsequent microembolism of the distal bed with its debris [1, 5, 11]. The relationship between the risk of developing CMVO and the lipid composition of the plaque, evaluated using modern methods of intravascular visualization, has been demonstrated in many studies in recent years [14, 15]. The cause of rupture and fragmentation of the plaque was intense mechanical stress during PCI [11], as indicated by the frequent use of multivessel stenting (9%, $p = 0.009$) in this group. Inflammation, on the other hand, apparently contributed to the development and destabilization of the atheroma [13, 16] and was a component of the pathogenetic cascade that exacerbated obstruction of the microvascular bed and led to the CMVO development [17]. Given the above, this cluster can be accurately referred to as an "atheroembolic" phenotype.

The above analysis of data and current literature allows us to assert that the described clusters can be considered as clinical and pathogenetic phenotypes of CMVO in MI. It should be stressed that the proposed names of the phenotypes were suggested to simplify the perception. These names are descriptive and reflect the leading, but not always the only factor in the CMVO development in the phenotype. In order to simplify the classification of patients with CMVO into a specific phenotype, we suggest using the algorithm presented in Fig. 4.

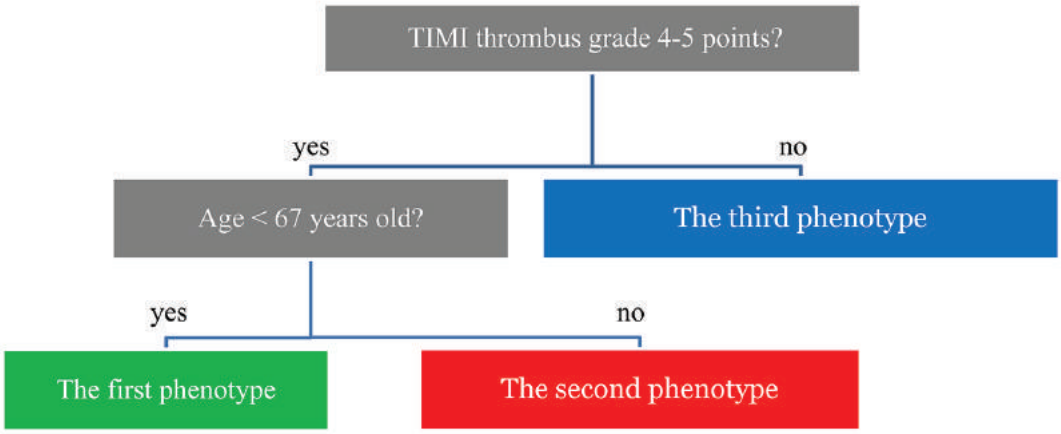

Fig. 4. Algorithm for attributing patients with CMVO to a specific phenotype.

It is worth noting that all identified phenotypes were mainly associated with different variants of distal peripheral microembolism after PCI. However, an important predictor of CMVO, the symptom-to-balloon time, was not included in the cluster model, although it is known that a delay in reperfusion is associated with severe ischemic damage [5, 11]. This can be explained by the fact that the time parameter is more associated with transportation and logistics. The delay in reperfusion definitely aggravates ischemic damage and contributes to the CMVO development, but it is not related to the patient's clinical profile [1, 4].

The identified phenotypes differed in the severity of hospital outcomes. The worst prognosis was observed in patients with the second phenotype (in-hospital mortality 13.5%, $p = 0.002$, LVEF at discharge – 42 [38; 48] %, $p = 0.01$, the Bonferroni correction threshold – 0.018), which was associated with severe MI, old age, severe atherosclerotic lesions of the CAs, frequent presence of AHF, and comorbidity.

These findings allow us to identify potential aims for targeted prevention and treatment of CMVO. In the case of the "microthromboembolic" phenotype, the most effective methods may be aimed at eliminating intracoronary thrombus: vacuum aspiration thrombectomy and the use of glycoprotein IIb/IIIa inhibitors [1, 3]. In CMVO with the "age-associated" phenotype, in addition to combating intracoronary thrombosis, effective methods may include perioperative correction of hyperglycemia, timely recognition of incipient cardiogenic shock, and the use of mechanical circulatory support [4, 18]. For patients with the "atheroembolic" phenotype, it is advisable to use minimally invasive interventions: performing PCI only in the IRA, performing post-dilation of the stent only if necessary, implanting the stent at moderate pressure, and using a deferred stenting strategy in selected patients [4, 19]. Early use of high-dose statins and anti-inflammatory drugs may also be effective in patients in this group [1, 20]. The

algorithm for such a selective approach is yet to be developed and tested in a prospective study.

LIMITATIONS OF THE STUDY

The presented study has several limitations, mainly related to the retrospective nature of the study. It is likely that some patients with moderate CMVO were not included in the study due to the retrospective inclusion of patients based on hospital database information, with subsequent verification of CMVO by angiography and ECG findings (as CMVO was not recorded in the primary documentation). The second limitation was that some laboratory tests associated with CMVO were not performed within the first day of hospitalization. Since the cluster analysis does not allow any missing data, a number of patients with CMVO who did not undergo the necessary tests had to be excluded from the study. Another factor that may have affected hospital outcomes in the studied group of patients was rare use of glycoprotein IIb/IIIa inhibitors.

CONCLUSION

Three phenotypes of CVMO were identified following the cluster analysis. The first phenotype is associated with severe thrombosis of the IRA and includes mostly men with moderate atherosclerotic lesions. The second phenotype is characterized by prevalence of elderly women with high hyperglycemia, advanced atherosclerotic lesions, severe AHF, impaired renal function, and thrombosis of the IRA. The third phenotype includes mostly men with mild atherosclerotic lesions, absence of severe thrombus burden, and preserved blood flow in the IRA before PCI, but with high levels of inflammatory markers and TC. The highest mortality rate and reduced LVEF were observed in patients of the second phenotype.

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Authors' contribution

Frolov A.A. – conception and design, analysis and interpretation of the data, justification of the manuscript, critical revision of the manuscript for important intellectual content. Frolov I.A. – conception and design, analysis and interpretation of the data. Ulanova N.D., Kuzmichev K.V. – analysis and interpretation of the data. Pochinka I.G. – analysis and interpretation of the data, justification of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Mukhin A.S., Sharabrin E.G. – justification of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

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Taking antibacterial drugs without a doctor's prescription in the Russian Federation

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ABSTRACT

Background. Antibiotic resistance is a global threat leading to ineffective treatment of many infectious diseases. One of the factors contributing to an increase in antibiotic resistance is over-the-counter sale of antibiotics.

The aim of this study was to establish the sources of antibiotic prescription and to determine the prevalence of self-medication and factors that cause it.

Materials and methods. The computer-assisted web interview (CAWI) methodology was used in the study. The questionnaire consisted of six blocks and 41 questions. For statistical analysis of the study results, Statistica for Windows version 10.0 and R-Studio software programs were used.

Results. The study involved 2,725 people. Only 50.9% of the respondents purchased antibiotics with a prescription or got them during hospitalization. Parameters associated with over-the-counter purchase of antibiotics included female gender (odds ratio (OR) = 1.4; 95% confidence interval (CI): 1.2–1.7), lack of higher education (OR = 1.6; 95% CI: 1.3–1.9), medical education (OR = 1.7; 95% CI: 1.2–2.5), lack of awareness of a ban on over-the-counter sale of antibiotics (OR = 1.6; 95% CI: 1.3–1.9), and relying on the knowledge (opinion) of family members or acquaintances as the main sources of information about the correct use of antibiotics (OR = 2.2; 95% CI: 1.7–2.9).

Conclusion. Antibiotic resistance can be reduced by propaganda and strict control over a ban on over-the-counter sale of antibiotics. It is also essential to update knowledge of medical professionals about antibacterial drugs and antibiotic resistance on a regular basis, also through raising their awareness of the development of antibiotic resistance in both patients and medical workers.

Keywords: antibiotics, antibacterial drugs, taking antibiotics without a doctor's prescription, antibiotic resistance, self-medication

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Прием антибактериальных препаратов без назначения врача в Российской Федерации

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РЕЗЮМЕ

Введение. Антибиотикорезистентность – глобальная угроза, приводящая к неэффективности лечения многих инфекционных заболеваний, причиной роста которой является ненадлежащее исполнение требований законодательства, а именно безрецептурная продажа антибиотиков.

Цель исследования. Установить источники назначения антибактериальных препаратов, распространенность самолечения и факторы, его обусловливающие.

Материалы и методы. В настоящей работе применялся метод онлайн-опроса (CAWI) с использованием анкеты, состоявшей из шести блоков и 41 вопроса. Статистический анализ данных проведен с использованием программ Statistica for Windows version 10.0 и R-studio.

Результаты. В исследовании приняли участие 2 725 человек. Только 50,9% респондентов приобретали (получали) антибактериальные препараты по рецепту врача. Параметрами, ассоциированными с приемом антибиотиков без назначения врача, являлись женский пол (отношение шансов (ОШ) = 1,4; 95%-й доверительный интервал (ДИ): 1,2–1,7), отсутствие высшего образования (ОШ = 1,6; 95%-й ДИ: 1,3–1,9), наличие медицинского образования (ОШ = 1,7; 95%-й ДИ: 1,2–2,5), отсутствие информации о запрете продажи антибиотиков без рецепта врача (ОШ = 1,6; 95%-й ДИ: 1,3–1,9) и применение знаний членов семьи или знакомых как основных источников информации о рациональном приеме антибактериальных препаратов (ОШ = 2,2; 95%-й ДИ: 1,7–2,9).

Заключение. Приоритетными направлениями по сдерживанию антибиотикорезистентности могут стать пропаганда и строгий контроль запрета продажи антибиотиков без рецепта врача. Кроме того, необходимо периодически актуализировать знания медицинских работников об антибактериальных препаратах и антибиотикорезистентности, в том числе для повышения настороженности специалистов сферы здравоохранения к развитию антибиотикорезистентности как у пациентов, так и самих работников.

Ключевые слова: антибиотики, антибактериальные препараты, прием без назначения врача, антибиотикорезистентность, самолечение

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов в связи с публикацией данной статьи.

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

Соответствие принципам этики. Исследование одобрено этическим комитетом по экспертизе социологических исследований в сфере общественного здравоохранения при ФГБУ «ЦНИИОИЗ» Минздрава России (заключение № 11/2022 от 07.10.2022).

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INTRODUCTION

Antibiotic resistance is a global threat leading to ineffective treatment, a rise in healthcare costs, and an increase in morbidity, mortality, and length of hospital stay [1, 2]. In the Russian Federation, the problem of antibiotic resistance is also relevant. According to O.Y. Kutsevalova et al. (2019), more than 50% of P. aeruginosa and K. pneumoniae strains and 90.9% of A. baumannii strains are resistant to carbapenems

[3]. V.V. Rafal'sky et al. (2018) found stable high resistance of E. coli strains to co-trimoxazole (19.3–26.2%) and ampicillin (33.1–41.5%) [4].

There are several causes of the rise in antibiotic resistance. One of them is non-compliance with legal regulations and over-the-counter sale of antibiotics. Foreign researchers report that this trend is observed in 51% of cases [5–7]. This results in overuse of antibacterial drugs, including self-medication, which ranges from 1 to 70% in some low- and middle-income countries [8, 9]: in Southeast Asia – 50%, in South America – 78%, in Italy – 32.3%, in Tanzania – 58% [10–13].

In the Russian Federation, sale of antibacterial drugs with a prescription and strict control over this process were introduced in 2017[1]. Unfortunately, in real practice, these recommendations are not always followed strictly. According to the results of the study by T.M. Klimova et al. (2017) that included 358 individuals, 73.4% took antibiotics without a doctor's prescription [14].

In order to better understand the sources of antibacterial drug prescription, the prevalence of self-medication with antibacterial drugs, and the factors that cause them, an observational study was carried out on a representative sample of the Russian Federation residents.

MATERIALS AND METHODS

The computer-assisted web interview (CAWI) methodology was used in the study. The questionnaire consisted of six blocks and 41 questions: characteristics of respondents, frequency and features of antibiotic intake, prescription and purchase, rationality of use, knowledge of the population and sources of information about antibacterial drugs and antibiotic resistance. To improve the questionnaire validity, the focus group method (n = 10 people) was used.

The statistical data analysis was carried out using the Statistica for Windows version 10.0 and R-Studio software. Qualitative variables were presented as absolute and relative frequencies. Quantitative variables were presented as the arithmetic mean and the standard deviation ($M \pm SD$).

The Shapiro – Wilk test was used to assess the distribution of the assessed variables. For non-normal distribution of the variables, the Mann – Whitney test was used to assess the significance of differences between two independent samples. For normal distribution of the variables, the Student's t-test was applied. The differences between the groups were considered statistically significant at $p < 0.05$. The odds ratio (OR) was used to assess the association of a particular outcome with its binary predictors. A logistic regression model was created to determine the probability of a certain event.

RESULTS

Characteristics of the respondents

The study included 2,725 people from all regions of the Russian Federation (45.6% men, n = 1,242; 54.4% women, n = 1,483). The average age of the participants was 42.4 years (± 14.4 years). More than 25% of the respondents were aged 25–34 years (n = 683), 22.7% of people were aged 35–44 years (22.7%, n = 619).

The respondents were asked a standard question for determining purchasing power: "How do you assess your financial situation?" According to the provided answers, the majority of the respondents (44.1%, n = 1,200) were attributed to the middle-income group, and 36.5% (n = 995) – to the upper middle group. Detailed socio-demographic characteristics of the participants are presented in Fig. 1–3.

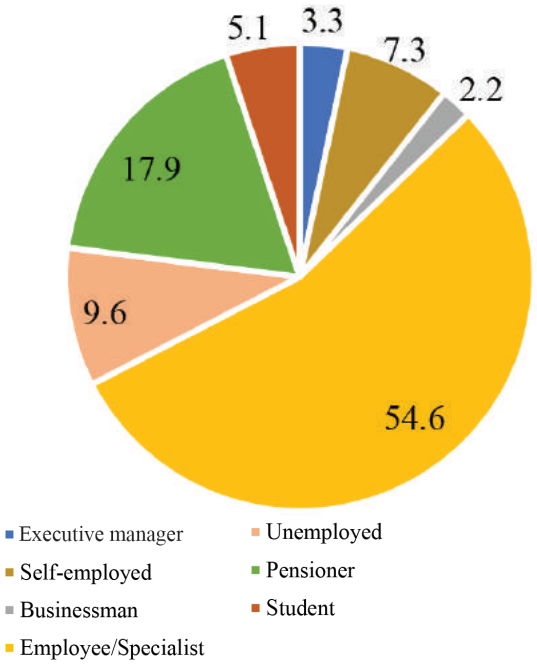

Fig. 1. Social and professional categories of the respondents, %

[1] Decree of the Government of the Russian Federation (2017) "On the Strategy for Preventing the Spread of Antimicrobial Resistance in the Russian Federation for the Period up to 2030" No. 2045-r of 25.09.2017

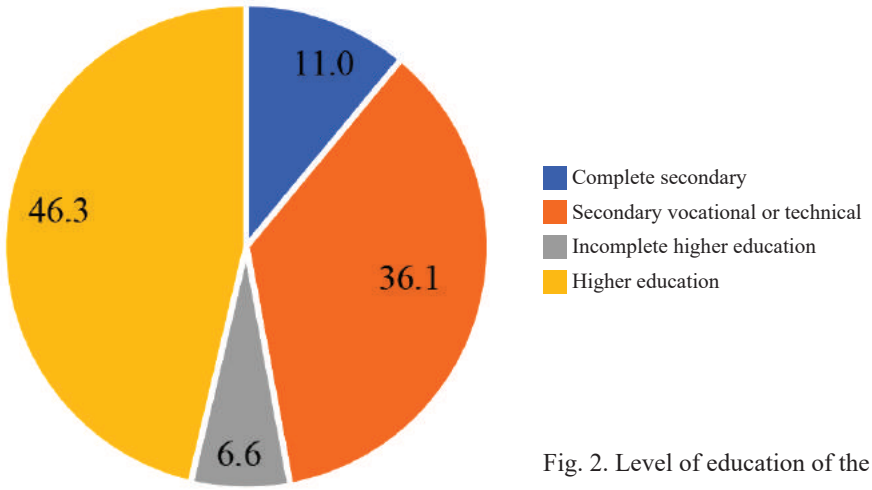

Fig. 2. Level of education of the respondents, %

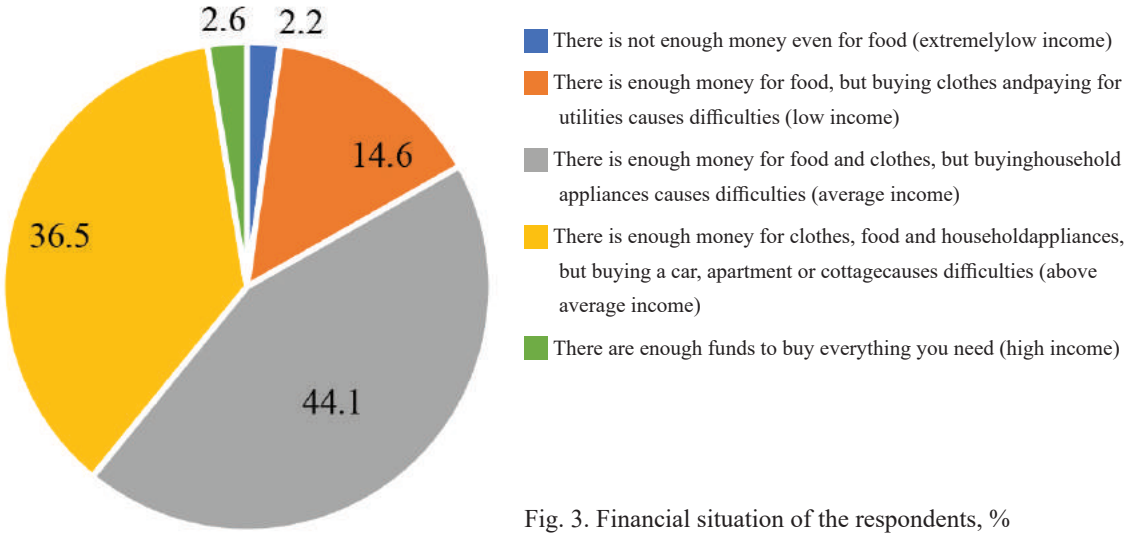

Fig. 3. Financial situation of the respondents, %

Prescription of antibacterial drugs

The study results indicate that 32.2% of the participants (n = 479) who took antibacterial drugs in the previous 12 months purchased them without a doctor's prescription. Of them, more than a third (34.0%, n = 163) decided to start taking antibiotics on their own, 33.2% did it following the advice of familiar medical professionals (n = 159), and 31.5% followed the advice of family members (n = 151) (Fig.4).

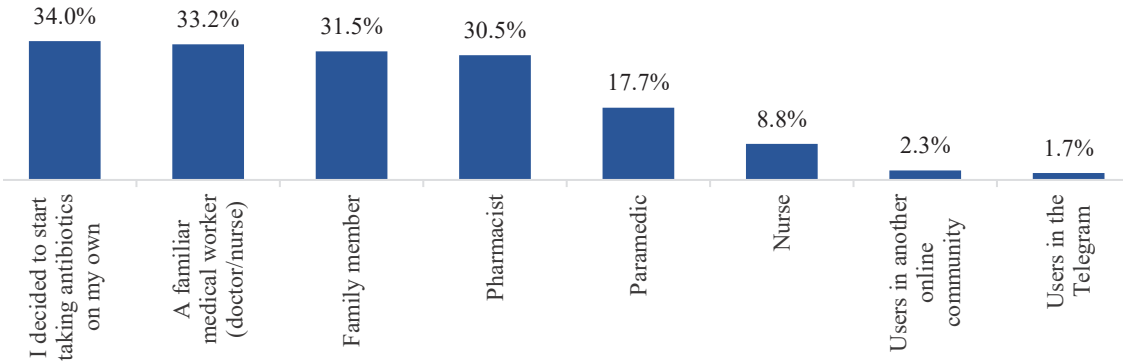

Fig. 4. Sources of prescription of antibacterial drugs other than the doctor

The percentage of the respondents who took antibacterial drugs prescribed by the doctor was the highest among people who had higher education (73.0%, n = 514) and the lowest among people with secondary vocational or technical education (63.6%, n = 330; p = 0.004). However, the analysis showed that social and professional groups and the financial situation of the respondents had no significant impact on taking antibiotics prescribed by the doctor ($p > 0.05$) (Fig. 5).

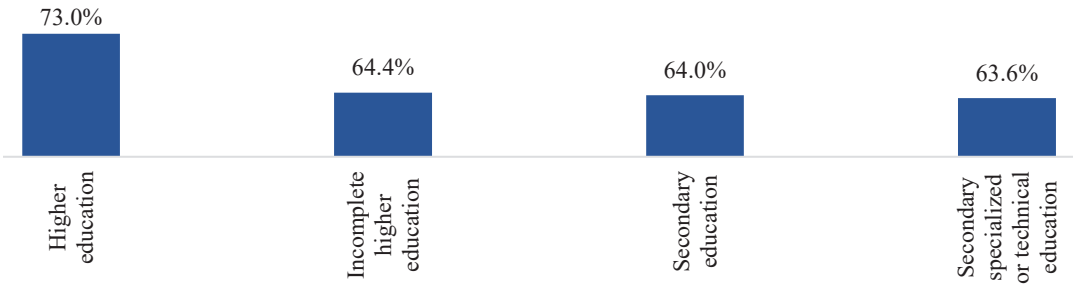

Fig. 5. The proportion of the respondents who took antibacterial drugs prescribed by the doctor, depending on the level of education: p = 0.004 when comparing respondents who had higher and secondary education

The parameters associated with more frequent use of antibiotics without a doctor's prescription were female gender (OR = 1.4; 95% confidence interval (CI): 1.2–1.7), lack of higher education (OR = 1.6; 95%CI: 1.3–1.9), medical education (OR = 1.7; 95%CI: 1.2–2.5), unawareness of a ban on over-the-counter sale of antibiotics (OR = 1.6; 95%CI: 1.3–1.9), and relying on the knowledge of family members or friends as the main sources of information about the rational use of antibacterial drugs (OR = 2.2; 95%CI: 1.7–2.9) (Table 1).

