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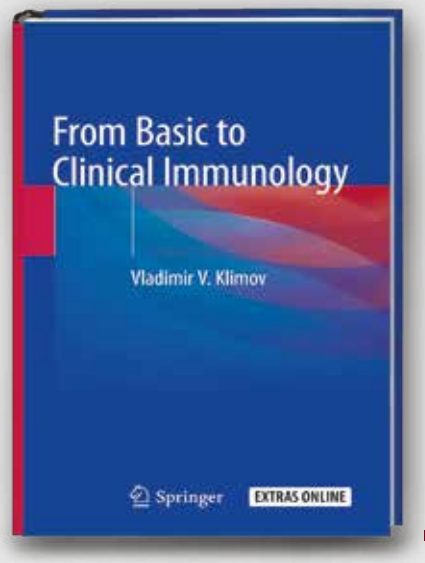

КНИГА УЧЕНОГО ИЗ СИБГМУ ЗАНЯЛА ВТОРОЕ МЕСТО В МЕЖДУНАРОДНОМ РЕЙТИНГЕ

Издание заведующего кафедрой иммунологии и аллергологии Сибирского государственного медицинского университета, доктора медицинских наук, профессора Владимира Климова заняло второе место в мировом рейтинге иммунологических изданий по версии независимого аналитического агентства научной литературы «BookAuthority»: https://bookauthority.org/award/From-Basic-to-Clinical-Immunology/3030033228/new-allergy-and-immunology-ebooks

Книга «FromBasictoClinicalImmunology» написана на английском языке, напечатана швейцарским издательством Springer (https://www.springer.com/gp/book/9783030033224) и распространяется многотысячным тиражом наряду с мировыми бестселлерами среди российских и зарубежных вузов, а также включена в каталог библиотеки конгресса США.

«Столь высокая оценка издания – это первый опыт для медицинского образования в России, что свидетельствует о высоком уровне и конкурентоспособности здравоохранения в нашей стране», – отметил Владимир Климов. В ближайшее время планируется перевод издания на китайский язык.

Уникальность книги заключается в том, что автору удалось объединить две смежные области знаний – фундаментальную иммунологию и клиническую. Специалисты обеих сфер до сих пор работали обособленно, так как теоретики не имели медицинской квалификации, а у врачей отсутствовал опыт фундаментальных исследований.

«Издание аккумулирует современные знания по базовой иммунологии и иммунопатологии с клиническими комментариями, которые дополняют общую картину», - добавил автор. По такому же сценарию профессор выстраивает образовательный процесс: обучающиеся осваивают ту теоретическую базу, которая в дальнейшем может быть применена ими в клинической практике.

«Лучший совет подрастающему поколению в медицинском университете – изучать английский язык и доводить это знание до совершенства, чтобы можно было читать научную литературу в оригинале», – считает заведующий кафедрой иммунологии и аллергологии СибГМУ Владимир Климов.

Ознакомиться с печатным вариантом издания можно в Научно-медицинской библиотеке СибГМУ. Книга входит в перечень учебных пособий по иммунологии для студентов, обучающихся на английском языке. Напомним, Владимир Климов является автором первого российского мультимедийного учебника «Основы общей иммунологии», который ранее также получил международное признание. Англоязычная версия мультимедийного курса была включена в каталоги более 30 университетов мира, в том числе таких престижных, как Кембридж, Гарвард, Йель и других. С момента публикации курса обучение по нему прошли иностранные студенты и врачи более чем из 130 стран. Учебник широко востребован благодаря краткости и доступности изложения сложных иммунологических процессов.

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Conformational features of lactate dehydrogenase: temperature effect in presence of small molecules, mathematical model

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ABSTRACT

The aim. To study the conformational changes of lactate dehydrogenase under the influence of different concentrations of intermediates (pyruvate, oxaloacetate) in the temperature gradient with the subsequent building of a mathematical model.

Materials and methods. Thermolability of lactate dehydrogenase was studied using the method of differential scanning fluorimetry to determine the change in endogenous fluorescence of tryptophan and tyrosine under the conditions of stable concentration of lactate dehydrogenase and changing concentrations of pyruvate and oxaloacetate. Further, a mathematical model was developed for a more in-depth consideration of the behavior of the catalytic protein.

Results. We found that pyruvate and oxaloacetate in low concentrations have a thermostabilizing effect on lactate dehydrogenase conformation; the effect of pyruvate is statistically more significant in comparison with oxaloacetate ($p < 0.05$). The studied ligands in high concentrations reduce the thermal stability of lactate dehydrogenase.

Conclusion. Understanding the role of small molecules in the regulation of biological and catalytic processes has long remained in the background of scientific interest, but today the work in this direction is reaching a new level. The data obtained indicate the possibility of small molecules acting as ligands when interacting with enzymes.

Key words: lactate dehydrogenase, conformation, differential scanning fluorimetry, oxaloacetate, pyruvate.

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Конформационные особенности лактатдегидрогеназы: влияние температурного фактора в присутствии малых молекул, математическая модель

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РЕЗЮМЕ

Цель. Исследовать конформационные изменения лактатдегидрогеназы под действием различных концентраций интермедиатов (пируват, оксалоацетат) в температурном градиенте с последующим построением математической модели.

Материалы и методы. Изучение термолабильности лактатдегидрогеназы проводили с использованием метода дифференциальной сканирующей флуориметрии по изменению эндогенной флуоресценции триптофана и тирозина в условиях стабильной концентрации лактатдегидрогеназы и изменяющихся концентраций пирувата и оксалоацетата. Далее была разработана математическая модель для более углубленного рассмотрения поведения каталитического белка.

Результаты. Было выявлено, что пируват и оксалоацетат в низких концентрациях оказывают термостабилизирующее воздействие на конформацию лактатдегидрогеназы, влияние пирувата статистически более значимо в сравнении с оксалоацетатом ($p < 0,05$). Изучаемые лиганды в высоких концентрациях снижают термостабильность лактатдегидрогеназы.

Заключение. Понимание роли малых молекул в регуляции биологических и каталитических процессов долгое время оставалось в тени научного интереса, но сегодня работа в данном направлении выходит на качественно новый уровень. Полученные данные свидетельствуют о возможности малых молекул выступать в качестве лигандов при взаимодействии с каталитическими белками.

Ключевые слова: лактатдегидрогеназа, конформация, дифференциальная сканирующая флуориметрия, оксалоацетат, пируват.

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

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INTRODUCTION

Recently, more attention has been paid to small molecules, unique chemical "fingerprints" that can change gene expression and flows in metabolic pathways, leading to changes in specific processes in intra- and intercellular spaces [1]. Signal proteins, such as catalytic ones, are therefore an interesting area for the study of protein-ligand interactions, as they are highly sensitive to external stimuli such as endogenous metabolites or small molecules [2].

Molecules with a small molecular weight, located at the intersection of metabolic pathways for the exchange of proteins, fats, carbohydrates and serving as the "metabolic currency" of a living cell are such key bioenergetic intermediates as pyruvic and oxaloacetic acids.

Oxaloacetate (OA), a four-carbon keto acid with tautomerism phenomenon, is a valuable and quite rare molecule; its concentration in mitochondria does not exceed 10^{-6} M, and it is involved in many metabolic pathways, including gluconeogenesis, citric acid cycle, glyoxylate cycle, urea cycle and amino acid metabolism. Oxaloacetate is a critical component in ATP production and should be continuously regenerated to maintain the required level of oxidation processes in the citric acid cycle and the electron transfer chain [3].

Pyruvic acid (pyruvate) is one of the intermediate components of metabolism, the precursor of the most important intermediates of anabolic and catabolic pathways, including gluconeogenesis, lipogenesis *de novo*, cholesterol synthesis, as well as maintenance of the citric acid cycle. Pyruvate is formed as a result of anaerobic glycolysis in pyruvate kinase reaction, and can also be synthesized from various precursors: lactate in lactate dehydrogenase reaction, malate in cytosol malate dehydrogenase reaction, from alanine [4]. Serine, threonine, glycine, cysteine and tryptophan can also be converted to pyruvate [5].

Lactate dehydrogenase (EC 1.1.1.27), a tetrameter protein with catalytic activity, belongs to the oxidoreductase class. It catalyzes reversible transformation of pyruvate into lactate with NADH oxidation. The following genes are known to encode this enzyme: LDHA, LDHB, LDHC and LDHD [6]. Gene transcription of this enzyme is regulated by more than 20 different factors, including HIF1 protein. Recently, it was shown that LDH, along with enzymatic activity, also regulates the cell cycle; suppression of LDH accelerates cell transition into the G2 stage, while an increase in the LDH activity delays the cell in S-period [7]. LDH molecules are found in the cytoplasm and the nucleus, where they play the role of a transcription factor and influence DNA synthesis [8]. There are indications that lactate dehydrogenase has a direct effect on potassium ion channels of myocardium and liver cells in hypoxia [9].

The aim of our work is to reveal conformational changes of lactate dehydrogenase under the influence of different intermediates (pyruvate, oxaloacetate) concentrations in a temperature gradient with the subsequent building of a mathematical model.

MATERIALS AND METHODS

The experiments were performed at the Department of Molecular and Radiation Biophysics of National Research Center "Kurchatov Institute".

The following reagents were supplied from Sigma-Aldrich, USA: lactate dehydrogenase (EC 1.1.1.27, LDH, L-Lactic Dehydrogenase) from rabbit muscle, type XI, lyophilizate, 848 U/mg protein; pyruvate, oxaloacetate, Tris-HCl buffer 50 mM, pH 7.5. The enzyme and small molecules were diluted in tris-HCl buffer.

Differential Scanning Fluorimetry (DSF) was performed on Prometheus NT.48 (NanoTemper Technologies, Germany). This device allows the quick and accurate evaluation of protein folding, as well as its chemical and thermal stability. The method is based on changes of tryptophan and tyrosine endogenous fluorescence at wavelengths of 330 and 350 nm. The result is recorded in degrees Celsius, corresponding to the temperature of protein melting (*Tm*) [10]. This parameter is dependent on the forces of non-covalent intermolecular interactions: electrostatic, hydrophobic, Van der Waals forces, as well as the presence and number of hydrogen bonds.

Six dilutions were prepared, in which the final LDH concentration remained unchanged: 1 μM, and the final concentration of pyruvate and oxaloacetate varied: 16 μM, 8 μM, 4 μM, 2 μM, 1 μM and 0.5 μM respectively. We placed 10 μM of the solution in Prometheus NT.48 capillaries (nanoDSF grade). Scanning fluorimetry was performed at laser

intensity of 30%, heating diapason from 20 °C to 95 °C, step 1 °C/min.

Two approaches were used to study the dependence of the relative fluorescence on the protein melting temperature and the ligand concentration: the pre-installed software (Promethus NT.48 software), which allowed the determination of the protein melting point and the maximum melting rate (the first derivative), and the nonlinear regression model in the SPSS 21 statistical package (IBM SPSS Statistics, USA, license No 20130626-3). We used a four-parameter S-shaped curve:

$$f(t) = d + \frac{c - d}{1 + e^{-a(t-b)}},$$

where: *f(t)* is a dependent variable, fluorescence ratio at wavelengths 330 and 350 nm; *t* – independent variable, temperature, °C; *a*, *b*, *c*, *d* – equation parameters, or regression coefficients with the following substantial interpretation.

This form of analytic dependence refers to sigmoidal curves, recommended for the approximation of the growth phenomena with saturation [11], including for various medical and biological needs [12–14]. The choice of the given mathematical model for the present research was determined by a convenient substantial interpretation of its parameters: *a* – reflects the melting rate; *b* – corresponds to the theoretical inflection point and the temperature when the melting rate is maximum; *c* – asymptotically minimal absorption ratio of wavelengths; *d* – asymptotically maximum absorption ratio of wavelengths.

Adequacy assessment of the built models was carried out by a graph-analytical method on the analysis of the obtained and estimated values by the regression equation; regression residues were analyzed and their distribution was normal. The quality of the approximation was estimated by the statistical significance of the model, determination coefficients and standard regression errors. After building regression models (one mathematical model for each intermediate in different concentrations), analysis of their parameters was performed. According to the estimated statistical package 95% confidence intervals of the regression coefficients were assessed to be statistically significant between the LDH melting curves with different ligand concentrations and two ligands

RESULTS

The enzyme in solution is simultaneously present both in a folded and partially unfolded state ("molten globule"). At high temperature, proteins, including enzymes with ordered structure, unfold [15, 16], which leads to changes in the orientation of aromatic residues. These changes can be detected by means of differential scanning fluorimetry.

Thermostability of lactate dehydrogenase when adding oxaloacetate and pyruvate had the following features (Fig. 1, Fig. 2).

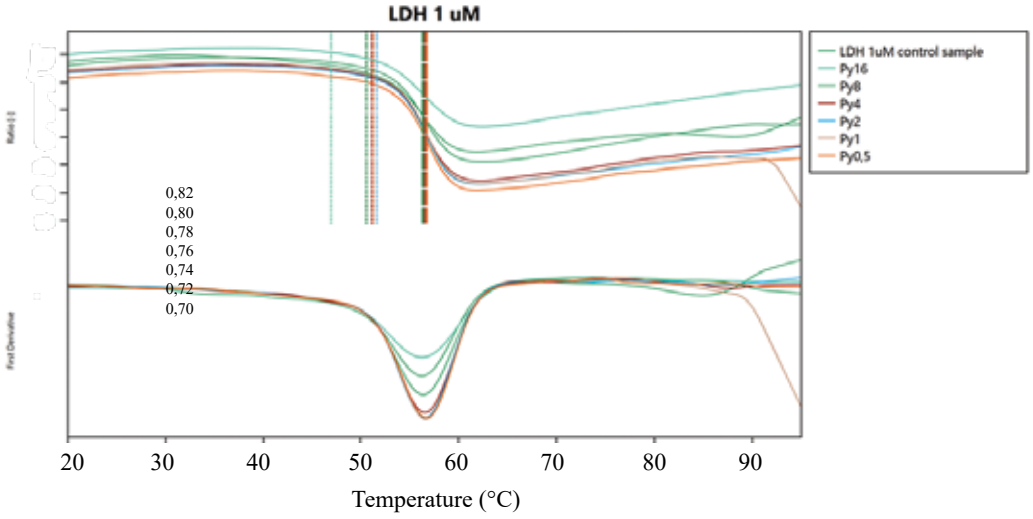

Fig. 1. Melting curves and the first derivate of lactate dehydrogenase (1 μM) in combination with oxaloacetate in different concentrations (upper chart). The concentration of oxaloacetate in the mixture was 16; 8; 4; 2; 1 and 0.5 μM, respectively. The fluorescence value (upper panel) at 350/330 nm (Y axis) is presented depending on temperature (X axis). The colored lines at the transition points correspond to the maximum peak *Tm* of the first derivate (bottom panel)

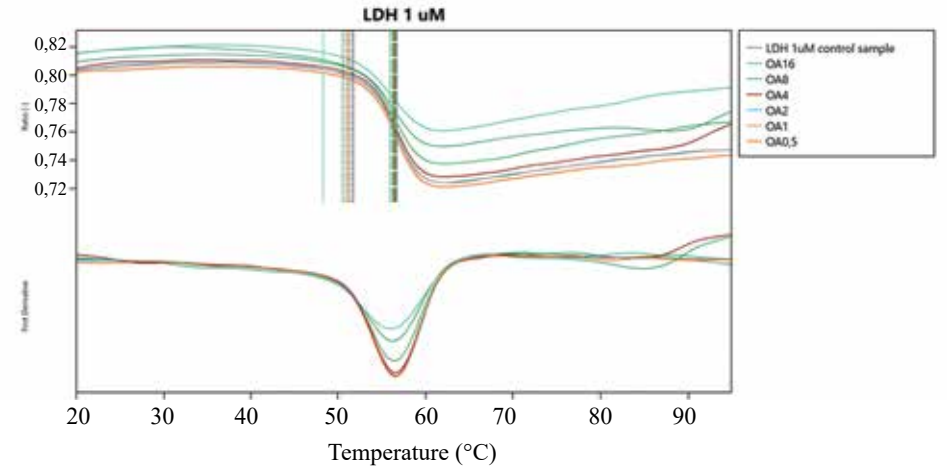

Fig. 2. Melting curves and the first derivate of lactate dehydrogenase (1 μM) in combination with pyruvate in different concentrations (upper chart). The concentration of oxaloacetate in the mixture was 16; 8; 4; 2; 1 and 0.5 μM, respectively. The fluorescence value (upper panel) at 350/330 nm (Y axis) is presented depending on temperature (X axis). The colored lines at the transition points correspond to the maximum peak *Tm* of the first derivate (bottom panel)

Increased concentrations of oxaloacetate and pyruvate (16 μM) promotes unfolding of the protein molecule, expressed in a decrease in the thermal stability: lower melting point (oxaloacetate – 48.3 °C, pyruvate – 47 °C compared to 50.5 °C for the reference sample), faster temperature inflection point (oxaloacetate – 56.0 °C, pyruvate – 56.2 °C compared to 56.3 °C for the reference sample). In contrast, the addition of oxaloacetate and pyruvate at the minimum concentration (0.5 μM) changes protein conformation to a more folded one, leading to an increase in the thermodynamic stability of lactate dehydrogenase. The starting melting point increased in comparison with the control sample and was 51.1 for oxaloacetate and 51.3 °C for pyruvate; there was a shift in the melting point of 56.6 °C for oxaloacetate, 56.7 °C for pyruvate. The total amplitude of the fluorescence signal during the unfolding process also changed significantly, indicating different conformational states of the complexes. We built a mathematical model for a more detailed characterization of catalytic protein condition in a temperature gradient when adding small molecules.

Before building a mathematical model that approximates the experimentally obtained points of the fluorescence ratio during protein heating, a visual analysis of initial scatterograms was carried out (Fig. 3). In contrast to the curves smoothed by the Prometheus NT.48 software shown earlier for oxaloacetate and pyruvate in Figures 1 and 2, it should be noted that fluorescence ratio spreads along the ordinate axis were higher before melting and after reaching the inflection point and decreased immediately when protein melting temperature was reached.

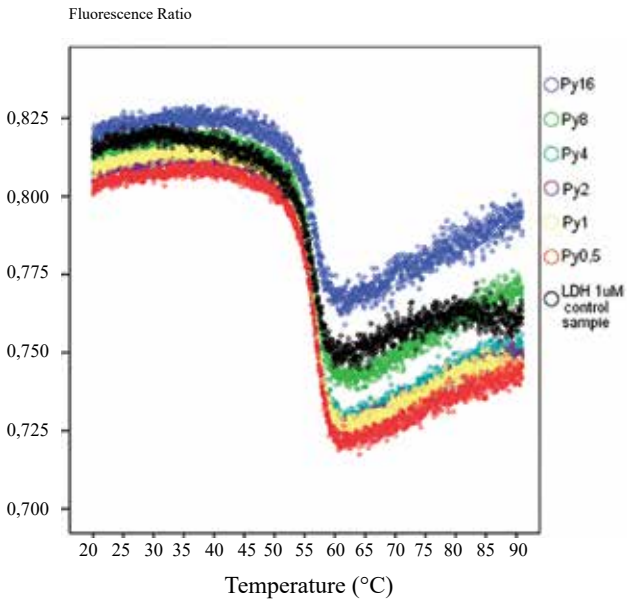

Fig. 3. Melting curves of lactate dehydrogenase (1 μM) in combination with pyruvate in different concentrations: non-smoothed fluorescence ratios

Temperature range from 48 to 63 °C was selected. Determination coefficients of the obtained models were in the range from 98.5% to 99.7%, and standard regression errors, respectively, from 0.0026 to 0.0018 (Table 1). The regression coefficients for different ligand concentrations are given in Table 2.

Table 1

Quality assessment of regression models approximating LDH melting curves in the presence of pyruvate and oxaloacetate in different concentrations, μM

Concentrations	Pyruvate		OA	
	Determination coefficient, R^2	Standard regression error	Determination coefficient, R^2	Standard regression error
16	0.985	0.0026	0.988	0.0025
8	0.995	0.0019	0.995	0.0021
4	0.996	0.0020	0.996	0.0021
2	0.997	0.0018	0.997	0.0018
1	0.997	0.0019	0.996	0.0020
0,5	0.996	0.0020	0.996	0.0019
0	0.992	0.0022	0.992	0.0022

Note: all built models are statistically significant at $p < 0.001$.

Table 2

Parameters of regression models approximating LDH melting curves in the presence of pyruvate and oxaloacetate in different concentrations, μM

Concentrations	Pyruvate				OA			
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*
16	0.724 (0.685–0.762)	56.2 (56.1–56.3)*	0.765 (0.765–0.766)*	0.819 (0.818–0.820)*	0.732 (0.697–0.767)	55.9 (55.8–56.0)	0.759 (0.758–0.759)	0.814 (0.814–0.815)
8	0.760 (0.737–0.783)	56.2 (56.2–56.3)	0.741 (0.740–0.741)*	0.811 (0.811–0.812)*	0.780 (0.756–0.804)	56.3 (56.2–56.3)	0.736 (0.735–0.736)	0.807 (0.807–0.808)
4	0.776 (0.755–0.797)	56.4 (56.4–56.4)	0.727 (0.726–0.727)	0.806 0.805–0.806)*	0.788 (0.768–0.809)	56.4 (56.3–56.4)	0.726 (0.726–0.727)	0.804 (0.803–0.804)
2	0.787 (0.768–0.806)	56.4 (56.4–56.4)	0.724 (0.724–0.725)*	0.805 (0.805–0.806)*	0.778 (0.759–0.797)	56.4 (56.4–56.4)	0.722 (0.722–0.723)	0.802 (0.802–0.803)
1	0.776 (0.757–0.796)	56.5 (56.4–56.5)*	0.724 (0.724–0.725)*	0.807 (0.806–0.807)*	0.767 (0.746–0.788)	56.3 (56.3–56.4)	0.722 (0.722–0.723)	0.802 (0.801–0.802)
0.5	0.761 (0.741–0.782)	56.5 (56.4–56.5)*	0.719 (0.719–0.720)	0.801 (0.801–0.801)	0.755 (0.735–0.775)	56.3 (56.3–56.4)	0.719 (0.719–0.720)	0.800 (0.799–0.800)
0	0.728 (0.698–0.757)	56.1 (56.0–56.1)	0.748 (0.747–0.748)	0.809 (0.808–0.809)	0.728 (0.698–0.757)	56.1 (56.0–56.1)	0.748 (0.747–0.748)	0.809 (0.808–0.809)

Note. The table shows regression coefficients and their 95% confidence intervals. Asterisks mark the statistically significantly different parameters between pyruvate and OA.