Table 1

Parameters associated with taking antibacterial drugs without a prescription

Parameter	OR (95% CI)
Female gender	1.4 (1.2–1.7)
Lack of higher education	1.6 (1.3–1.9)
Medical education	1.7 (1.2–2.5)
Unawareness of a ban on over-the-counter sale of antibiotics	1.6 (1.3–1.9)
The main source of information about the rational use of antibacterial drugs is the knowledge (opinions) of family members or friends	2.2 (1.7–2.9)

Sources of buying (getting) antibacterial drugs

Almost half of the respondents (49.1%, n = 732) who took antibiotics in the previous 12 months took these without a prescription. Of these, the vast majority (86.3%, n = 632) bought antibiotics over the counter in a pharmacy and a fifth (22.8%, n = 167) took leftover antibiotics from a previous prescription (Fig. 6).

A significantly lower proportion of the respondents who bought antibiotics with a prescription was observed among self-employed (31.4%, n = 38; $p < 0.05$). During in-patient treatment, students and persons with extremely low income received antibacterial drugs less than other groups (19.1%, n = 13 and 3.8%, n = 1, respectively; $p < 0.01$).

Besides, the age group of 25–34 years old (28.1%), self-employed people (50.4%), and people with low income (53.8%) had the biggest proportion of the respondents who bought antibacterial drugs over the counter; the smallest proportion was noted for the age group of 18–24 years (13.3%), students (26.5%), and people with high income (18.9%).

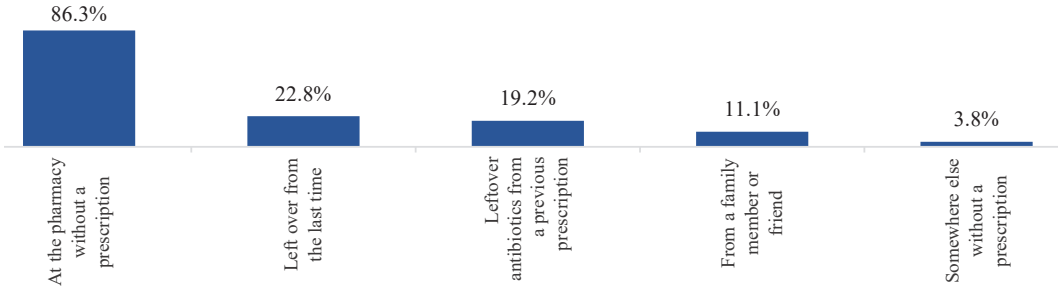

Fig. 6. Distribution of the respondents by sources of buying (getting) antibacterial drugs other than from the doctor

The proportion of the respondents who got antibacterial drugs from family members or friends was significantly bigger in the age group of 18–24 years (7.8%) and among students (20.6%), but it was significantly smaller in the age group of 55–64 years (1.3%) and among businessmen (0.0%) (Fig. 7).

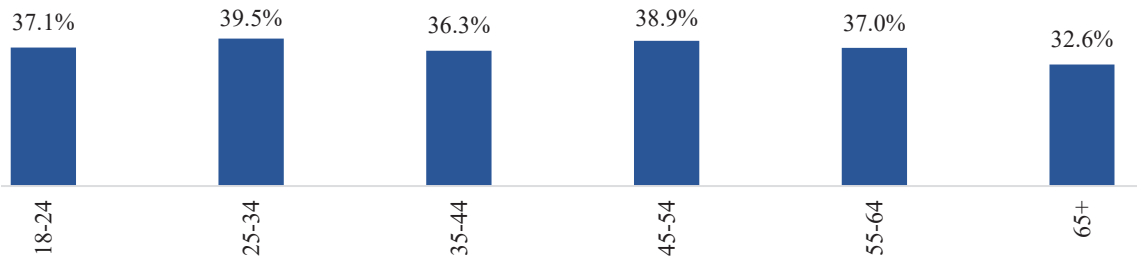

Fig. 7. Percentage of the respondents who took antibacterial drugs prescribed by the doctor depending on the age group, %, $p = 0.04$ when comparing the age group of 25–34 years and the group over 65 years

What is more, it was discovered that the respondents with a very poor financial situation were more likely than other people to buy antibiotics over the counter in a local pharmacy (53.8%) or in an online pharmacy (23.1%, $n = 6$). The level of education did not have a significant impact on the choice of a certain source of getting antibiotics (Table 2).

Among those who bought (got) antibacterial drugs over the counter, there were significantly fewer people who took drugs in the form of injections (11.1% vs. 19.4%; $p < 0.01$), completed a full course of antibiotics (71.1% vs. 85.8%; $p < 0.01$), underwent tests before the antibiotic therapy to identify the cause of the disease (25.6% vs. 53.3%; $p < 0.01$), and who were aware of and supported the ban on over-the-counter antibiotic sale (59.0% vs. 75.3% and 39.0% vs. 56.1%; $p < 0.01$). Besides, the abovementioned group used antibacterial drugs on average for fewer days (5.5 and 3.5 days vs. 6.7 and 3.6 days; $p < 0.01$) compared to people who bought (got) antibiotics with a prescription.

At the same time, there were significantly more people in this group who took antibiotics following the recommendation of family members and friends who were medical professionals (14.8% and 14.5% vs. 5.3% and 6.7%; $p < 0.01$), as well as those who relied on the knowledge (opinions) of family members or friends and the Internet as the main sources of information about antibacterial drugs (18.9% and 26.6% vs. 13.7% and 20.4%, respectively; $p = 0.004$ and $p = 0.02$) (Table 3).

Table 2

Sources of buying (getting) antibacterial drugs for different groups of respondents

Parameter		With a prescription, % ($n = 657$, 44.1%[1])	During in-patient treatment, % ($n = 101$, 6.8%[1])	Without prescription, % ($n = 631$, 42.4%[1])	Leftover drugs from a previous prescription, % ($n = 167$, 11.2%[1])	Online pharmacy, % ($n = 140$, 9.4%[1])	Family or friends, % ($n = 81$, 5.4%[1])	Avito, Ozon and other platforms, % ($n = 28$, 1.9%[1])
Age groups, years	18-24	24.7	6.2*	13,3*	7.5	5.2	7.8*	2.3
	25-34	27.7	3.2*	28.1*	6.1	4.8	2.9*	1.5
	35-44	24.9	3.5	23.9*	5.2	5.2	2.9*	0.6
	45-54	23.4	3.0	27.3	7.1	4.7	1.5*	0.9
	55-64	19.4	2.8	20.5*	6.7	6.2	1.3*	0.7
	65+	20.4	4.9	16.7	4.2	4.9	2.7*	0.0
Social and professional category	Executive manager	44.8	10.3	34.5	17.2	13.8	5.2*	5.2
	Self-employed	31.4*	5.8*	50.4*	14.9	9.1	4.9*	1.7
	Businessman	50.0	8.8	47.1	2.9	11.8	0.0*	2.9
	Employee/Specialist	46.8*	5.4*	43.1	9.9	4.7	2.8*	1.5
	Unemployed	32.1*	3.7*	50.0*	11.9	8.9	5.2*	2.2
	Pensioner	45.5	9.6	36.4	11.5	12.4	4.8*	1.0
	Student	48.5	19.1*	26.5*	17.6	11.8	20.6*	5.9

Table 2 (continued)

Parameter		With a prescription, % (n = 657, 44.1%[1])	During in-patient treatment, % (n = 101, 6.8%[1])	Without prescription, % (n = 631, 42.4%[1])	Leftover drugs from a previous prescription, % (n = 167, 11.2%[1])	Online pharmacy, % (n = 140, 9.4%[1])	Family or friends, % (n = 81, 5.4%[1])	Avito, Ozon and other platforms, % (n = 28, 1.9%[1])
Financial situation	Extremely low income	26.9	38.4	53.8*	15.4	23.1*	3.8	0.0
	Low income	37.9	6.4	44.8*	11.3	5.9*	4.4	1.5
	Middle income	46.9	6.3	41.3*	12.3	8.3	5.1	1.9
	Upper middle income	43.3	7.4	43.7*	10.0	11.2	6.1	1.9
	High income	54.1	10.8	18.9*	8.1	10.9	8.1	2.7

* $p < 0.01$ when comparing responses within a population group; [1] percentage of those who have taken antibiotics in the last 12 months

Table 3

Characteristics of the groups depending on the sources of buying (getting) antibacterial drugs

Parameter	Buying (getting) antibiotics with a prescription	Buying (getting) antibiotics without a prescription	p
Form of taking antibacterial drugs – injections, %	19.4	11.1	<0.01
Source of prescribing (recommendations) about antibiotics – family members or friends, %	5.3	14.8	<0.01
The source of prescription (recommendations) of antibiotics – a friend who is a medical worker, %	6.7	14.5	<0.01
Duration of taking antibiotics, days	6.7 ± 3.6	5.5 ± 3.5	<0.01
Completed a course of antibiotics prescribed by the doctor, %	85.8	71.1	<0.01
Underwent tests before taking antibiotics to identify the cause of the disease, %	53.3	25.6	<0.01
Knowledge about the ban on the over-the-counter sale of antibiotics, %	75.3	59.0	<0.01
Considered it right to ban over-the-counter sale of antibiotics, %	56.1	39.0	<0.01
Source of information about antibiotics – family members or friends, %	13.7	18.9	0.006
Source of information about antibiotics – Internet, %	20.4	26.6	0.004
Source of information about antibiotics – radio, %	1.8	0.5	0.02

A larger percentage of the respondents who completed a full course of antibiotic therapy was noted among the group of people who took antibacterial drugs prescribed by the doctor (83.9%, n = 847) compared to the rest of the respondents (66.6%, n = 319; $p < 0.01$).

A logistic regression model was created according to the following equation to evaluate the probability of self-medication with antibacterial drugs for various groups of people:

The probability self-medication with antibiotics = $\beta_0 + \beta_1$*Female gender + β_2*Male gender + β_3*Source of information – doctor + β_4*Source of information – personal experience + β_5*Source of information – nursing staff or paramedic + β_6*Source of information – Internet

Thus, the probability that women will self–medicate with antibacterial drugs was 12.2% (95% CI: 10.2–14.8), while similar probability for men was 9.0% (95%CI: 7.0–11.5). The probability of self-medication decreased from 19 to 6.3% (95% CI: 1.3–23.0) when the Internet was the main source of knowledge about antibacterial drugs. If the doctor was the main source of information, the probability decreased from 19 to 3.8% (95% CI: 2.5–5.9), whereas in case of personal (past) experience, it increased from 19 to 32.3% (95%CI: 22.6–42.5) (Table 4).

Table 4

Probability of self- medication

Parameter	Probability of self-medication, % (95% CI)	p
Female gender	12.2 (10.2–14.8)	< 0.001
Male gender	9.0 (7.0–11.5)	< 0.001
Source of information – a doctor	3.8 (2.5–5.9)	< 0.001
Source of information – personal (past) experience	32.3 (22.6–42.5)	< 0.001
Source of information – nursing staff or a paramedic	11.5 (6.3–19.0)	< 0.001
Source of information – Internet	6.3 (1.3–23.0)	< 0.001

The parameters associated with self–medication with antibacterial drugs were female gender (OR = 1.4; 95%CI: 1.0–1.9), medical education (OR = 2.1; 95%CI: 1.3–3.5), and unawareness of a ban on over-the-counter sale of antibiotics (OR = 8.4; 95%CI: 5.3–13.3) (Table 5).

Table 5

Parameters associated with self-medication with antibacterial drugs

Parameter	OR (95% CI)
Female gender	1.4 (1.0–1.9)
Medical education	2.1 (1.3–3.5)
Unawareness of a ban on over-the-counter sale of antibiotics	8.4 (5.3–13.3)

DISCUSSION

The results obtained in this work are comparable with other foreign studies. So, in Saudi Arabia, almost 51% of respondents took antibiotics without a doctor's prescription, including for the prevention of infections, 37.5% bought them at a pharmacy without a prescription and 42% of the participants discontinued taking drugs after symptoms had been relieved [15]. In China, the percentage of respondents who bought antibacterial drugs in the pharmacy without a doctor's prescription reached 47%, in Ethiopia – 67.3%, in the USA – 66%, and in Brazil – 19.0% [8,16–18].

The parameters associated with taking antibacterial drugs without a doctor's prescription in the Russian Federation differ from other countries. Thus, in a Chinese study by X. Yin et al. (2022), a multifactorial logistic regression analysis showed that people aged 30–44 years with higher education and low self-evaluation of health were more likely to take antibiotics without a doctor's prescription [19]. In studies from Lebanon and Ethiopia, age, financial status, level of education, and awareness of antibiotics and antibiotic resistance were significantly correlated with self-medication practices [17, 20].

Similar data were obtained in the work by Y. Ateshim et al. (2019), where the results of the multivariate logistic regression analysis showed that the factors associated with self-medication were gender ($p = 0.046$), education level ($p = 0.019$), and the attitude of citizens to the problem of antibiotic resistance ($p < 0.001$) [21]. Also in the Cameroon study, male gender (OR = 2.32, 95%CI: 1.24–4.34) and higher education (OR = 2.05, 95%CI: 1.08–3.89) were significantly associated with self-medication [5].

CONCLUSION

The results obtained both in the present and other domestic and foreign studies demonstrate the importance and relevance of the problem of self-medication with antibacterial drugs. Moreover, the fact that self-medication is associated with irrational medication intake (since the patient is not aware of the principles of rational antibiotic therapy) doubles the severity of this phenomenon. Since the main source of buying (getting) antibiotics was a pharmacy without a doctor's prescription, the propaganda and strict control over the ban on over-the-counter purchase of antibiotics can become a priority measure to curb antibiotic resistance, which in particular should be directed to the following categories of the population:

– medical and pharmaceutical workers (access channels: professional websites, medical journals, websites of medical organizations and regional executive authorities in the field of healthcare, professional communities);

– citizens without higher education (access channels: general education institutions, educational institutions of primary vocational education, social networks, television, metro, other types of public transport);

– self-employed, entrepreneurs, unemployed, citizens with a poor financial situation (access channels: social networks and Telegram channels, television, public transport).

At the same time, first of all, it is necessary to concentrate on the age group of 25–34 years. This age is the beginning of economic activity of citizens, when they do not want or do not have the opportunity to visit a doctor and get a sick leave, so they independently begin taking the most common and well-known antibacterial drugs for diseases not always caused by bacteria.

A special category characterized by an increased risk of self-medication is medical workers. So, according to the results of the study, the presence of medical education increased the chances of self-treatment twice. In this regard, it is necessary to regularly update the knowledge of medical workers about antibacterial drugs and antibiotic resistance, for example, by holding the "Global Week of Rational Consumption of Antibiotics" in medical organizations and pharmacies on a regular basis. It will help, among others, to increase awareness of healthcare professionals about the development of antibiotic resistance in both patients and medical workers themselves.

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Effect of M-CSF on the expression of endothelial progenitor cell markers in blood mononuclear cell culture in coronary heart disease

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ABSTRACT

Aim. To evaluate the nature of changes in the expression of markers of endothelial progenitor cells (VEGFR2, CD34, CD14) and endothelial cells (CD146) in association with the expression of the leukocyte common antigen CD45 in the culture of blood mononuclear cells in the presence of M-CSF in patients with coronary heart disease (CHD) and healthy donors.

Materials and methods. The study included 12 patients with CHD with class III–V angina pectoris and 10 healthy donors, from whom 30 ml of venous blood was taken on an empty stomach in the morning and stabilized with heparin. Blood mononuclear cells were isolated by Ficoll density gradient centrifugation (1.077 g / cm^3) and subject to immunomagnetic separation using CD14-MicroBeads and CD34-MicroBead Kit (Miltenyi Biotec B.V. & Co. KG, Germany). The resulting $CD14^+$ and $CD34^+$ culture of mononuclear cells was incubated for 6 days in a complete nutrient medium with and without M-CSF 50 ng / ml (Cloud-Clone Corp., USA) with complete replacement of the medium and repeated application of M-CSF on day 3. After 6 days, the proportions of $CD45^+$, $CD14^+$, $CD34^+$, $VEGFR2^+$, and $CD146^+$ cells in the culture were assessed by flow cytometry using CD14-FITC, CD34-PE, VEGFR2-Alexa Fluor 647; CD45-FITC and CD146-PerCP antibodies (BD Biosciences, USA).

Results. It was shown that in healthy donors, the proportion of $CD146^+$ cells in the co-culture of blood mononuclear cells with M-CSF exceeded their number in the sample without it, with comparable expression rates of CD45, CD14, and VEGFR2 markers between the control and stimulated cultures. In CHD patients, the number of CD146+ and $VEGFR2^+$ cells did not change when M-CSF was added to the mononuclear cell culture; however, the proportion of $CD14^+$ cells increased and the proportion of $CD45^+$ cells decreased compared to the control sample. The number of $CD34^+$ cells was comparable both between control and stimulated samples, and between the groups of examined individuals. At the same time, in patients with CHD, an increased proportion of $VEGFR2^+$ cells was found in the control and stimulated samples compared to healthy individuals, while an increased proportion of $CD14^+$ cells was detected only in the stimulated culture.

Conclusion. The development of CHD disrupts the response of blood mononuclear cells to the effect of M-CSF, increasing the number of $CD14^+$ and reducing the proportion of $CD45^+$ cells in the culture in the absence of stimulating effects on the expression of endothelial cell marker CD146. At the same time, M-CSF does not affect the expression of CD34 and VEGFR2 in endothelial progenitor cells both in patients with CHD and in healthy individuals.

Keywords: endothelial progenitor cells, monocytes, M-CSF, vascular repair, endothelial cells, coronary heart disease

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Влияние M-CSF на экспрессию маркеров прогениторных эндотелиальных клеток в культуре мононуклеаров крови при ишемической болезни сердца

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РЕЗЮМЕ

Цель: оценить характер изменений экспрессии маркеров эндотелиальных прогениторных клеток (VEGFR2, CD34, CD14) и эндотелиоцитов (CD146) в ассоциации с экспрессией панлейкоцитарного маркера CD45 в культуре мононуклеаров крови в присутствии M-CSF у больных ишемической болезнью сердца (ИБС) и здоровых доноров.

Материалы и методы. В исследование вошли 12 больных ИБС со стенокардией напряжения III–V функционального класса и 10 здоровых доноров, у которых утром натощак забирали венозную кровь в количестве 30 мл и стабилизировали гепарином. Мононуклеары крови выделяли на градиенте фиколла 1,077 г/см^3 и подвергали иммуномагнитной сепарации с применением антител CD14-MicroBeads и CD34-MicroBead Kit (Miltenyi Biotec B.V. & Co. KG, Германия). Полученную смешанную по CD14 и CD34 культуру мононуклеаров инкубировали 6 сут в полной питательной среде с добавлением M-CSF 50 нг/мл (Cloud-Clone Corp., США) и без него с полной заменой среды и повторным внесением M-CSF на 3-и сут. Через 6 сут оценивали долю позитивных по CD45, CD14, CD34, VEGFR2, CD146 клеток в культуре с помощью антител CD14-FITC, CD34-PE, VEGFR2-Alexa Fluor 647; CD45-FITC и CD146-PerCP (BD Biosciens, США) методом проточной цитофлуориметрии.

Результаты. Показано, что у здоровых доноров доля CD146$^+$ клеток в смешанной культуре мононуклеаров крови при добавлении M-CSF превышает их количество в пробе без его внесения при сопоставимых показателях экспрессии маркеров CD45, CD14 и VEGFR2 между контрольной и стимулированной культурами. У больных ИБС численность CD146$^+$ и VEGFR2$^+$ клеток не изменялась при добавлении M-CSF в культуру мононуклеаров, однако доля CD14$^+$ клеток возрастала, а CD45$^+$ клеток снижалась относительно контрольной пробы. Количество CD34$^+$ клеток было сопоставимым как между контрольной и стимулированной пробами, так и между группами обследованных лиц. При этом у больных ИБС установлено превышение доли VEGFR2$^+$ клеток относительно здоровых доноров в контрольной и стимулированной M-CSF пробах, а для CD14$^+$ мононуклеаров – только в стимулированной культуре мононуклеаров.

Заключение. Формирование ИБС нарушает реакцию мононуклеаров крови на действие M-CSF, увеличивая число CD14$^+$ и уменьшая долю CD45$^+$ клеток в культуре при отсутствии стимулирующего влияния на

экспрессию маркера CD146 эндотелиальных клеток. При этом M-CSF не влияет на экспрессию маркеров CD34 и VEGFR2 ЭПК как у больных ИБС, так и у здоровых лиц.

Ключевые слова: моноциты, прогениторные эндотелиальные клетки, M-CSF, репарация сосудов, эндотелиоциты, ишемическая болезнь сердца

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

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследования проведены с разрешения локального этического комитета СибГМУ (протокол № 9299 от 28.11.2022).

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INTRODUCTION

For many decades, cardiovascular diseases have been the main cause of death in many countries of the world [1, 2]. Studies of the pathogenesis of vascular pathology are often limited to studying vasomotor endothelial vascular dysfunction [3, 4], however, its angiogenic form which includes impairment of angiogenesis and reparative processes in the vessels is insufficiently studied [5].