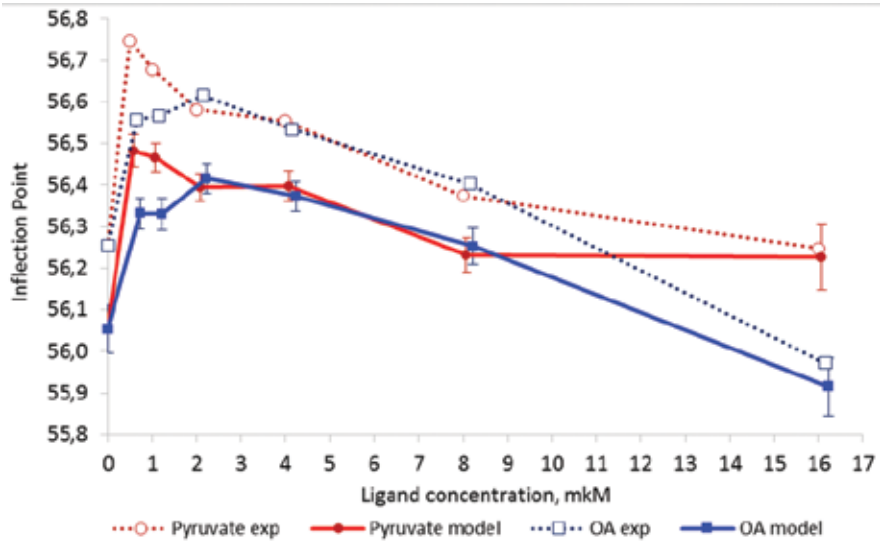

Fig. 4. Position of inflection points in the melting curves of lactate dehydrogenase in combination with pyruvate and oxaloacetate in different concentrations

For the convenience of comparison of the parameters between different ligand concentrations, we have drawn dependency plots.

The parameter *b*, numerically equal to the inflection point, is considered in Figure 4. The data obtained for the mathematical model, as well as data from Prometheus NT.48 Software, are presented. The nature and magnitude of the inflection point shift, estimated by different methods, are the same. Concentrations of ligands from 0.5 to 8 μM cause an increase in the melting point of the LDH, and a concentration of 16 μM leads to a decrease in the thermal stability of the protein. The maximum change of the melting temperature when adding pyruvate was noted at its concentration of 0.5 μM and in the case of oxaloacetate it was at a concentration of 2 μM. Shift estimates obtained by modeling the nonlinear regression and the Prometheus NT.48 software differ slightly: the discrepancy with the data obtained by the software is 0.2 °C.

When comparing the influence of two ligands on the melting point shift, it was found that the influence of oxaloacetate and pyruvate differs statistically significantly in the area of low concentrations (0.5 and 1 μM) and high concentrations (16 μM). Pyruvate at low concentrations causes a statistically significant ($p < 0.05$) increase in the melting point than oxaloacetate, while at high concentrations it causes a greater reduction in the inflection temperature. In concentrations of 2–8 μM the ligands' effect on the melting point shift according to the regression model built was indistinguishable.

To analyze the maximum melting rate of LDH in the presence of ligands (Fig. 5), the first derivatives of the inflection points were obtained from the instrument software report.

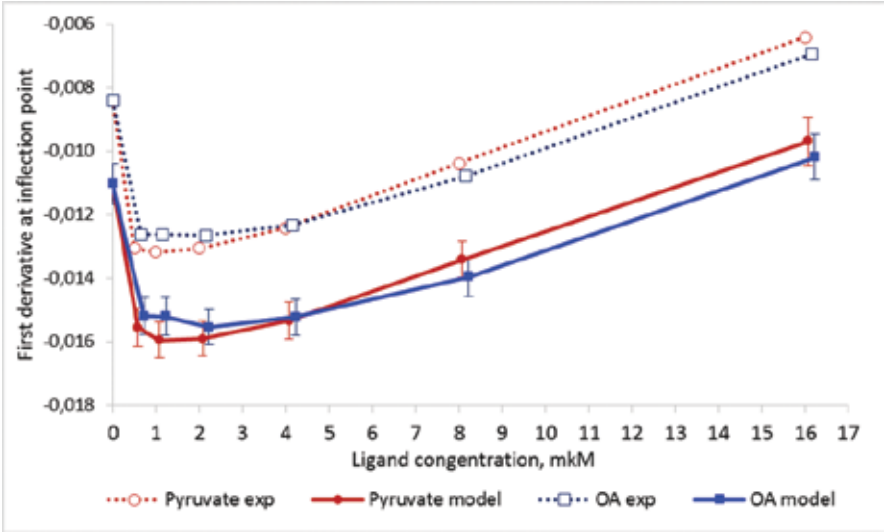

Fig. 5. Maximum melting rates of lactate dehydrogenase in combination with pyruvate and oxaloacetate in different concentrations

First derivatives of the temperature of reaction mixture $t = b$ were calculated from the parameters of the built mathematical models by the formula $f'(t) = -0.25a(d–c)$. The errors of the received results are estimated by the rules of errors calculation in arithmetic operations. Negative values of the derivatives reflect a decrease in the fluorescence ratio during protein unfolding. The higher the absolute value of the first derivative, the higher the rate of protein melting.

Both studied ligands caused a significant increase in the melting rate of LDH in concentrations of 0.5–4 μM ($p < 0.05$ compared to no ligands) and its subsequent decrease in concentrations of 8–16 μM. The effect of different concentrations of pyruvate and oxaloacetate on the maximum melting rate was the same: no statistically significant differences between the parameters *a*, or between the first derivatives at the inflection point for pyruvate and oxaloacetate were found ($p > 0.05$).

DISCUSSION

When comparing the maximum melting velocities obtained by different methods (according to software and mathematical modeling), a systematic difference of 0.003 on average was revealed. We

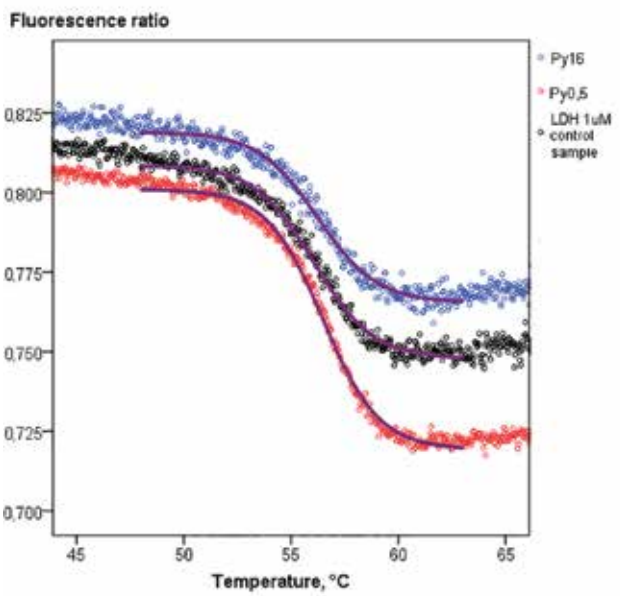

Fig. 6. Empirical and theoretical melting curves of lactate dehydrogenase without pyruvate in combination with pyruvate in concentrations of 16 and 0.5 μM.

assume that this is due to the fact that the proposed form of the analytical dependence is well consistent with the empirical data only in the area with the maximum melting rate, but not before and after it. It is because of this fact that when constructing the regression model, a rather narrow temperature range was chosen at which melting occurs. Figure 6 shows the fluorescence ratios observed in the experiment and the theoretically calculated points for pyruvate concentrations of 0.5 and 16 μM according to the models constructed. The figure shows that the beginning of the theoretical curves has more divergence with the experimentally observed points than in the LDH melting section. Therefore, insufficient estimations of the parameter *d* of the developed regressions lead to an insignificant shift in the estimation of the maximum melting rate. However, from the point of view of this study these corrections have no statistically significant effect.

CONCLUSION

When studying the conformational state of lactate dehydrogenase by differential scanning fluorimetry with the subsequent building of a mathematical model, it was found that pyruvate and oxaloacetate in low concentrations (0.5–2 μM) have a thermostabilizing effect on the LDH structure, and at high concentrations (16 μM), on the contrary, they reduce the thermal stability of LDH. Despite the co-directed effects of the studied metabolites, the effect of pyruvate was more pronounced in comparison with oxaloacetate ($p < 0.05$).

The ability of intermediates to take part in parametabolic interactions and, in particular, to bind and influence the conformation of catalytic proteins, determines a wide range of their biological action, the mechanisms of which have yet to be studied in more detail. Of undoubted interest is the further study of pyruvate and oxaloacetate as molecules of protectors and stabilizers, an increase of the studied concentrations and protein partners, which is especially promising in view of bioenergetical and "mitochondrial" medicine concepts.

These are the unknown pages of a known enzyme.

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The study of platelet reaction on a-C:H:SiO_x coatings obtained via plasma enhanced chemical vapor deposition with bipolar bias voltage

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ABSTRACT

Aim. To study platelet adhesion to a-C:H:SiO_x film on titanium in an *in vitro* experiment to evaluate its antithrombogenic potential.

Materials and methods. Thin (less than 1 μm) a-C:H:SiO_x films were deposited on VT-6 titanium plates with a size of 10 × 10 mm^2 and a thickness of 0.2 mm using a vacuum ion-plasma unit using pulsed bipolar bias. The surface roughness was evaluated according to GOST 2789-73 using an atomic force microscope. The test samples were cultured at 37 °C for 30 min in platelet-rich human blood plasma, prepared for scanning electron microscopy, after which the distribution density of blood plates adhering to the test coating was calculated.

Results. With the same roughness index of the studied a-C:H:SiO_x samples, the film decreased 116 times (in comparison with untreated titanium) the platelet count per 1 mm^2 of the surface.

Conclusion. The deposition of a-C:H:SiO_x thin film on the surface of VT-6 titanium alloy by PACVD method using pulsed bipolar bias significantly reduces the distribution density of platelets in comparison with an untreated metal surface. *In vitro* data suggest a significant antithrombogenic potential of this type of coating on the surface of devices in contact with blood.

Key words: human platelet adhesion, *in vitro*, carbonic surface modified by silicon oxides, scanning electron microscopy, atomic force microscopy.

Conflict of interest. Authors declare no actual or potential conflict of interest related to publication of this article.

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Conformity with the principles of ethics. The work was carried out in accordance with the principles of the Helsinki Declaration upon receipt of voluntary informed consent for blood sampling.

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Исследование реакции тромбоцитов на a-C:H:SiO$_x$ покрытие, полученное методом плазмохимического осаждения с использованием импульсного биполярного смещения

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РЕЗЮМЕ

Цель. Изучить в эксперименте *in vitro* адгезию тромбоцитов к a-C:H:SiO$_x$ пленке на титане для оценки ее атромбогенного потенциала.

Материалы и методы. Тонкие (менее 1 мкм) a-C:H:SiO$_x$ пленки наносили на титановые пластины марки ВТ-6 размером 10 × 10 мм^2 и толщиной 0,2 мм с помощью вакуумной ионно-плазменной установки с использованием импульсного биполярного смещения. Шероховатость поверхности оценивали согласно ГОСТ 2789-73 с помощью атомно-силового микроскопа. Исследуемые образцы культивировали при 37 °С в течение 30 мин в плазме крови человека, обогащенной тромбоцитами, подготавливали для сканирующей электронной микроскопии, после чего подсчитывали плотность распределения кровяных пластинок, адгезирующих к исследуемому покрытию.

Результаты. При одинаковом индексе шероховатости исследуемых образцов a-C:H:SiO$_x$ пленка в 116 раз снижала (в сравнении с необработанным титаном) количество тромбоцитов на 1 мм^2 поверхности.

Заключение. Формирование на поверхности титанового сплава ВТ-6 тонкой пленки состава a-C:H:SiO$_x$ методом плазмохимического осаждения с использованием импульсного биполярного смещения значительно снижает плотность распределения тромбоцитов в сравнении с необработанной металлической поверхностью. Полученные *in vitro* данные предполагают существенный атромбогенный потенциал данного вида покрытий на поверхности устройств, контактирующих с кровью.

Ключевые слова: адгезия тромбоцитов человека, *in vitro*, углеродная поверхность, модифицированная оксидами кремния, сканирующая электронная микроскопия, атомно-силовая микроскопия.

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

Источник финансирования. Работа выполнена при финансовой поддержке Российского научного фонда, проект № 19-19-00186.

Соответствие принципам этики. Работа выполнена в соответствии с принципами Хельсинкской декларации при получении добровольного информированного согласия на забор крови.

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INTRODUCTION

The interaction of implants with the biological environment of the body largely depends on their surface properties, which play a direct role in various post-implantation biological reactions, including the precipitation of various minerals, protein adsorption, cell adhesion and proliferation [1, 2]. In the application to devices (mechanical pumps) and stents for surgical treatment of coronary heart disease, redundancy of inflammatory cell-molecular reactions at the artificial surface/tissue interface increases the risk of thrombus. In this regard, there is renewed interest in surface modification methods for biocompatible artificial materials contributing to their bioinertness [3].

One widely discussed solution to the problem of deterministic biocompatibility in the bioinertness/bioactivity range is the application of thin diamond-like carbon coatings (diamond-like coating, DLC). Since the beginning of the 2000s, it has been shown that DLC films are bioinert, resistant to mechanical stress and corrosion, and not cytotoxic to monocytes/macrophages, fibroblasts, and osteoblasts [4]. Owing to the optimal ratio of sp^3/sp^2 hybridized carbon atoms, they have good hemocompatibility [5, 6]. In the last 5 years, due to some dissatisfaction with the results of biomedical testing of DLC coatings, publications have been accumulating on their physicochemical modification (in particular, silicon and its oxides), which improves the consumer properties of a-C:H:SiO_x surfaces on medical materials and products [7].

On the basis of the Institute of High Current Electronics of the Siberian Branch of the Russian Academy of Sciences (IHCE SB RAS), a new approach has been developed for plasma enhanced chemical vapor deposition of a-C:H:SiO_x films on the internal surfaces and moving parts of auxiliary circulatory devices, based on the use of pulsed bipolar substrate bias.

The aim of the work was to study in an *in vitro* experiment the adhesion of platelets to a-C:H:SiO_x film on titanium to evaluate its antithrombogenic potential.

MATERIALS AND METHODS

VT-6 titanium plates with a size of 10 × 10 mm and a thickness of 0.2 mm coated with a thin a-C:H:SiO_x film (less than 1 μm thick) were used for the research (five «T2» samples). Titanium samples without a-C:H:SiO_x coating were used as control samples (five «T1» samples). The film was deposited on a vacuum ion-plasma installation with the technological deposition parameters described in Grenadyorov [8].

The standard deviation of the R_q profile according to GOST 25142-82 was determined according to GOST 2789-73 using an atomic force microscope (AFM) Solver P47 (NT-MDT, Russia) with an area of 5 square micrometers.

To perform the platelet adhesion test from the blood of a healthy adult male donor (intended for blood transfusion), 50 ml of platelet-rich plasma was obtained by centrifugation and separation of blood cells [9, 10].

The resulting plasma was diluted with 0.9% sodium chloride in a ratio of 1:1. The tested samples were immersed in the platelet suspension and incubated at 37 °C for 30 minutes. Then, the samples were washed with distilled water to remove nonadherent cells. The platelets remained on the surface were fixed with a 2% glutaraldehyde solution at room temperature for 1 hour and dried at 37 °C.

The samples were coated with a 20 nm thick chromium layer in an argon atmosphere at an ion current of 6 mA and a pressure of 0.1 mm Hg using the Q150T ES setup (Quorum Technologies, UK) and were subjected to scanning electron microscopy using a Mira3 microscope (Tescan, Czech Republic). On each sample, the number of adherent platelets in 20 random fields of view was calculated according to the principles of morphometry [11].

Statistical processing was performed using the software Statistica10.0 software (StatSoft, USA). The normality of the distribution was checked using the Shapiro-Wilk criterion with subsequent assessment of the equality of variances according to the Leven criterion. In the case when the distribution in the experimental groups was normal, and the intergroup equality of variances was observed, further processing was carried out using the method of parametric statistics, the Newman-Cales test. For a distribution other than normal and non-compliance of the intergroup equality of variances, the methods of nonparametric statistics

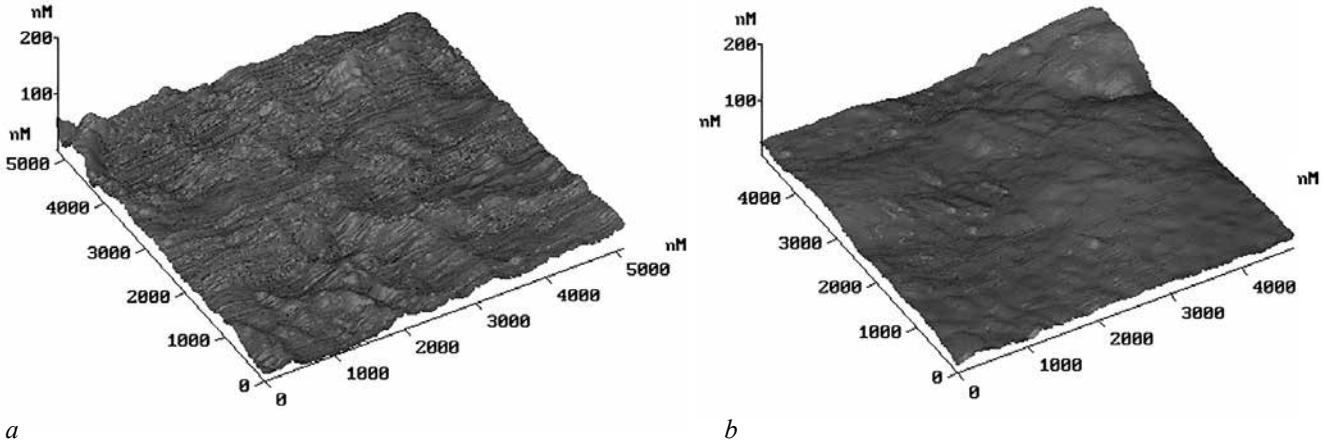
were used via the Kruskal-Wallis test. Results are presented as mean (M) and standard error of the mean (m). Differences between groups were considered significant at $p < 0.05$.

RESULTS

The results of atomic force microscopy showed a certain smoothing of the surface roughness of the VT-6 titanium alloy after the formation of the a-C:H:SiO$_x$ film (Fig. 1). However, differences in the roughness index R_q did not reach statistical differences (Table 1). The data obtained correspond to the results published previously [12].

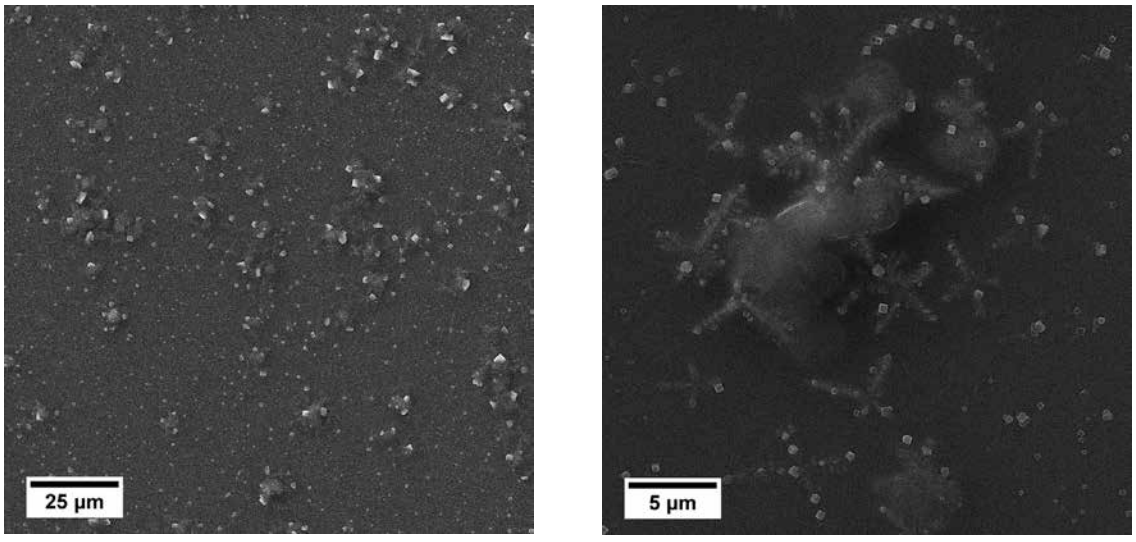
In the biological part of the study, the use of whole blood plasma enriched with platelets led to the formation of their microconglomerates and crystallization of dissolved salts on the surface of the samples (Fig. 2), which made it difficult to count the number of individual cells. Dilution of plasma with an isotonic solution of sodium chloride in a ratio of 1 : 1, followed by washing the samples with a solution of distilled water, allowed us to obtain images available for morphometric analysis (Fig. 3).