In atherosclerosis, which underlies coronary heart disease (CHD), monocytes can have both a negative and a protective effect. On the one hand, plaque macrophages, maintaining chronic inflammation, prolong vascular alteration with the help of matrix metalloproteinases (MMPs) [1, 6, 7] and promote vascularization in atheroma, which increases the risk of plaque hemorrhage with subsequent plaque destabilization [3, 8]. On the other hand, monocytes containing a population of endothelial progenitor cells (EPCs) [9] can participate in the angiogenesis induction, which is necessary for the formation of collateral blood flow and repair of damaged vessels, which has a protective and adaptive value in CHD.

Angiogenesis is realized by early and late EPCs, which have monocytic VEGFR2$^+$CD34$^+$CD14$^+$ and non-monocytic VEGFR2$^+$CD34$^+$CD14$^-$ immunophenotypes, respectively [2, 9]. Early EPCs stimulate the mature endothelial cells survival in a paracrine way, can acquire their markers, but have limited proliferative activity; late EPCs have a high proliferative activity and differentiate into endotheliocytes [2, 9]. It is known that circulating EPCs can be isolated from peripheral blood mononuclear cells. Moreover, when monocytes are cultured in the presence of vascular endothelial growth factor (VEGF), they transform into an intermediate cell phenotype and further differentiate in endotheliocytes, losing the CD45 pan-leukocyte marker [10]. The ability of other growth factors to stimulate angiogenesis has also been demonstrated. Thus, the cultivation of bone marrow cells with granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF) increased the expression of endothelial markers CD31 and CD146 [11], and the cultivation of EPCs isolated from blood monocytes with granulocyte – macrophage colony-stimulating factor (GM-CSF) increased their proliferative activity [12]. Considering the above, it can be assumed that blood monocytes (CD14$^+$) enriched with hematopoietic stem (CD34$^+$) cells [2] can modulate their phenotype under the influence of various stimuli. Since early EPCs are monocytic cells, it is possible that the cultivation of a mixed culture of monocytes (CD14$^+$) and hematopoietic stem cells (CD34$^+$) in the presence of M-CSF can affect the expression of markers characteristic of EPCs.

The aim of the study was to evaluate the nature of changes in the expression of markers of endothelial progenitor cells (VEGFR2, CD34, CD14) and endotheliocytes (CD146) in association with the expression of the leukocyte common antigen CD45 in CD14$^+$ and CD34$^+$ blood mononuclear cell culture in the presence of M-CSF in patients with CHD and healthy individuals.

MATERIALS AND METHODS

A single-stage, case-control, single-center, observational study was conducted from December 2022 to May 2023. The study included 12 patients with CHD (10 men and 2 women, mean age 62.0 [56.5; 64.0] years) with class II–IV angina pectoris and mainly NYHA class II–III heart failure, who had a history of myocardial infarction and were admitted to the Cardiology Research Institute of Tomsk National Research Medical Center to undergo coronary bypass grafting. Patients received standard anti-anginal therapy with long-acting nitrates, β1-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, as well as antiplatelet therapy with acetylsalicylic acid preparations or P2Y12 receptor blockers and lipid-lowering therapy with statins. The comparison group consisted of 10 apparently healthy donors (7 men and 3 women, mean age 57.5 [48.0; 65.5] years), who did not have any cardiovascular diseases and complaints of a corresponding nature.

The exclusion criteria were as follows: age over 70 years, autoimmune diseases, an allergic process in the acute phase, a tumor process, viral hepatitis, syphilis, HIV infection, anemia, treatment with iron-containing drugs, erythropoietin or immunosuppressive therapy, acute infections within less than 3 weeks before the study, as well as the patient's refusal to participate in the study.

The studies were carried out in accordance with the ethical principles set out in the Declaration of Helsinki (1975) and approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 9299 of 28.11.2022).

The material of the study was blood from the cubital vein in a volume of 30 ml, taken in the morning on an empty stomach before exercise, diagnostic and therapeutic procedures, which was stabilized with heparin (25 IU / ml). Blood mononuclear cells were isolated by Ficoll density gradient centrifugation (1.077 g /cm^3) (PanEco LLC, Moscow). After double washing of mononuclear cells with 0.5% PBS (PBS, pH=7.2), immunomagnetic separation was performed using CD14 MicroBeads and CD34 MicroBead Kit antibodies (Miltenyi Biotec B.V. & Co. KG, Germany), MS separation columns (Miltenyi Biotec B.V. & Co. KG, Germany), and a MiniMACS magnet (Miltenyi Biotec B.V. & Co. KG, Germany) according to the manufacturer's instructions. The purity of isolation, i.e. the proportion of CD14+ and CD34+ cells in the culture, was 80–85% and 5–7%, respectively. Cell viability was determined in a test with 0.1% trypan blue (PanEco LLC, Moscow). If it was at least 96%, cells were added to 2 wells of a 24-well plate, 106 cells each. The samples were incubated for 6 days with 5% CO_2 in a complete nutrient medium (nutrient medium RPMI-1640 (PanEco LLC, Moscow) supplemented with fetal calf serum, L-glutamine, penicillin – streptomycin) with the addition of 50 ng / ml of recombinant human M-CSF (Cloud-Clone Corp., USA) to one of the wells. After 3 days of incubation, the medium was partially replaced and the stimulant was re-introduced at the same dose. The sample with recombinant M-CSF was considered stimulated, and the sample without M-CSF was considered control. After 6 days, the cells were removed from the plate surface by incubating them with 500 μl of 0.05% trypsin – EDTA solution (PanEco LLC, Moscow) per well for 5 min at 37 °C. After washing the cells with 500 μl of 0.5% PBS, the pellet was resuspended, and the cells were used for flow cytofluorometry.

To determine the expression of CD45, CD14, CD34, VEGFR2 (KDR; CD309), and CD146 molecules in the co-culture culture of blood mononuclear cells, flow cytometry was performed using monoclonal antibodies with two combinations of labels: CD14-FITC, CD34-PE, VEGFR2(KDR; CD309)-Alexa Fluor 647 and CD45-FITC, CD146-PerCP, VEGFR2(KDR; CD309)-Alexa Fluor 647, according to the manufacturer's instructions (BD Biosciences, USA). Dead cells were excluded from the analysis using DAPI staining (Wuhan Servicebio Technology Co., Ltd, China). The fluorescence intensity was measured on the CytoFLEX flow cytometer (Beckman Coulter International S.A., USA), and the data obtained were analyzed using the CytExpert 2.3 software (Beckman Coulter International S.A., USA). The positivity boundaries of the label luminescence were established using fluorescence minus one (FMO) controls. We estimated the proportion of cells positive for each marker as a proportion (%) of the total number of cases, excluding the area of small objects (FSC less than 100×10^4).

Statistical data was analyzed using the Statistica 10.0 program. In the statistical description of the results, the median and interquartile range were calculated (*Me* [*Q*1; *Q*3]). The Mann – Whitney test (for independent samples) and the Wilcoxon test (for dependent samples) were used to perform comparative analysis of sample data. The results of the statistical analysis were considered statistically significant at $p < 0.05$.

RESULTS

The comparative analysis of control and M-CSF-stimulated samples of $CD14^+$ and $CD34^+$ blood mononuclear cell co-cultures in healthy donors revealed a statistically significant increase in the proportion of $CD146^+$ cells only in the sample with M-CSF compared to the sample without M-CSF with comparable expression parameters for CD45, CD14, and VEGFR2 markers (Table). On the contrary, in patients with CHD, the number of $CD146^+$ cells did not change when M-CSF was added to the culture, as did the number of $VEGFR2^+$ cells, but the proportion of $CD14^+$ mononuclears increased significantly, and $CD45^+$ decreased compared to the control sample. In the meantime, CD34 expression did not differ between the control and stimulated samples, or between the groups of examined individuals.

The analysis of the expression parameters of the studied markers between the groups of examined patients revealed an excess of the relative number of $VEGFR2^+$ cells in CHD patients compared to healthy donors in both control and stimulated M-CSF samples, and for $CD14^+$ mononuclear cells – only in the stimulated sample (Table).

Table

Expression of markers of endothelial progenitor cells and endotheliocytes, as well as CD45 molecules in the $CD14^+$ and $CD34^+$ blood mononuclear cell co-culture stimulated and not stimulated with M-CSF in patients with CHD and healthy donors, *Me* [Q_1; Q_3]

The proportion of cells expressing the marker	Healthy donors		CHD patients	
	Control sample	Sample with M-CSF	Control sample	Sample with M-CSF
CD45, %	56.18 [40.55; 64.20]	30.93 [23.40; 47.12] $p_c = 0.288$	30.79 [19.66; 46.60] $p = 0.135$	17.68 [11.62; 23.90] $p = 0.217$ $p_c = 0.049$
CD14, %	25.82 [18.34; 31.10]	19.17 [12.32; 26.80] $p_c = 0.627$	26.43 [15.93; 30.12] $p = 0.916$	40.36 [25.14; 51.69] p = 0.046 $p_c = 0.031$
CD34, %	22.71 [18.14; 27.58]	19.18 [15.07; 23.76] $p_c = 0.743$	15.84 [10.86; 24.11] $p = 0.566$	17.32 [14.24; 22.31] $p = 0.920$ $p_c = 0.855$
VEGFR2, %	4.38 [1.75; 9.25]	5.62 [2.51; 11.43] $p_c = 0.315$	21.16 [13.05; 28.56] $p = 0.002$	25.47 [13.80; 32.16] $p = 0.013$ $p_c = 0.407$
CD146, %	1.79 [0.94; 2.70]	2.82 [1.63; 5.40] $p_c = 0.023$	1.44 [0.90; 3.82] $p = 0.821$	2.36 [1.59; 4.27] *p = 0.763* $p_c = 0.194$

Note: p_c – the level of statistical significance of the differences in the parameters compared with the cell content in the control sample, p – in healthy donors.

DISCUSSION

The study of the M-CSF effect on the marker expression inherent in early EPCs (VEGFR2, CD34, CD14) and more differentiated endotheliocytes (CD146) established the differences in the effects of this cytokine on the culture of blood mononuclear cells mixed in $CD14^+$ and $CD34^+$ in CHD patients and healthy donors (Table). It was shown that the physiological response of these cells to M-CSF consists in increased CD146 expression, which does not occur in patients with CHD.

The CD146 molecule is expressed on endotheliocytes and pericytes, promoting the formation of cell – cell interactions between them, increasing endothelial adhesiveness and endothelial cell survival, and also contributing to pericyte recruitment, EPC homing, vessel architectonics, and their stabilization [13, 14]. Its soluble form sCD146 was shown to enhance the angiogenic properties of EPCs, and sCD146 injection improved neovascularization in a murine ischemia model, which was mediated by VEGFR1, VEGFR2, angiomotin, and the shCD146 isoform [13]. Therefore, an increase in CD146 expression in the blood mononuclear culture in healthy individuals can be considered as a positive effect of M-CSF, which could have a protective effect on vessels of the ischemic myocardium affected by atherosclerosis

in patients with CHD, but it is not realized in CHD (Table).

Using a model of chronic obstructive pulmonary disease, it was shown that CD146 deficiency in pulmonary endothelial cells was associated with its increased permeability and tissue infiltration by monocytes, and the addition of sCD146 increased monocyte transmigration *in vitro* [13]. Accordingly, impaired CD146 expression under the effect of M-CSF in patients with CHD can not only inhibit angiogenesis, but also enhance monocyte migration into the vessel wall and tissues, contributing to inflammation and fibrosis. CD146 deficiency is associated with suppression and enhancement of the non-canonical and canonical Wnt pathways, which leads to a profibrotic state [15].

Since CD146 is more present on mature endotheliocytes and is often used as a marker of desquamated endothelial cells [14, 16, 17] and to a lesser extent – on EPC [9, 14], in healthy individuals, a mature phenotype of endotheliocytes is probably more likely to form under the M-CSF influence in blood mononuclear cell culture. At the same time, the expression of VEGFR2, which is inherent in EPCs and endotheliocytes, did not change in blood mononuclear cell culture in the presence of M-CSF in both healthy individuals and patients with CHD (Table).

Binding of VEGFR2 to its ligands VEGF-A and VEGF-C stimulates the expression of adhesion molecules, vascular permeability, cell survival (through activation of the PI3K/AKT pathway), cell attachment and migration (through activation of p38MAPK and focal adhesion kinase FAK), and proliferative response (through activation of mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK)) [18]. Therefore, it can be assumed that early $VEGFR2^+CD34^+CD14^+$ and late $VEGFR2^+CD34^+CD14^-$ EPCs present in the mononuclear cell culture (because they were separated by $CD34^+$) were transformed under the M-CSF influence in healthy donors into a more mature phenotype with CD146 expression, but without increasing the proliferative potential of cells through VEGFR2 signaling. This transformation obviously did not occur in CHD. Since the expression of VEGFR2 and CD34 in the blood mononuclear cell culture did not increase in the M-CSF presence in both healthy individuals and CHD patients (Table), it cannot be argued that M-CSF promotes EPC differentiation or proliferation. However, the proportion of $VEGFR2^+$ cells in the co-culture of blood mononuclear cells in CHD patients was higher than in healthy individuals, both with M-CSF and without it (Table). This fact is obviously associated with the initially greater $VEGFR2^+CD34^+$ cell separation in patients due to the high $VEGFR2^+$ cell content in the blood of CHD patients, which we discussed earlier [17].

A significantly higher percentage of $CD34^+$ cells in both groups of examined individuals after mononuclear cell culture (Table) is worth noting compared to that obtained using immunomagnetic separation (see Materials and Methods section). It has been shown that blood mononuclear cells in the culture can transform not only into endothelial cells and macrophages, but also into fibrocytes expressing CD34 [19]. We observed the spindle cell formation in culture (the results are not presented, they are being statistically processed), the shape of which is inherent in both fibrocytes [19] and terminal endothelial cells [2, 10]. However, without studying specific fibrocyte markers, such as the presence of intracellular collagen and CD34 in the absence of CD33, CD35, and CD93 expression [19], it is impossible to discuss the M-CSF effect on fibrocyte differentiation.

The unchanged proportion of $CD34^+$ mononuclear cells in the culture under the M-CSF effect in the examined individuals of both groups (Table) can also be associated with the induction of multi-directional processes by this cytokine: stimulation of its formation from monocytes ($CD14^+$) that do not express $CD34^+$ into fibrocytes carrying it, and with the transition of $CD34^+$ EPCs into mature endothelial cell forms that lose $CD34^+$. Thus, $CD34^+$ cells with high expression of endothelial cell markers, surrounded by spindle cells, were found in the blood monocyte culture *in vitro*.

Over time, two types of colony-forming units (CFU) were isolated in the monocyte culture: CFU-Hill cells and endothelial colony-forming cells. The former are phagocytic and express CD14, CD45, CD115, but do not have proliferative and vasculogenic activity, while the latter do not have CD14, CD45, CD115 and express markers of endothelial cells and form capillary-like structures *in vitro* and vessels *in vivo* [2]. Exposure to M-CSF caused $CD14^+$ cell accumulation (Table) in CHD patients compared to healthy individuals, which indicates increased CFU-Hill cell formation. At the same time, the decrease in the expression of the leukocyte common antigen CD45 on the cultured blood mononuclear cells in CHD patients with M-CSF can probably be explained by its stimulating effect on the transformation of monocytes into various cells not carrying CD45 (endothelial [2])

and weakly expressing it (fibrocytes and macrophages [19]).

CONCLUSION

In CHD, the nature of the response of $CD14^+$ and $CD34^+$ blood mononuclear cells to the M-CSF action changes, which is manifested by an increase in the CD14 marker expression and inhibition of CD45 molecule expression. At the same time, the physiological response of these cells to M-CSF stimulation in the form of increased endothelial marker CD146 expression is lost. Meanwhile, M-CSF does not change the EPC marker (VEGFR2, CD34) expression on $CD14^+$ and $CD34^+$ blood mononuclear cells both in healthy individuals and in CHD patients. The obtained knowledge creates the concept of the effectiveness of cytokine and cell therapy using M-CSF for the angiogenesis induction in CHD patients and the mechanisms of its possible therapeutic effect. These data can become the grounds for developing a new method for treating this disease. Provided that the recombinant M-CSF side effects are excluded, further research is required *in vivo*.

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Authors' contribution

Chumakova S.P. – conception and design, literature review, statistical processing of the research results and their interpretation, drafting and formatting of the manuscript. Urazova O.I. – conception and design, supply and equipment support for laboratory research, interpretation of the data, editing of the manuscript. Shipulin V.M. – consulting on research planning and interpretation of the clinical aspects and results obtained in cardiac patients. Gladkovskaya M.V. – sample preparation and carrying out of cell culture research. Andreev S.L. – interaction with cardiac patients, provision of clinical material. Nevskaya K.V. – carrying out of flow cytometry. Zima A.P. – supply and equipment support for laboratory research, interpretation of the data. Nikulina E.L. – statistical processing of the research results.

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Adipokine imbalance and its role in the pathogenesis of novel coronavirus infection

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ABSTRACT

The review summarizes and analyzes the results of major foreign studies on the role of adipokine imbalance in the development of a severe course and complications of novel coronavirus infection (COVID-19). Adipokines are biologically active compounds produced by adipose tissue cells and involved in the regulation of metabolism and the functioning of the immune system.

Obesity is a proven risk factor for severe COVID-19 due to high hormonal and metabolic activity of visceral adipose tissue. A deep understanding of COVID-19 pathogenesis from the point of view of the role of adipokine imbalance in it can provide the grounds for the development of effective pathogenetic approaches to the prevention of a severe course and complications of novel coronavirus infection.

Keywords: novel coronavirus infection, SARS-CoV-2, metabolic syndrome, obesity, adipokines, adipokine imbalance

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Дисбаланс адипокинов и его роль в патогенезе новой коронавирусной инфекции

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РЕЗЮМЕ

В обзоре обобщены и проанализированы результаты крупных зарубежных исследований, касающихся изучения роли адипокинового дисбаланса в развитии тяжелого течения и осложнений новой коронавирусной инфекции (COVID-19). Адипокины – биологически активные соединения, которые вырабатываются клетками жировой ткани и участвуют в регуляции обмена веществ и работы иммунной системы.

Известно, что ожирение является доказанным фактором риска тяжелого течения COVID-19 в связи с высокой гормональной и метаболической активностью висцеральной жировой ткани. Глубокое понимание

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патогенеза COVID-19 с позиций участия в нем адипокинового дисбаланса может лечь в основу разработки эффективных патогенетических подходов к профилактике тяжелого течения и осложнений новой коронавирусной инфекции.

Ключевые слова: новая коронавирусная инфекция, SARS-CoV-2, метаболический синдром, ожирение, адипокины, адипокиновый дисбаланс

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

Novel coronavirus infection (COVID-19), caused by the SARS-CoV-2 virus, has become a serious medical and social problem throughout the world, as confirmed by epidemiological studies in recent years [1]. COVID-19 has significantly affected the level and structure of disability and mortality among the working-age population [2]. Despite the fact that the World Health Organization (WHO) has declared the end of the pandemic, the incidence of COVID-19 remains significant. The infectious process in COVID-19 is manifested by a variety of symptoms and pronounced clinical presentation, from an asymptomatic course to fulminant forms of the disease [3]. The scientific community's understanding of the mechanisms of the SARS-CoV2 effect on the body continues to expand, and currently there are sufficient data indicating not only damage to the respiratory system, but also the involvement of other body systems in the pathological process [4].

The severity of the problem of COVID-19 is determined not only by the unprecedented level of prevalence, a trend toward a severe course, and the frequency of fatal complications, but also by the lack of a comprehensive understanding, due to the short observation period, of the leading links in the pathogenesis of this infection, which determine the prognosis. In the scientific literature of recent years, the search for predictors of the severe course and adverse outcomes of COVID-19, including mortality, has been widely discussed.

METABOLIC SYNDROME AS A RISK FACTOR FOR A SEVERE COURSE OF COVID-19

To date, a large amount of evidence has been accumulated convincingly indicating that the components of metabolic syndrome (MS) should be taken into account as predictors of a severe and complicated course of COVID-19. MS is characterized as a complex of metabolic, hormonal, and clinical disorders associated with a large number of socially sensitive chronic non-communicable diseases and also determines the severe course of a number of infections. Insulin resistance, dyslipidemia, abdominal obesity, and arterial hypertension (AH) are considered as the main components of MS. Each of these components underlies the development and the severe and complicated course of associated pathology, worsens the disease prognosis, and negatively affects the quality of life of patients [5].

The prevalence of MS in recent years has become pandemic in nature and is maintaining an upward trend, covering, according to recent estimates, up to 50% of the population in the Russian Federation [6]. Chronic systemic low-grade inflammation is considered to be one of the key pathogenetic factors that unites MS and associated diseases. This is illustrated by numerous studies showing a direct relationship of the level of systemic inflammation markers with the severity of metabolic disorders and the risk of developing a number of socially sensitive non-communicable diseases [5, 7].

Since the beginning of the COVID-19 pandemic, data have been accumulated on the impact of metabolic disorders on the course of the infection. Researchers' attention was drawn to the fact that the presence of obesity, AH, and carbohydrate metabolism disorders increased the risk of hospitalization and a severe course of COVID-19 [8, 9].