Platelet counts showed that a-C:H:SiO$_x$ coating on a titanium substrate dramatically reduced their surface adhesion (Table 1).



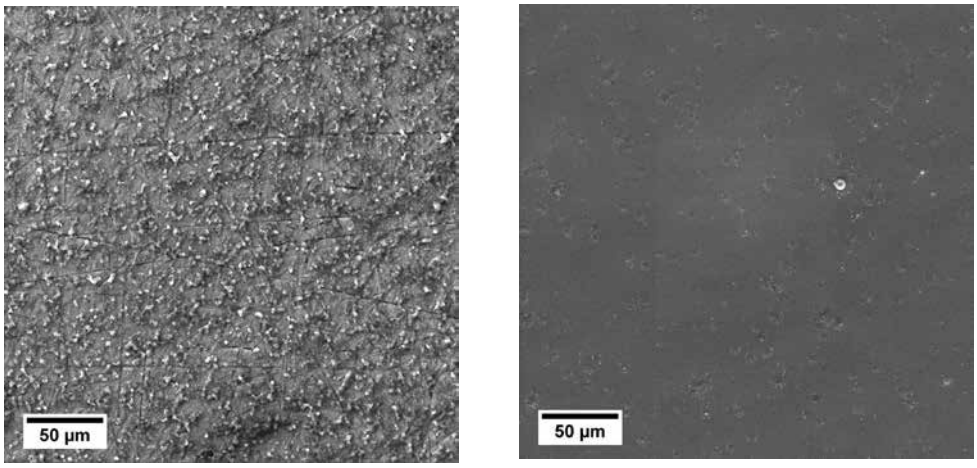
a *b*

Fig. 1. AFM images of the surface morphology of titanium (*a*) and titanium coated with a-C:H:SiOx film (*b*)



a *b*

Fig. 2. SEM image of platelet conglomerates and salt crystals on the surface of a VT-6 titanium sample. Scale bar 25 and 5 μm



a *b*

Fig. 3. SEM images of the surface of a titanium sample T1 (*a*) and T2 with deposited a-C:H:SiOx film (*b*) after a platelet adhesion test. Scale bar 50 μm

Table 1

The content of adherent platelets in the studied samples according to scanning electron microscopy, *M ± m*

Group marking	Group name, $n = 5$	The average number of cells per surface area 250 μm^2	The surface roughness index of the samples, R^q, nm
T1	VT-6 titanium without film deposition	290 ± 72 $n_1 = 20$	7.9 ± 0.8
T2	VT-6 titanium with a-C:H:SiO$_x$ film deposition	2.5 ± 1.8 $n_1 = 20$ <0.001	7.2 ± 0.7

Note: n is the number of samples studied in each group; n_1 is the number of studied fields of view on each sample.

In the T2 group (coated titanium), the number of blood platelets was 116 times less than that on the surface of the samples from the T1 group (without plasma-chemical treatment). It should be emphasized that there are no statistically significant differences in the roughness index of the studied surfaces (Table 1), since the relief of implants and other medical devices has significant biological significance.

DISCUSSION

Studies of DLC coatings modified with silicon and its oxides have focused mainly on the study of their physicomechanical properties [13]. Thus, the formation of Si–C bonds significantly increases the adhesion of the coating to substrates while maintaining the high tribological characteristics of DLC films [12]. At the same time, the physicochemical processes of improving the hemocompatibility of materials due to both DLC [14] and Si-DLC films remain in the hypothesis area. Due to the identical and insignificant roughness of the studied samples (Table 1), required for products in contact with blood, from the whole variety of biologically active physicochemical factors (surface energy, phase and elemental composition, solubility, presence of biologically active (medicinal) molecules in the composition of the surface) [15] the charge (zeta (ξ) potential) of the surface can come to the fore in determining hemocompatibility. Sawyer et al. [16] suggested that the anticoagulant properties of implantable materials can give an electrostatic charge to their surface. Ikada et al. [17] hypothesized that in biological fluids there is a relationship between the surface ξ potential and the anticoagulant properties of the surface of medical devices. Indeed, the introduction of silicon into the composition of thin films significantly changes their electrical and biological characteristics [18].

In this regard, the *in vitro* established antithrombogenicity of a-C:H:SiO$_x$ film on titanium is a valuable consumer property for devices and products contacted with blood, and requires further study of the electrokinetic and other physicochemical characteristics of its biological inertness.

CONCLUSION

The formation on the surface of the VT-6 titanium alloy of a thin a-C:H:SiO$_x$ film obtained by plasma-chemical deposition using pulsed bipolar displacement of the substrate reduces the distribution density of human platelets by more than 100 times in comparison with an untreated metal surface. *In vitro* data suggest a significant antithrombogenic potential of a-C:H:SiO$_x$ coatings on the surface of blood contact devices.

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Grenadyorov A.S. – deposition of coatings, carrying out of the research, drafting of the article.
Korneev D.S. – carrying out of the research, processing of the obtained data.
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Aberrations of the number of copies (CNA) in the genome of luminal B breast tumor

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ABSTRACT

Aim. To describe the CNA (Copy Number Aberration) landscape of luminal B breast tumor before treatment.

Materials and methods. The study included 100 patients with breast cancer (BC) of luminal B subtype for which a biopsy of the tumor material was performed prior to neoadjuvant chemotherapy (NAC). The tumor DNA was examined using a CytoScan HD Array microarray (Affymetrix, USA). The obtained microarray data were correlated with NAC efficacy.

Results. The study showed that loci 1q32.1–32.3, 1q41–42.2, and 8q24.21 had the highest frequency of amplifications (in more than 65% of patients). The highest deletion frequency (in more than 60% of patients) was found in loci 16q21, 16q22.1, 16q23.1–24.1, 17p13.1, and 17p12. Trisomy was most often observed in chromosomes 7, 8, 12, and 17, and monosomy in chromosomes 3, 4, 9, 11, 18, and X-chromosomes. The CNA landscape of luminal B subtype breast tumors is different from triple-negative breast cancer. The largest difference in the frequency of amplifications between patients with an objective response to NAC and patients with no response to NAC was shown in 1q24.2–42.2 loci (46%), and the largest difference in the frequency of deletions (more than 30%) between groups was in regions 6q16. 3, 11p15.4, 11q23.1, and 16q22.2–22.3. These loci can be considered potential predictive markers.

Conclusion. The research determined loci with the highest amplification and deletion frequencies for luminal B breast cancer. Potential predictive markers for the given molecular subtype were identified.

Key words: breast cancer, microarray analysis, deletions, amplifications, neoadjuvant chemotherapy.

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. The study was carried out in compliance with the ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association Ethical Principles for Conducting Scientific Medical Research with Human Participation as amended in 2000 and the Rules of Clinical *Practice in the Russian Federation*, approved by the Order of the Ministry of Health of the Russian Federation of 19.06.2003, No. 266. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Cancer Research Institute, Tomsk National Research Medical Center.

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Аберрации числа копий в геноме опухоли молочной железы люминального подтипа B

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РЕЗЮМЕ

Цель. Описание ландшафта Copy Number Aberration (CNA) опухоли молочной железы люминального подтипа B до лечения.

Материалы и методы. В исследование включены 100 больных раком молочной железы (РМЖ) люминального подтипа B, для которых проведен забор биопсийного материала опухоли до проведения неоадъювантной химиотерапии (НХТ). ДНК из опухоли исследована при помощи микроматрицы CytoScan HD Array (Affymetrix, США). Полученные микроматричные данные соотнесены с эффективностью НХТ.

Результаты. Показано, что наибольшая частота амплификаций (более чем у 65% больных) наблюдается в следующих локусах: 1q32.1-32.3, 1q41-42.2, 8q24.21. Наибольшая частота делеций (более чем у 60% больных) была обнаружена в локусах 16q21, 16q22.1, 16q23.1-24.1, 17p13.1, 17p12. Трисомия чаще всего наблюдалась в 7-, 8-, 12- и 17-й хромосомах, моносомия – в 3-, 4-, 9-, 11-, 18-й и X-хромосомах. Ландшафт CNA опухоли молочной железы люминального подтипа B отличается от трижды негативного РМЖ. Наибольшая разница частоты встречаемости амплификаций между больными с объективным ответом на НХТ и больными с отсутствием ответа на НХТ показана в 1q24.2-42.2 локусах (46%), а наибольшая разница частоты встречаемости делеций (более 30%) – между группами в регионах 6q16.3, 11p15.4, 11q23.1, 16q22.2-22.3. Данные локусы могут быть рассмотрены в качестве потенциальных предиктивных маркеров.

Заключение. Установлены локусы с наибольшей частотой амплификаций и делеций для рака молочной железы люминального подтипа B. Идентифицированы потенциальные предиктивные маркеры для данного молекулярного подтипа.

Ключевые слова: рак молочной железы, микроматричный анализ, делеции, амплификации, неоадъювантная химиотерапия.

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

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INTRODUCTION

Thanks to the Cancer Genome Atlas Program (TCGA), it became clear that tumors of many localizations contain not only point mutations of oncogenes and tumor suppressor genes but also a large number of various chromosomal abnormalities that play a key role in carcinogenesis and tumor progression [1]. The most common chromosomal abnormalities are found in solid tumors [2]. Deletions or amplifications of chromosomal regions and individual chromosomes are referred to as aberrations of the number of DNA copies or CNA (Copy Number Aberration). These types of cytogenetic disorders can affect gene expression; as a rule, in deletions, the expression of genes localized in the deleted region is reduced, while in amplifications it is increased [3]. Breast cancer (BC) is no

exception. Despite the fact that breast tumors have a high degree of intra-tumoral heterogeneity, the most common chromosome aberrations have been identified for them. In breast cancer (according to TCGA data), CNA in 1q, 8q, 8p, 11q, 13q, 16q, 17q and 20q regions have a high frequency of occurrence [4–6].

To date, the detailed elaboration of changes in tumors has begun and CNA genetic characteristics of specific tumor localizations and related patterns of gene expression are being described. In fact, CNA tumor-specific landscapes with large changes in the number of genomic copies lead to global deregulation of tumor cell transcriptomes. In addition, the molecular characterization of cytogenetic abnormalities has made it possible to gain insight into the mechanisms of oncogenesis and in some cases has led to the clinical implementation of effective diagnostic and prognostic tools, as well as treatment strategies aimed at a specific genetic anomaly.

The study by J.Y. Goh et al. determined that amplification of 1q21.3 chromosome is a new biomarker and an effective target for breast cancer. This amplification is present in 10–30% of primary tumors, and in more than 70% of recurrent tumors, regardless of breast cancer subtype. The molecular mechanism by which 1q21.3 amplification is associated with a relapse of breast cancer, including the functional relationship between S100A7/8/9 and IRAK1, was investigated. Using ddPCR, the authors developed a molecular analysis based on a blood test to detect 1q21.3 amplification in extracellular DNA and showed that this amplification can serve as a circulating biomarker to predict early relapse and monitor the breast tumor response to chemotherapy [7].

Currently, there are studies of the CNA association and clinical and morphological parameters of the tumor for individual subtypes of breast cancer, in particular, for triple negative breast cancer. According to these studies, 10p and 12q chromosomal regions show the highest amplification frequency, which corresponds to an increase in the number of copies of the GATA3 and MDM2 genes. Amplifications in chromosomes 1q (MDM4), 3q (PIK-3CA), 6p (CCND3), 8q (MYC) and 18 (BCL2 and SMAD4) are less frequent, and frequent deletions include chromosomes 4p (FGFR3), 5q (PIK3R1), 8p (DBC2), 9p (NR4A3), 12 (MDM2) and 22 (CHEK2) [8].

However, most studies do not determine the CNA of the entire genome, investigating only the key genes involved in tumor pathogenesis. No full-genome CNA landscape description is provided for luminal B subtype of breast cancer.

The study aims to describe the CNA landscape of luminal B breast tumor before treatment.

MATERIALS AND METHODS

In the course of this study, a bank of biological material was collected from 100 patients with a morphologically verified diagnosis of luminal breast cancer (11 of them are luminal B HER2+) and a detailed register of clinical and morphological data was compiled (average age 46.2 ± 0.4 years) (Table 1). Biopsy material was collected from each patient before treatment with the help of ultrasound-guided pistol biopsy. DNA was isolated from samples using the QIAamp DNA miniKit kit (Qiagen, Germany) in accordance with the manufacturer's instructions.

Table 1

Clinical and morphological parameters of the examined patients with breast cancer

Clinical and morphological parameters		Number of patients (%)
Age, years	≤45	41 (41%)
	>45	59 (59%)
Menstrual status	Premenopause	58 (54%)
	Postmenopause	42 (42%)
Histological type	Invasive ductal carcinoma	85 (85%)
	Invasive lobular carcinoma	8 (8%)
	Medullary carcinoma	1 (1%)
	Other types	7 (7%)
Tumor size	T_1	13 (13%)
	T_2	78 (78%)
	T_3	4 (4%)
	T_4	5 (5%)

Table 1 (continued)

Clinical and morphological parameters		Number of patients (%)
Lymph node metastasis	N_0	45 (45%)
	N_1	43 (43%)
	N_2	4 (4%)
	N_3	8 (8%)
Molecular subtype	Luminal B	100 (100%)
Epidermal growth factor receptors HER2	0/+	89 (89%)
	++/+++	11 (11%)
Histological form	Unicentric	70 (70%)
	Multicentric	30 (30%)
NAC regimen	CAX	19 (19%)
	FAC/AC	31 (31%)
	Taxotere	20 (20%)
	AT/ACT	9 (9%)
	CP	12 (12%)
	Not carried out	9 (9%)
Response to NAC	Progression	4 (4%)
	Stabilization	22 (22%)
	Partial regression	53 (53%)
	Complete regression	12 (12%)
	Not carried out	9 (9%)

Note. NAC – neoadjuvant chemotherapy; CAX – cyclophosphamide, adriamycin, xeloda; FAC – 5-fluorouracil, adriamycin, cyclophosphamide; CP – cyclophosphamide, cisplatin; AT – adriamycin, docetaxel; AC – adriamycin, cyclophosphamide; ACT – adriamycin, cyclophosphamide, docetaxel.

To study CNA in tumor cells, a high density microarray CytoScan HD Array (Affymetrix, USA) was used, which allows a full-genome format to evaluate DNA deletions and amplifications in all tumor cells at the same time and quantitatively analyze the representation of mutation (or a clone carrying mutation) against normal DNA.

The effectiveness of pre-surgery chemotherapy was evaluated according to the criteria of the WHO and the International Union Against Cancer with the help of ultrasound examination and / or mammography. Full regression (100% decrease in tumor), partial regression (decrease in tumor volume by more than 50%), stabilization (decrease in tumor volume by less than 50% or increase by no more than 25%) and progression (increase in tumor volume by more than 25%) were recorded. According to international recommendations, during pre-surgery chemotherapy breast cancer patients with stabilization or progression constituted a group with no response to NAC, and patients with partial and complete regression formed a group with an objective response. The program "Chromosome Analysis Suite 4.0" (Affymetrix, USA) was used to process the results of microchipping (bioinformatic analysis).

RESULTS

The first stage saw the analysis of CNA occurrence frequency carried out for all 862 cytobands for each patient included in the study. The amplification and deletion frequencies are shown in Figure 1 and in the appendix in the form of tabular data. Table 2 presents data on the genomic regions with a high incidence of CNA and their absence in the group of breast cancer patients.

In most cases, amplifications and deletions were absent in the pericentromeric regions of chromosomes 13, 14, 15, 21, and 22. Moreover, the study showed that the absence of amplifications in loci 10q23.32–24.33, 11q23.1–23.2, and 13q14.11–14.3 was accompanied by the presence of deletions in more than 30% of patients, and, conversely, the absence of deletions in loci 8q12.1 and 8q24.11–24.21 was accompanied by the presence of amplifications in more than 40% of patients (Fig. 1, appendix). Numerical chromosomal abnormalities were calculated. Trisomy was most often observed in chromosomes 7, 8, 12, and 17; monosomy was most often observed in chromosomes 3, 4, 9, 11, 18 and X chromosomes.

Next, we studied the association of the response to NAC with the CNA occurrence frequency. Before treatment patients were divided into two groups: group

1 included patients with stabilization and progression of the tumor process after NAC (n = 26), while group 2 consisted of patients with partial and complete tumor regression after treatment (n = 65).

Table 2

Data on the genomic regions with a high incidence of CNA and their absence in the group of breast cancer patients

Parameter	Locus
Frequency of amplifications >65%	1q32.1-32.3, 1q41–42.2, 8q24.21
Frequency of deletions >60%	16q21, 16q22.1, 16q23.1–24.1, 17p13.1, 17p12
Absence of amplifications	10q23.32–24.33, 11q23.1–23.2, 13p12–11.1, 13q14.11–14.3, 14p13–11.1, 14q11.1, 15p13–11.1, 15q11.1, 21p13–11.1, 21q11.1, 22p13–11.1
Absence of deletions	8q12.1, 8q24.11–24.21, 13p13–11.1, 14p12–11.1, 14q11.1, 15p12–11.1, 15q11.1, 21p13–11.1, 21q11.1, 22p13–11.2
Absence of amplifications and deletions	13p13–11.1, 14p12–11.1, 15p12–11.1, 21p13–11.1, 21q11.1, 22p13–11.2

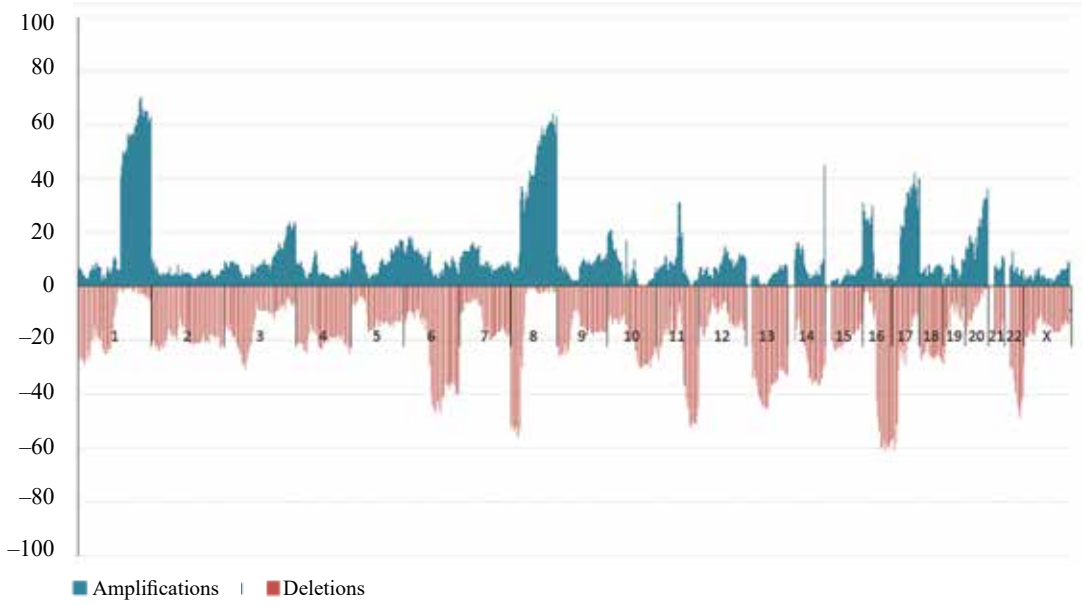

Fig. 1. CNA frequency in a luminal B molecular subtype breast tumor

The highest frequency of amplifications (over 60%) in the group of patients with stabilization and progression after NAC was found only in loci 8q23.1–24.3. It is interesting to note that in the presence of more than 60% of amplifications in regions 8q23.1–24.3, there was a complete absence of deleted sites in these loci. The maximum deletion rate (more than 50%) in group 1 was observed in loci 8p23.3, 16q21, 16q23.1–24.2, and 17p13.3–11.2. At the same time, locus 8p23.3 with the highest deletion frequency demonstrated the absence of amplifications. A general picture of the incidence of CNA in patients with stabilization and progression of the tumor process is presented in Figure 2.

For the 2nd group of patients, the highest amplification frequency (84%) was found in locus 1q32.2. The amplification frequency of more than 60% was found in the long arm of chromosome 1, loci 1q23.2–25.3 and 1q31.1–44, and the long arm of chromosome 8, 8q22.1–24.3. At the maximum frequency of amplifications in these regions, deletions were practically absent. The maximum deletion rate (72%) was observed in locus 17p13.1. A deletion rate of more than 50% was found in a large number of loci: 6q14.1–16.3, 6q21–22.1, 8p23.3–21.1, 11q21–25, 13q14.11–14.3, 13q21.1, 16q11.2–13, 16q21–24.3, 17p13.3–11.2, and 22q12.3–13.2. A general picture of the CNA incidence in patients of group 2 is also presented in Figure 2.

In the joint analysis of the two groups, cytobands were found in which the difference in the frequency of occurrence of chromosomal abnormalities in the groups with the presence and absence of an objective response to NAC reached a maximum value of 30% or more. The largest difference in the frequency of occurrence of amplifications between groups is shown

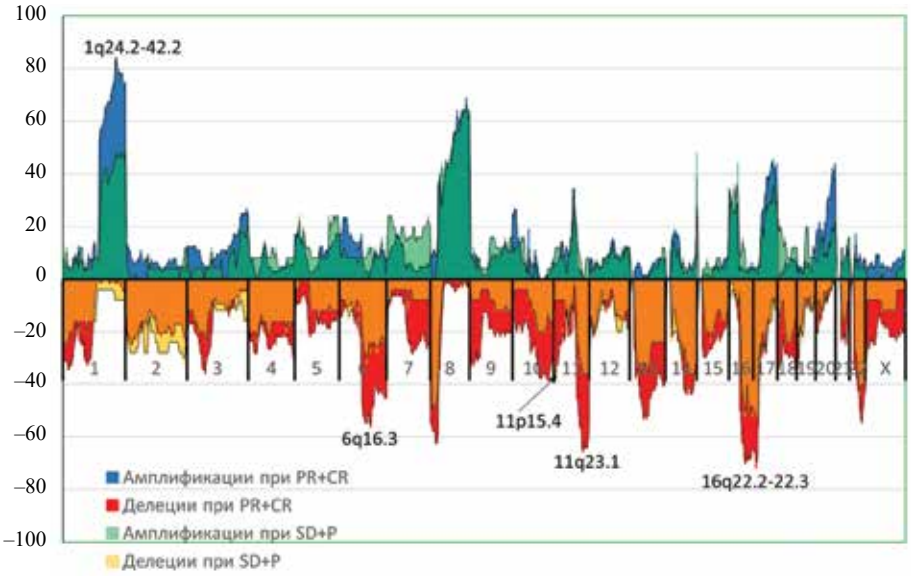

Fig. 2. The ratio of amplification and deletion frequencies in the tumor before treatment, depending on the effect of neoadjuvant chemotherapy: loci with the largest difference in amplification and deletion frequencies are signed. SD + P – stabilization + progression; PR + CR – partial regression + complete regression

in loci 1q24.2–42.2 (46%), and the largest difference in the frequency of occurrence of deletions (more than 30%) between groups is in regions 6q16.3, 11p15.4, 11q23.1, 16q22.2–22.3 (Fig. 2). These loci have potential predictive significance for luminal breast cancer, which must be validated in prospective studies.

DISCUSSION

Given the fact that breast cancer is a genetically heterogeneous disease, it is now necessary to conduct studies to identify the spectrum of molecular and genetic features of this tumor in order to develop new approaches to the treatment of breast cancer patients. Therefore, features of the genetic landscape of the breast tumor need to be described in detail, with division into molecular subtypes and based on the main clinical indicators.

Currently, data on the frequency analysis of chromosomal aberrations for a small sample of patients ($n = 12$) with triple negative breast cancer have already been published. Microarray analysis determined chromosome regions with the most frequent amplifications (1q, 3q, 6p, 8q), frequent trisomy of chromosome 18, regions with the most frequent deletions (4p, 5q, 8p, 9p) and monosomy of chromosomes 12 and 22. Moreover, many unique amplifications that occurred exclusively in individual patients were identified [8].

Similar data were obtained by Matthew D. Burstein et al. in a large sample of patients ($n = 278$) with triple negative breast cancer. Thus, the features of CAN occurrence frequency were characterized, which show that chromosomes 1q31.2, 3q26.1 and 8q23.3 demonstrated the highest frequency of amplification occurrence (more than 84%), and the highest deletion frequency was found in chromosomes 8p23.2, 9p21.3 and 10q23.31 [9]. In contrast to triple negative breast cancer [9], in the case of luminal B breast cancer the telomeric part of the long arm of chromosome 1 has a high frequency of amplifications, but in 3q26.1 the frequency of amplifications is much lower, and in the long arm of chromosome 8 the highest frequency (66%) is observed in 8q24 .21, where one of the most famous oncogenes, -c-MYC, is located. Triple negative and luminal B subtype breast cancer also differ in loci with the highest deletion frequency (Table 2), in particular, loci in the long arm of chromosome 16 and the short arm of chromosome 16 (17p13.1, 17p12) are most often deleted in luminal B BC. Locus 17p13.1 contains one of the most famous tumor suppressor genes TP53. These data indicate that the CNA landscape of breast tumor is dependent on its molecular subtype.

In addition to the description of the CNA landscape itself, it is important to understand that such data can form the basis to develop new markers of treatment efficacy for patients with breast pathology. The study by Kazantseva et al. examined molecular and genetic markers of effectiveness of neoadjuvant chemotherapy with anthracyclines in patients with breast cancer, where a sample of 46 patients with breast cancer showed that deletions of 18p.11.21; 11q22.1 and amplifications of loci 1q24.1–43 can be considered

as predictive criteria for high efficiency of NAC. The presence of at least one of the markers makes it possible to predict a high efficacy of pre-surgery treatment with anthracyclines in 85.3% of cases [10]. In the present study on luminal B subtype breast cancer, the long arm of chromosome 1 in patients with an objective response also showed a relatively high frequency of amplifications. At the same time, deletions were more often observed in patients with an objective response in loci 6q16.3, 11p15.4, 11q23.1, 16q22.2 – 22.3, which differs from the work of Kazantseva and her co-authors.

CONCLUSION

The study described the CNA landscape of luminal B breast tumor before treatment as well as the CNA landscape in patients with an objective response to NAC and its absence. The incidence rates of aberrations in all cytobands were established; aneuploidy and cytobands with the highest frequency of CNA occurrence and their absence were detected. Differences in the CNA landscape of luminal B subtype breast tumor and triple negative breast cancer were discussed.

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The effect of major salivary gland hypertrophy on rat's spermatogenic epithelium ultrastructure of rats

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ABSTRACT

Purpose. The aim of this study was to ascertain the characteristics of major salivary glands endocrine effect on spermatogenesis.

Materials and methods. Mature white outbred male rats (2 months, 153 ± 18 g) consisted of the following groups (each containing 30 rats): intact, control, and group of rats subjected to multiple amputation of incisors. To achieve hypertrophy of major salivary glands multiple amputation of incisors was performed: incisors were cut to a level of 1-2 mm above the gingival margin under ether anesthesia once every 3 days within 2 weeks. Animals of the control group were anesthetized with ether at the same time. Rats were sacrificed by CO2 asphyxia after 2, 3, 4, 6, 8 and 10 weeks after the first amputation of incisors. Fragments of the rat testes were examined on a JEM-1400 "JEOL" (Japan) transmission electron microscope. On electron microscopy images the specific vacuolization of the cytoplasm of Sertoli cells, spermatogonia, spermatocytes and spermatids (standard units) was analyzed by the point counting method. In spermatogenic cells the proportion of mitochondria (%) with morphological signs of swelling was assessed.

Results. Transient ultrastructural changes of Sertoli and spermatogenic cells develop in the rats convoluted seminiferous tubules as a result of multiple amputation of the incisors, such as phagosomes and pronounced vacuolization in the Sertoli cells cytoplasm, cytoplasm vacuolization and mitochondrial swelling in spermatogenic cells. Sporadic spermatogenic cells with signs of nuclear (chromatin fragmentation, its condensation on the periphery of the nucleus) and cytoplasm (destruction of membrane organelles) destruction appeared as a result of multiple incisors' amputation. Ultrastructural changes of Sertoli and spermatogenic cells are most pronounced at 2-3 weeks, decrease at 4 week and are completely leveled by the 6th week of the experiment.

Conclusion. Hypertrophy of major salivary glands, caused by multiple amputations of incisors, has similar to sialoadenectomy effect on the spermatogenic epithelium. Multiple incisors' amputation cause transient depression of granular convoluted cells function. Probably submandibular gland granular convoluted tubules cells endocrine factors make the greatest contribution to the regulation of spermatogenesis in rats.

Key words: spermatogenesis, Sertoli cells, salivary glands, hypertrophy.

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Влияние гипертрофии больших слюнных желез на ультраструктуру сперматогенного эпителия крыс

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РЕЗЮМЕ

Цель. Выяснение особенностей эндокринного влияния больших слюнных желез на сперматогенез половозрелых крыс.

Материалы и методы. Половозрелые белые беспородные самцы крыс (возраст 2 мес, масса тела (153 ± 18) г) составили три группы (по 30 особей): интактная, контрольная и крысы, подвергшиеся многократной ампутации резцов. Для оценки эндокринного влияния эпителиоцитов ацинусов и протоков больших слюнных желез моделировали их гипертрофию путем многократной ампутации резцов. Крыс выводили из эксперимента на 2-, 3-, 4-, 6-, 8- и 10-ю нед после первой ампутации резцов. Семенники животных оценивали при помощи трансмиссионной электронной микроскопии. На электронограммах анализировали удельный объем вакуолизации цитоплазмы сустентоцитов, сперматогоний, сперматоцитов и сперматид (усл. ед.), в сперматогенных клетках оценивали количество митохондрий (%) с морфологическими признаками набухания.

Результаты. В ранние сроки эксперимента в извитых семенных канальцах крыс развивается вакуолизация цитоплазмы сустентоцитов, а также ультраструктурные изменения сперматогенных клеток (вакуолизация цитоплазмы, альтерация митохондрий, разрушение мембранных органелл, фрагментация хроматина). Изменения структуры сперматогенных клеток и сустентоцитов максимально выражен на 2–3-й нед, снижаются на 4-й нед и полностью нивелируются к 6-й нед эксперимента.

Заключение. Гипертрофия больших слюнных желез, вызванная многократной ампутацией резцов, оказывает на сперматогенный эпителий влияние, схожее с эффектом сиалоаденэктомии. В результате многократной ампутации резцов угнетается функциональное состояние клеток гранулярных извитых трубок поднижнечелюстных желез. Эндокринные факторы, которые вносят наибольший вклад в регуляцию сперматогенеза у крыс, вырабатываются клетками гранулярных извитых трубок.

Ключевые слова: сперматогенез, сустентоцит, слюнные железы, гипертрофия.

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

Источники финансирования. Авторы заявляют об отсутствии финансирования.

Соответствие принципам этики. Исследование одобрено решением локального этического комитета ФГБОУ ВО СибГМУ Минздрава России (протокол № 4253 от 28.09.2015).

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INTRODUCTION

The major salivary glands are duacrine glands [1]: their exocrine function is associated with digestion, taste reception, non-specific immune defense, excretion and speech production. They have a proven endocrine effect on the organs of the hematopoietic and immune systems, skin, nephron epithelial cells, cartilage, and the gonads [2, 3]. The mutual influence of the salivary glands and gonads is present in many animals, including humans. It has been proved that a complete medical examination makes it possible to diagnose the interstitial form of sialadenosis (sialosis) in 100% of both male and female patients with hypogonadism [4]. Rodents are the most convenient model

for studying the mutual influence of the gonads and salivary glands because of the pronounced morphological and biochemical sexual dimorphism of their major salivary glands. Thereby, the aim of this study was to elucidate the features of the endocrine effect that the major salivary glands have on spermatogenesis of rats.

MATERIALS AND METHODS

Mature, white, outbred male rats (age 2 months, body weight 153 ± 18 g) constituted the following groups (each containing 30 rats): intact (I), control (C), and the group of rats subjected to repeated amputation of incisors (RA). The hypertrophy for RA rats was simulated through repeated amputation of incisors. Lower and upper incisors were trimmed under ether anesthesia to a level of 1–2 mm above the gingival margin once every 3 days within 2 weeks (5 amputations in total). Animals of the control group were narcotized with diethyl ether with the same periodicity. Removal from the experiment was carried out by CO2 asphyxiation during the 2nd, 3rd, 4th, 6th, 8th and 10th weeks after the first amputation of incisors.

Fragments of the rat testes were fixed for 24 hours in 4% paraformaldehyde (Serva, Germany), then for 3 hours in 1% OsO4 (SPI, USA) at 4 ° C, pH 7.4. The samples were immersed in a mixture of epoxy resins Epon 812: Araldite 502: DDSA (SPI, USA). Ultrathin sections (80 nm) were obtained on an ultratome (eica EM UC 7 (Leica, Austria) and contrasted with uranyl acetate and lead citrate for examination with a JEM-1400 transmission electron microscope (JEOL, Japan).

On electron diffraction patterns, the specific volumes of cytoplasmic vacuolization of Sertoli cells, spermatogonia, spermatocytes, and spermatids (standard units) were analyzed using the program ImageJ 1.48 (NIH Image, USA). In spermatogonia, spermatocytes, and spermatids the number of mitochondria (%) with morphological signs of swelling was assessed (calculations were based on the analysis of 200 mitochondria).

Statistical processing of quantitative data was performed using the Shapiro – Wilk, Mann – Whitney, and Kruskal – Wallis tests and SPSS 17.0 (IBM, USA). The results of the morphometric study are presented as the median and interquartile range *Me* (Q_1; Q_3), the significance level is taken as $p < 0.05$.

RESULTS

In the convoluted seminiferous tubules of rats of the intact (I) and control (C) groups, Sertoli cells and all populations of germ cells were determined throughout the period of study. However, in animals of the RA group, spermatozoa were found in the lumens of the convoluted seminiferous tubules starting from the 3rd week of the experiment. During the study period, the rats of the C group showed no difference in the ultrastructure of Sertoli and spermatogenic cells from those of the group I (Tables 1, 2).

Table 1

The specific volume of cytoplasmic vacuolization, standard units, *Me* (Q_1; Q_3)

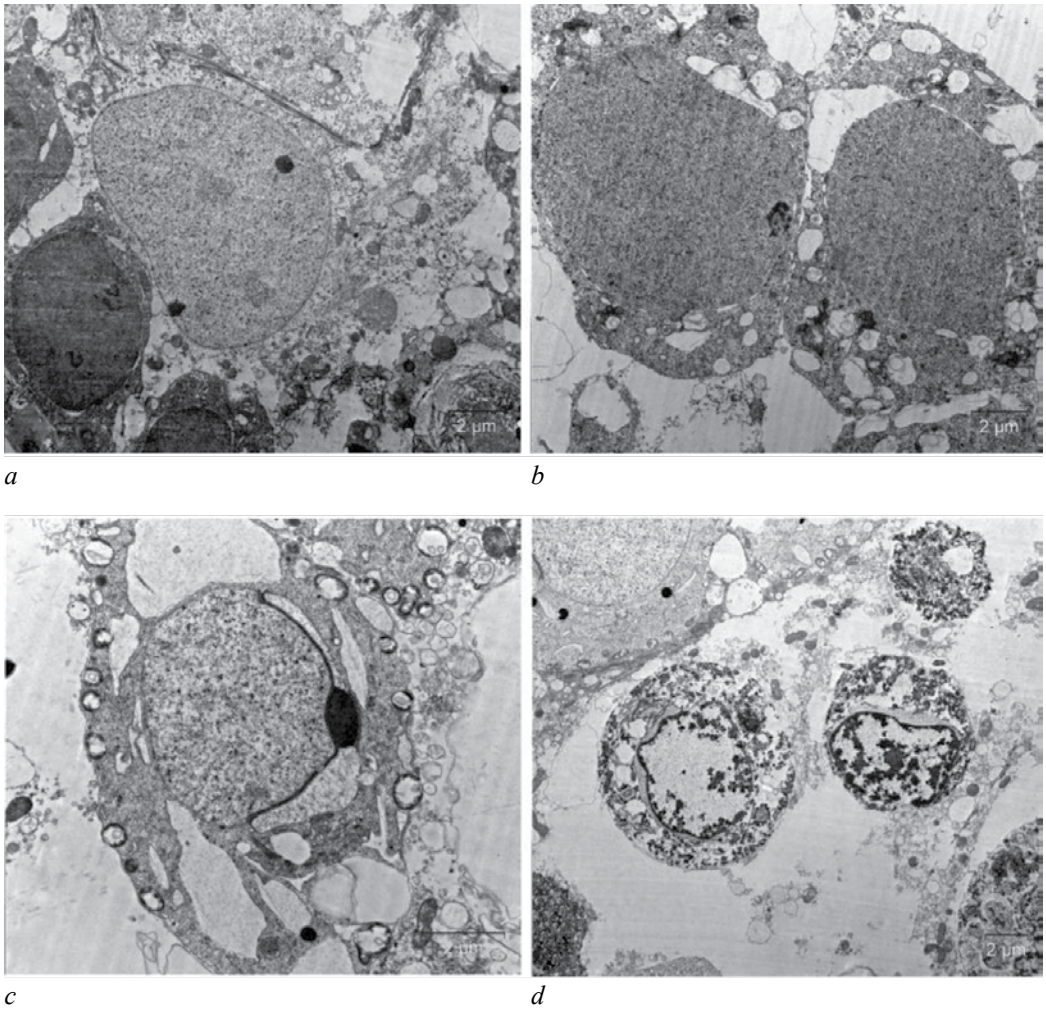
Experiment duration, week	Sertoli cells			Spermatogonia			Spermatocytes			Spermatids		
	I	C	RA	I	C	RA	I	C	RA	I	C	RA
2nd	0 (0; 1.5)	0 (0; 1.2)	36.8 (24.7; 45.0)*	0 (0; 4.8)	0 (0; 3.6)	9.9 (5.6; 18.1)*	0 (0; 4.2)	0 (0; 1.6)	10.2 (7.1; 14.4)*	0 (0; 1.6)	0 (0; 2.1)	10.0 (7.3; 25.9)*
3rd	0 (0; 2.1)	0 (0; 2.6)	37.9 (14.5; 44.2)*	0 (0; 1.6)	0 (0; 4.5)	4.2 (2.4; 9.7)	0 (0; 2.6)	0 (0; 2.0)	6.9 (0.9; 9.9)	0 (0; 2.5)	0 (0; 1.0)	10.7 (5.1; 22.8)*
4th	0 (0; 1.2)	0 (0; 1.6)	16.1 (14.7; 21.6)*#	0 (0; 3.7)	0 (0; 0.6)	2.0 (0; 4.1)	0 (0; 1.4)	0 (0; 1.6)	3.8 (0; 16.0)	0 (0; 1.5)	0 (0; 2.1)	6.2 (1.0; 14.8)#
6th	0 (0; 0.8)	0 (0; 1.4)	0 (0; 6.7)#	0 (0; 0.7)	0 (0; 1.0)	0 (0; 1.6)	0 (0; 2.0)	0 (0; 0.7)	0 (0; 1.6)	0 (0; 0.9)	0 (0; 1.2)	0 (0; 3.1)
8th	0 (0; 0.6)	0 (0; 1.6)	0 (0; 1.1)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 1.3)	0 (0; 2.3)	0 (0; 0)	0 (0; 0)	0 (0; 1.6)
10th	0 (0; 1.0)	0 (0; 0.6)	0 (0; 0.8)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0.7)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)

Note. I – intact group, C – control group, RA – the group of rats subjected to repeated amputation of incisors (here and in Table 2).
*difference between the indicator and the corresponding indicator of the intact group, $p < 0.05$.
#difference between the present indicator and that during the previous period within the same group, $p < 0.05$ (here and in Table 2).

At 2–4 weeks of the experiment, phagosomes, phagolysosomes and cytoplasmic vacuolization were detected in the cytoplasm of the Sertoli cells in rats of the RA group (Fig., *a*). The observed vacuoles were dilated cisterns of the endoplasmic reticulum (EPR). The severity of vacuolization was maximal at 2–3 weeks, though decreased over time and completely leveled off by the 6th week of the experiment ($p < 0.05$; see Table 1). Vacuolization of the cytoplasm was observed in spermatogonia, spermatocytes (order I and II) in rats of the RA group during the 2nd week of the experiment ($p < 0.05$; see Table 1).

Mitochondrial swelling, which was identified as a decrease in the number and size of cristae, expansion of matrix and the appearance of vesicular structures in it [6], was observed in spermatogonia at 2–3 weeks, and in spermatocytes, at 2–4 weeks after the first amputation of incisors ($p < 0.05$; see Table 2; Fig., *b*). The ultrastructural changes in spermatogonia described above and developing in response to repeated amputation of incisors completely leveled off by the 4th week, and in spermatocytes, by the 6th week of the experiment.