Thus, a cohort study conducted by J.L. Denson et al., including 29,040 patients hospitalized with COVID-19, revealed that the presence of MS significantly increased the risk of hospitalization

in the intensive care unit (ICU), the frequency of using mechanical ventilation, and the development of acute respiratory distress syndrome (ARDS) and death. At the same time, the severity of metabolic disorders determined the risk of complications [10]. It was found that MS proved to be a more significant prognostic factor for severe COVID-19 than each of its components separately [11]. However, each of the components makes its own negative contribution and aggravates the course of the infectious process, which is also confirmed by the results of a number of studies. A 2021 meta-analysis combining data from 186 studies showed an increase in the relative risk of death from COVID-19 in patients with diabetes mellitus (DM) by 54%, in patients with hypertension – by 42%, and in patients with obesity – by 45%. [12].

CARBOHYDRATE METABOLISM DISORDERS AND COVID-19

Long before the COVID-19 pandemic, results were published characterizing hyperglycemia and DM as independent predictors of a severe course and mortality for respiratory infections, such as SARS [13]. As the COVID-19 pandemic spread, researchers from different countries pointed out that the presence of DM significantly increases the risk of death in COVID-19 [14–16]. It was noted that not only DM was associated with the development of adverse outcomes, but also an increase in fasting blood glucose levels was associated with a high risk of mortality after adjustments for age, gender, and comorbidities [17].

The results from meta-analyses also showed that hyperglycemia at admission was a strong independent predictor of a severe course and mortality in COVID-19. In particular, an increase in glucose concentration by 1 mmol / l increased the risk of a severe disease course by 33% [18, 19]. It is now known that HbA1c level is also associated with increased mortality and severity of COVID-19 [20].

ARTERIAL HYPERTENSION AND COVID-19

An analysis of multiple observational studies found that the risk of death for patients with AH during COVID-19 was 11% higher than for patients without AH [12]. Some reports have suggested that AH may represent a risk factor for increased susceptibility to COVID-19 infection, a more severe course of the disease, and increased mortality. However, the independent role of AH remains a matter of debate, since this syndrome is often associated with older age and other cardiovascular risk factors in the general population, which may also aggravate the course of COVID-19. It is believed that high and uncontrolled systolic blood pressure may contribute to a more severe course of COVID-19, likely due to the presence of vascular remodeling, which may exacerbate endothelial dysfunction, endothelial lesions, and endotheliitis caused by COVID-19 [21].

DYSLIPIDEMIA AND COVID-19

Researchers' attention was drawn to the nature of dyslipidemia, which has distinctive features in COVID-19. At the beginning of the pandemic, Chinese scientists described changes in the lipid profile characteristic of COVID-19: a significant decrease in the level of total cholesterol (TC), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) in the blood serum compared to the controls [22]. At the same time, hypolipidemia, which occurred in patients even with a mild infection, tended to progress as the severity of COVID-19 increased [23, 24]. Another group of researchers noted that patients admitted to the ICU had higher triacylglycerol (TAG) levels [25]. Subsequently, as clinical data accumulated, large meta-analyses were conducted that confirmed the association of dyslipidemia with the severity of COVID-19 and the risk of death [26, 27].

OBESITY AND COVID-19

Obesity as an unfavorable prognostic factor in respiratory infectious diseases was considered long before COVID-19. Back in 2009, during the influenza A (H1N1) pandemic, obesity was first characterized as a predictor of a severe disease course and high mortality in infected individuals [28]. In further clinical studies of H1N1 flu, obesity was associated with an increased risk of hospitalization, transfer to ICU, and a need for mechanical ventilation [29, 30]. Since the beginning of the COVID-19 epidemic, researchers have noted that a significant proportion of SARS-CoV-2-infected patients with respiratory failure admitted to the ICU were overweight. The strong relationship of abdominal obesity with adverse outcomes of COVID-19 infection (the risk of hospitalization, the severity of clinical and paraclinical symptoms, the development of respiratory failure, the need for mechanical ventilation, and mortality) has been confirmed by a number of independent studies conducted in different countries [31–33]. A meta-analysis of several large-scale epidemiological studies suggests that with increasing body weight, the severity of the disease increases significantly [34–36].

Therefore, the association of obesity with adverse clinical outcomes from COVID-19 is robust across the board and persists even after adjusting for confounding variables, such as age, sex, and comorbidity. Various mechanisms are currently being discussed to explain why obese patients are most susceptible to respiratory infections, including COVID-19. The role of abdominal obesity in the pathogenesis of undesirable outcomes of associated diseases is explained by high endocrine and metabolic activity of adipose tissue. Visceral adipose tissue secretes biologically active substances in large quantities, such as adipokines. They realize their systemic effect by participating in the regulation of a variety of body functions [37]. The biological effects of adipokines and the molecular mechanisms of their action require careful study [28]. Recently, close attention of researchers has been directed to studying the role of adipokine imbalance not only in the pathogenesis of diseases associated with MS, but also in the mechanisms of development of COVID-19.

ADIPOKINE IMBALANCE AND COVID-19

The most studied adipokines to date include leptin and adiponectin.

Leptin is an adipokine synthesized primarily by adipocytes in response to food intake, acting as a signaling molecule for the brain in the regulation of appetite and metabolism. Circulating leptin levels are directly proportional to body adipose tissue mass and are altered under conditions of both chronic negative and positive energy balance, whereby undernutrition promotes hypoleptinemia, and obesity promotes hyperleptinemia [39]. In addition to its main role in regulating appetite and maintaining body weight due to the induction of anorexigenic factors, suppression of orexigenic neuropeptides in the hypothalamus, and influence on energy metabolism, leptin plays an important role in the functioning of the immune, hematopoietic, neuroendocrine, and reproductive systems [40]. Leptin is characterized by many researchers as an inducer of inflammation and oxidative stress, since its level in the blood is associated with the proinflammatory and prooxidant activity of immunocompetent cells in the blood and adipose tissue [41–43]. In this regard, the scientific interest in the role of this adipokine in the mechanisms of COVID-19 development is justified.

Thus, it was found that the average level of leptin in the blood was higher in patients with novel coronavirus infection who were critically ill, in contrast to a group of patients with critical illness not associated with COVID-19, while leptin levels correlated with body mass index (BMI) [34, 44]. Along with the level of proinflammatory cytokines, the level of leptin is closely associated with the progression and severity of the infectious process [45] and the need for transfer to ICU [46]. Changes in the leptin concentration in the blood of patients with COVID-19 were determined: peak values were observed on day 1 with a gradual decrease over the next 28 days of illness, which coincided with the changes in the level of inflammatory biomarkers interleukin (IL)-1β and tumor necrosis factor (TNF)α [47].

However, a number of researchers studying the pathogenetic role of leptin did not find statistically significant associations of leptin levels with either the severity of the disease or inflammatory factors (C-reactive protein (CRP), IL-6) in the blood of patients [48], or with the need to transfer patients with COVID-19 to the ICU [25], as well as with in-hospital mortality [49]. Researchers' attention was also drawn to the fact that the concentration of leptin was significantly higher in women than in men, which confirms the results of other authors who showed gender characteristics of hyperleptinemia [50].

Adiponectin is a polypeptide that is mainly produced by subcutaneous adipose tissue and exhibits a wide range of protective effects: increasing insulin sensitivity, oxidizing fatty acids in adipose tissue and free fatty acids in skeletal muscles, reducing glucose release from the liver, increasing glucose uptake, and activating adipogenesis [51, 52]. The effects of adiponectin are realized through its receptors, which are found in adipose tissue, liver, and skeletal muscles [53, 54]. The level of adiponectin increases with a decrease in body weight in the context of taking antidiabetic drugs; in obesity, insulin resistance, and inflammation, its secretion decreases [55]. It is known that this adipokine exhibits anti-atherosclerotic effects, inhibiting the migration of monocytes / macrophages into the vessel wall and preventing the formation of foam cells, as well as a selective anti-inflammatory effect [56]. The anti-inflammatory effects of adiponectin were confirmed by negative relationships between its concentration and the level of some proinflammatory cytokines and markers of oxidative stress [50, 57–59].

Scientific interest in the role of adiponectin in the pathogenesis of COVID-19 has been demonstrated by a large number of publications. Some studies in which the level of adiponectin was determined once

at admission did not show its relationship with the severity of the disease or with adverse outcomes in COVID-19 [25, 48, 60, 61]. Italian researchers found that serum adiponectin levels were significantly lower in hospitalized patients with COVID-19 compared to healthy controls. Moreover, patients in both groups differed significantly in BMI, which may explain the observed difference in adiponectin concentrations. It was also noted that high molecular weight (HMW) oligomeric complexes, considered to be the most active form of adiponectin, were negatively correlated with the degree of lung injury (as measured by ultrasound, LUS score), while serum adiponectin levels generally did not show a prognostic value for adverse outcomes in patients with COVID-19 [44].

Danish researchers showed that the level of adiponectin in patients with community-acquired pneumonia of various etiologies, including SARS-CoV-2, despite higher values of inflammatory biomarkers in patients with novel coronavirus infection, did not differ significantly [62]. Experts from the University of Virginia School of Medicine (USA) found that patients with COVID-19 complicated by respiratory failure had lower adiponectin values, in contrast to patients with respiratory failure associated with infections of other etiologies [63]. The study conducted within the CRACoV-HHS project in Poland included the assessment of the levels of adiponectin and other cytokines in a cohort of patients with COVID-19 with varying degrees of severity not only at admission, but also on day 7 and 28 of hospitalization. Severe infection was associated with low adiponectin values throughout the observation period and increased levels of inflammatory biomarkers (TNFα, IL-1β, PTX3) [47]. Dutch colleagues in a multicenter prospective study found that patients with severe and extremely severe COVID-19 had lower adiponectin values [64]. In a recently published retrospective study, doubling circulating adiponectin levels was associated with a 38% reduction in the odds of 90-day mortality (OR 0.62, CI 0.43–0.89) and a 40% reduction in the risk of developing respiratory failure (OR 0.60, CI 0.42–0.86) [65]. This reflects the protective and anti-inflammatory properties of adiponectin, previously described by many researchers, and may serve as a reason for continuing studies in larger cohorts of patients.

Resistin is a protein predominantly synthesized by monocytes and macrophages [66]. Currently, resistin is considered as an adipokine involved in the formation of insulin resistance and inflammatory responses [67]. Resistin is believed to influence obesity and insulin homeostasis through paracrine and endocrine signaling pathways. Resistin has a proinflammatory effect because it stimulates the expression of TNFα and IL-6 by mononuclear leukocytes [68], and it is considered as a marker of inflammation in atherosclerosis [69]. Studies on the effects of resistin in individuals with metabolic disorders, including obesity, have shown conflicting results [70, 71].

In few studies examining the role of resistin in the pathogenesis of the inflammatory response in COVID-19, it has shown itself to be a promising marker that can be considered as a predictor of not only the severity of COVID-19, but also of adverse outcomes. A number of researchers found a significant increase in the level of resistin in the serum of patients with COVID-19, which correlated with the level of proinflammatory cytokines, the severity of the disease, and the frequency of adverse outcomes, including mortality [44, 47, 61, 64, 72]. Resistin levels were associated with the severity of clinical symptoms, the need for oxygen therapy, and the need for mechanical ventilation [73]. Besides, it was also associated with a worse prognosis of COVID-19 and was characterized as a diagnostically significant predictor for transfer of patients to ICU [74].

Chemerin is a protein that is synthesized primarily by hepatocytes and adipocytes. Researchers' interest in this adipokine has appeared relatively recently. It was found that chemerin is involved in the regulation of a large number of biological processes: it affects the differentiation of adipocytes and regulates adipogenesis, glucose homeostasis, oncogenesis, inflammation, angiogenesis, myogenesis, and migration of immunocompetent cells, acting as a chemoattractant [75–79]. The question of whether chemerin is a pro- or anti-inflammatory protein is the subject of active scientific debate, as conflicting results have been obtained in cell culture studies and biological models [80, 81], which opens up prospects for further basic research.

Currently available studies on chemerin in COVID-19 also show mixed results. Thus, despite the fact that in patients with novel coronavirus infection, the concentration of chemerin in the blood serum was significantly lower than in healthy people, the relationship with the severity of clinical symptoms, the severity of the course, and the need for hospitalization in the ICU has not been established [82]. Polish scientists drew attention to the fact that patients with severe and moderate COVID-19 had the

lowest concentrations of chemerin in the blood on day 7, while in patients with mild COVID-19, the level of chemerin did not change throughout the observation period. Researchers believe that the decrease in the chemerin concentration one week after the onset of symptoms may be associated with inflammatory activity in severe COVID-19 [47].

Belgian researchers found the opposite results: the concentration of chemerin in the blood was significantly higher in patients hospitalized in the ICU. Moreover, chemerin levels were associated with the severity of the disease and positively correlated with inflammatory biomarkers, such as CRP and TNFα. The univariate and multivariate logistic regression analysis showed that high chemerin levels on day 14 of hospitalization were an independent risk factor for death [83].

CONCLUSION

Despite the great interest of the scientific community in studying the role of adipokine imbalance in the mechanisms of development of COVID-19 and a significant number of publications on this topic, contradictory results associated with different study designs, the relevance of the novel coronavirus infection, and the need to search for diagnostically significant predictors of adverse outcomes open up prospects for further basic research in this area. A deep understanding of the pathogenesis of this infection from the standpoint of the role of adipokine imbalance in it can form the grounds for the development of effective pathogenetic approaches to the prevention of severe disease and complications.

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Lipidomic markers of obesity and their dynamics after bariatric surgery

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ABSTRACT

Obesity is considered as a chronic progressive disease, heterogeneous in its etiology and clinical manifestations, and characterized by excess in body fat mass and its deposition in the body. The term "morbid obesity" refers to excessive deposition of adipose tissue with a body mass index (BMI) ≥40 kg / m^2 or with a BMI ≥ 35 kg / m^2 in the presence of serious complications associated with obesity. Along with obesity, the frequency of type 2 diabetes mellitus and cardiovascular diseases closely associated with it has increased. It results from the progression of metabolic disorders, including insulin resistance, which is inextricably linked with the accumulation of visceral fat and plays a key role in the pathogenesis of obesity-related diseases.

The study of lipidomic signatures in obesity and associated conditions is a promising branch of fundamental medicine, which makes it possible to significantly and at a new conceptual level stratify a cohort of obese patients into various phenotypes, including a metabolically healthy and metabolically unhealthy obesity phenotypes. Dynamic changes in the lipidome both in the context of diet, drug treatment, and after various bariatric surgeries are of great interest for developing personalized strategies for the treatment of this disease. Currently available studies and their results suggest that we are only at the very start of studying this promising biomedical field.

Keywords: obesity, mass spectrometry, metabolic profiling, lipidome, lipids, clinical markers, biomarkers, bariatric surgery

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Липидомные маркеры ожирения и их динамика после бариатрических операций

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РЕЗЮМЕ

Ожирение рассматривается как хроническое прогрессирующее заболевание, гетерогенное по своей этиологии и клиническим проявлениям, характеризующееся избыточным и отложением жировой массы в организме. Под термином «морбидное ожирение» понимают избыточное отложение жировой ткани с индексом массы тела (ИМТ) ≥ 40 кг/м^2 или с ИМТ ≥ 35 кг/м^2 при наличии серьезных осложнений, связанных с ожирением. Одновременно с ожирением возросла частота тесно ассоциированных с ним сахарного диабета второго типа и сердечно-сосудистых заболеваний, представляющих собой итог прогрессирования метаболических нарушений, в том числе инсулинорезистентности, которая неразрывно связана с накоплением висцерального жира и играет ключевую роль в патогенезе сопряженных с ожирением заболеваний.

Исследование липидомных сигнатур при ожирении и ассоциированных с ним состояний – перспективный раздел фундаментальной медицины, позволяющий существенно и на новом понятийном уровне стратифицировать когорту пациентов с ожирением на различные фенотипы, в том числе на метаболически здоровый и нездоровый фенотип. Динамические изменения липидома, как на фоне диетических воздействий, медикаментозного лечения, так и после различных бариатрических операций, также интересны с точки зрения разработки персонализированных стратегий лечения данного заболевания. Имеющиеся на данный момент исследования и их результаты позволяют считать, что мы находимся только в самом начале этого перспективного биомедицинского направления.

Ключевые слова: ожирение, масс-спектрометрия, метаболические профилирование, липидом, липиды, клинические маркеры, биомаркеры, бариатрия

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

According to data released in 2021 by the World Health Organization, more than 1.9 billion adults over 18 years of age are overweight, of which over 650 million are obese. In Russia, as of the end of 2016, 23.5 million obese people were registered, which accounts for 16% of the population [1].

Obesity is considered as a chronic progressive disease, heterogeneous in its etiology and clinical manifestations, characterized by excessive deposition of fat in the body. The term “morbid obesity” refers to excessive deposition of adipose tissue with a body mass index (BMI) ≥ 40 kg / m^2 or with a BMI ≥ 35 kg / m^2 in the presence of serious complications associated with obesity.

Along with obesity, the incidence of closely associated type 2 diabetes mellitus and cardiovascular diseases (CVD) has increased, resulting from the progression of metabolic disorders, including insulin resistance, which is inextricably linked with the accumulation of visceral fat and plays a key role in the pathogenesis of obesity-related diseases [2, 3].

To control the obesity pandemic in the 21st century, it is necessary to develop not only effective treatment methods, but also to pay attention to comprehensive research aimed at searching for metabolic markers and predictors of the development of pathological conditions associated with obesity, such as prediabetes and type 2 diabetes mellitus, arterial hypertension, dyslipidemia, non-alcoholic fatty liver disease with progression to the stage of fibrosis and cirrhosis, infertility and cancer.

The main approaches of modern medicine are developing within a philosophical paradigm the main direction of which is the transition from classical clinical diagnosis to personalized regular monitoring of health status to make forecasts containing an assessment of the risks of developing both new diseases and specific complications.

Currently, there is no single approach to the treatment of obesity and metabolic syndrome that would make it possible to create a universal method of personalized monitoring that facilitates formulating a prognosis for the development of obesity based on information about genetic predisposition and risk factors for a particular patient. In routine clinical practice, obesity is diagnosed by assessing BMI, which is calculated as the ratio of body weight (kg) to height squared (m^2), which allows doctors to classify people from class 1 obesity to class 3 or high-risk obesity. For the most part, BMI only provides an indirect assessment of the risks associated with obesity. The assessment of waist circumference reflects to a slightly greater extent the biological cause of fat deposits, insulin resistance, and angiogenesis of adipose tissue, however, it does not fully characterize all its metabolic features.

The assessment of the component composition of the human body using bioelectrical impedance vector analysis and dual-energy X-ray absorptiometry is not objective for comparing the lipotoxicity of tissues of different topologies. Such a diagnosis cannot assess metabolic changes in obesity because in patients the expression of many genes associated with cellular metabolism and adipokine production changes significantly, so currently, it is impossible to reliably divide a cohort of overweight and normal BMI individuals into metabolically healthy and metabolically unhealthy phenotypes using existing tools and approaches.

OMICS

In modern medicine, the field of omics research is intensively developing. Metabolomic analysis, which is one of the most promising molecular methods in systems biology, makes it possible to evaluate the structure and quantitatively characterize molecules that can serve as products or substrates of enzymatic reactions involved in physiological and pathophysiological processes.

Metabolomics is a technological tool for monitoring the general condition of the patient and stratifying the risk of possible metabolic disorders. This is a unique way to determine the metabolic fingerprint (metabolomic signatures) of a recognizable chemical pattern specific to a particular sample.

Clinical lipidomics is a branch of metabolomics, the main analytical tool of which is gas chromatography–mass spectrometry. Due to the wide analytical coverage of lipids, together with high sensitivity and molecular specificity, it becomes possible to detect lipid imbalances in altered cell membranes and lipid droplets, including the stage of early diagnosis of clinically silent conditions [4, 5].

The main focus of this review is lipidomics, the identification of the quantitative and qualitative composition of lipids in different biological environments. In a healthy person, lipid metabolism is in balance, but various trigger factors can change homeostasis. The information presented in the review concerns lipidome studies in the context of the characteristics of obesity and its complications.

Lipid annotation is necessary to interpret the results, as well as to relate the data to other levels of biological information. The most modern and annually updated nomenclature was developed by the LIPID MAPS consortium [6], it is convenient for annotating data obtained using mass spectrometry. The lipid structure database is divided into 8 main groups:

– fatty acylites (FAc), including fatty acids (FA), eicosanoids, fatty alcohols, aldehydes, esters, acylcarnitines, acyl-CoA, wax esters and others;

– glycerolipids (GL);

– glycerophospholipids (GP), including glycerophosphocholines (PC),

– glycerophosphoethanolamines (PE), glycerophosphoserines (PS), glycerophosphoglycerophosphates (PG), glycerophosphoinositol (PI), glycerophosphates (PA);

– sphingolipids (SP), including ceramides, acylceramides, sphingomyelins, sphingosine and others;

– sterol lipids (ST). Most of the cholesterol in the plasma is esterified. Among the cholesteryl esters (CEs) of human plasma, CE (18:2) and CE (20:4) contribute to the major fraction, prenol lipids (PR). The two main prenol lipids in plasma are dolichol and ubiquinone;

– sugar lipids (SL);

– polyketides (PK).

LIPIDOMIC BLOOD MARKERS IN OBESITY

Fatty acids

The molecular mechanisms underlying lipotoxicity include endoplasmic reticulum (ER) stress, oxidative stress, mitochondrial dysfunction, impaired autophagy, and inflammation [7]. Relative hypoxia of adipose tissue is also a factor in its dysfunction. Fatty acid synthesis in adipocytes is activated by signals induced by hypoxia-inducible factor (HIF). Carnitine palmitoyltransferase I (CPTI) is inhibited by HIF, reducing the transport of fatty acids into mitochondria and channeling fatty acids into lipid droplets for storage. Increased droplet counts in cells are associated with increased lipotoxicity and altered metabolism, which contribute to further cellular dysfunction of adipose tissue.