At 2–4 weeks of the experiment, early and late spermatids in rats of the RA group were characterized by mitochondrial swelling ($p < 0.05$; see Table 2) and membrane destruction. Expansion of the EPR cisterns and the Golgi apparatus was also detected in early spermatids at 2–3 weeks ($p < 0.05$; Table 1; Fig., *b*). Structural changes in spermatids caused by repeated amputation of incisors leveled off by the 6th week of the experiment.



a *b*

c *d*

Figure. Fragment of the convoluted seminiferous tubule of a mature rat subjected to repeated amputation of incisors: *a* – cytoplasmic vacuolization of a Sertoli cell; *b* – cytoplasmic vacuolization, expansion of the perinuclear space and mitochondrial alteration in first-order spermatocytes; *c* – cytoplasmic vacuolization and destruction of mitochondrial membranes in an early spermatid; *d* – spermatogenic cells with signs of destruction of the nucleus and cytoplasm; transmission electron microscopy; 2nd week (*a*, *d*) and 4th week (*b*, *c*) of the experiment

Table 2

The number of mitochondria with signs of swelling, %, *Me* (Q_1; Q_3)

Experiment duration, week	Spermatogonia			Spermatocytes			Spermatids		
	I	C	RA	I	C	RA	I	C	RA
2nd	0 (0; 2.8)	0 (0; 2.5)	8.0 (5.0; 11.0)*	0 (0;2.4)	0 (0; 2.5)	25.6 (17.2; 39.2)*	0 (0; 4.1)	0 (0; 6.4)	36.8 (30.4; 47.0)*
3rd	0 (0; 2.5)	0 (0; 2.2)	10.0 (6.6; 11.9)*	0 (0; 2.1)	0 (0; 4.4)	32.0 (20.4; 37.3)*	1.4 (0; 5.3)	0 (0; 2.8)	31.8 (26.2; 39.1)*
4th	0 (0; 3.2)	0 (0; 1.3)	0 (0; 3.7)#	0 (0; 3.2)	0 (0; 4.0)	14.7 (6.0; 25.2)*#	0 (0; 7.1)	0 (0; 2.2)	24.5 14.1; 29.8)*#
6th	0 (0; 2.8)	0 (0; 3.1)	0 (0; 1.6)	0 (0; 1.9)	0 (0; 3.8)	4.0 (1.5; 8.9)#	0 (0; 3.1)	0 (0; 6.5)	6.0 (1.5; 10.3)#
8th	0 (0; 2.2)	0 (0; 2.0)	0 (0; 2.6)	0 (0; 1.5)	0 (0; 0.8)	0 (0; 4.6)	0 (0; 1.6)	0 (0; 2.0)	0 (0; 0.5)
10th	0 (0; 0.5)	0 (0; 1.0)	0 (0; 1.5)	0 (0; 0.5)	0 (0; 0.5)	0 (0; 0)	0 (0; 0.5)	0 (0; 0.7)	0 (0; 0)

At 2–3 weeks of the experiment, the spermatogenic epithelium of the RA group demonstrated individual germ cells with signs of nuclear destruction (fragmentation of chromatin, its condensation along the periphery of the nucleus) and cytoplasm (destruction of membrane organelles). Cells with signs of destruction are round in shape and have adluminal localization (see Fig. 1, d), which allows researchers to identify them as spermatocytes or early spermatids. Starting from the 4th week of the experiment, no spermatogenic cells with signs of destruction were detected in the convoluted seminiferous tubules of the RA group.

During the study period, we did not observe changes in the morphology of spermatozoa and peritubular myoid cells in rats in response to repeated amputation of incisors.

DISCUSSION

We have previously made a conclusion that the removal of the major salivary glands leads to ultrastructural changes in spermatogenic epithelium of immature rats [3]. However, it remains unclear which structures of the major salivary glands are the source of factors that have the greatest influence on testes. For example, sialorphin and parotin are produced by the acini of the submandibular and parotid glands respectively [7]. Epidermal growth factor, transforming growth factor α and β are produced by cells in the ducts of the submandibular glands [8]. All of the above and possibly some other biologically active factors of the major salivary glands affect spermatogenesis and steroidogenesis. Repeated amputation of incisors causes hypertrophy exclusively in epithelial cells of the acini in the major salivary glands, though it is not accompanied by hyperfunction [9]. On the contrary, repeated amputation of incisors inhibits the functional state of cells in the granular convoluted tubules of the submandibular glands [9]. Thus, the chosen experimental model will make it possible to assess the contribution of the acini and ducts of the major salivary glands to the endocrine regulation of spermatogenesis.

Cytoplasmic vacuolization of Sertoli cells is a non-specific response to damage [10] and indicates a violation of cell metabolism [11]. Sertoli cells are involved in the regulation of spermatogenesis in the paracrine way (inhibin, activin, anti-Müllerian hormone), as well as through contact with germ cells [12]. Sertoli cells are a labile element of the blood-testis barrier and take part in the formation of the microenvironment for developing germ cells [12]. Dysfunction of Sertoli cells inevitably leads to dysregulation of spermatogenesis.

Ultrastructural changes in spermatogenic cells that develop in response to repeated amputation of incisors affect the energy and synthetic apparatuses of cell. Swelling and destruction of mitochondria (2–4 weeks) in spermatogenic cells of the RA group indicate a decrease in the intensity of energy processes in them. Mitochondria in germ cells perform many functions: they are involved in the initiation of apoptosis in defective germ cells, ensure the motility of spermatozoa, and their controlled production of active radicals is necessary for proper capacitation and acrosome reaction [13]. Defects in the ultrastructure of mitochondria are associated with impaired sperm functioning [13]. Mitochondrial alteration is associated with excessive production of active radicals, which are potential inducers of cytoplasmic vacuolization. Damage to the membrane and enzyme systems of the granular EPR is the cause of the violation of protein folding and deg-

radation, which leads to the expansion of the lumen of its cisterns [14, 15].

Spermatids and spermatocytes are the most sensitive spermatogenic cells when it comes to changes caused by repeated amputation of incisors. In a number of spermatogenic cells, ultrastructural changes become irreversible. The presence of germ cells with signs of destruction (2–3 weeks) and the absence of spermatozoa (2 week) in the convoluted seminiferous tubules indicate the impossibility of a proper maturation phase and formation of spermatogenesis in rats shortly after reaching hypertrophy of the major salivary glands through repeated amputation of incisors. Phagolysosomes detected in the cytoplasm of Sertoli cells at 2–4 weeks of the experiment are likely the result of absorbing the fragments of destroyed spermatogenic cells.

The ultrastructural changes in Sertoli and spermatogenic cells observed in response to repeated amputation of incisors are similar to those developing after sialoadenectomy [3]. Since repeated amputation of incisors leads to the acini hypertrophy, the reduced number of cells in the ducts of the submandibular glands and their inhibited functional activity [9] suggests the following: it is the granular convoluted tubules of the submandibular glands that produce factors having the greatest effect on spermatogenic epithelium. The suppression of synthetic and secretory activity in cells of the granular convoluted tubules of the submandibular glands leads to the development of ultrastructural changes in cells of the convoluted seminiferous tubules. Epidermal growth factor, transforming growth factor α and β, and other biologically active factors of epithelial cells in the ducts of the submandibular glands can have a direct effect on germ cells or an effect mediated by Sertoli cells and interstitial endocrinocytes of testis. It is worth noting the potential endocrine action of the major salivary glands on spermatogenesis indirectly, through the central and peripheral endocrine glands. Biologically active substances of the granular convoluted tubules of the submandibular glands in rats are also produced by the major salivary glands of humans. Elucidation of the endocrine interactions between the human salivary glands and gonads is a long-term objective.

Changes in the morphology and functional status of the epithelial cells of the acini and ducts in the major salivary glands of rats in response to repeated amputation of incisors are transient [9]. This explains the gradual decrease in the severity of morphological changes in the spermatogenic epithelium and complete normalization of the ultrastructure of Sertoli and germ cells by the 6th week of the experiment.

CONCLUSION

We have shown that repeated amputation of incisors causes transient ultrastructural changes in Sertoli and spermatogenic cells of mature rats, similar to those observed after sialoadenectomy. The endocrine factors that make the greatest contribution to the regulation of spermatogenesis in rats are produced by cells of the granular convoluted tubules. Substances produced by the epithelial cells of the acini in the major salivary glands of rats probably have a less potent effect on spermatogenic epithelium.

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Authors contribution

Ivanova V.V. – analysis and interpretation of data, justification of the manuscript. Tikhonov D.I., Serebrjakova O.N. – analysis and interpretation of data. Mil'to I.V. – conception and design of the study. Gereng E.A. – critical revision of the manuscript for important intellectual content. Pleshko R.I. – final approval of the manuscript for publication.

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Influence of clinical and therapeutic indicators on the severity of neurocognitive deficits in patients with schizophrenia

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ABSTRACT

Aim. To assess the association of clinical and therapeutic parameters with the severity of the neurocognitive deficits in patients with schizophrenia.

Materials and methods. We examined 118 patients with schizophrenia, aged 34 [29; 41] years, and with a disease duration of 10 [4; 16] years. 33 patients (28%) received conventional antipsychotic drugs (CAD), and 85 (72%) patients received atypical antipsychotic drugs (AAD). As concomitant therapy, 58 people (49.1%) took trihexyphenidyl, 60 people did not take it (50.9%). Assessment of cognitive functions was carried out for all patients using the Brief Assessment of Cognition in Schizophrenia (BACS), and clinical psychopathological symptomatology was evaluated using the Positive and Negative Syndrome Scale (PANSS). Statistical analysis of the data was performed using the Kruskal – Wallis test ANOVA with the multiple comparison procedure, the Pearson's chi-squared test, and K-means cluster analysis.

Results. Neurocognitive deficits formed three clusters of disturbances that differ in clinical severity: 1) mild, 2) moderate, 3) severe. According to the subscale of positive PANSS symptoms, patients with mild neurocognitive deficits had a lower average total score compared to patients with severe neurocognitive deficits ($p = 0.011$), who, in turn, received significantly longer antipsychotic therapy compared with patients with moderate ($p = 0.014$) and mild ($p = 0.01$) neurocognitive deficits. Herewith, the duration of CAD treatment did not differ between clusters; consequently, the obtained results on antipsychotics as a whole were obtained due to AAD ($p = 0.005$ and $p = 0.001$, respectively). Trihexyphenidyl did not affect the severity of neurocognitive deficits.

Conclusion. The severity of positive symptoms of schizophrenia was lower in patients with mild neurocognitive deficits. The most pronounced neurocognitive deficits are observed in patients receiving AAD.

Key words: schizophrenia, neurocognitive deficits, antipsychotics, trihexyphenidyl.

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Conformity with the principles of ethics. All the people included in the study gave their written informed consent. The study was approved by the ethics committee of Mental Health Research Institute of Tomsk National Research Medical Center (Protocol No. 99 of 17.04.2017).

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Влияние клинических и терапевтических показателей на выраженность нейрокогнитивного дефицита у пациентов с шизофренией

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РЕЗЮМЕ

Цель. Оценить связь клинических и терапевтических показателей с выраженностью нейрокогнитивного дефицита у пациентов с шизофренией.

Материалы и методы. Были обследованы 118 пациентов с шизофренией в возрасте 34 [29; 41] лет, с длительностью заболевания – 10 [4; 16] лет. Конвенциональные антипсихотические препараты (КАП) получали 33 пациента (28%), атипичные антипсихотическое препараты (ААП) – 85 (72%) пациентов. В качестве сопутствующей терапии 58 человек (49,1%) принимали тригексифенидил, не принимали его 60 человек (50,9%). Оценка когнитивных функций проведена всем пациентам по шкале краткой оценки когнитивных функций у пациентов с шизофренией (Brief Assessment of Cognition in Schizophrenia, BACS), клинико-психопатологической симптоматики – с использованием шкалы позитивных и негативных синдромов (Positive and Negative Syndrome Scale, PANSS). Статистический анализ полученных данных выполнен с использованием критерия Краскела – Уоллиса ANOVA с процедурой множественного сравнения, критерия $\chi 2$ Пирсона и кластерного анализа методом *K*-средних.

Результаты. Нейрокогнитивный дефицит образовал три кластера нарушений, отличающихся между собой клинической выраженностью: 1) легкий, 2) умеренно выраженный, 3) выраженный. По субшкале позитивных симптомов PANSS пациенты с легким нейрокогнитивным дефицитом имели меньший средний суммарный балл по сравнению с больными с выраженным нейрокогнитивным дефицитом ($p = 0{,}011$), которые, в свою очередь, значимо дольше получали антипсихотическую терапию по сравнению с пациентами с умеренным ($p = 0{,}014$) и легким ($p = 0{,}01$) нейрокогнитивным дефицитом. При этом длительность приема КАП не различалась между кластерами, следовательно, имеющиеся результаты по антипсихотикам в целом получены за счет ААП ($p = 0{,}005$ и $p = 0{,}001$ соответственно). Тригексифенидил не оказал влияния на выраженность нейрокогнитивного дефицита.

Заключение. Выраженность позитивных симптомов шизофрении была ниже у пациентов с легким нейрокогнитивным дефицитом. Наиболее выраженные нейрокогнитивные нарушения отмечаются у пациентов, получающих ААП.

Ключевые слова: шизофрения, нейрокогнитивный дефицит, антипсихотики, тригексифенидил.

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

Источник финансирования. Исследование выполнено при поддержке гранта РФФИ 18-315-20019 «Новые подходы к генетике клинического полиморфизма и нейрокогнитивного дефицита при шизофрении».

Соответствие принципам этики. Все лица, вошедшие в исследование, подписали письменное информи-

рованное согласие. Исследование одобрено этическим комитетом НИИ психического здоровья, Томского НИМЦа (протокол № 99 от 17.04.2017).

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INTRODUCTION

Contemporary antipsychotics are primarily antagonists of second type dopamine receptors and can induce extrapyramidal adverse events [1]. Anticholinergics are widely used for their relief in psychiatric practice. However, anticholinergic agents precipitate different peripheral side effects like dry mouth, urination disturbances, and constipation; as well as central side effects: cognitive deficits, worsening of tardive dyskinesia and emergence of delirium [2]. Cognitive deficits occur at the earliest stages of schizophrenia process and account for the main part of functional problems related to the disorder. Likely, the long-term combined use of antipsychotics and anticholinergics intensifies the basic cognitive deficits in patients with schizophrenia, which eventually affects their quality of life [3]. Thus, modern guidelines for the treatment of schizophrenia do not normally recommend preventive and long-term use of anticholinergic agents. Nevertheless, the widespread long-term use of anticholinergics in the combination with antipsychotics has taken place in several countries [3–5]. The results of the study of this problem are debatable. The number of surveys of the previous decade shows the positive effect of anticholinergic agents on cognitive functions in patients with schizophrenia [4, 6, 7]. An earlier study dedicated to the possible effect of anticholinergics on residual symptoms of schizophrenia showed that antipsychotics and trihexyphenidyl in combination have a positive effect on the memory and attention of patients [8].

However, modern studies contain more data indicating the adverse effect of anticholinergics on the cognitive functions of patients with schizophrenia, or the absence of such an effect [5, 9, 10]. Thus, S. Ogino et al. [3] defined that cancellation of long-term use of anticholinergics can improve objective indicators of cognitive functions and subjective characteristics of the quality of life for patients with chronic schizophrenia.

S. Eum et al. [10] investigated the influence of the total anticholinergic burden arising from the combined use of anticholinergic and antipsychotic drugs on the cognitive functions of patients with psychotic disorders and schizophrenia. According to their data, the common anticholinergic burden was inversely proportional to the level of cognitive activity; especially, it affected the impairment of verbal memory. Despite the similar cumulative anticholinergic burden in groups with various psychotic disorders, increased cognitive susceptibility to anticholinergic agents was revealed in patients with schizophrenia.

A number of studies have shown improvement in cognitive functions of varying degrees with the use of second-generation antipsychotics in the treatment of long-term schizophrenia or the first psychotic episode. The positive effects of clozapine, risperidone, olanzapine, quetiapine, sertindole, and aripiprazole on various cognitive functions were noted. At the same time, some authors have the opinion that there is currently no convincing evidence of the greater effectiveness of second-generation antipsychotics compared to first-generation antipsychotics for cognitive impairment [4, 6].

In routine clinical psychiatric practice in various countries, psychiatrists continue to widely use a combination of antipsychotics and anticholinergics for the treatment of schizophrenia. In this connection, there is a necessity to further study the effects of prolonged combined use of antipsychotics and anticholinergic agents for the treatment of schizophrenia.

The aim of the study was to assess the association of clinical and therapeutic parameters with the severity of neurocognitive deficits in patients with schizophrenia.

MATERIALS AND METHODS

The study included inpatients of the hospital of Mental Health Research Institute of Tomsk National Research Medical Center of the Russian Academy of Sciences and Tomsk Clinical Psychiatric Hospital. The criteria of inclusion in the study were age from 18 to 60 years, verified diagnosis of schizophrenia

according to the ICD-10 Classification of Mental and Behavioral Disorders – Diagnostic Criteria for Research [11], and the ability to write informed consent. The criteria for non-inclusion were the presence of organic mental disorders, brain and severe somatic disorders leading to organ failure, and refusal to take part in the study.

Thus, we examined 118 patients with schizophrenia, aged 34 [29; 41] years, and with a disease duration of 10 [4; 16] years and the average age of onset of 23 [20; 29] years. All patients included in the study received antipsychotics as basic therapy in therapeutic dosages approved by the Russian Ministry of Health.

Based on the receptors profile of basic antipsychotic therapy patients were divided into two groups: 33 patients (28%) received conventional antipsychotic drugs (CAD), and 85 (72%) patients received atypical antipsychotic drugs (AAD). All dosages of various antipsychotics were brought to uniformity in the equivalent of chlorpromazine (CPZeq) [12], common antipsychotic burden was 320 [160; 598.5]; for CAD – 416.9 [160; 1000], for AAD – 300 [199.9; 428.1].

As concomitant therapy, 58 people (49.1%) took trihexyphenidyl and 60 people did not (50.9%). The duration of receiving trihexyphenidyl was 2 [0.5; 4] years. In this study, due to its observational nature, the reasons and purpose of trihexyphenidil prescription in the course of treatment of patients were not taken into account, but the fact of prescribing an anticholinergic drug was assessed as likely to have an effect on cognitive deficit in patients with schizophrenia.

Assessment of cognitive functions was carried out for all patients on the Brief Assessment of Cognition in Schizophrenia (BACS) [13], in the adapted Russian version [14]. A set of tasks on this scale ("List learning", "Digit sequencing task", "Token motor task", "Verbal fluency", "Symbol coding", "Tower of London") allows us to evaluate the parameters according to the sequence of the list: verbal memory, working memory, motor speed, processing speed, attention and processing speed, executive functions.

The psychopathological assessment was carried out using a Positive and Negative Syndrome Scale (PANSS) [15] in the adapted Russian version – SCI-PANSS [16].

The obtained data were tested for normal distribution with the SPSS Kolmogorov – Smirnov Test for Normality (with the Lilliefors significance correction) and the Shapiro – Wilk Test. Data with a normal type of distribution are presented as mean and standard deviation ($M \pm SD$), in the absence of a normal distribution; the data are presented as the median and quartiles (Me [Q_1; Q_3]). Qualitative data are presented by frequency indicators (n (%)). Comparing several independent samples of quantitative data having a non-normal distribution, we used the Kruskal – Wallis ANOVA test with the multiple comparison procedure. To compare frequencies, the Pearson's chi-squared criterion was used. To reveal the variants for the severity of neurocognitive deficits (NCD), K-means clustering was used. Statistical analysis was performed using the software Statistica for Windows (V. 12.0). The threshold value of the achieved significance level of p was taken equal to 0.05.

RESULTS

Neurocognitive deficits are formed by three clusters of impairment registered in accordance with all BACS subscales, which differ in clinical severity: 1) mild, 2) moderate, 3) severe. Consequently, patients were stratified according to the level of severity as follows (Fig., Table 1): cluster 1 (37 (31.4%)), cluster 2 (51 (43.2%)) and cluster 3 (30 (25.4%)). The results of the analysis of variance for all clusters represent the good quality of clustering (< 0.0001).

Then, the assessment of the connection between the severity of NCD and clinical and therapeutic indicators was made in the selected clusters.

The average age of schizophrenia onset and the duration of the disorder had no differences between the clusters; however, the average age of the patients in the study had significant differences (Table 2) between cluster 2 and cluster 3 ($p = 0.024$).

An assessment of the effect of the actual mental state on the severity of NCD (Table 3) showed that according to the subscale of positive symptoms PANSS patients with mild NCD had a lower average total score of positive symptoms compared with patients with severe NCD, who had a more pronounced predominance of the positive symptom complex ($p = 0.011$).

For assessing the effect of trihexyphenidyl on the severity of NCD, the Pearson's chi-squared criterion was used; statistically significant differences were not established. The duration of receiving trihexyphenidyl was evaluated in 58 patients; the evaluation was based on interviews and medical records including medical histories. The duration of receiving was presented in years. Differences between the clusters were not revealed. Also, the clusters of the severity of NCD had

no differences depending on the type of basic antipsychotic therapy: patients receiving CAP or patients receiving AAP. Assessment of the total antipsychotic burden revealed that CPZeq had no differences in all clusters as well as in groups receiving CAP and AAP.

The duration of antipsychotic therapy had differences (Table 4) between cluster 1 and cluster 3 ($p = 0.01$), as well as between cluster 2 and cluster 3 ($p = 0.014$). These results suggest that patients receiving long-term antipsychotic therapy have more severe NCD.

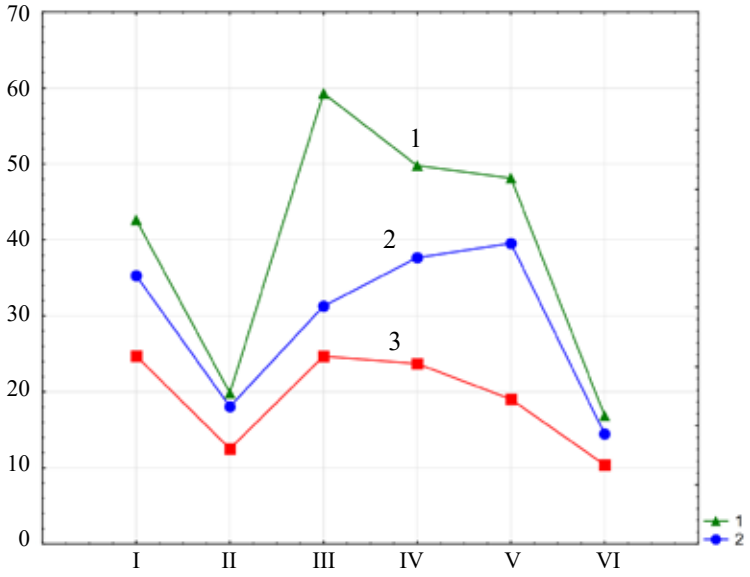

Figure. "Cognitive profile" of the selected variants of the severity of neurocognitive deficits according to BACS in the group of patients with schizophrenia: I – List Learning Test; II – Digit Sequencing Task; III – Token Motor Task; IV – Verbal Fluency; V – Symbol Coding; VI – Tower of London Test; 1 – cluster 1; 2 – cluster 2; 3 – cluster 3.

Table 1

Descriptive statistics for identified clusters in accordance with BACS, *M* ± *SD*

Tasks of BACS	Cluster 1 $n = 37$ (31.4%)	Cluster 2 $n = 51$ (43.2%)	Cluster 3 $n = 30$ (25.4%)
List learning	42.6 ± 11.4	35.3 ± 9.5	24.7 ± 9.1
Digit sequencing task	19.8 ± 3.8	18.1 ± 4.7	12.5 ± 4.8
Token motor task	59.2 ± 11.6	31.3 ± 13.3	24.6 ± 11.6
Verbal fluency	49.7 ± 11.5	37.6 ± 9.7	23.7 ± 8.1
Symbol coding	48.1 ± 12.4	39.6 ± 9.7	19.0 ± 8.9
Tower of London	16.8 ± 2.3	14.5 ± 4.7	10.4 ± 5.8

Table 2

Clinical indicators depending on the severity of NCD according to BACS, *Me* [Q_1; Q_3]

Clinical indicators	Cluster 1 $n = 37$ (31.4%)	Cluster 2 $n = 51$ (43.2%)	Cluster 3 $n = 30$ (25.4%)	p (1–2; 1–3; 2–3)
Age, years	35 [29; 39]	32 [28; 38]	37.5 [33; 53]	1.0; 0.167; 0.024
Age of onset, years	26 [20; 29]	22 [19; 27]	23.5 [20; 29]	0.588; 1.0; 1.0
Duration of the disorder, years	10 [4; 14]	8 [2; 16]	13.5 [5; 22]	1.0; 0.118; 0.073

Table 3

PANSS scores depending on the severity of the NCD according to BACS, *Me* [Q_1; Q_3]

PANSS subscales	Cluster 1 $n = 37$ (31.4%)	Cluster 2 $n = 51$ (43.2%)	Cluster 3 $n = 30$ (25.4%)	p (1–2; 1–3; 2–3)
Positive symptom subscale score	19 [16; 22]	21 [15; 25]	23 [20; 27]	0.626; 0.011; 0.166
Negative symptom subscale score	25 [23; 28]	25 [21; 28]	26.5 [22; 30]	1.0; 1.0; 0.485
General psychopathology subscale score	54 [48; 60]	54 [48; 58]	55.5 [49; 61]	1.0; 1.0; 0.807
Total score	100 [92; 107]	100 [86; 109]	107.5 [94; 114]	1.0; 0.227; 0.199

Table 4

Duration of basic antipsychotic therapy depending on the severity of NCD according to BACS, *Me* [Q_1; Q_3]

Parameter	Cluster 1 *n* = 37 (31.4%)	Cluster 2 *n* = 51 (43.2%)	Cluster 3 *n* = 30 (25.4%)	*p* (1–2; 1–3; 2–3)
Duration of antipsychotic therapy	3 [0.5; 5]	3 [1; 5]	7 [3; 17]	1.0; 0.01; 0.014

The duration of CAP administration had no differences in the clusters of the severity of NCD, it means that the available results on antipsychotics were obtained due to AAP generally: 1–3 clusters – $p = 0.001$; 2–3 clusters – $p = 0.005$ (Table 5).

Table 5

Severity of NCD according to BACS depending on the duration of the therapy with the use of conventional and atypic antipsychotics, *Me* [Q_1; Q_3]

Duration of antipsychotic therapy	Cluster 1 *n* = 37 (31.4%)	Cluster 2 *n* = 51 (43.2%)	Cluster 3 *n* = 30 (25.4%)	*p* (1–2; 1–3; 2–3)
Duration of receiving CAP	5.5 [2.5; 10.5]	3 [1; 8]	8.5 [0.3; 20]	1.0; 1.0; 1.0
Duration of receiving AAP	3 [0.3; 4]	3 [0.8; 5]	7 [4; 13.5]	1.0; 0.001; 0.005

DISCUSSION

At present, in addition to the existing dichotomic theory of schizophrenia dividing positive and negative syndromes, the cognitive symptom complex is considered as the third component of the disorder [2], while cognitive functioning of patients is increasingly in view of the researchers, not only in the field of clinical and biological psychiatry [17, 18] but also in the sphere of somatic medicine [19].

The use of anticholinergic agents in the treatment of adverse movement phenomena of antipsychotic therapy has a negative effect on cognitive functions in patients with schizophrenia [9, 20], just like the general index of anticholinergic burden [10]. Attention to this issue should be strengthened with consideration of the physical status of the patients and the potential influence of anticholinergics in combination with other agents used in psychiatry on the cardiovascular system [21], as cardio-vascular diseases themselves induce cognitive impairment. The obtained results did not show the influence of the use of trihexyphenidyl on the intensity of NCD in patients with schizophrenia. The attained results have some limitations, as the influence of a particular medication was assessed, but not the total cholinergic burden. The use of somatic drug groups with similar effect was not taken into consideration either. Nevertheless, in contemporary literature, this issue has hardly been discussed and the studies on the influence of the combination of antipsychotics and anticholinergic agents have been few [3, 6, 22].

The existing assumption on the influence of antipsychotics on the cognitive functions in patients with schizophrenia took root in the psychiatric community; for example, the use of CAP is thought of being connected with the negative influence on the cognitive functions, while the use of AAP is thought to be connected with their improvement [7, 23], although this fact is controversial [4]. In our study, we have not observed differences in the intensity of NCD in patients who received CAP and AAP as basic therapy.

Meta-study of A.L. Mishara, T.E. Goldberg [24] demonstrated that CAP, in general, has a moderately positive effect on cognitive functions, while the dosage of the medicine does not have any effect on cognitive functions, which was also observed in the course of our study. The use of agents of the third generation is connected with multiple advantages, including their positive effect on cognitive functions [25].

It was revealed that the longer the patient receives antipsychotic therapy, the higher the intensity of NCD; although the length of the disorder, which is closely linked to the duration of receiving supporting therapy, in our sampling did not have such significant influence. The length of use of AAP is also linked to the higher intensity of NCD, which was not found in patients receiving CAP, although they were used for a longer period. The revealed data on the higher intensity of NCD in view of increasing the length of the basic antipsychotic therapy in patients receiving AAP precisely appears interesting, as the long-term treatment observation of patients with schizophrenia showed that after 8 weeks of treatment there did not appear to be any dissimilarities in cognitive indicators in patients with schizophrenia receiving CAP and AAP [26].

The existence of negative symptoms and neurocognitive deficits in clinical evidence of schizophrenia has a relatively constant character, unlike the positive symptoms which can fluctuate in the course of the disorder. Lowering of the score according to the subscales of negative and positive symptoms PANSS was connected with the improvement in BACS task performance in patients with schizophrenia [27]. It should be emphasized that the surveyed patients were in the active phase of the disorder, so the PANSS

scores must be cross-referenced with the results of similar studies conducted in the period of remission [28]. The received dissimilarities in the higher score according to the PANSS subscale of positive symptoms in patients with significant NCD in comparison with the patients with mild NCD correspond with the data referring to the length of use of basic antipsychotic therapy, as the choice of AAP was possibly made taking into account the grave mental condition and the predominance of delusions and hallucinations.

CONCLUSION

The influence of trihexyphenidyl and atypical antipsychotics of the second generation on cognitive indicants in patients with schizophrenia remain an open question and warrant further investigation and examination of the issue taking into account the combined drug burden. As expected, patients performed the BACS tasks better against the background of AAP usage, although long-term use of agents of this group was connected with the higher level of NCD in patients with schizophrenia.

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Kornetova E.G. – conception and design of the study, drafting of the article, critical revision for important intellectual content. Goncharova A.A. – clinical-psychopathological and psychometric examination of the sampling, processing of statistical data, analysis of literature on the research topic, drafting of the article. Dmitrieva E.G. – psychometric examination of the sampling, drafting of the article, analysis of literature on the research topic. Arzhanik A.A. – processing of statistical data. Kornetov A. N. – critical revision for important intellectual content. Semke A.V. – final approval of the manuscript for publication.

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Evaluation of factors influencing adherence to treatment with sodium-glucose cotransporter type 2 inhibitor

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ABSTRACT

Background. Despite the emergence of new groups of drugs for the treatment of type 2 diabetes mellitus (DM2), the issue of optimal adherence to treatment remains of interest.

The aim of this study was to investigate the factors that influence the adherence to treatment with sodium glucose co-transporter type 2 inhibitor, empagliflozin (Jardiance, Boehringer Ingelheim, Germany), in patients with DM2.

Materials and methods. The study included 102 patients with DM2 (58 of them were women); the observation time was 24 weeks. The mean age was 58.3 ± 10.4 years.

Results. Patients without cognitive impairment had a lower level of glycated hemoglobin (HbA1c) (7.76%, 6.18–9.34) than patients with mild dementia (8.51%, 7.02–10; $p = 0.032$). In the group of patients who noted the impossibility of purchasing even a part of the drugs, the level of HbA1c was 9.73% (8.95–10.51), while patients who had no difficulties in purchasing drugs HbA1c was 8.83% (7.85–9.81; $p = 0.036$). Empagliflozin was discontinued in 38.2% of patients for the following reasons: cost of the drug (16.6%), development of side effects (10.7%), lack of effectiveness (7.8%), other reasons (2.9%).

Conclusion. Thus, the main factors influencing the adherence to treatment were the cost of the drug, development of adverse events, and lack of effectiveness from the therapy. At the same time, the opportunity of purchasing all the necessary drugs was associated with both better glycemic control and a higher quality of life.

Key words: diabetes mellitus, adherence to treatment, sodium glucose co-transporter type 2 inhibitor, empagliflozin.

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

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Оценка факторов, влияющих на приверженность к лечению ингибитором натрий-глюкозного ко-транспортера 2-го типа

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РЕЗЮМЕ

Актуальность. Несмотря на появление новых групп препаратов для лечения сахарного диабета 2-го типа (СД2), вопрос оптимального соблюдения режимов терапии остается в центре внимания.

Цель. Изучить факторы, влияющие на приверженность к лечению ингибитором натрий-глюкозного ко-транспортера 2-го типа – эмпаглифлозином (Джардинс, Берингер Ингельхейм, Германия) у пациентов с СД2.

Материалы и методы. В исследование были включены 102 пациента с СД2 (из них 58 женщин), время наблюдения – 24 нед. Средний возраст составил (58,3 ± 10,4) лет.

Результаты. Пациенты без нарушения когнитивных функций имели меньший уровень гликированного гемоглобина (HbA1c) – 7,76 (6,18–9,34)%, чем пациенты с деменцией легкой степени выраженности – 8,51 (7,02–10)%, p = 0,032. В группе пациентов, которые отметили невозможность приобретения даже части препаратов, уровень HbA1c составил 9,73 (8,95–10,51)%, в то время как пациенты, не испытывающие финансовых затруднений в приобретении препаратов, имели HbA1c 8,83 (7,85–9,81)%, p = 0,036. Терапия эмпаглифлозином была прекращена у 38,2% пациентов по следующим причинам: стоимость препарата – 16,6%, развитие побочных эффектов – 10,7%, отсутствие эффективности от терапии в виде улучшения гликемического контроля – 7,8%, другие причины – 2,9%.

Заключение. Таким образом, основными причинами, влияющими на продолжение лечения, оказались финансовые затруднения в приобретении препарата, возникновение побочных эффектов и отсутствие эффективности от терапии. При этом возможность приобретать всю необходимую терапию ассоциировалась как с лучшим гликемическим контролем, так и более высоким качеством жизни.

Ключевые слова: сахарный диабет, приверженность к лечению, ингибиторы натрий-глюкозного ко-транспортера 2-го типа, эмпаглифлозин.

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

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INTRODUCTION

Diabetes mellitus (DM) is one of the most widespread non-infectious diseases worldwide. In 2017, 425 million people worldwide were reported to have DM, which is a global prevalence of 8.3% [1]. At the same time, the majority of cases are of DM2, which represents a problem in modern healthcare because of a significant social burden and major financial cost of treatment for chronic complications like nephropathy, retinopathy, and neuropathy that can finally lead to terminal kidney failure, blindness, and amputation of lower extremities. The risk of death from cardiovascular or cerebrovascular events in patients with DM2 is significantly higher in comparison with people without diabetes [2].

In clinical practice, the maintenance of target values of glycated hemoglobin (HbA1c) remains one of the main therapeutic tasks to prevent complications as-

sociated with DM2, especially microvascular events. This data is based on the results of three major experimental studies (ACCORD, ADVANCE and VADT) and UKPDS study that showed that lower levels of HbA1c are associated with later onset and progression of microvascular complications [3–6].

Although drug therapy for DM2 achieved significant development in the past years, some factors decrease the effectiveness of this therapy. Among them are low awareness of the disease and insignificant adherence to the therapy [7, 8].

Adherence to therapy is an important factor that determines the disease outcome in patients with chronic diseases. In 2003, the WHO reported that "an increase in the effectiveness of interventions to the adherence to therapy can have a more beneficial effect on the population health than the improvement of single types of treatment" [9]. Still, the majority of studies indicate that the adherence to drug therapy remains suboptimal for patients with multiple chronic diseases, including DM2 [10–14]. In 2004, Cramer published a systematic review aimed at comparing the parameters of adherence to anti-hyperglycemic drugs and insulin [15]. The results of the review showed that many patients with diabetes did not adhere to the recommended pharmacotherapy, which led to non-optimal control of glycemia. Besides, the review on the adherence to DM2 showed that the level of education in patients, especially regarding self-management during treatment, was the most important factor that predicted the improvement of glycemic control, the quality of life and possible outcomes [16]. Among the reasons for non-optimal adherence to the treatment of DM2, there were factors like the complexity of the dose regimen and adverse events associated with the treatment. A meta-analysis that included 40 studies published from 2005 to 2015 showed that only 67.9% of patients with DM2 adhered to their anti-hyperglycemic drugs [17]. Still, the adherence to treatment of diabetes mellitus is known to be associated with better glycemic control, lower rates of hospitalizations, lower expenses on health care, and lower mortality rate [18].

Despite the emergence of numerous anti-hyperglycemic drugs on the market that are characterized by high efficiency and good tolerance, the issue of adherence remains acute. Although it is suggested that there is a positive association between the adherence to treatment, patients' awareness of their disease and their levels of HbA1c, few studies on anti-hyperglycemic drugs evaluate the association between the described variables factually and performed the analysis of factors that influence these processes [19, 20]. This study is focused on the factors that influence the adherence to treatment with sodium-glucose co-transporter type 2 inhibitor (SGCI2) empagliflozin (Jardiance, Boehringer Ingelheim, Germany) in patients with DM2.

MATERIALS AND METHODS

The study included patients with DM2 (men and women aged 18 to 70) who started their therapy with sodium-glucose co-transporter type 2 inhibitor (SGCI2) empagliflozin at a daily dose of 10 mg. Signing the patient's informed consent form for participation in the study and stable anti-hyperglycemic therapy for a minimum of 12 weeks before being included in the study were obligatory study entry criteria. The criteria of exclusion were diabetes mellitus type 1, acute coronary syndrome, acute cerebrovascular condition within the past two months, verified kidney diseases (glomerulonephritis, pyelonephritis, amyloid disease) or chronic kidney disease with GFR < 45 ml/min/1.73 m^2. Additionally, the study excluded patients with leg ulcers in cases of diabetic feet and patients that underwent bariatric operations and treatment with medications for weight loss within the past 3 months.

In the beginning of the study, the authors collected patients' personal information like age, sex, level of education, height and body weight (BMI), duration of diabetes, chronic complications with the stage of retinopathy and nephropathy, if any, and a history of infections of the urinary tract and reproductive organs. The authors evaluated such parameters as arterial blood pressure (BP), heart rate (HR), glycated hemoglobin (HbA1c), fasting plasma glucose, and creatinine in the blood with the calculation of GFR. During the first visit, patients filled in the EQ5D and life quality evaluation forms. The Hospital Anxiety and Depression Scale (HADS) was used to identify the presence and severity of depression. The cognitive condition of every patient was evaluated with Mini-Mental State Examination (MMSE). Patients additionally filled in a questionnaire that included the questions on peculiarities of disease development and the presence or absence of diabetic chronic complications. The questionnaire also included questions that allowed the authors to evaluate the level of patients' trust toward the attending physician and the awareness of their disease.

The observation time was 24 weeks. During the study, the authors evaluated the dynamics of laboratory parameters (HbA1c, creatinine, alanine amino-

transferase (ALT), aspartate aminotransferase (AST), low-density lipoproteins (LDL), and clinical parameters (BP, HR). Patients filled in the same questionnaires that they had during the first visit. The authors analyzed medical documentation provided by patients to reveal the development of urinary tract and reproductive organ infections, cardiovascular conditions (acute myocardial infection), acute coronary syndrome, acute cerebrovascular conditions, and hospitalizations for diabetic ketoacidosis. During the study, the authors recorded data on the development of adverse events and discontinuation of therapy with specifying the reason.

Statistical analysis was performed with the software package STATISTICA 10 (StatSoft Inc, USA). Qualitative parameters were presented as *n* (%), quantitative parameters in cases with normal distribution were presented as an arithmetic average and standard deviation ($M \pm SD$), in cases with asymmetric distribution as a median and quartiles (*Me*, 25% quartile; 75% quartile). To compare quantitative data, the authors used the Mann – Whitney U-test. The Spearman's rank correlation coefficient was used to identify correlations between the factors. The obtained results were considered statistically significant at $p < 0.05$.

RESULTS

The study included 102 patients with DM2 (Table 1). The mean age of patients was 58.3 ± 10.4 years old, 56.8% of patients were women. By the time of inclusion into the study, 36.2% of the patients received insulin drugs along with anti-hyperglycemic drugs and 23.5% of patients received metformin as a monotherapy.

Table 1

Initial features of patients

Parameter, units of measurement	Value
Average age, years	58.3 ± 10.4
Women, *n* (%)	58 (56.8%)
Men, *n* (%)	44 (43.2%)
Duration of diabetes, years, $M \pm SD$	9.2 ± 4.5
BMI, kg/m^2	28.6 ± 5.5
Glomerular filtration rate (eGFR), ml/min/1.73 m^2, $M \pm SD$	69.7 ± 20.7
> 60 ml/min/1.73 m^2, *n* (%)	58 (55.8%)
< 60 ml/min/1.73 m^2, *n* (%)	44 (44.2%)
HbA1c, %, $M \pm SD$	8.8 ± 1.6

64.7% of patients had completed a higher education course, 29.4% of patients had secondary vocational education. The comparison of levels of glycated hemoglobin after 24 weeks in these two groups did not reveal any significant differences with 8.2% (7.3–9.1) and 8.4% (7.4–9.3), respectively ($p > 0.05$). However, patients without cognitive disorders (24–30 points under the MMSE inventory) had lower levels of HbA1c with 7.7% (6.2–9.3) than patients with mild dementia (20–23 points) who had HbA1c levels of 8.5% (7.1–10) ($p = 0.032$).

The mean level of HbA1c after 24 weeks of treatment was 8.4% (7.2–8.9) in patients that did not miss the doses and 9.3 % (7.6–10.8) in patients that missed doses several times per month and more often ($p = 0.026$). Moreover, there was a correlation revealed between the baseline level of HbA1c and the possibility to buy drugs. In the group of patients who had financial difficulties in buying even a part of the drugs, the level of HbA1c was 9.7 % (8.9–10.5), while in patients who did not have financial difficulties, the level of HbA1c was 8.8 % (7.8–9.8), ($p = 0.036$). Similar differences remained after 24 weeks of the therapy: 9.5% (8.7–10.3) and 7.9 (6.8–8.7), respectively ($p = 0.027$). The lipid profile evaluation showed that the baseline level of LDL was 3.9 mmol/L (2.7–4.7) in the group of patients who had financial difficulties when purchasing drugs, and 2.8 mmol/L (2.2–3.3) in patients who had none.

The quality of life self-estimated by patients under the visual-analogue scale of EQ-5D inventory positively correlated with the total score according to the MMSE inventory ($p = 0.002$; $r = 0.69$). At the same time, the quality of life in patients who had no financial difficulties was significantly higher (62.5 (48.4; 75.3)) than in patients who had those difficulties (43.9 (30.1; 59.7)), ($p < 0.001$). In turn, a negative correlation was obtained between the level of HbA1c and the data obtained from EQ-5D inventory ($p < 0.001$; $r = 0.51$). The analysis of the results obtained from HADS inventory revealed a positive correlation between the level of anxiety and HbA1c ($p < 0.001$; $r = 0.51$). A weak but statistically significant positive correlation was found between the level of depression under the HADS scale and the level of HbA1c ($p = 0.016$; $r = 0.31$).

Empagliflozin therapy was discontinued in 39 patients (38.2%) due to the following reasons: cost of the drug (17 patients, 16.6%), adverse effects (11 patients, 10.7%), lack of effectiveness of the therapy as an improvement of the glycemic control (8 patients, 7.8%), other reasons (3 patients, 2.9%). Among patients that discontinued therapy because of financial difficulties, 70% stopped purchasing the drug within the first

90 days of the therapy. The most common adverse effects were urogenital conditions (15.7% of them were registered in female patients – 76.4% of all urogenital infections), mild hypoglycemia (8.8%), and hypotension (5.8%). In 4 patients, the recurrence of urogenital infections (more than 1 time) was observed. Discontinuation of therapy because of recurrent vulvovaginitis was required in 2 female patients.