In metabolic disorders, lipid metabolic intermediates accumulate intracellularly, leading to cellular dysfunction and apoptosis of cells in various tissues, including kidneys, brain, skeletal muscles, and heart. Intermediates of lipid metabolism, such as ceramides, diacylglycerides, and acylcarnitines, disrupt intracellular signaling cascades and are largely considered as toxic lipid signaling molecules.

Saturated fatty acids are thought to be particularly harmful to all cell types by inducing a wide range of adverse cellular responses: apoptosis, inflammation, accumulation of reactive oxygen molecules, and oxidative stress [8]. Short-chain fatty acids (SCFA), including acetate, butyrate, and propionate, inhibit lipolysis and promote adipogenesis in visceral adipose tissue, because they are substrates for glucose and lipid synthesis. SCFAs act on G protein-coupled receptors, which leads to inhibition of lipolysis and a decrease in free fatty acid levels in plasma [9]. SCFAs have no more than six carbon atoms and are the main metabolites of the intestinal microbiota, and modern science confirms their role as biomarkers of central obesity [10].

A meta-analysis of randomized clinical trials on obesity [11] showed a characteristic pattern of the lipid profile in this disease: an increase in palmitic, palmitoleic, stearic, and oleic acids, as well as stearoylcarnitine [12]. Also in obesity, the pattern of disturbances in the structure of triacylglycerols (TAGs) was determined; a shorter carbon chain length and fewer double bonds were associated with a higher BMI, while a relatively long acyl chain and a larger number of double bonds were associated with a lower BMI [13].

In a cross-sectional study involving 1,443 Spanish women, it was shown that with increased BMI, the relative concentration of total saturated fatty acids increased in the phospholipid fraction of blood serum, and an increase was also observed in the concentration of palmitoleic, dihomo-γ-linolenic, arachidonic (AA), and α-linolenic acids, while the concentration of oleic, gondoic, trans-vaccenic, linoleic, and γ-linolenic acids decreased [14]. Patients with metabolic syndrome have elevated plasma levels of C16:0, C18:0, C21:0, C16:1, C18:1, C18:2, C18:3n6, C20:3n6, C20:4n6, C22:4n6, C22:5n6 [15], as well as lactic and β-hydroxybutyric acids [16].

Thus, an increase in the level of saturated fatty acids relative to unsaturated fatty acids in plasma correlates with the trend toward an increase in BMI. The results of studies on fatty acids from tissues are thought-provoking, since the ratio of saturated / unsaturated fatty acids in the TAG fraction from visceral and subcutaneous adipose tissue decreased in patients with metabolic syndrome, while it was higher in the control group [17].

Free fatty acids (C14:0, C18:1, C20:2, C20:3, C20:5 and C22:6) were significantly increased in both obesity and type 2 diabetes mellitus, and the C22:6 level was determined as an independent risk factor for type 2 diabetes mellitus [18]. Another meta-analysis of plasma lipidomic studies in obesity showed an association of higher concentrations of circulating fatty acids 20:0, 22:0, and 24:0 with a lower risk of type 2 diabetes mellitus [19].

ACYLCARNITINES

Beta-oxidation of intracellularly stored lipids leads to the production of acetyl-CoA through oxidative degradation of fatty acids. Acetyl-CoA produced by each β-oxidation cycle can subsequently be incorporated into the tricarboxylic acid cycle to generate NADH and FADH2 for the electron transport chain and ATP products [20].

Cell oversaturation with fatty acids and overload of mitochondria with them leads to incomplete

β-oxidation of fatty acids and accumulation of carnitine esters and fatty acids-acylcarnitine in the cell, which was shown in patients with obesity and type 2 diabetes mellitus [21]. Also, the accumulation of a wide range of acylcarnitines with an even number of carbon atoms (from C6 to C22) is specific for obese individuals [22].

Decreased fatty acid oxidation leads to elevated circulating lipid levels, which further increases oxidative stress. Therefore, acylcarnitine (C18:2) has got a negative association with the phenotype of metabolically healthy obesity [23] and a positive association with increasing BMI [24]. Acylcarnitine accumulation is associated with increased insulin resistance in obese patients and the development of a higher risk of CVD [25, 26].

The hypothesis of an association of increasing cardiovascular risk with the accumulation of acylcarnitines is confirmed by the identification of high levels of short-chain acylcarnitines (C2, C3, C4DC), free carnitine (C0), and long-chain acylcarnitines (C16, C18OH) in individuals with metabolic syndrome [27]. A 2020 meta-analysis established a similar pattern with a high degree of evidence; elevated concentrations of acylcarnitine (14:2) were associated with increasing age and BMI of patients [28].

SPHINGOLIPIDS

Research over the past ten years has shed light on the role of changes in lipid metabolism, namely bioactive sphingolipids, in the development of obesity and complications associated with it. Obesity is characterized by a decrease in sphingomyelin [29] and an increase in ceramide synthesis due to stimulation of the so-called salvage pathway, which leads to the production of ceramides through the catabolism of hexosylceramides [30].

Long-chain saturated non-esterified fatty acids (NEFAs) are the main source of ceramide synthesis (palmitic acid is involved in the synthesis of ceramide C16:0, stearic Cer-C18:0, arachidonic Cer-C20:0, and linoceric Cer-C24:0). An increased content of the substrate, long-chain saturated fatty acids, promotes increased synthesis of ceramides and their accumulation in the cell. As is known, C16:0-ceramide has the highest pathogenic potential [31].

In the development of the disease, it is the localization of the ceramide accumulation and not the total mass of ceramides in adipose tissue that matters most (if it is a specific intracellular localization or in specific pools). For example, an increase in the content of ceramides in mitochondria, endoplasmic reticulum (ER), and nucleus inversely correlated with insulin signal transduction, while the accumulation of ceramides in the cytosolic fraction did not affect insulin signaling [32].

The largest population-based study of plasma sphingolipidome conducted by W.S. Chew in 2019 revealed a positive correlation of ceramide levels with BMI and a negative correlation with hexosylceramide levels [33]. This was confirmed by other studies, which showed that the content of sphingomyelins, on the contrary, was inversely associated with the waist-to-hip ratio and BMI [34, 35].

The study by J.M. Weir et al. found a strong specific relationship between ceramide 18:0 and BMI, as well as an increase in all types of dihydroceramides in obese patients [36]. These data are also supported by another more recent study in a large population-based cohort using a targeted lipidomic approach, which found that Cer (18:1/18:0) and Cer (18:1/20:0) levels increased proportionally with BMI [28]. At the same time, W.H. Tell-Hansen et al. found no significant differences in sphingomyelin levels in the plasma of metabolically healthy and metabolically unhealthy obese patients [30].

A 2020 meta-analysis described a positive association of person's age with the C18:1 / 21:0 ceramide content [28]. A study of the serum lipidomic profile in children with abdominal obesity showed that elevated levels of sphingomyelin (d21:1) were associated with central obesity and might mediate the relationship between abdominal obesity and dysregulation of glucose homeostasis [37].

PHOSPHOLIPIDS

Data on changes in the metabolism of the glycerophospholipid (GPL) group are debatable. Obesity is characterized by an increase in the total concentration of GPL in blood plasma. For example, a metabolomics study от serum profiles of diabetic and obese patients found increased concentrations of glycerol, which was positively associated with the established phenotype of type 2 diabetes mellitus and BMI [16].

An Australian metabolomics study in patients with diabetes and obesity found a positive association of glycerolipids with waist circumference [24]. However, phospholipids and most types of lysophospholipids were negatively associated with BMI [28]. An increase in BMI is associated with a significant decrease in the levels of circulating phosphatidylcholines and lysophosphatidylcholines [29, 35, 38].

Another study observed a significant reduction in the levels of five lysophosphatidylcholine (LPC) species (LPC18:2, LPC18:1, LPC20:2, LPC20:1 and LPC20:0) in obese adolescents [39]. PC (15:0/0:0), PE (18:0/0:0), LPC (15:0), LPE (0:0/18:0), and PI (14:0/22:2) were also reduced [40]. Lysophosphatidylcholines LPC 18:2, PC 18:1 were negatively correlated with BMI [41]. However, acyl-lysophosphatidylcholine C16:1, diacyl-phosphatidylcholine, and LPCa C16:1 had the highest correlation indices with high BMI [42]. Phosphatidylcholines, which contain polyunsaturated omega-6 fatty acids, such as 20:3, 20:4 or 22:4, were also positively associated with BMI [28].

One clear signal of plasma lipid ratios associated with BMI was the plasma alkenylphosphatidylethanolamine/ alkylphosphatidylethanolamine ratio; for example, the ratio PE (P-16:0/22:6)/ PE (O-16:0/22:6) [29, 43, 44]. Glycerophosphoethanolamines PE P-16:0/20:3 showed a significant positive association with BMI [41]. LPE with shorter carbon length and fewer double bonds were associated with lower BMI [13].

Metabolically healthy obese patients had elevated diacyl-phosphatidylcholines C32:1 and C38:3, while acyl-lysophosphatidylcholine C18:1 and C18:2 were inversely associated with the patients' condition. In the metabolically unhealthy obesity phenotype, the content of acyl-lysophosphatidylcholine C16:1 was higher, and the level of acyl-lysophosphatidylcholines C18:1 and C18:2 was reduced [45].

Current results of lipidome studies in obesity and diabetes in animals suggest that overexpression of lipoprotein lipase (LPL) may lead to increased activity of the Krebs cycle and proteinogenic amino acid metabolic pathways in skeletal muscle, and these improvements may play an important role in the biological mechanisms underlying antidiabetic features of LPL overexpression [46]. This is confirmed by another study by P.J. Ferrar et al. which revealed that mice with skeletal muscle-specific knockout of lysophosphatidylcholine acyltransferase 3 (LPCAT3), an enzyme involved in phospholipid transacylation, demonstrated an increase in the lysophosphatidylcholine/phosphatidylcholine ratio and an increase in skeletal muscle insulin sensitivity [47].

LIPIDOMIC TISSUE MARKERS IN OBESITY

To assess adipose tissue, we can rely on the in-depth human lipidome atlas AdipoAtlas, which includes the lipidomic profile of adipose tissue from patients with obesity and normal body weight. Quantitative analysis of subcutaneous and visceral adipose tissue samples from the studied cohort allowed to divide the global lipidome into main classes. The lion's share of the total amount belongs to TAG (96.2 nmol / μg of protein), containing mainly saturated and monounsaturated fatty acyl chains, with an average of two double bonds per three chains. The second most common class is cholesterol esters. In terms of quantity, non-polar lipids were followed by phosphatidylcholines, phosphatidylethanolamines, and sphingomyelin. Phosphotidylethanolamines are characterized by a higher concentration of polyunsaturated fatty acids. Ceramids are another class of lipids of high metabolic importance, with C16:0 and C18:1 being the most abundant species. A large number of potentially lipotoxic deoxyceramides are detected in the fat depot, accounting for more than 10% of all ceramide subclasses [48].

LIVER BIOPSY SPECIMENS

In a small cohort of obese patients , the absolute amount of ceramides, SM, PC, PE, PE(e), Lyso (tot) and LPC was higher in the liver compared to adipose tissue. The amount of PC(e), LPE, LPE(e), and triacylglycerols was lower in the liver than in adipose tissue. DAG concentrations in the liver were comparable to those in adipose tissue. In subcutaneous and visceral adipose tissue, TAGs accounted for 99.2% of lipids, phospholipids – for 0.8%, while in the liver, this distribution was 75.5 and 24.5%, respectively [49].

In obese patients, the lipid composition of triglycerides, phosphatidylcholines and sphingomyelins in liver biopsy specimens correlated with sphingomyelins in LDL [50].Significant positive correlations were revealed between the proportions of ceramide C14:0, C18:0, C20:0, and C24:1 in liver and total plasma. These subspecies may be markers of the species composition of hepatic ceramides in obese patients [51].

BIOPSY SPECIMENS OF EPICARDIAL FAT

When analyzing the lipidomic profile of blood plasma, 9 species of lipids were identified that were associated with an increase in epicardial fat: triacylglycerol, hydroxylated acylcarnitine, deoxyceramide, alkyldiacylglycerol, ubiquinone, diacylglycerol, dihydroceramide, phosphatidylinositol, and phosphatidylglycerol. The strongest associations observed were with two species of deoxyceramides [52]. Cer (m18:1/18:0) and Cer (m18:1/20:0) and sphingosine are also elevated, confirming an earlier study of biopsy specimens in obesity [53].

Lipidomic analysis of subcutaneous and epicardial adipose tissue in patients with coronary artery disease and type 2 diabetes revealed multiple changes in the content of fatty acids with an odd number of carbon atoms (15:0, 15:1, 17:0, 17:1). More pronounced changes were found in epicardial adipose tissue compared to subcutaneous adipose tissue [54].

COMPARISON OF SUBCUTANEOUS AND VISCERAL FAT

The extent of lipidome changes depending on fat topology was analyzed by N. Al-Sari et al. [55]. Lipidomic analysis was performed on adipose tissue collected from the abdomen, chest, thigh, and lower back. Levels of triacylglycerols (TAGs) with long-chain polyunsaturated fatty acids were higher in thigh tissue. The difference between thigh and lower back adipose tissue was generally similar to the difference between the thigh and abdomen. Minor changes in the lipid spectrum were observed in the tissue of the lower back and chest, while more significant ones were observed in the adipose tissue of the thigh and abdomen [55]. It should be noted that the lipidome of muscle tissue, unlike adipose tissue, does not have such pronounced differences between patients [56].

In obese patients without diabetes, the subcutaneous fat lipidome contains high concentrations of long-chain FAs and ceramides, in particular ceramide C18:1/24:1 [57]. And in patients with obesity and type 2 diabetes mellitus-*, there is an increase in the level of ceramide C16:0 in subcutaneous fat tissue rather than in visceral deposits [58]. These changes are also confirmed by a more recent study [59].

In the visceral tissue of patients with diabetes and prediabetes, the content of Cer (d18:1/16:0), Cer (d18:1/18:0), Cer (d18:1/18:1), Cer (d18:1/20:0), Cer (d18:1/24:1) is increased in contrast to healthy individuals [60]. A similar result was obtained by Choromańska et al. in patients with metabolic syndrome, and it was also observed that saturated palmitic and stearic acids were the most abundant fatty acids of the ceramide fraction in both adipose tissues [17].

An increase in visceral tissue ceramides in obese patients with diabetes was confirmed by a previous study. The length of the acyl chain of ceramides in adipose tissue (C16–20) is shorter than in plasma (C16–24). Lower sphingomyelin concentrations were observed in obese patients [56].

Excessive accumulation of lipids in adipose tissue is observed mainly in the form of TAG. Physiological stimuli lead to hydrolysis of TAG in adipocytes, which is accompanied by an increase in plasma long-chain fatty acids and subsequent accumulation of lipids in ectopic tissues. In the group of patients with metabolic syndrome, the ratio of saturated to unsaturated fatty acids composing the TAG fraction decreased significantly in visceral and subcutaneous adipose tissues, and the pool of free fatty acids in plasma increased mainly due to palmitic, stearic, arachidonic, and nervonic acids [17].

Patients receiving fish oil supplements for 12 months were included in the research cohort of a multicenter study in which adipose tissue biopsy specimens and blood plasma were collected. Fatty acids with a carbon chain length of more than 22 carbon atoms prevail in subcutaneous adipose tissue, while polyunsaturated fatty acids prevail in visceral fat deposits [61].

Adipose tissue stores significant amounts of cholesterol in the human body, and obesity is associated with decreased serum cholesterol concentrations. In patients with metabolic syndrome, a direct relationship was found between the content of oxidized cholesterol metabolites in adipose tissue – oxysterols – and blood insulin levels, as well as resistance to the hormone. Tissue cholesterol correlates more with 27-hydroxycholesterol in subcutaneous adipose tissue and with 24S-hydroxycholesterol in visceral adipose tissue [62].

A 2022 study in which 26 obese patients without type 2 diabetes underwent bariatric surgery is of particular interest [63]. Biopsy specimens were obtained from subcutaneous adipose tissue of the thigh, subcutaneous abdominal adipose tissue, deep subcutaneous abdominal adipose tissue, intra-abdominal adipose tissue, two areas of muscle tissue (vastus lateralis muscle; rectus abdominis muscle), and liver (wedge of the right lobe). The study revealed that plasma lipidomic profiles more closely reflect the liver profile than other tissues examined. All four fat depot localizations showed similarities in their metabolic relationships with plasma; it is impossible to distinguish between different depots in terms of their metabolic relationships with plasma, but the plasma pool better reflects the TAG deposition in deep adipose tissue rather than subcutaneous adipose tissue.

Those sphingomyelin and ceramide fractions that correlate between plasma and liver also show chain length specificity, i.e. these are sphingomyelins and ceramides that contain long-chain fatty acids with an acyl chain of 22 or more carbon atoms, such as

C22:0, C24:0 and C24:1. The liver is the main source of ceramides, but given that their content in the liver correlates well with plasma fractions, it proves that plasma sphingolipids may reflect their abnormal metabolism in the liver. Sphingomyelins, which contained a smaller even total number of carbon ($C \leq 34$) and monounsaturated chain fatty acids, and their concentration correlated with indicators of intra-abdominal adipose tissue, liver, and muscles [63].

CONDITIONS ASSOCIATED WITH OBESITY

The multiplicity of organ damage in obesity includes frequent pathology of the hepatobiliary system. According to autopsy data, non-alcoholic fatty liver disease is diagnosed in 70–93% of obese patients with type 2 diabetes [64]. A recent study found that plasma levels of certain lipid fractions (saturated and monounsaturated TAG) indicate early stages of fat accumulation in the liver [65].

TAG species containing low total carbon and fewer unsaturated bonds were significantly associated with steatohepatosis, vascular disease, and an increased risk of diabetes, while species containing a high amount of carbon and a higher number of unsaturated bonds were associated with a reduced risk of diabetes [66–68].

Long-term persistent obesity contributes to the development of focal segmental glomerulosclerosis, chronic kidney disease, and diabetic nephropathy. In patients with progressive diabetic nephropathy and obesity, a relative abundance of TAGs with longer chain polyunsaturated acyls and a lower content of C16–C20 acylcarnitines was identified. The increase in their levels had a compensatory adaptive mechanism for converting more toxic lipids (saturated non-esterified fatty acids) into less toxic lipids (polyunsaturated long-chain TAG). Unsaturated free FAs and TAGs with short-chain acyls and low double bond content predicted the progression of diabetic kidney disease [69].

This is confirmed by another more recent study which revealed that obese patients with chronic kidney disease (CKD) tended to have a decrease in the number of carbons in the acyl chain of predominantly unsaturated TAGs. The content of lysophosphatidylcholines is also increased in these patients, but the balance of saturation and unsaturation is preserved. LysoPC (18:0), LysoPC (20:3), and PC (35:3) had the greatest predictive ability to distinguish between obese patients and obese patients with nephropathy [64].

Other negative consequences of uncorrected systemic inflammation in obesity include damage to nerve cells and fibers. According to the results of global metabolomics and targeted lipidomics, plasma FFA levels are increased in patients with obesity and polyneuropathy, mostly due to long-chain fatty acids (more than 19 atoms). In this clinical cohort, metabolomic profiles between obese and lean individuals were most strongly correlated with gamma-glutamino acid and branched-chain amino acid metabolism. Moreover, the plasma level of gamma-glutamino acid is positively correlated with TAG, BMI, and blood pressure and is associated with oxidative stress in obesity and metabolic syndrome [70].

In another study, patients with diabetes and polyneuropathy had increased concentrations of medium- and long-chain saturated fatty acids from 8 to 18 carbons [71]. Patients with significantly reduced total medium-chain (C6–C14) acylcarnitines had a correlation with the development of peripheral neuropathy over 10 years. These patients were characterized by a decrease in plasma levels of medium-chain acylcarnitines (C2–26) and phosphatidylcholines and an increase in lysophosphatidylcholines [72]. Low plasma sphingomyelin (SM) levels may correlate with poorer neurological outcomes [71].

CHANGES IN THE LIPIDOME AFTER BARIATRIC SURGERY

Bariatric surgery is the most effective way to treat obesity. According to 2018 Russian guidelines, bariatric surgery is indicated for patients with a BMI of more than 40 kg / m^2 and a BMI of 35–40 kg / m^2 in the presence of diseases associated with obesity, in which improvement should be expected as body weight is reduced (type 2 diabetes, CVD, obstructive sleep apnea, joint damage). American clinical guidelines refer to prospective and retrospective studies confirming improved quality of life and life expectancy of patients with a BMI of 30–34.9 kg / m^2 after bariatric surgery.

Bariatric surgery includes four main procedures: biliopancreatic diversion (BPD), Roux-en-Y gastric bypass (RYGB), adjustable gastric banding, sleeve gastrectomy, in which the main effects are achieved through malabsorption and restriction.

A meta-analysis of metabolomic profiling of blood plasma in patients after surgical procedures (Roux-en-Y and gastric banding) describes the change in insulin resistance after surgical treatment of obesity.

Thus, 92 metabolites are associated with varying degrees of reduction in HOMA-IR up to –40% of the baseline [73]. A recent study found greater accelerated weight loss in metabolically unhealthy obese patients than in metabolically healthy obesity [74].

The analysis of blood plasma proved that the content of short-chain fatty acids, mainly metabolites of the intestinal microbiota, decreases after bariatric surgery, while the level of branched fatty acids increases. Changes in short-chain fatty acid content are associated with weight loss. Elevated plasma BCFA levels have been shown to be associated with increased insulin sensitivity [75, 76]. A decrease in the concentration of free fatty acids in blood plasma after gastric bypass surgery is associated with a decrease in the distance of food passage in the intestine and, accordingly, with a decrease in fat absorption compared with laparoscopic sleeve gastrectomy, where the absorption surface is larger [77].