DISCUSSION

Poor adherence to treatment was associated with a number of factors that can be grouped in social-economic, therapy-associated, and medical personnel-associated factors [21]. Many of these factors can be interconnected, which makes it difficult to identify the main reasons for failure to adhere to the treatment. For example, patients may report that they simply forgot to take the drug because of "being busy" with their everyday life when, in reality, they lack the motivation to take the drug. This can be associated with failure to understand its importance, concern about unfavorable events, and lack of a possibility to purchase it or any other reason that overweighs the benefits that, in their mind, the therapy would bring. Even the fact of taking the drug on a daily basis can negatively affect adherence since it reminds the patients that they are sick [22].

Since the patients primarily reported good connections with their physician and the study did not include interviewing the medical personnel, it was impossible to evaluate the impact of medical personnel on the adherence to treatment. One of the studies conducted in northern California that included 9 thousand patients showed that patients who had a lower level of trust to their doctor were found to have poorer adherence to the treatment [23]. Some studies showed that there were medical personnel-associated factors that decreased the effectiveness of the treatment as the lack of involvement of patients into making decisions during the therapy and lack of understanding of issues that might arise during the therapy. The results of the present study showed that only 34.3% of doctors discussed financial aspects of the treatment with their patients. Besides, such factors as openness, emotional support, clear and complete information, and possibility to ask questions contribute to the establishment of a trustful relationship between doctors and patients [24].

Social and economic factors influence the adherence to therapy by patients with chronic diseases, including diabetes mellitus. A recent study (a telephone survey) was aimed at evaluating the rate of refusals to continue the treatment with anti-hyperglycemic drugs. Around 16% out of 1200 patients with DM2 reported on the discontinuation of therapy because of the cost of the drugs (patients were not divided into groups) [25]. Meanwhile, the cost of different drugs varies widely and patients with financial difficulties refuse expensive therapy more often. In this study, the authors evaluated the impact of this factor on the adherence to the therapy with empagliflozin. The rate of refusal because of financial issues was 16.6%, which corresponded to a moderate rate. At the same time, patients who had financial difficulties when purchasing drugs had significantly higher levels of glycated hemoglobin both in the beginning of the study and after 24 weeks of empagliflozin therapy. They also had higher levels of LDL and worse quality of life. The obtained results show that the cost of drugs is an important factor that negatively affects the adherence to the treatment.

The complexity of the drug regimen and high daily rate of drug intake also determine the adherence to the therapy. Several studies described the influence of the rate of dosing on adherence to the treatment recommendations [22, 26]. The analysis of the data of patients with atrial fibrillation and arterial hypertension showed that the adherence to the therapy in patients with single daily administration of a drug was 26% higher than in patients that had to take a drug twice a day [26]. This data indicates a significant impact of the drug regimen on the adherence to the therapy in patients with diabetes mellitus and other chronic diseases. This study did not evaluate the dose regimen because empagliflozin is always administered once a day and this can positively influence the adherence to the treatment.

The tolerance and safety of drugs also influence the adherence to the treatment. The influence of adverse events provoked by anti-hyperglycemic drugs on the adherence to the treatment was evaluated by RECAP-DM [25]. The study included 1709 patients that received monotherapy with metformin as well as sulphonylurea or thiazolidinedione-containing drugs. The study results showed that patients with hypoglycemic episodes missed the doses of the drug and discontinued the therapy more often. The patients assessed the effectiveness, convenience, and satisfaction with the treatment significantly lower than patients that did not have any hypoglycemic conditions. The results of this study show that hypoglycemic conditions in patients who received empagliflozin were observed in 8.8% of patients. All those patients received

insulin therapy and needed dose correction of insulin after the beginning of empagliflozin therapy. Still, the development of hypoglycemic conditions did not lead to discontinuation of the therapy. Urogenital infection was the most common adverse effect (15.7% of patients). In clinical studies, the morbidity rate with genital infections in patients who received empagliflozin was 5% in comparison with the placebo group (1%). The incidence rate of urinary tract infection in the groups that received empagliflozin 10 and 25 mg and the placebo group was similar (9.8%, 10.4%, 9.3%) [27]. The present study revealed a higher rate of urogenital infections in comparison with a randomized controlled study. This can be explained by the fact that only 64.5% of attending physicians told the patients about possible adverse effects and advised on how to prevent them. Among patients who discontinued the therapy because of adverse effects, 72.7% of patients stopped taking the drug after a single event of urogenital infection, although it is recommended to discontinue the therapy with SGCI2 only in case of recurrent urogenital infection.

Apart from social and economic factors, specifics of therapy and factors associated with attending physicians and individual characteristics of patients also have an impact on the low level of adherence to the treatment. Some authors see a low level of awareness as a potential barrier for optimal adherence to the therapy. A survey conducted among 405 patients with DM2 showed that patients with a high level of awareness and strong belief that anti-hyperglycemic drugs are necessary had better adherence to the therapy [28]. And vice versa, patients with a high level of concern about adverse events showed a lower level of adherence to the therapy. In this study, less than a half of the patients were aware of their level of glycated hemoglobin before being included into the study. It should be mentioned that cognitive condition of patients also influenced the adherence to the therapy and the quality of life. Patients without cognitive disorders had lower levels of glycated hemoglobin in comparison with patients with mild dementia. The MMSE score positively correlated with the quality of life according to patients' self-estimation. Depression can be one of comorbid chronic diseases associated with the level of adherence to the therapy. Symptoms of depression were associated with a lower level of adherence in one of the studies [29], which agrees with the results obtained by the authors. Furthermore, it was shown that the level of anxiety and depression (HADS inventory) positively correlated with the level of glycated hemoglobin, which can prove the influence of psychological welfare on adherence to the therapy. There are also other factors that influence the adherence to the therapy, such as alcohol abuse [30] and severity of comorbid diseases [31], but they were not evaluated in this study.

The authors obtained data on adherence to SGCI2 (empagliflozin) therapy in clinical practice. The main reasons that influence the duration of the treatment were financial difficulties, development of adverse effects, and lack of effectiveness of the therapy. The possibility for purchasing the required drug in a volume needed for the therapy was associated with better glycemic control and better quality of life. Among factors that influence the adherence to the therapy, a decrease in the quality of cognitive functions plays an important role. Preventive measures aimed at the maintenance of the cognitive status of patients with chronic diseases, including DM2, are important to improve the adherence to the therapy. At the same time, the obtained data indicates that the psychological status of the patient should be taken into account when evaluating the adherence to the therapy. Therefore, psychotherapy may have a positive influence on adherence to the recommended treatment.

CONCLUSION

Low adherence to treatment is a crucial issue for patients with chronic diseases like diabetes mellitus. Understanding the factors associated with the failure to adhere to recommendations can help resolve this issue while improving the adherence to the therapy reduces long-term negative consequences in patients with diabetes mellitus. All the factors that influence the adherence can be divided into those that can be corrected (awareness, adverse events) and those that cannot be corrected (cognitive condition, comorbid diseases). When choosing the therapy, doctors should work with factors that can be corrected: educate and instruct patients and discuss financial aspects and adverse events, as well as ways to prevent and treat them. Wide implementation of the above-mentioned inventories can help significantly when evaluating the risk factors of the low level of adherence and developing individual measures to improve it. At the same time, many factors that cannot be corrected tend to have a different nature in the beginning of the therapy. To improve the interaction between doctors and patients, teaching doctors how to communicate with patients and take action to improve the adherence to the therapy should become compulsory in modern

medical education. Patients with diabetes mellitus meet numerous barriers trying to adhere to the therapy, therefore, the researchers need more data to systematize all the factors that influence the adherence and to develop practical advice on how to improve the adherence to the treatment.

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Age-specific comparison of some morphological parameters of the proximal phalanges of the hand in male children and adolescents from Tajikistan and Western India

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ABSTRACT

The aim of the study was (a) to establish ethnicity-specific differences in such morphological parameters of the proximal phalanges (PP) as the bone length and the width of diaphysis in male children and adolescents from Tajikistan and Western India and (b) to develop regression equations for determining their age based on the size of the PP.

Materials and methods. Three hundred and sixty-two X-ray images of the right hand of male subjects were examined. All subjects originated from Tajikistan and Mumbai, India, and aged from 6 to 17 years. The relationship between the subjects' age and the length of the PP (LPP) and the width of the diaphysis of the PP (WPP) was investigated using a simple linear regression and correlation analysis. The LPP and WPP dependence on age was determined using one-way ANOVA and the Kruskal–Wallis test followed by post-hoc analysis by age groups.

Results. LPP and WPP of the subjects from Tajikistan and India correlated with age, with the correlation coefficient exceeding 0.5. In both ethnic groups, the correlation coefficients for LPP vs. age was greater than 0.8. The correlation coefficient for WPP vs age ranged from 0.68 to 0.77 in Tajiks and from 0.58 to 0.69 in Indians. Simple linear regression models were developed to predict the age from LPP ($R^2 > 0.6$), except for LPP 5 for Tajiks. The LPP 5 values in Tajiks and the WPP values in both ethnic groups showed weak R^2, which ranged from 0.35 to 0.53. Eleven significant differences were identified between the ethnic groups of the same age with respect to LPP and WPP.

Conclusion. PP length was a better age predictor than the diaphysis width. The most reliable predictor for both ethnic groups was LPP 2. The PP parameters did not change uniformly over time. The PP demonstrated especially intensive growth between 12 and 15 years. The most significant differences in LPP and WPP between two ethnic groups were found for the ages of 8 and 15–16 years, with LPP and WPP in Tajiks exceeding those in Indians.

Key words: hand, phalanges, identification from bones, age estimation, ethnic features.

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Conformity with the principles of ethics. Parents of minors signed an informed consent for the use of the obtained data in the research work. The study was approved by the local Ethics Committee of the Pavlov First St.-Petersburg State Medical University.

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Сравнительная оценка некоторых линейных параметров проксимальных фаланг кисти у детей и подростков мужского пола Таджикистана и Западной Индии в возрастном аспекте

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РЕЗЮМЕ

Цель. Установить этнические различия в линейных параметрах длины проксимальных фаланг (ПФ) и ширины диафизов ПФ у детей и подростков мужского пола Таджикистана и Западной Индии. Разработать регрессионные уравнения для определения возраста по размерам ПФ.

Материалы и методы. Исследовано 366 рентгенограмм правой кисти лиц мужского пола Таджикистана (г. Канибадам) и Западной Индии (г. Мумбаи) 6–17 лет. Зависимость возраста от длины ПФ (ДлПФ) и ширины диафиза ПФ (ШПФ) изучалась с помощью парного линейного регрессионного анализа и корреляционного анализа. Влияние возраста на ДлПФ, ШПФ определялось с помощью однофакторного дисперсионного анализа, критерия Краскела – Уоллиса и последующего post-hoc анализа по возрастам.

Результаты. ДлПФ и ШПФ представителей Таджикистана и Индии коррелируют с возрастом, коэффициент корреляции больше 0,5. В обеих этнических группах коэффициенты корреляции между возрастом и ДлПФ превышали 0,8. Коэффициент корреляции возраста и ШПФ у таджиков колеблется в диапазоне 0,68–0,77, у индийцев – 0,58–0,69. Построены парные линейные регрессионные модели для прогноза возраста по ДлПФ с коэффициентом детерминации R^2, большим 0,6, за исключением ДлПФ5 для таджиков. ДлПФ5 для таджиков и значения ШПФ для обеих этнических групп показали слабые R^2 (0,35–0,53). Найдено 11 значимых различий между равновозрастными этническими группами по ДлПФ и ШПФ.

Заключение. ДлПФ является лучшим предиктором возраста, чем ширина диафиза. Самым надежным предиктором для обеих национальностей является ДлПФ2. Увеличение параметров ПФ происходит неравномерно с возрастом. Интенсивный рост ПФ наблюдается преимущественно в интервале 12–15 лет. Больше всего значимых отличий ДлПФ и ШПФ между этническими группами найдено в 8 и 15–16 лет, при этом ДлПФ и ШПФ таджиков превышали индийские.

Ключевые слова: кисть, фаланги, идентификация по костям, определение возраста, этнические особенности.

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

Источник финансирования. Автор заявляет об отсутствии финансирования.

Соответствие принципам этики. Родители несовершеннолетних подписывали информированное согласие на использование полученных данных в исследовательской работе. Исследование одобрено локальным этическим комитетом ПСПбГМУ.

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INTRODUCTION

The study of the human skeleton with respect to ethnicity and origin-specific features remains an important research area. We need to accumulate data on bone growth patterns in people of different origins because, due to the considerable increase in migration observed over the past decades, more and more people arrive from countries with different climates. The study of bone growth in people coming from other climate zones and areas with a different population structure is of considerable interest to morphologists, as well as pediatricians, traumatologists and forensic experts [1–3]. The need to identify a victim, i.e. to

establish their gender and age, sometimes using only bones or even just bone fragments, arises after industrial and natural disasters with mass casualties, as well as in criminal cases. X-ray is commonly used for identifying badly damaged remains [4]. When analyzing X-ray images, one should remember that the pattern of bone formation and growth may vary in people originating from different countries and even different areas of the same country. Possible reasons responsible for such discrepancies include climatic factors, ethnic characteristics, environmental and geographical factors, which change over time [5]. In developing countries, such as India, age estimation is an important task because illiterate people may not keep proper birth records [6]. In South Asia, up to 65% of children under five years old do not have their birth registered [7]. Regression equations for the single-bone age estimation yield much better results, i.e. closer to real values, if the estimation takes population-specific characteristics into account [8]. Errors are likely to occur if a person is being identified from bones without population-specific morphologic parameters being considered [9]. For instance, a study of the size of the second metacarpal bone in Guamanians and white Americans revealed a difference in the length of this bone between the compared groups [10]. Data collected by the Institute of Demography of the National Research University Higher School of Economics show that during the period from January to July 2018 Tajikistan contributed the most to the Russian Federation's net migration gain [11]. People from Tajikistan come to the Russian Federation together with their children of preschool and school age. This fact provides the rationale for deepening our knowledge on various body structures of Tajik children and their ethnic-specific characteristics. S.S. Mirzoev points towards the ethnic specificity of Tajiks, their specific genotypic and phenotypic features [12]. Osteological studies of people from Tajikistan were carried out as far back as the 1960s [13], so it has been a long time since those results were obtained. The literature search among available sources showed works neither on the growth patterns of the proximal phalanges (PP) of the hand of men from Tajikistan, nor on the approaches for estimating their age from the size of the PP.

The aim of this study was (*a*) to establish ethnicity-specific differences in such morphological parameters of the proximal phalanges as the bone length and the width of the diaphysis in male children and adolescents from Tajikistan and Western India and (*b*) to develop regression equations for determining their age based on the size of the PP.

MATERIALS AND METHODS

To identify ethnicity-specific characteristics in the morphological parameters of the PP, a comparison was performed among 115 Tajik boys and adolescents aged 6 to 17 years from Konibodom (Tajikistan), which is an area with a continental subtropical climate and a continental mild climate, and 251 boys and adolescents from Mumbai (Western India), which is an area with a tropical monsoon climate. In Tajikistan, the study was conducted in Konibodom, inhabited by 96% of the indigenous Tajik people (Viloyati), which belong to the ethnic group of Tajiks of the cities and oases [14]. In India, the study was conducted in Mumbai, whose inhabitants represent the Indo-Arabian ethnic group. All the X-ray images were obtained in the presence of the author during his trips to India and Tajikistan. Data on the age distribution are presented in Table 1.

Table 1

Distribution of the examined male children and adolescents between the geographical regions and among the age groups

Region	Age, years											
	6	7	8	9	10	11	12	13	14	15	16	17
Western India	14	14	10	10	17	20	27	36	25	37	22	19
Tajikistan	5	10	9	8	10	10	11	13	7	15	7	10

X-ray examinations were performed in patients with suspected fractures and in apparently healthy children who complained of pain in the joints of the hand. The study included the images of the right hand obtained from children and adolescents who did not have any skeletal disorders. Parents of the underage subjects signed letters of informed consent for the use of the obtained data in the research work.

X-ray images were obtained at a 60-cm distance from the anode area of the X-ray tube to the film. The length of the PP and the width of the PP diaphysis (at the middle) were measured in the X-ray images of the hand using a sliding caliper with an accuracy of 0.05 mm. The length of each PP was measured from the middle of the semilunar contour of their base to the very distal contour of the head.

The study data were processed statistically. Regression analysis was performed to determine the relationship between the age of the study subjects and the morphological parameters of their PP. The quality of the regression model was evaluated using the determination coefficient R^2 and the overall significance assessed by the *F*-test. The residual normality was assessed using the Kolmogorov-Smirnov normality test. The residuals were tested for having expectation zero using the one-sample t-test. For normally distributed data, the relationship between the age of the examined children and the length of the PP and the width of the PP diaphysis was assessed using Pearson's correlation coefficient (*r*). The Spearman rank-order correlation coefficient (*rs*) was used for non-normally distributed data. The significance of the correlation coefficients was assessed using Student's t-test. The Shapiro–Wilk normality test was used for small samples of the PP length and diaphysis width values within each age group.

The age-specific comparison of the PP length and diaphysis width was carried out using either a parametric analysis (one-way analysis of variance, or one-way ANOVA) or a non-parametric analysis (Kruskal – Wallis test, or *H*-test), depending on the data distribution in a given age group. Further post-hoc pairwise comparisons between the age groups were carried out using the Student's *t*-test after one-way ANOVA or the Mann – Whitney *U*-test if the Kruskal – Wallis *H*-test had been previously used. In both cases, multiple comparisons were processed using the Benjamini – Hochberg procedure (false discovery rate, or FDR). The morphological parameters of the PP in children and adolescents of the same age from Tajikistan versus Western India were also compared using *t*-test for normal distribution and *U*-test for non-normal distribution.

Descriptive statistics were given as *M (SD)* for normally distributed PP length and diaphysis width values and as $Me(Q_1–Q_3)$ for non-normally distributed values, where *M* is the mean, *SD* is the standard deviation, *Me* is the median, Q_1 and Q_3 are the first and third quartiles, respectively. The threshold for statistical significance was set at 0.05 for all hypothesis testing criteria.

RESULTS AND DISCUSSION

An age-specific comparison of the length of the PP and the width of the PP diaphysis was performed in male subjects from Tajikistan and Mumbai. In this paper, LPPX refers to the length of a proximal phalanx and WPPX refers to the width of the diaphysis of a proximal phalanx, with X being the finger number. Study data are presented in Figures 1 and 2 for India and Tajikistan, respectively. The interquartile ranges show that the PP length values in the subjects from both ethnic groups had a smaller dispersion compared to the PP diaphysis width values.

The PP length values in the subjects from both ethnic groups have a smaller dispersion compared to the PP diaphysis width values (Fig. 1, 2).

The PP length and diaphysis width did not grow uniformly over time (all significant results were obtained using the Benjamini – Hochberg procedure [FDR correction]). The growth periods were as follows:

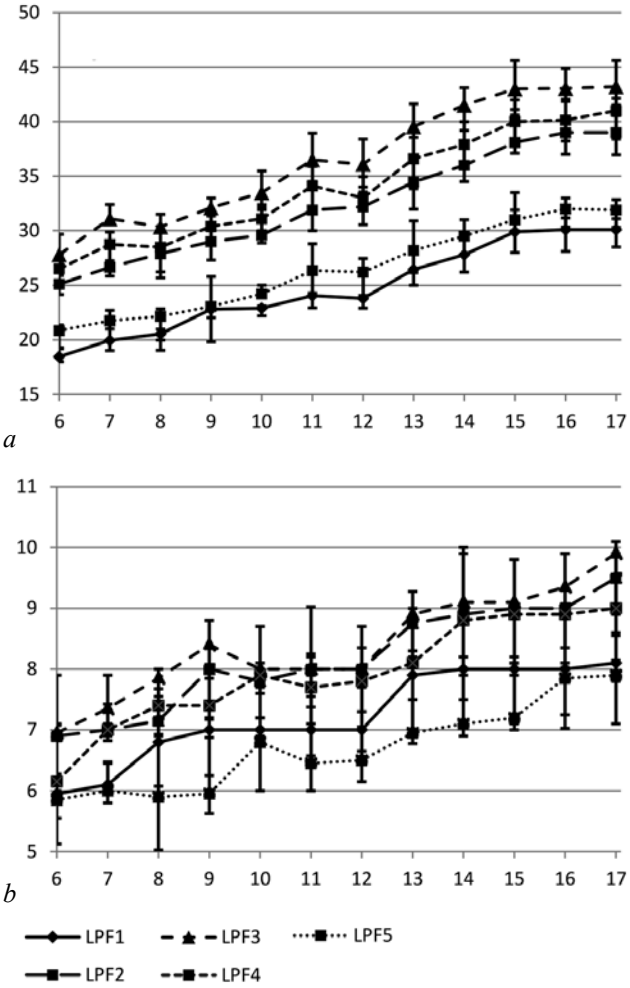

Fig. 1. Changes in morphological parameters of the proximal phalanges of the hand in boys and adolescents from Western India: *a* – length of a proximal phalanx (LPP); *b* – width of the diaphysis of a proximal phalanx (WPP), mm. The horizontal axis shows age (years); the vertical axis shows morphological parameters of the bones (mm). The curve represents median values; the whiskers show the first and third quartiles. PP1 to 5 denote proximal phalanges of the 1st to 5th finger.