It was found that in plasma the cluster of phosphatidylcholines, especially phosphatidylcholines with the sum of diacyl residues C42:Ys, as well as SM (OH) C16:1, SM C26:1, lysoPC a C16:0, glutamine, glycine, citrulline, and histidine, were enriched only in patients who underwent Roux-en-Y, but not in patients who changed their lifestyle and diet [78]. Since some groups of fatty acids are considered beneficial, such as n-3 polyunsaturated FAs (PUFAs), while others are detrimental to human health, such as saturated FAs (SFAs), each change in their levels can have an important impact on the metabolic outcome of bariatric surgery.

Blood was collected from patients before and after gastric bypass surgery with a single anastomosis. A decrease in the total amount of fatty acids with an odd number of carbon atoms, branched chain and polyunsaturated fatty acids was observed in patients with morbid obesity before surgery compared to the control group. Monounsaturated fatty acid content was increased, which was mainly caused by a higher level of oleic acid (18:1). The content of monounsaturated fatty acids in triglycerides increased in patients after surgery, mainly due to a higher level of oleic acid. There was no noticeable increase in the level of polyunsaturated fatty acids. Due to the anti-inflammatory, cardioprotective, and anticancer properties of PUFAs, a decrease in their content in the long-term postoperative period makes it necessary to prescribe dietary supplements. At the same time, the content of α-linolenic acid and eicosapentaenoic acids in the blood serum did not differ significantly before and after surgery [79].

Gas chromatography was used to study the blood of patients before and after laparoscopic sleeve gastrectomy. The surgery lead to a long-term decrease in serum α-linolenic acid and eicosapentaenoic acid levels in the first year [80].

Two weeks after gastric bypass with one anastomosis, a decrease in the content of branched-chain fatty acids and an increase in the level of monounsaturated fatty acids (MUFA) were detected in the blood plasma. Obese patients also showed decreased plasma levels of some PUFAs, including linolenic acid (18:3 n-3) and eicosatetraenoic acid (EPA; 20:5 n-3 and 20:4 n-3) [81].

Blood analysis before and after Roux-en-Y revealed significant changes in the content of six metabolites (3-indoleacetic acid, 2-hydroxybutyric acid, valine, glutamic acid, 4-hydroxybenzeneacetic acid, and alpha-tocopherol), while changes in the content of the identified metabolites were associated with the changes in lipid, insulin, and glucose levels [82].

The first study using capillary electrophoresis – mass spectrometry in obese patients after laparoscopic sleeve gastrectomy found that the relative content of tricarboxylic acid cycle metabolites, including citric acid, succinate, and malic acid, was significantly increased in blood plasma after surgery [83].

Patients with CKD and severe obesity after Roux-en-Y gastric and sleeve gastrectomy showed a dramatic weight loss with a significant decrease in proteinuria, albuminuria, uric acid levels, a decrease in the degree of glomerular hyperfiltration, and an increase in HDL levels. The lipid profile and metabolome of the blood serum in patients changed significantly after surgery: the level of diacylglycerols, triacylglycerols, and branched-chain amino acids decreased. A significant decrease in their levels was positively correlated with uric acid levels, while the levels of PC (39:0) and PC (44:5) increased, and only PC (36:3) decreased [84]. Interestingly, bariatric surgery did not restore all types of lipids; some of them were decreased, and, therefore, they were considered as potential targets for early diagnosis or therapeutic intervention.

CONCLUSION

The study of lipidomic signatures in obesity and associated conditions is a promising branch of fundamental medicine, which makes it possible to significantly and at a new conceptual level stratify a cohort of obese patients into various phenotypes, including metabolically healthy and metabolically

unhealthy phenotypes. Dynamic changes in the lipidome, both after dietary changes, drug treatment, and various bariatric surgeries, are also interesting from the point of view of developing personalized strategies for treating this disease. The current studies and their results suggest that we have just begun research in this promising field in biomedicine.

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Quality of life and mental disorders in the post-COVID period (systematic review)

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ABSTRACT

The 2019 novel coronavirus infection (COVID-19) pandemic has been a great burden for all of humanity. Soon after it began, researchers noticed that elimination of the virus from the body and recovery are not the end of the disease, since many patients did not return to their previous state of health, continued to complain of pathologies of various organs and systems, could not work, and some of them developed mental disorders.

The aim of the review was to analyze and summarize published data on the quality of life and mental disorders in the post-COVID period. Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations, 7,374 scientific works were found, of which 176 English-language and 276 Russian-language relevant publications were selected for analysis. The review included 17 (including 2 Russian-language) articles relevant to the topic of this review. Complaints of decreased memory and attention, appearing no later than 6 months after recovery from COVID-19, were reported by 3.2–9.1% of patients. Asthenic symptoms during the first month after the elimination of the novel coronavirus infection occurred in 55–70% of patients, and six months later – in every fifth patient.

At the same time, post-viral fatigue more often affected women discharged from respiratory hospitals and persons with chronic bronchopulmonary pathology. Quite often, those who recovered from COVID-19 experienced insomnia and emotional disturbances, the frequency of which also correlated with the female sex and the severe course of the disease, which required hospitalization in the intensive care unit (ICU). In the post-COVID period, the development of depressive symptoms is not excluded, but sufficient evidence for this has not been obtained. The quality of life in these patients decreased. After discharge from the hospital, some patients remained unable to work, and some began to experience difficulties with self-care. However, over time, there is a trend toward restoration of the quality of life, which is especially evident in young people. In patients who have been in the ICU for more than 7 days, the rehabilitation potential is much lower. Psychopathological symptoms contribute to a decrease in the quality of life along with physical factors (persistent dyspnea, decreased exercise tolerance).

Keywords: novel coronavirus infection, prolonged COVID-19, post-COVID syndrome, quality of life, anxiety, depression, cognitive impairment

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Качество жизни и психические расстройства в постковидном периоде (систематический обзор)

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РЕЗЮМЕ

Большим бременем для всего человечества явилась пандемия новой коронавирусной инфекции 2019 г. (COVID-19), и в скором времени после ее начала исследователи обратили внимание, что элиминация вируса из организма и реконвалесценция не являются завершением болезни. Многие заболевшие не вернулись к прежнему состоянию здоровья и продолжали испытывать жалобы, отражающие патологию разных органов и систем, не могли работать, а некоторые из них столкнулись с психическими расстройствами.

Цель обзора заключалась в проведении анализа и обобщении опубликованных данных о качестве жизни и расстройствах психической сферы в постковидном периоде. Используя рекомендации «Предпочтительные элементы отчетности для систематических обзоров и метаанализов» (PRISMA) были обнаружены 7 374 научные работы, из которых анализу подлежали 176 англоязычных и 276 русскоязычных подходящих публикаций. В обзор включили 17 (в том числе 2 русскоязычные) статей, соответствующих теме данного обзора. Жалобы на снижение памяти и внимания, появляющиеся не позднее чем через 6 мес после выздоровления от COVID-19 предъявляли 3,2–9,1% пациентов. Астенические симптомы в течение 1-го мес после элиминации нового коронавируса встречались у 55–70% пациентов, а спустя 6 мес – у каждого 5-го.

При этом поствирусной астенией чаще страдали женщины, выписанные из респираторных госпиталей и лица с хронической бронхолегочной патологией. Нередко выздоровевшие от COVID-19 сталкивались с бессонницей и эмоциональными нарушениями, частота которых также коррелировала с женским полом и тяжелым течением заболевания, потребовавшим госпитализации в отделение реанимации и интенсивной терапии (ОРИТ). В постковидном периоде не исключено развитие депрессивной симптоматики, но достаточных доказательств этому не получено. Качество жизни у этих пациентов заметно снижается, после выписки из стационара часть больных оставалась нетрудоспособна, а у некоторых начались трудности с самообслуживанием. Однако с течением времени имеется тенденция к восстановлению качества жизни, что особенно прослеживается у лиц молодого возраста. У больных, пребывавших в ОРИТ более 7 сут, реабилитационный потенциал гораздо ниже. Психопатологическая симптоматика вносит вклад в снижение качества жизни наряду с физическим компонентом (персистирующее диспноэ, снижение толерантности к физической нагрузке).

Ключевые слова: новая коронавирусная инфекция, длительный COVID-19, постковидный синдром, качество жизни, тревога, депрессия, когнитивные нарушения

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

There have been 765 million confirmed cases and more than 6.9 million deaths during the New Coronavirus Infection 2019 (COVID-19) pandemic. Overall, the mortality rate for COVID-19 was 0.9% (according to WHO data as of May 4, 2023) [1]. According to the report of the Ministry of Health of the Russian Federation [2], during the period of circulation of SARS-CoV-2 strains associated with the greatest severity and mortality, 34% of patients required hospitalization, of which 11% needed intensive care in the intensive care unit (ICU). Morbidity and mortality rates changed in waves and maintained an upward trend for a prolonged period. Temporary guidelines for the treatment of COVID-19, including diagnostic and therapeutic innovations aimed at reducing mortality and disability, have been updated with sufficient frequency [3].

It is known that tissue damage is not limited to the respiratory tract in this infectious disease. Antigenic structures of the virus and characteristic pathomorphological changes are found in the gastrointestinal tract, genitourinary system, vascular endothelium, nervous tissue, and kidney tissue. Non-respiratory symptoms of COVID-19 include enterocolitis, vasculitis, skin lesions, delirium, and an increased risk of thrombosis, cardiac arrhythmias, myocardial infarction, and stroke have been noted [4]. At the same time, the problem of COVID-19 is not confined to the acute period of the disease lasting 3-6 weeks; its consequences can also be observed in a more delayed period, which is currently challenging to estimate in terms of duration [5].

A small number of studies conducted on COVID-19 survivors have shown that only 10.8% of patients remain asymptomatic after their illness. The most common symptom in the post-COVID period is fatigue (72.8%). Other manifestations were also noted: anxiety (38%), joint pain (31.4%), persistent headache (28.9%), chest pain (28.9%), dementia (28.6%), depression (28.6%), and dyspnea (28.2%). At the same time, 32.4% of those examined in the post-COVID period had persistent (long-term) disorders that significantly affected the quality of life (QoL) and the state of health [6].

It should be noted that only a limited number of studies have focused on mental disorders in COVID-19. However, all researchers pay attention to the frequent detection of significant depressive disorder, anxiety spectrum disorders, and cognitive changes not only during hospitalization but also in the delayed period after discharge. There are concerns about an increase in suicidal tendencies among survivors of COVID-19. Thus, as a comparison, it is often mentioned that in a study of people who suffered SARS-CoV infection in 2003, more than half of the survivors had mental disorders [7].

It has been established that after a SARS-CoV-2 infection, elderly people often experience a decrease in functional activity and the ability to self-care [8]. Also noteworthy is the long period of disability in a number of patients after COVID-19 [9].

Currently, the mechanisms of development of mental disorders in COVID-19 and their impact on the course and outcome of the disease, both in acute and post-COVID periods, are still completely unknown. These changes significantly worsen the quality of life of patients and require further research to develop comprehensive algorithms for rehabilitation care aimed at both treating organic pathology associated with COVID-19 and correcting mental status.

The aim of this systematic review was to analyze and summarize published data regarding the study of quality of life and mental disorders in the post-COVID period.

MATERIALS AND METHODS

Using the electronic search system PubMed and the scientific electronic library eLIBRARY, a search was conducted for original studies regarding the decline in quality of life in various areas, as well as the detection of mental disorders after COVID-19. The review includes original articles published between February 1, 2020, and October 6, 2022, in English and Russian. All PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were adhered to when identifying potential studies [10]. The analysis was carried out using an algorithm comprising four stages (Fig. 1).

Stage 1. A primary search for publications dedicated to the study of quality of life and psychopathological symptoms in patients who have had COVID-19. Logical operators were used to combine the following keywords: ((long COVID) OR (post-COVID) OR (post-acute COVID-19) OR (prolonged COVID) OR (COVID-19 survivor) OR

(survivors of COVID-19)) AND ((quality of life) OR (mood disorder) OR (affective disorder) OR (depressive disorder) OR (depression) OR (anxiety) OR (cognitive disorder) OR (cognitive impairment) OR (asthenia) OR (sleep disturbance) OR (suicide) OR (physical activity) OR (psychiatric outcomes) OR (fatigue) OR (mental health) OR (psychopathology). The search yielded a total of 7,374 publications: 7,049 in English and 325 in Russian.

Stage 2. From the aforementioned articles, 176 English-language and 276 Russian-language publications were selected, containing original research data and available in full-text version.

Stage 3. Upon examining the abstracts of the selected articles, 148 publications from the electronic search engine PubMed and 274 publications from the scientific electronic library eLIBRARY that were not related to the topic of this systematic literature review were excluded.

Stage 4. A detailed analysis of the full text of publications was conducted. Articles that did not meet the inclusion criteria were excluded. Two studies focused on the acute period of a new coronavirus infection; three other published studies did not assess functional indicators, quality of life, or mental disorders in post-COVID syndrome. Another publication reviewed contained information about the social consequences of the pandemic on a population of people who did not have COVID-19. Three studies with small sample sizes (less than 30 patients) were also excluded. One article appeared twice, in two articles the full results of the research were not presented, in another article access to the results was closed. For the review, 17 articles (including 2 in Russian) that met the inclusion criteria were included in the analysis (Fig. 1).

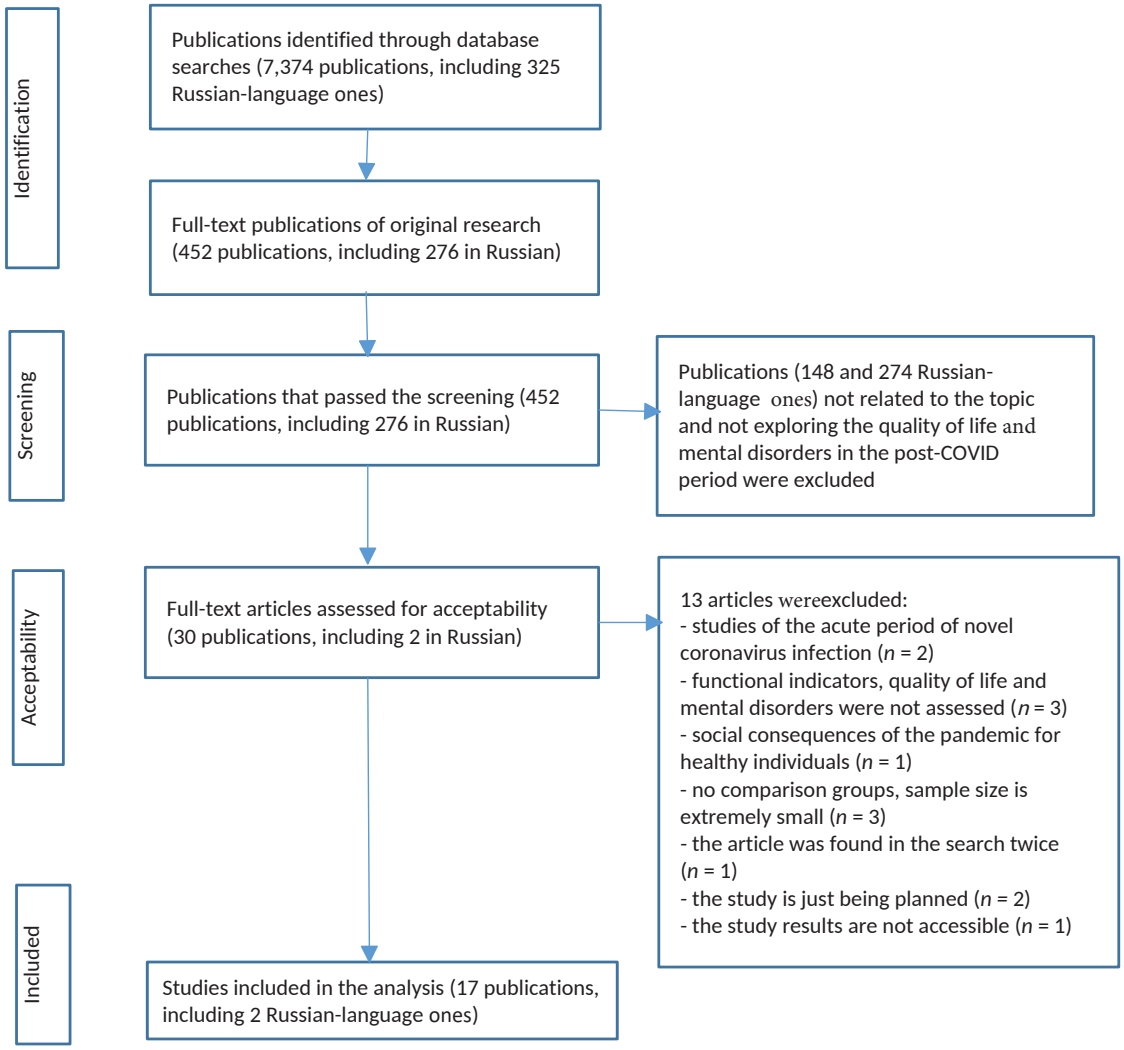

Fig. 1. Algorithm for identifying studies included in the review

MODERN CONCEPT OF POST-COVID SYNDROME, ITS RELEVANCE AND PREVALENCE

An analysis of the geography of published studies reveals that the issue of post-COVID syndrome is addressed worldwide. This review encompasses studies conducted in Russia, the USA, the countries of the European Union, China, India, Israel, and Iran.

In all the analyzed studies, authors report that individuals, after suffering a new coronavirus infection, face a range of complex health problems affecting the cardiovascular, respiratory, nervous systems, as well as the mental sphere. Consequently, these patients often seek medical attention and require rehabilitation measures, despite many having no pre-existing health issues before COVID-19.

The terms long COVID (Long-COVID) and post-COVID syndrome have been proposed for detailed research, clinical diagnosis, and the development of management tactics for patients experiencing symptoms beyond the acute phase of the SARS-CoV-2 infection. D. Munblit [11] defines long COVID (a post-COVID-19 condition, subacute consequences of SARS-CoV-2 infection) as a broad spectrum of symptoms occurring within several weeks or months after SARS-CoV-2 infection. According to R. Mahmud et al. [12], "post-coronavirus disease syndrome" includes persistence of symptoms after viral clearance, new development of symptoms after convalescence, or exacerbation of chronic diseases within a month after initial clinical and virological cure of the disease.

The National Institute for Health and Care Excellence (NICE) provides classifications based on the duration of the main clinical manifestations of COVID-19: "acute COVID" (<4 weeks), "continuing COVID" (4 weeks to 12 weeks), and "post-Covid syndrome" (symptoms lasting >12 weeks without an explanation by other illnesses). NICE also introduces the term "Long-COVID," encompassing both ongoing COVID and post-Covid syndrome [13]. Soriano J.B. et al. (2022) describe the post-COVID state as symptoms developing after 3 months of initial infection, lasting at least 2 months, including fatigue, shortness of breath, and cognitive dysfunction. These symptoms may persist after acute COVID-19 or occur for the first time after recovery [14]. Presently, post-COVID syndrome is relevant and classified under the International Classification of Diseases ICD-10 code U 09.09 as "Condition after COVID-19, unspecified" [15].

In a study conducted by L.G. Jacobs et al. (USA, 2020) among patients with a favorable outcome of hospitalization for COVID-19, confirmed by PCR, persistent symptoms on the 35th day after discharge were observed in 72.7% of patients [16].

According to the findings of another study led by D. Munblit et al. (Russia, 2021), among individuals discharged with a diagnosis of COVID-19, confirmed either by PCR or established clinically, 47.1% of patients reported enduring symptoms after an average of 218 days. Notable complaints included fatigue (21.2%), dyspnea (14.5%), and memory impairment (9.1%) [11].

R. Mahmud et al. (Bangladesh, 2021) published results from a study in which 46% of patients developed symptoms within 1 month after recovering from COVID-19, with "post-viral" fatigue (asthenic syndrome) being the most prevalent complaint, occurring in 70% of cases [12].

In a study by A. Pérez-González et al. (Spain, 2022), involving hospitalized patients with a laboratory-confirmed diagnosis of COVID-19, 48.0% of individuals reported one or more persistent complaints six months after discharge. The most prevalent issues included extrathoracic symptoms (39.1%), symptoms associated with chest discomfort (27%), dyspnea (20.6%), and fatigue (16.1%) [17].

Analyzing publications dedicated to the post-COVID syndrome, it can be inferred that after recovering from COVID-19, symptoms and syndromes of psychiatric pathology are frequently discovered, necessitating correction and treatment. Among these disorders, asthenic, affective, and cognitive disorders hold a particular prominence.

COGNITIVE IMPAIRMENTS IN COVID-19

Cognitive functions represent the brain's ability to engage in the process of rational cognition of the world, encompassing attention, memory, speech, perception (gnosis - recognition of information from the senses), praxis (the ability to acquire, retain, or use various motor skills), and control (regulatory) functions (planning and monitoring the execution of actions). Cognitive impairment is defined as a subjective and/or objective decline compared to

the initial level of cognitive functions, affecting professional, social, and everyday activities [18].

Traditionally, clinicians focused primarily on severe cognitive disorders, such as dementia, which significantly impairs daily functioning, work capacity, self-care, communication, and independence [19]. Subsequently, researchers turned their attention to cognitive activity disorders detected through patient complaints (e.g., forgetfulness, decreased concentration) or identified during in-depth neuropsychological examinations. These disorders, not causing signs of social maladjustment, are now termed non-dementia cognitive disorders [20].

D. Munblit et al. analyzed data from 2649 COVID-19 patients discharged from four respiratory hospitals in Moscow. The average age was 56 years, with 51.1% being women. Notably, 34% of patients required supplemental oxygen therapy, and invasive respiratory support was necessary in 2.6% of cases. After 218 days post-hospitalization, researchers assessed patients' conditions via a telephone survey using the ISARIC Long-term Follow-up Study form (long-term follow-up study protocol). According to the survey conducted 7 months after COVID-19 recovery, 9.1% of respondents complained of forgetfulness [11].

Information regarding the prevalence of cognitive disorders in the post-COVID period is found in the results of a study by A. Pérez-González et al. (Spain, 2022). This prospective cohort study included 248 patients with a positive SARS-CoV-2 PCR test result. Participants averaged 57 years of age, with 59.7% being men. Hospitalization was required for 69.4% of patients, and 10.2% were in critical condition. Surveys conducted 1, 3, and 6 months after the COVID-19 diagnosis identified 3.2% of patients with complaints of impaired memory and attention six months after the diagnosis [17].

The study led by I.A. Zolotovskaya et al. (Russia, 2021) examined 12 outpatients with COVID-19, aged 49.8±8.9 years, presenting with asthenic complaints. The study assessed general weakness, fatigue, decreased concentration, non-systemic dizziness, headache, sleep disturbances, and decreased cognitive function. Results revealed that, among patients not using nootropics, neurometabolics, or antihypoxants, 80.0% reported impaired attention, and 58.5% reported memory impairment. Cognitive impairment was assessed using the Mini-Mental State Examination (MMSE), indicating a pronounced decrease in cognitive function in relatively young patients, attributed to pseudocognitive deficit amid severe asthenic syndrome. The study also identified a tendency towards spontaneous regression of complaints related to memory and attention over time, with no significant impact of nootropic therapy on symptom resolution speed or intensity [21].

In another study conducted by S. Zilberman-Itskovich et al. (Israel, 2022), the condition of 83 adult patients (average age 48 years, men – 34.9%) with cognitive complaints after COVID-19, negatively impacting quality of life and persisting for more than three months post a positive PCR test, was assessed. The study excluded individuals with previously diagnosed cognitive impairment and brain pathology before COVID-19. Conditions were evaluated twice: at baseline and 1–3 weeks after the last treatment session (hyperbaric oxygen therapy or placebo). Assessments included the SF-36 scale (The Short Form-36), PSQI (Pittsburgh Sleep Quality Index) for sleep research, Brief Symptom Inventory-18 (BSI-18) for assessing depression, anxiety, and somatization, Brief Pain Inventory (BPI), Mindstreams computerized cognitive testing program, and MRI brain scanning. The study found improved attention and executive functions (p = 0.04, p = 0.05, respectively) during hyperbaric oxygen therapy compared to the control group. Both groups showed improved memory, attributed by researchers more to the natural course of the disease than hyperbaric oxygen therapy [22].

Thus, the studies presented indicate a cognitive complaint prevalence ranging from 3.2–9.1% among recovered patients, with symptoms such as decreased memory and concentration persisting for at least 6 months post-clinical and laboratory recovery from acute SARS-CoV-2 infection. It is noteworthy that these symptoms tend to resolve spontaneously without drug treatment as part of the natural course of the disease.

ASTHENIC SYNDROME AND COVID-19

Asthenic syndrome is characterized by a loss of strength, feelings of overwork, excessive exhaustion, fluctuations in neuropsychic excitability, weakened attention, unstable mood, and a general decline in mental activity. Unlike ordinary fatigue, asthenia is

persistent, not directly linked to overexertion, and may persist even after extended periods of rest. In severe cases, weakness may be profound, hindering movement or medication intake. Symptoms typically exacerbate in the afternoon, towards the end of the workweek, and before vacations [23].

At the initial stage, neuropsychic excitability rises, sensitivity to visual, auditory, tactile, and other stimuli intensifies ("the clock is ticking loudly," "the text appears bold and voluminous"), and visceral sensitivity increases, resulting in headaches, heaviness, and body aches. Patients find waiting challenging, display impatience in queues, and often experience mood swings, expressed as irritability, tearfulness, anxiety, and moodiness. Objectively, examinations reveal exhaustion and distractibility of attention. Asthenic syndrome frequently presents with sleep disturbances, including difficulty falling asleep and frequent awakenings [23].

Numerous studies indicate the presence of asthenic syndrome in the post-COVID period. Information on the prevalence of asthenic complaints is reported in a study by L.G. Jacobs et al. (USA, 2020), involving 183 adult patients hospitalized for PCR-confirmed COVID-19. The average patient age was 57 years, with 61.5% being men. Immediately after completing inpatient treatment, 56.8% of respondents reported fatigue, and after 35 days, during a follow-up phone conversation, 55.0% still reported fatigue [16].

In a study by D. Munblit et al. (Russia, 2021), conducted 218 days after the end of the acute period of COVID-19, 21.2% of respondents reported fatigue, with chronic lung diseases being associated with chronic fatigue [11].

R. Mahmud et al. (Bangladesh, 2021) identified asthenia in 70.7% of respondents within a month after the resolution of the acute period of COVID-19. Furthermore, asthenic symptoms were statistically significantly associated with the female gender (p = 0.03).

In another study by A. Pérez-González et al. (Spain, 2022), hospitalized patients with laboratory-confirmed COVID-19 exhibited a higher prevalence of asthenic manifestations 6 months after discharge (20.9% versus 5.3%) compared to non-hospitalized patients (p = 0.001) [17].

According to S. Zilberman-Itskovich et al. (Israel, 2022), asthenic syndrome was recorded in 77% of patients with cognitive deficit 3 months after the detection of SARS-CoV-2 [22].

I.A. Zolotovskaya et al. (Russia, 2021), while studying asthenic syndrome in the post-COVID period, observed a positive effect of nootropic therapy. Improvement was noted in research scales, and the differences before and after treatment were statistically significant. The self-assessment of asthenia on a visual analogue scale in the treatment group changed from 8.4 ± 2.5 to 3.9 ± 1.2 cm over three visits, while the control group changed from 8.2 ± 2.7 to 5.1 ± 1.4 cm. On the MFI-20 scale, the treatment group changed from 70.85 ± 10.1 to 49.03 ± 10.1 points, while the control group changed from 69.99 ± 11.5 to 56.18 ± 11.25 points. Notably, the severity of asthenic syndrome decreased even without treatment during the natural course of the disease [21].

Thus, the prevalence of asthenic manifestations in the post-COVID period is consistently reported (approximately 55–70% of patients in the first month after recovery and 20% six months later). Additionally, post-COVID asthenia tends to resolve in most patients over time. There is a statistically significant association between asthenic symptoms in the post-COVID period and female gender, the presence of chronic bronchopulmonary diseases, and the experience of hospitalization for acute COVID-19.

DEPRESSIVE SYNDROME AND COVID-19

Classically, depressive syndrome includes low mood, mental retardation and decreased activity. In addition to these symptoms, there are also hypochondriacal ideas, sleep disturbances, appetite disorders, feelings of guilt, decreased self-esteem, and vegetative manifestations [23]. The ICD-10 diagnostic criteria contain an important indication that the main manifestation of affective disorders is a change in physical activity; these diseases are cyclical, that is, periods of exacerbations and remissions are observed. The main symptoms of a depressive episode are low mood, loss of pleasure and loss of energy. This state is monotonous and changes little for at least two weeks [24].

S. Zilberman-Itskovich et al. (Israel, 2022) revealed in patients with cognitive disorders 3 months after the resolution of the acute period of COVID-19 complaints of insomnia, the level of which, according

to the results of the PSQI questionnaire, was 10.6 ± 4.0 points; however, the level of distress (BSI-18) in the global indicator indicates the absence of anxiety-depressive symptoms in these patients (the average BSI-18 score was 25.1 ± 13.6 points, while a value above 63 points is considered critical) [22].

P. Sadeghipour et al. (Iran, 2022) in their study showed that female gender was associated with higher odds of developing depressive disorders (Patient Health Questionnaire-2 score ≥3) three months after resolution of acute COVID-19 symptoms. In individuals at risk for depressive disorder, the researchers found no statistically significant changes after 3 months (the proportion of patients with a PHQ-2 score ≥3 points decreased from 26.1% at day 30 to 16.6% at 90 days of follow-up ($p = 0.05$)) [8].

A. Pérez-González et al. (Spain, 2022) found sleep disturbances in 5.2% of hospitalized patients in the post-COVID period, while in the outpatient group there were no patients with such complaints, although the differences between the groups on this basis were not statistically significant [17].

R.G. Khabchabov et al. (Russia, 2021) in their study using the World Health Organization Quality of Life Questionnaire (QOL-100) found that depressive symptoms appeared in 68.6% of patients who had recovered from COVID-19, of which 24.8% noticed this family members [9].

S. Zhu et al. (China, 2020) examined 432 patients (average age 49 years, 49% women, a third of cases had severe COVID-19) upon discharge from hospital after COVID-19 using the Tsung scale for anxiety symptoms. 28.7% of patients showed positive test results for clinical anxiety. However, severe COVID-19 (adjusted risk ratio (RR) 2.533; 95% confidence interval (CI) 1.693–3.788) was the strongest risk factor for the development of clinically significant anxiety. Moreover, there was a trend towards an increased relative risk of anxiety in survivors who remained in hospital for more than 14 days (adjusted RR 1.482; 95% CI 0.998–2.200) [25].

Summarizing the analyzed studies, we can note quite clear indications that patients after COVID-19 are faced with insomnia, anxiety, emotional disturbances, which may reach the delineated depressive episodes, given that changes in the mood of patients are noticed by their close people. These emotional disturbances show a correlation with female gender and the fact of hospitalization for acute COVID-19. Clinically significant anxiety correlates with COVID-19 severity and length of hospital stay. Further study of the prevalence of affective disorders in people with post-COVID syndrome is required in order to qualify the mental state of patients according to the current ICD criteria.

QUALITY OF LIFE IN PATIENTS WITH POST-COVID SYNDROME

Currently, Quality of Life (QOL) is understood as a characteristic of a person's physical, psychological, emotional, and social functioning, based on their subjective perception [26]. The primary method for assessing QoL is questionnaires, with the SF-36 questionnaire being the most widely used in clinical studies. This questionnaire comprises 8 scales reflecting the main components of QoL: physical functioning, role functioning, bodily pain, general health, vitality, social functioning, emotional state, and mental health [27].

In a study by L.G. Jacobs et al. (USA, 2020), information regarding the quality of life of patients who had COVID-19 35 days after discharge from the hospital is presented. Among them, 13.8% of respondents rated their physical activity as low, 24.9% as moderate, and 61.3% as complete. 3.3% of patients reported great difficulty getting dressed, and 29.6% reported no or slight difficulty getting out of bed. At hospital discharge, 52% of participants were employed, but by day 35, only 29.9% had returned to work. Social relationships worsened for 60.4% of patients, and only 39.6% rated their social life as excellent [16].

J. Li et al. (China, 2021) conducted a study of 120 previously hospitalized adult patients diagnosed with COVID-19, persistently complaining of dyspnea. The average age was 50.6 years; 44.5% were men, and 86.6% received oxygen therapy or non-invasive respiratory support during treatment. Exclusion criteria included chronic diseases in the decompensation stage, dyspnea at rest, or tachycardia (heart rate > 100/min). The condition was assessed at baseline (on average 70 days after hospital discharge) and then after 6 weeks and six months. The 6-minute walk test value increased by 17.1 m over 6 weeks, indicating improvement. The physical component of QOL according to the SF-12 questionnaire improved from 39.69 to 43.53 points, and the mental component improved from 44.13

to 48.3 points, reflecting an overall betterment in patients' quality of life over time [28].

The results of the study by M.A. McNarry et al. (USA, 2022) contain information on the quality of life using the K-BILD (The King's Brief Interstitial Lung Disease) scale in 281 adult patients 9 months after acute COVID-19. The average age was 46.6 years, with 88% being women. The average quality of life score at the beginning and after 8 weeks remained virtually unchanged (at the beginning 59.7 points, after 8 weeks – 59.8 points). However, improvements were noted in individual domains: shortness of breath and activity increased from 40.6 points to 41.9 points, the psychological component from 56.9 points to 59.2 points, and chest symptoms from 56.6 to 59.5 points, indicating an enhancement in the health status of patients over time [29].

S. Dhooria et al. (India, 2022) conducted an analysis of the condition and quality of life of 130 individuals within 3–8 weeks from the onset of acute COVID-19, confirmed by PCR. The average age of the patients was 57 years, with 32% of study participants being female. Notably, 98% of patients experienced a severe form of COVID-19, and 43% of cases required invasive respiratory support or high-flow oxygen therapy. A distinctive aspect of this study was the examination of the health of patients with severe COVID-19. Consequently, inclusion criteria comprised persistent dyspnea (mMRC $\geq$ 2 points), resting hypoxemia (oxygen saturation $\leq$ 94%), or exercise desaturation ($\geq$4% drop in oxygen saturation during exercise) at screening; diffuse abnormalities affecting $\geq$ 20% of the lung parenchyma. All patients in this study received prednisolone, categorized into two groups with high and low doses of glucocorticosteroids (GCS). Six weeks after the first symptoms of COVID-19, the group receiving high doses of GCS exhibited an average physical activity score of 59.4 ± 26.3 points on the Health-Related Quality of Life (HRQoL) scale, while the low-dose group scored 62.9 ± 28.5 points (p = 0.49). The indicator of social adaptation after 6 weeks in the high-dose GCS group on the HRQoL scale was 76.4 ± 25.4 points. In the group with low doses of GCS, this indicator was 69.2 ± 29.9 points (p = 0.15). Additionally, in this study, no statistically significant differences were observed between the two groups in X-ray dynamics, changes in spirometry parameters, saturation levels, and the severity of shortness of breath. Consequently, the authors did not identify any advantage of high doses of prednisolone over low doses [30].

S. Zilberman-Itskovich et al. (Israel, 2022) conducted a study assessing Quality of Life (QoL) using the SF-36 scale. The results indicated an improvement in QoL characteristics such as physical functioning and vitality in the group of patients receiving hyperbaric oxygen therapy [22].

P. Sadeghipour et al. (Iran, 2022) undertook a study involving 375 patients diagnosed with COVID-19 who received treatment in the ICU. The average age of participants was 62 years, with 42% being women. Mechanical ventilation was administered to 20.8% of patients, non-invasive ventilation to 33.2%, and oxygen therapy to 46.1%. The study employed the Post-COVID-19 Functional Status Scale (PCFS) to evaluate functional status and the Patient Health Questionnaire-2 (PHQ-2) to assess depressive symptoms. From days 30 to 90, the proportion of patients with moderate to severe functional limitations (PCSF grade 3 or 4) decreased from 20.0% to 4.8% ($p < 0.001$). Concurrently, the percentage of patients without functional limitations (grade 0 PCFS) increased from 4.2 to 15.4% ($p < 0.001$). Statistical analysis revealed that younger age predicted functional recovery at 90 days, while a longer ICU stay (>7 days) was associated with increased odds of severe functional limitation at the 3-month follow-up [8].

E. Zasadzka et al. (Poland, 2022) evaluated 30 adult patients admitted to a neurological rehabilitation unit after severe COVID-19. The examination, including the Functional Independence Measure (FIM), showed a change in FIM values from 89 to 117 points, indicating a gradual increase in the ability for self-care (independence) among recovered COVID-19 patients [31].

In another work (Germany, 2022), a comparative analysis was presented, examining 206 patients who had COVID-19 on an outpatient basis and individuals who had never contracted COVID-19 (negative test for antibodies to SARS-CoV-2). The assessment included indicators such as hospitalization rates over 7 months and quality of life assessment (EQ-5D-5L, SF-36, PCFS, 6-minute walk test). The average age was 47 years, with women comprising 58.2% of the participants. Hospitalization rates were low and comparable in both groups (2.4% in the COVID-19

group versus 2.9% in the control group). However, the analysis revealed a decrease in QoL on the EQ-5D-5L scale in patients who had COVID-19 (average VAS values 83.6 ± 15.2 mm versus 88.6 ± 12.4 mm in the control group; $p < 0.05$) [32].

Keir E.J. Philip et al. (UK, 2022) analyzed the condition of 150 adult patients who had recovered from COVID-19 with ongoing shortness of breath. The average age was 49 years, with 81% being female. After 320 days from the onset of the first symptoms of COVID-19, quality of life (SF-36, SF-6D), severity of shortness of breath (VAS), and anxiety level (GAD-7) were assessed. Despite the performance of breathing exercises in one of the groups, there were no differences in the domain of the physical component of QoL compared to the control group. However, patients in the main group showed better scores in the psychological component ($p = 0.047$) [33].

In another study that examined Quality of Life (QoL) in the post-COVID period, S.I. Filippchenkova et al. (Russia, 2022) included 87 adult patients (average age 44.8 years; 29% of them were men) and conducted a survey using the SF-36. The study excluded individuals with traumatic brain injuries, diseases of the central nervous system, mental disorders, oncological, endocrine, and somatic pathologies, as well as bad habits (alcohol and drug abuse). According to the survey results, approximately 50% of patients demonstrated average indicators of the physical component of health, and almost 30% of study participants experienced difficulties in performing daily tasks [34].

R.G. Khabchabov et al. (Russia, 2021) conducted a special assessment of the quality of life in 121 patients aged 41–76 years (68.6% men) one month after discharge from the cardiology hospital, where they were treated for COVID-19. Participants were individuals who had been under the supervision of doctors for more than two years with functional class III–IV angina pectoris, arterial hypertension, and type 2 diabetes mellitus. All of them suffered from COVID-19, while in 3.4% of patients, the degree of lung damage varied from 30% to 60%, in 15.7% of patients – 10–30%, in 38.0% of patients, the extent of damage to the pulmonary parenchyma was < 10%, and 43.0% of patients were diagnosed with mild COVID-19. A survey of patients using the QOL-100 questionnaire showed that a month after suffering from COVID-19, the proportion of patients with insufficient energy for everyday life increased by 23.2% ($p = 0.001$); those dissatisfied with their health increased by 31.4% ($p = 0.0016$); anxious and depressive experiences began to be detected 33.9% more often ($p = 0.0018$). 17.3% more patients reported insomnia ($p = 0.0005$), and personal relationships with friends and relatives worsened by 63.6% ($p = 0.0027$). At the same time, the number of patients with temporary disability after COVID-19 increased by 15.7% ($p = 0.0003$), compared with their number before COVID-19 [9].

Thus, the results of assessing various domains of QoL in patients after clinical recovery from COVID-19 remain at a statistically significantly lower level than in persons who have not had COVID-19 for an extended period. There is a decrease in both the physical and psychological components of QoL. After completing hospitalization for at least 35 days, a significant proportion of patients remained unable to work. Some patients, after completing the acute phase of COVID-19, experienced limitations in physical functioning, difficulty getting dressed, getting out of bed, and restrictions in social functioning. Many studies provide evidence that the deterioration in the quality of life in the post-COVID period is temporary and is characterized by a tendency towards gradual recovery. Some authors find a relationship between the low severity of functional impairments and high rehabilitation capabilities in young people; they also note that a worsening functional prognosis in patients is associated with a length of stay in the ICU of more than 7 days.

CONCLUSION

This review delves into a critical public health concern – the medical and social repercussions of COVID-19, shaping the so-called post-COVID syndrome.

Through the analysis of published data, it can be inferred that individuals recovering from COVID-19 experience cognitive impairment [11] [17][21], asthenia, and insomnia [17]. However, these issues tend to spontaneously resolve over time [21] and exhibit partial responsiveness to currently available methods of pharmacotherapy and physical rehabilitation [22].

It is noteworthy that post-COVID asthenic manifestations were more frequently associated

with female gender [12], the presence of chronic bronchopulmonary diseases [11], and hospitalization for COVID-19 [17]. Additionally, anxiety symptoms correlated with the severity of COVID-19 and the length of hospital stay [25]. Emotional disorders were also identified in the post-COVID period, but further clarification is needed [8, 9].

The literature analysis results lead to the assertion that there is a decline in the quality of life in patients after the resolution of acute COVID-19 [9, 32]. Importantly, unlike many other infectious diseases (e.g., bacterial pneumonia), individuals completing hospitalization may not be able to return to work and remain disabled even 35 days after leaving the hospital [16]. Simultaneously, there is a degradation in results across all domains contributing to the Quality of Life (QoL). However, with time, QoL indicators gradually improve, particularly in young individuals [8]. Conversely, an ICU stay exceeding 7 days correlates with a poorer rehabilitation prognosis [8].

Despite substantial progress in post-COVID mental disorders and quality of life research, several unresolved questions necessitate further investigation. Extended observation of psychopathological symptoms in individuals recovering from COVID-19, continuous assessment of their functional status, identification of criteria for permanent disability in people with long-term COVID, and a comprehensive understanding of affective sphere pathology remain areas of interest.

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Challenges in the diagnosis of cervical pathologies

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ABSTRACT

This review deals with the current state of affairs in the diagnosis of cervical squamous intraepithelial lesions. Transformation of classifications of cervical pathologies is considered. The role of cytological (liquid-based and conventional cytology), molecular biological (Digene Hybrid Capture test), immunohistochemical (p16INK4α, Ki-67), and histologic methods in the diagnosis of cervical lesions is discussed. Particular attention is paid to the diagnosis of human papillomavirus infection. Performance indicators of screening programs based on primary determination of human papillomavirus (HPV) DNA in comparison with common cytological methods are presented. Tropism of HPV to various parts of the cervix, which predisposes to the formation of deep multifocal lesions, as well as the influence of the physical status of HPV on the treatment strategy and risks of relapse are considered.

Keywords: cervical squamous intraepithelial lesions, cervical intraepithelial neoplasia, HPV, diagnosis

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Проблемы диагностики патологий шейки матки

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РЕЗЮМЕ

Статья посвящена современному состоянию проблемы диагностики плоскоклеточных интраэпителиальных поражений шейки матки. Рассмотрены вопросы трансформации классификаций и терминологии патологий шейки матки. Обсуждается роль цитологических (жидкостная и традиционная цитология), молекулярно-биологических (Hybrid Capture Diegene Test), иммуногистохимических (p16INK4α, Ki-67) и гистологических методов в диагностике поражений шейки матки. Особое внимание уделено диагностике папилломавирусной инфекции, приведены показатели эффективности скрининговых программ, основанных на первичном определении ДНК вируса папилломы человека (ВПЧ) в сравнении с общепринятыми

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цитологическими методами. Рассмотрена тропность ВПЧ к различным отделам шейки матки, предрасполагающая к формированию глубоких мультифокальных поражений, а также влияние физического статуса ВПЧ на лечебную тактику и риски рецидивов.

Ключевые слова: плоскоклеточные интраэпителиальные поражения шейки матки, цервикальная интраэпителиальная неоплазия, ВПЧ, диагностика

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

Squamous intraepithelial lesions of the cervix are some of the most common pathologies among women of reproductive age in clinical practice. The leading etiological factor in these pathologies is human papillomavirus (HPV) infection and its direct cytopathic effect on the cervical epithelium. The main challenge in the diagnosis and treatment of cervical pathologies is high frequency of persistent latent HPV infection involving endocervical glandular lesion, which, in turn, leads to incomplete elimination of the virus, incomplete excision of altered tissues, and, as a consequence, a high relapse rate. Currently, the search for optimal approaches to diagnosing and assessing the prognosis of these pathologies is underway, also through improving screening programs.

CHRONOLOGY OF CHANGES IN CERVICAL PATHOLOGY TERMINOLOGY

The term “dysplasia” in relation to cervical pathology was coined in 1953, and in 1956, it was proposed for the histologic classification of cervical diseases [1]. Dysplasia is characterized by intense atypical cell proliferation in the basal and parabasal layers (cellular and nuclear polymorphism, increased mitosis) with impaired stratification of the epithelium, but without involving the surface layer and stroma. Depending of the altered epithelial layer, weak, moderate, and severe dysplasia are differentiated. Carcinoma *in situ* was considered separately and, according to the FIGO classification, belonged to stage 0 cervical cancer. It was subsequently proven that there are no clear histologic differences between carcinoma *in situ* and severe dysplasia, and a possible similarity of cytological changes was confirmed [2].

In 1973, R.M. Richart proposed a unified concept of neoplastic changes in the cervix. On this basis, the term “cervical intraepithelial neoplasia” (CIN) was proposed to describe the stages of tumor progression [3]. Inclusion of carcinoma *in situ* in the group of severe dysplasia, based on similarities in genetic cell abnormalities, prognosis, and clinical outcomes, made a significant difference for the classification compared to the previous approach.

In 1974–1976, doubt was put on the concept of CIN as obligate precancer following the H. Hausen’s definition of the etiological role of HPV in the development of cervical dysplasia and cancer [4], the pathogenesis of viral damage, the regenerative potential of tissues, and the possibility of self-elimination of the virus.

In 1988, the National Cancer Institute (USA) developed and introduced a new classification for cervical smears – The Bethesda System (TBS) for reporting cervical pathology, heralding the introduction of the term “squamous intraepithelial lesion” (SEL) [5]. According to this approach, dysplastic changes in the cervix are divided into “low-grade squamous intraepithelial lesion (L-SIL)”, corresponding to CIN I, and “high-grade squamous intraepithelial changes” (H-SIL), corresponding to CIN II–III, including carcinoma *in situ* (CIS).

The negative for intraepithelial lesion or malignancy (NILM) category was introduced to indicate a normal cytological pattern. The new classification is etiologically and pathogenetically

substantiated and correlates with the mechanisms of initiation and course of the disease, depending on the high- or low-risk HPV types. Later it was proposed to use TBS categories for the morphological description of the histologic material. Therefore, the use of a unified terminology for histologic and cytological impressions increases the evidence-based diagnosis.

Currently it is recommended to use L-SIL as a diagnostic category to describe changes associated with transient HPV infection, while H-SIL is used to define a true precancerous lesion. However, some ambiguous morphological findings may be included in the Atypical squamous cells (ASCs) category, depending on qualitative and quantitative criteria. This category includes atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells – cannot exclude H-SIL (ASC-H), differentiated according to the expected lesion grade (L-SIL or H-SIL, respectively) [6]. To describe endocervical epithelial cell abnormalities, the terms "atypical glandular cells" (AGC), "atypical glandular cells, favor neoplastic", and "adenocarcinoma *in situ*" (AIS) were proposed.

According to the International Classification of Diseases (ICD), 11th revision (2022), the abbreviation CIN was completely excluded from use. CIN I was replaced by the term "low-grade squamous intraepithelial lesion of the cervix". The description of L-SIL states that it is a "a condition of the cervix caused by chronic infection," so L-SIL is not currently considered as cervical dysplasia. CIN II and CIN III are grouped in the category of high-grade squamous intraepithelial lesion of the cervix and represent the class *Carcinoma in situ of cervix uteri*. The headings "Cervical intraepithelial neoplasia grade II" and "Cervical intraepithelial neoplasia grade III" were completely excluded from the new edition [7]. The abbreviation SIL has officially replaced the abbreviation CIN in clinical practice.

Thus, over the past 70 years, several approaches to cervical lesion classification have been proposed. Despite the shift in the emphasis toward the development of a clinically oriented classification (TBS) and attempts to standardize the terminology, the existing uncertainty in the use of CIN and SIL demonstrates the lack of unified approaches to the diagnosis and treatment of cervical pathologies.

EPIDEMIOLOGY

The Information Center on HPV and Cancer of the Catalan Institute of Oncology and the International Agency for Research on Cancer have published epidemiological data on the global prevalence of HPV infection caused by HPV types 16 and 18 in women over 15 years, depending on the results of cytological screening. Women with normal cytology results were infected by HPV in 3.9% of cases, and HPV was detected in 25.8% of women with L-SIL. Almost every second woman with H-SIL was infected by HPV (51.9%) [8].

According to D. Egemen et al. (2020), 87% of L-SIL, 95% of H-SIL, and 54% of ASC-US cases are associated with other highly oncogenic HPV serotypes, including those mentioned before [9]. The prevalence of HPV types 16 and 18 in the Russian Federation was 9.4% among women with NILM, 35.1% in women with L-SIL, and 56% in women with H-SIL [10]. It should be mentioned that these highly oncogenic serotypes are the most common both in the Russian Federation and worldwide.

Carriers of HPV infection are most often detected in the group of women aged 20–29 and 30–39 years, as well as in the age group of 40–49 years [11]. The prevalence of cervical lesions varies depending on the population and averages 1.5–7.7% for L-SIL and 0.4–1.5% for H-SIL [12–14]. About 1.7% of cytological smears are classified as L-SIL, 0.3% – as H-SIL, 4.1% – as ASC, and 0.21% – as AGCs [9].

THE ROLE OF HPV INFECTION

The predominant HPV-associated etiology of cervical cancer is proven [4]. Along with common knowledge about the pathogenetic processes caused by HPV persistence, it is necessary to understand the physical status of HPV and its tropism to various parts of the cervix. It is known that high-risk HPV affects pluripotent stem and proliferating cells in the emerging transformation zone, as well as in the superior canal and endocervical crypts [15].

According to J.Y. Chen et al. (2018), glandular lesions were observed in 82.57% of HPV-infected women. The persistence of HPV in the cervical crypts in 80.77% of cases was combined with positive margins after resection and leads to relapse after surgery. Relapse in the initial absence of glandular lesions was observed only in 18.23% of patients with

positive margins [16]. The depth of crypt lesions in 94% of cases did not exceed 5 mm laterally to the cervical canal, however, their location at a distance of up to 4 cm from the ectocervix may be the cause of incomplete excision of the endocervical component [17].

Women with positive endocervical margins are at high risk for recurrent lesions. In addition, negative margins after cold-knife conization do not guarantee the absence of future relapses [18].

According to M. Arbyn et al. (2017), the presence of high-risk HPV after treatment increased the relapse risk to 28.4%, while a negative result reduced the risk to 0.8%. When positive resection margins were combined with a positive HPV status, the risk of relapse was 53%. With negative resection margins and persistent HPV, the risk of relapse was 13%, and 1% with a negative HPV status, regardless of the resection margin state [19].

CYTOLOGY, HISTOLOGY, IMMUNOHISTOCHEMISTRY

A cytological examination of the cervical epithelium with interpretation of the results according to TBS is used in all cervical screening programs. The sensitivity and specificity of the cytological method for L-SIL detection is 80.31 and 68.46%, respectively, while for H-SIL, it is 97.14 and 85.58%. respectively [21]. However, approximately 30% of newly diagnosed cases of cervical cancer occur among women who were screened negative due to misinterpretation or sampling errors [22]. Comparing liquid-based and conventional cytology methods, a lot of authors state that liquid-based cytology improves the quality of cytological material, is more preferable in terms of cost-effectiveness, and also makes it possible to use the sample for subsequent HPV testing [22–24]. Positive predictive value (PPV) of these methods is comparable. Negative predictive value (NPV) is higher for liquid-based cytology [25]. A systematic review carried out in 1991–2007 as part of the European Quality Assurance Guidelines for Cervical Cancer Screening found that although liquid-based and conventional cytology had equal sensitivity and specificity in detecting dysplasia from stage CIN II (H-SIL), the specificity of liquid-based cytology in case of ASC-US was lower [26].

Cytological screening does not reveal any pathology in most HPV-positive women. It was established that among HPV-positive women with normal cytology results, dysplasia was diagnosed within 5 years in 6.4% of cases [27]. The affected glands often located deep in the cervical canal make obtaining adequate material for the cytological examination difficult. That is why the cytological examination is limited in the diagnosis of precancer and cancer of the cervix. Pathological changes in the obtained material may be mild or absent, since the process is often located deep in the crypts, while the squamous epithelium is practically intact [28].

In this case V.G. Cherenkov et al. (2019) insisted on obtaining the material for cytological and molecular studies not only from the transformation zone, but also from the crypts in the endocervix [29]. In addition, it is recommended to perform a biopsy involving the endocervical component before elaborating a treatment strategy, since it is persistence of HPV in the crypts that may cause subsequent progression of lesions as well as relapses, especially in case of H-SIL [30, 31].

The histologic method is the gold standard for cervical pathology diagnosis. Comparing the results of the cytological examination and the final histologic diagnosis, several authors noted significant discrepancies. In 20% of patients with L-SIL cytology, the presence of dysplasia was not confirmed, in 45.52% of cases, CIN I (L-SIL) was detected, in 20.89% of cases – CIN II (H-SIL), and in 04.47% of cases – CIN III (H-SIL). On the other hand, in 0.9% of H-SIL cases, the presence of dysplasia was not confirmed, CIN I (L-SIL) was detected in 15.09% of cases, CIN II (H-SIL) – in 16.98% of cases, CIN III (H-SIL) – in 50.94% of cases. Cervical cancer was detected in 5.6% of H-SIL cases [32].

A comparative analysis of the histologic findings after primary excisional biopsy in patients with previous targeted cervical biopsy showed a higher positive correlation with CIN I (L-SIL) and CIN III (H-SIL) compared to CIN II (H- SIL). Cervical cancer was revealed by excisional methods in 1.8% of patients with CIN III (H-SIL), diagnosed previously by targeted biopsy [33].

Applying immunohistochemical markers of cell proliferation, such as ki-67 and p16/INK4a, can help to improve the accuracy and information value

of a routine histologic examination and reduce the frequency of false-positive and false-negative results. HPV DNA replication begins with the formation of oncoproteins E6 and E7 promoting the functional inactivation of the retinoblastoma protein (pRb) genes, which has an antiproliferative effect through the expression of the p16/INK4a protein [34].

The transforming effect of HPV leads to overexpression of this protein, controlled by pRb through negative feedback [35]. Ki-67 is a nuclear non-histone protein actively expressed during cell division (cell proliferation marker) [36]. Overexpression of Ki-67 is associated with the severity of dysplastic changes in the cervix, but not with HPV infection [37]. It is known that an increase in dysplastic changes of the cervix is associated with an increase in the expression of these markers [38]. Most L-SIL cases are associated with elevated levels of Ki-67. Approximately in one third of L-SIL cases, strong diffuse expression of p16INK4a can be found in the lower part of the epithelial layer [39]. However, it has been proven that not every p16INK4a-associated damage of the cervical epithelium is caused by HPV.

P.P. Pereira et al. (2022) demonstrated the possible immunopositivity for p16 and Ki-67 in healthy tissues, as well as in areas of tubal metaplasia of the cervix and cervical endometriosis [40]. Thus, simultaneous detection (coexpression) of both p16INK4α and Ki-67 proteins (dual stain) has the greatest diagnostic value. Simultaneous detection of p16/Ki-67 can be a reliable method for risk stratification among HPV-positive women, especially in cases of CIN II–III (H-SIL) [41]. The degree of coexpression correlates with the severity of cervical lesions and indicates the disease progression [42].

UNRESOLVED ISSUES IN THE DIAGNOSIS OF CERVICAL PATHOLOGY

The issues of diagnosis, treatment, and prevention of squamous and glandular cervical lesions remain highly relevant in the Russian Federation and around the world. It is explained by many factors, including the need to optimize screening, treatment, and prevention programs [43].

The International Federation of Gynecology and Obstetrics (FIGO) recommends a cytological examination (up to 60 years old) and testing for HPV (up to 65 years old) as screening every 5 years. The Society of Gynecologic Oncology and American Society for Colposcopy and Cervical Pathology (ASCCP) have recommended only primary HPV screening for women aged 25 years and older since 2015. The World Health Organization (WHO, 2014) recommends HPV testing, a cytological examination, and a visual inspection with acetic acid at least once for every woman aged 30–49 years [25]. PCR methods for HPV diagnosis are increasingly replacing cytology as the main screening test in the USA, Australia, and England [44–46].

The mainstay for the diagnosis of persistent HPV infection is the detection of viral DNA in cervical scraping by PCR (Hybrid Capture Digene HPV test) with determination of the critical DNA concentration (viral load) and assessment of the physical status, the oncogenic risk (high or intermediate), and the type of infection (single or multiple).

Despite the described cases of HPV-negative lesions of the cervix, including malignant ones, L. Rodríguez-Carunchio et al. (2015) in most cases associated the detection of such lesions with diagnostic artifacts and false-negative results. This fact was confirmed by the detection of viral DNA when using more sensitive PCR diagnostic methods [47].

According to T. Malagón et al. (2020), the use of Hybrid capture-based technology for the HPV diagnosis reduces the risk of subsequent occurrence and progression of cervical dysplasia to a greater extent than the use of other PCR methods among women with normal cytology findings [27].

According to a Cochrane Library review, the sensitivity of Hybrid capture-based detection of precancerous lesions of the cervix is higher than that of cytological diagnostic methods. Precancerous lesions will be diagnosed in 20 screened women out of 1,000. The HPV test will identify 18 women in this group, while the cytology test will identify 15. A negative HPV test result is more reassuring than a negative cytology result, because the cytology test has a higher chance of being false-negative [48].

There are controversies regarding the choice of the screening method for the age group of 25–30 years old. The results of numerous studies indicate that transient HPV is common among this age group, and self-elimination of the virus reaches 90%. HPV persistence is more often observed for 2 years or

more in the age group of 30 years and older, which is one of the risk factors for lesion progression [49–51]. Consequently, HPV screening before the age of 30 might be associated with a high rate of false-positive results, which can lead to unnecessary treatment. Despite this, primary HPV screening among women of this age group is associated with higher CIN III (H-SIL) detection compared to cytological methods. According to O. Feldstein et al. (2023), it should be considered as the main diagnostic approach in this age [52].

Y.J. Tai et al. (2017) assessed the links between the risk of disease progression and the diagnostic and treatment approaches among 53,000 women with L-SIL [53]. These approaches included repeated cytology, colposcopy, cervical biopsy or cervical curettage, cryotherapy, and excisional methods. It revealed that cryotherapy and excisional procedures significantly reduced the risk of lesion progression, presumably due to the effective elimination of HPV. C. Firnhaber et al. (2017) and G. St-Martin et al. (2021) also supported the active diagnostic and treatment strategy in cases of L-SIL. [54,55].

On the other hand, C. Buick et al. (2020) and C. J. Min et al. (2020) indicated that there is no need for in-depth screening and subsequent treatment of young women with L-SIL due to a low risk of malignancy and a high probability of spontaneous elimination of HPV in this age group [56, 57].

CONCLUSION

Direct pathogenetic links of squamous intraepithelial and glandular lesions with the progression of cervical cancer dictate the need to search for new diagnostic approaches in order to timely identify and treat these conditions and reduce the risk of their progression.

Long-term latent HPV persistence, especially in the cervical crypts, leads to an increased risk of occurrence and recurrence of squamous intraepithelial and glandular lesions. Modern diagnostic and treatment approaches should be aimed at increasing the efficiency of cervical lesion diagnosis by assessing the degree of involvement of the cervix and especially cervical crypts in the pathological process, as well as by determining the optimal volume of excision. These issues can be solved by a comprehensive assessment of cytological, molecular biological, histologic, and immunohistochemical findings that have high sensitivity and specificity for the diagnosis of cervical pathologies.

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AUTHOR INDEX FOR VOLUME 22

L

M

N

O

P

R

S

T

U

V

Y

Z

135 ЛЕТ СИБИРСКОМУ ГОСУДАРСТВЕННОМУ МЕДИЦИНСКОМУ УНИВЕРСИТЕТУ

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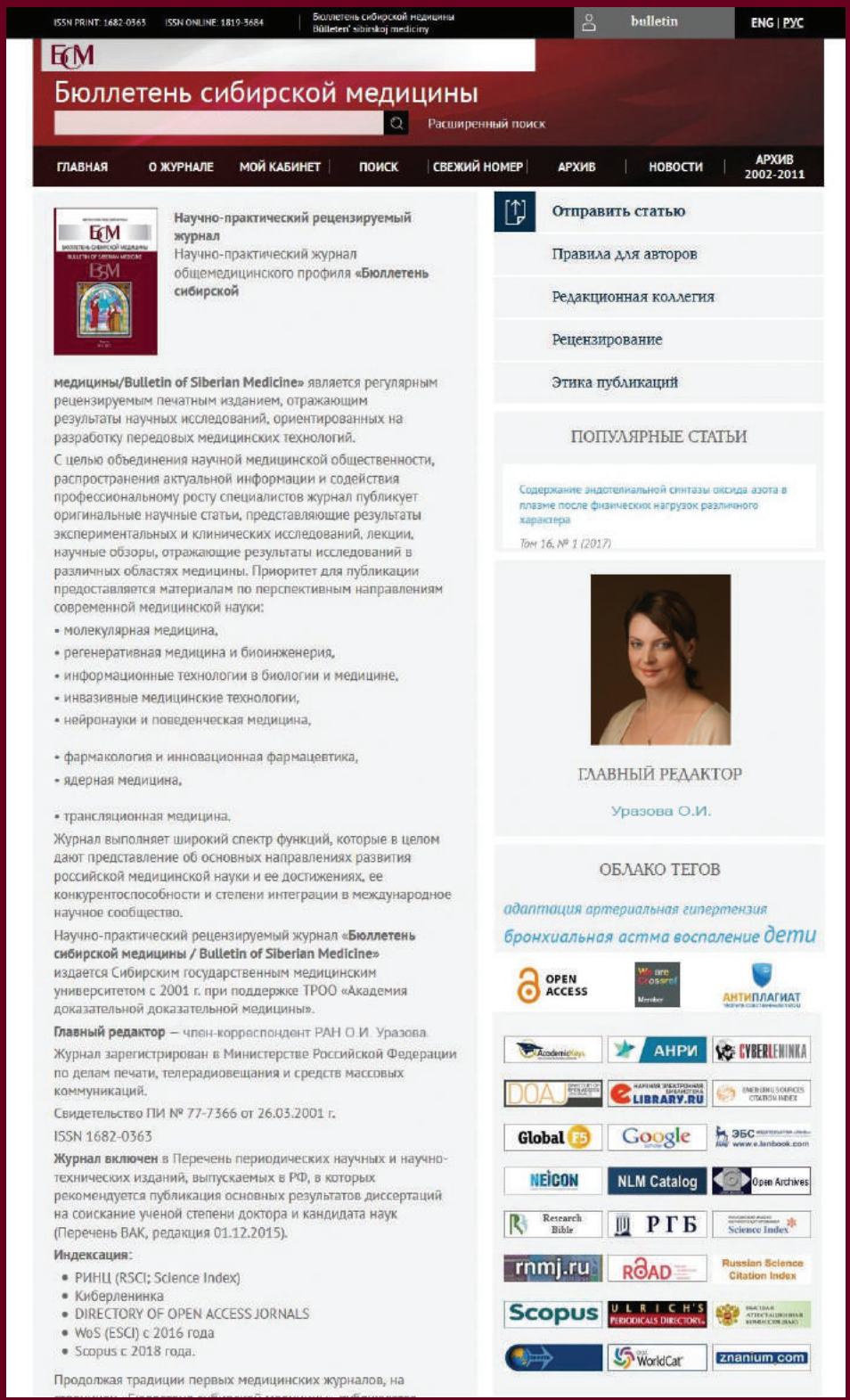

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