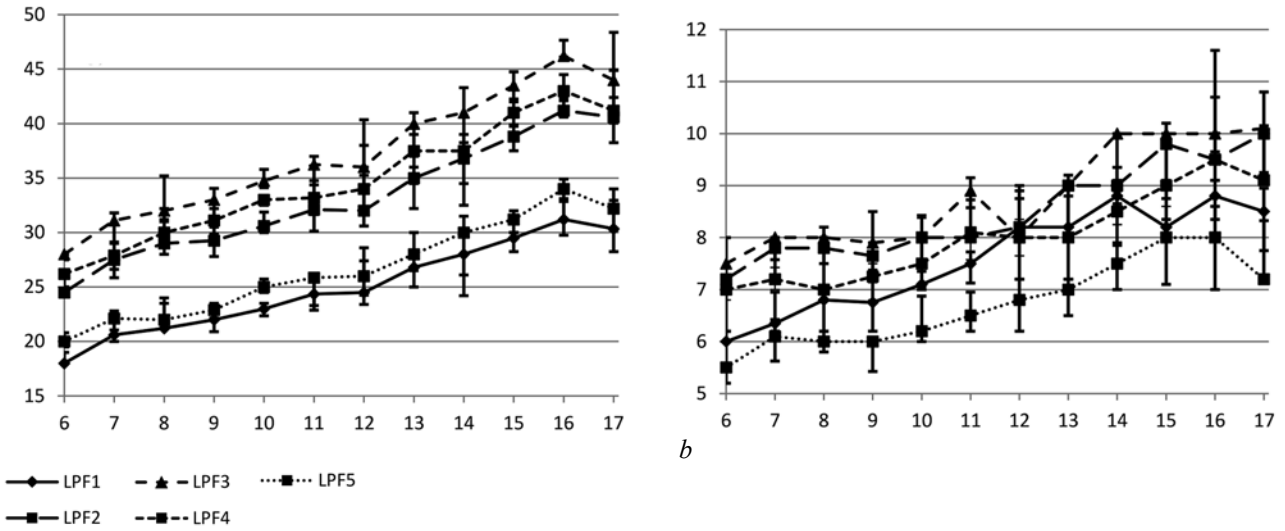

Fig. 2. Changes in morphological parameters of the proximal phalanges of the hand in boys and adolescents from Tajikistan: *a* – length of a proximal phalanx (LPP); *b* – width of the diaphysis of a proximal phalanx (WPP), mm. The horizontal axis shows age (years); the vertical axis shows morphological parameters of the bones (mm). The curve represents median values; the whiskers show the first and third quartiles. PP1 to 5 denote proximal phalanges of the 1st to 5th finger.

– Indian subjects from Mumbai, PP length. LPP1, LPP2, LPP3, LPP4: 6–7, 12–13 and 14–15 years; LPP4 also 8–9 years; LPP5: 10–11, 12–13, 14–15 years;

– Indian subjects from Mumbai, PP diaphysis width. WPP1 to 5: 12–13 years;

– Tajik subjects, PP length. LPP1: 14–15 years; LPP2: 6–7, 7–8, 15–16 years; LPP3, LPP5: 9–10, 15–16 years; LPP4: 9–10, 14–15, 15–16 years;

– Tajik subjects, PP diaphysis width: no significant differences between the adjacent age groups.

The Kruskal – Wallis *H*-test and ANOVA showed a significant increase in the studied morphological parameters of the PP with age. However, the post-hoc analysis revealed that the statistically significant difference in the morphological parameters assessed between age pairs was greater for the PP length compared to the diaphysis width both in Indians (by 20.6% on the average) and Tajiks (by 32.9%) (Table 2).

The correlation analysis of individual osteometric parameters of the PP of the hand was performed using the Spearman rank-order correlation.

Table 2

Changes in LPP and WPP with respect to age and post-hoc analysis results

Parameter	Mumbai (India)			Tajikistan		
	Multiple and pairwise comparison	*p*-value	Number of pairwise differences between age groups	Multiple and pairwise comparison	*p*-value	Number of pairwise differences between age groups
LPP1	H-test, U-test	< 0.001	54	one-way ANOVA, Tukey's test	< 0.001	37
LPP2	H-test, U-test	< 0.001	56	H-test, U-test	< 0.001	50
LPP3	H-test, U-test	< 0.001	55	H-test, U-test	< 0.001	50
LPP4	H-test, U-test	< 0.001	57	H-test, U-test	< 0.001	53
LPP5	H-test, U-test	< 0.001	55	H-test, U-test	< 0.001	44
WPP1	H-test, U-test	< 0.001	42	H-test, U-test	< 0.001	34
WPP2	H-test, U-test	< 0.001	43	H-test, U-test	< 0.001	37
WPP3	H-test, U-test	< 0.001	45	one-way ANOVA, Tukey's test	< 0.001	24
WPP4	H-test, U-test	< 0.001	42	H-test, U-test	< 0.001	35
WPP5	H-test, U-test	< 0.001	48	H-test, U-test	< 0.001	27

Note. LPP1–5: length of the first to fifth proximal phalanges of the hand; WPP1–5: width of the diaphysis of the proximal phalanges of the hand; *H*-test: Kruskal – Wallis test; *U*-test: Mann – Whitney test; one-way ANOVA: one-way analysis of variance.

Linear regression equations were developed to be used as a method for determining the age of male subjects in Tajikistan and Western India.

If all the bones of the hand are available, multiple linear regression is the best method for age estimation. If only separate bones are available for testing, a single bone can be used to create the regression equation [8].

The regression models were developed during the correlation and regression analysis that can be used to estimate the age of 6 to 17-year-old boys and adolescents from Mumbai and the indigenous Tajik people (Viloyati) (Table 3).

The length of the PP in boys and adolescents from Tajikistan and Western India showed a closer correlation with age than the width of the PP diaphysis (Table 3). All rank correlation coefficients for age versus bone length exceed 0.8. In contrast, the correlation coefficients for the PP diaphysis width ranged from 0.58 to 0.75 in both study groups. It was found that nine out of ten correlation coefficients for Tajik boys and adolescents exceeded those of Indians by an average of 8%.

Table 3

Regression models for the age estimation of 6 to 17-year-old children and adolescents in Mumbai (India) and Tajikistan developed using data on the length of the PP and the width of the PP diaphysis of the hand

Parameter	Regression model	*F*-test, *p*-value	Adjusted R^2	Correlation coefficient *rs*	Applicability of the model*
Mumbai (India)					
LPP1	Age = – 3.049 + 0.596 × LPP1	< 0.001	0.682	0.820	yes
LPP2	Age = – 5.538 + 0.529 × LPP2	< 0.001	0.718	0.827	yes
LPP3	Age = – 5.327 + 0.466 × LPP3	< 0001	0.712	0.822	yes
LPP4	Age = – 5.518 + 0.507 × LPP4	< 0.001	0.705	0.822	yes
LPP5	Age = – 3.681 + 0.583 × LPP5	< 0.001	0.659	0.814	yes
Tajikistan					
LPP1	Age = – 4.576 + 0.645 × LPP1	< 0.001	0.706	0.848	yes
LPP2	Age = – 5.401 + 0.516 × LPP2	< 0.001	0.745	0.874	yes
LPP3	Age = – 3.457 + 0.404 × LPP3	< 0.001	0.664	0.879	yes
LPP4	Age = – 4.213 + 0.454 × LPP4	< 0.001	0.6915	0.879	yes

Note. LPP1–5: length of PP 1 to 5; age is given in years.
*The practical applicability of the model.
All the correlation coefficients shown in Table 3 are statistically significant. All the residuals follow a normal distribution and have zero expectation.

M.A.Grigoryeva, E.S.Anushkina [15] point out that the choice of the optimal regression model may be complicated even if the object is not damaged. Mathematical models yield the most accurate results when the proportional composition of the studied population resembles as much as possible the composition of the population used to develop the equations [5].

Although multivariate tests showed a significant increase in the PP length and diaphysis width with age, and the correlation analysis confirmed these relationships for all the morphological parameters studied, well-fitting regression models ($R^2 > 0.6$) could be developed only for the length of PP1 to 4.

The models developed for the age estimation from the length of the PP in boys and adolescents from Western India were of approximately the same quality: the determination coefficients ranged from 0.66 (PP5) to 0.72 (PP2). The best regression model for the age estimation in Indian boys (Mumbai) was developed using LPP2 as the independent variable ($p < 0.001$).

LPP2 was also the best predictor of age in boys and adolescents from Tajikistan. The determination coefficient of the LPP2 model was 0.75 ($p < 0.001$) and it significantly exceeded the coefficients for other models.

When comparing the regression models developed for the two ethnic groups studied, it was found that the length of the PP between 6 and 17 years grew faster in Tajik boys and adolescents, whose multipliers of the independent variables exceeded those in the Indian subjects of the same age in four of the five models (PP2-4) (Table 3). The growth in width of PP1 to PP3 diaphysis in children and adolescents of Tajikistan also exceeded that in the Indian subjects of the same age. However, the diaphysis width of PP4 and PP5 grew faster in the Indian subjects from Mumbai.

A comparative analysis of the PP sizes in boys from Western India and Tajikistan revealed several differences (Table 4).

Table 4

Age-specific differences in the morphological parameters of PP between two ethnic groups

No.	Parameter	Age, years	P-value	Mumbai (India)	Tajikistan
1	LPP1	8	0.005	20.6 (19.0 21.0)	22.2 (1.9)
2	LPP2	8	0.021	27.2 (1.7)	29.4 (2.0)
3	LPP3	8	0.029	30.1 (2.0)	32.6 (2.4)
4	LPP4	8	0.022	28.0 (2.1)	30.5 (2.2)
5	WPP1	12	0.020	7.1 (0.9)	8.1 (1.2)
6	WPP2	15	0.040	9.0 (8.1 9.8)	9.5 (8.8 10.0)
7	WPP4	15	0.027	8.9 (8.1 9.0)	9.2 (0.9)
8	LPP2	16	0.021	38.6 (2.3)	40.9 (1.9)
9	LPP3	16	0.002	43.3 (2.3)	46.6 (1.8)
10	LPP4	16	0.005	40.2 (2.2)	43.2 (2.0)
11	WPP4	16	0.023	8.7 (0.9)	9.9 (1.1)

LPP1–4: length of the proximal phalanges of the first to fourth fingers; WPP1–4: diaphysis width of the proximal phalanges of the first to fourth fingers.

Eight-year-old Tajik boys had longer PP of fingers 1, 2, 3, 4 compared to the Indian boys of the same age. In the age range between 9 and 14 years, no significant differences in the studied morphological parameters of the PP were found, except for WPP1, which was significantly greater in the subjects from Tajikistan. The analysis of X-ray images showed that the width of the PP diaphysis in the first and second fingers was significantly greater in 15-year-old boys from Tajikistan compared to the Indian boys of the same age. Also, the length of PP2, PP3, and PP4, as well as the diaphysis width of PP4, were greater in 16-year-old subjects from Tajikistan versus 16-year-old Indian boys.

CONCLUSION

The most accurate estimation of the age of children and adolescents between 6 and 17 years is achieved when a researcher uses data from the same ethnic group. The bone length is the better predictor of the age of Tajik and Indian (Mumbai) children and adolescents than the diaphysis width if the age is estimated based on the size of the proximal phalanges of the hand. The length of the proximal phalanx of the second finger is the most reliable predictor of age for both ethnic groups. An osteometric study conducted in boys and adolescents aged between 6 and 17 years revealed that the proximal phalanges of the hand did not grow uniformly over time. The PP demonstrated intensive growth between 12 and 15 years. Most of the significant between-group differences in the length and diaphysis width were found for the ages of 8 and 15–16 years, with the greater length and width values observed in Tajiks compared to the Indian (Mumbai) subjects of the same age.

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Late results and health-related quality of life in patients after endovascular treatment for multiple intracranial aneurysms

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ABSTRACT

Aim. To assess the results of endovascular treatment in patients with multiple intracranial aneurysms (MIA) in the late postoperative period according to health-related quality of life (HRQoL) concept.

Materials and methods. 172 cases of patients having undergone endovascular MIA repair were examined. The evaluation of patient health-related quality of life was carried out using the SF-36 (The Short Form (*36*) Health Survey), the ICF (the International Classification of Functioning), and the modified Rankin Scale (mRS).

Results. The complication of subarachnoid hemorrhage (*SAH*) appears in approximately 1,2% of cases in the late postoperative period. When assessing the health-related quality of life according to the SF-36 domains in patients with Subarachnoid hemorrhage (SAH), the QoL showed a decrease in "Social Functioning" ($p = 0.03$). In patients with pseudotumor cerebri (PTC) a decrease was seen in "Role-Physical Functioning" (RP) ($p = 0.004$), while "General Health" (GH) ($p = 0.049$), "Social Functioning" (SF) ($p = 0.005$) and "Mental Health" (MH) ($p = 0.009$) subscales also saw decreases.

Having more than two inpatient surgical procedures is also associated with the health-related quality of life of patients ($p < 0.05$). Assessment of activity with ICF showed the *intensity* of irregularities on the d4501 domain – "walking short distance" – depended on the existing SAH ($p < 0.05$). Procedural complications affected the *patient's* daily *activities* on the domains d4501 – "walking long distance" ($p = 0.03$), and d640 – "doing household chores" ($p = 0.01$).

Conclusion. The assessment with ICF allows the specification of patient activity and participation in public life. The SF-36 scale provides additional information on the patients' subjective perception of their condition. Considering the quality of life in the late postoperative period is not completely restored in all patients, ongoing rehabilitation measures, diagnostic cerebral angiographies and improvements in the surgery techniques are required.

Key words: cerebral aneurysms, multiple aneurysms, postoperative period, long-term results, endovascular treatment, quality of life.

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Отдаленные результаты и качество жизни у пациентов с множественными церебральными аневризмами после эндоваскулярного лечения

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РЕЗЮМЕ

Цель. Оценить результаты лечения больных после эндоваскулярных вмешательств по поводу множественных церебральных аневризм (МнА) в отдаленном послеоперационном периоде с учетом качества жизни пациентов.

Материалы и методы. Проанализированы данные 172 наблюдений с МнА, оперированных эндоваскулярно. Исследование качества жизни производилось с использованием общего опросника The Short Form-36 (SF-36), Международной классификации функционирования (МКФ), шкалы Рэнкина.

Результаты. В отдаленном послеоперационном периоде субарахноидальное кровоизлияние (САК) отмечено в 1,2% случаев. При оценке по шкале SF-36 у больных, перенесших в анамнезе САК, качество жизни снижалось по субшкале «Социальное функционирование» ($p = 0,03$), у больных с псевдотуморозным типом течения – по субшкалам «Ролевое функционирование, обусловленное физическим состоянием» (RP) ($p = 0,004$), «Общее состояние здоровья» (GH) ($p = 0,049$), «Социальное функционирование» (SF) ($p = 0,005$), «Психическое здоровье» (MH) ($p = 0,009$). Число оперативных вмешательств >2 также ассоциировано с качеством жизни пациентов ($p < 0,05$). При оценке активности по МКФ выраженность нарушений по домену d4501 – ходьба на близкие расстояния – зависела от перенесенного САК ($p < 0,05$). Осложнения эндоваскулярного лечения оказывали влияние на активность пациентов по доменам d4501 – ходьба на дальние расстояния ($p = 0,03$), d640 – выполнение работы по дому ($p = 0,01$).

Заключение. Уточнить, каковы активность и участие в общественной жизни пациента, позволяет проведение оценки по МКФ. Дополнительную информацию о субъективном восприятии пациента своего состояния позволяет получить шкала SF-36. Учитывая, что качество жизни в отдаленном послеоперационном периоде не у всех пациентов восстанавливается полностью, возникает необходимость в дальнейшем проведении реабилитационных мероприятий, контрольных церебральных ангиографий, а также в усовершенствовании методов хирургического лечения.

Ключевые слова: артериальные аневризмы, множественные церебральные аневризмы, отдаленные результаты, эндоваскулярное лечение, качество жизни, результаты эндоваскулярного лечения.

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

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INTRODUCTION

On average, cerebral arterial aneurysms (CAA) in a healthy adult population occur in approximately 3.2% of cases [1]. Multiple cerebral aneurysms (MCA) of blood vessels of the brain among CAA occur according to various sources of literature in 2–44.9% of cases [2–3]. The average prevalence of CAA is 20.1% [4]. CAA is the most common cause of subarachnoid hemorrhage (SAH) [5]. Almost 30% of monitored patients with MCA have SAH [6]. Case mortality rate from repeated CAA rupture is as high as 68–70% [7–8]. The choice of tactical decisions in surgical treatment in patients with MCA is personalized. It considers the size, shape and localization of CAA, the course of the disease, and the daily functioning of a patient. Treatment of MCA, as a rule, requires combined methods applied in several stages [9–10]. According to various sources, decrease in quality of life (QoL) occurs in one third of MCA survivors 1 year after SAH [11–13]. There are studies comparing QoL of patients according to the type of surgery (microsurgical and endovascular management) [14] that do not identify any differences. They, moreover, compare preoperative and postoperative QoL [15]. At the same time, the available literature is not yet sufficient to draw conclusions for the problem of QoL studying and clinical manifestations in patients after endovascular treatment of MIA.

Having several AAs and the need for repeated surgical interventions and angiographic control determine the relevance of the QoL examination in such patients [16]. The objective of the work is to evaluate the results of endovascular surgical treatment of patients with MIA in the late postoperative period, regarding the dynamics of neurological disorders and QoL.

MATERIALS AND METHODS

The information from 172 cases of MIA patients after endovascular repair was examined. These patients received endovascular therapy at the Department of Surgery for Brain and Spinal Cord Vascular Pathology at Polenov Neurosurgical Institute between 2012 and 2018. A comprehensive follow-up examination of patients, operational outcomes, and QoL after endovascular treatment in the long-term postoperative period was carried out.

The cases included adults aged 26–77, the median age (the sample mean +/- a margin of error ($M \pm m$), sample size $n = 172$)) was about 54.19 ± 0.83 years. 81.4% of patients were women (140/172), 18.6% were men (32/172). The male to female gender ratio was 1:4.4. A total of 172 patients were diagnosed with 441 MIA. Patients with 2 AAs accounted for 62.2% (107/172), 25.6% had 3 AAs (44/172), 7.5% had 4 (13/172), 3.5% had 5 (6/172), 0.6% had 6 (1/172), and 0.6% had 7 (1/172).

The size of the identified AA was of the following types: miliary (up to 3mm) (22.7%, 100/441), regular size (4–15 mm) (71.7%, 316/441), large (16–25 mm) (2.0% ,9/441), and giant (>25mm) (3.6%, 16/441). 50% of patients (86/172) had one or more SAHs in their history, 5.8% (10/172) of patients had AAs with prognosis of pseudotumor cerebri. All patients underwent 1 to 5 endovascular surgeries.

In general, 172 patients underwent 354 endovascular aneurysm repairs. One-stage operations were performed in 30.8% of patients (53/172). In 15.1% (8/53) of cases, AAs were totally excluded from the bloodstream within the one stage. In 41.5% of patients (22/53), AAs were shut down; however, there were miliary AA and/or aneurysmal expansion of the visceral patches, requiring intensive monitoring. 37.7% of patients (20/53) underwent the first step of surgical management from the planned multistage treatment. 5.7% of patients (3/53) are under the follow-up monitoring. 69.2% (119/172) of patients were treated with multistage surgery: all AAs were excluded from the bloodstream in 55.5% (66/119) of the patients, 44.5% (53/119) of the patients underwent two or more surgical procedures, however, reoperation is planned on other AAs (patients are at the stage of elective surgery for AA exclusion from the bloodstream). The evaluation of surgical treatment occurred over the following 6- to 24-month period. The follow-up was specified during repeated hospitalizations for control angiography or coronary intervention, outpatient visits and clarifying correspondence. Patients were asked to fill out a questionnaire designed for this purpose. The data of a standard diagnostic neurosurgical complex was also evaluated: examination by a neurologist, neuro-ophthalmologist and a therapist, MRI + MR angiography of the brain, CT scan of the brain, selective cerebral angiography, EEG. The research of QoL in the late postoperative period was carried out using the general SF-36 (The Short Form-36 Health Status Survey) questionnaire and ICF (International Classification of Functioning). The functional status of patients before and after surgery was evaluated using the modified Rankin scale (J. Rankin, 1957). Data were analyzed using Statistica software integrated for Windows; frequency indicators of the qualitative indi-

cators were compared using non-parametric statistical criteria (Pearson's chi-squared test (χ^2), Fisher's exact test). Comparison of frequency indicators was carried out using nonparametric statistics (Mann – Whitney *U*-test, Analysis of variance (ANOVA)). The assessment of the studied dynamics parameters after the received treatment was carried out using the Wilcoxon signed-rank test. The criterion for the level of statistical significance of differences was $p < 0.05$.

RESULTS

Procedural complications of endovascular treatment occurred in 3.4% (12/354) of patients. Coronary complications: in 9 of 12 cases, intraoperative rupture of AA in 3 of 12 cases. According to the follow-up data, in the late postoperative period, SAH was verified in 1.2% (2/172) of patents – rupture of unoperated AA; ischemic complications (with regard to self-discontinuation of antiplatelet therapy) in 1.2% (2/172) of patients, and stent migration in 0.6% (1/172) of cases. According to the control cerebral angiogram performed in the late postoperative period (between 6 months and 2 years after the surgery), the development of de novo AA or the unoperated AA growth occurred in 8.7% (15/172) of cases, AA recurrence or recanalization was found in 19.2% (33/172) of cases – 9.3% of operations performed (33/354). In 5.2% (9/172) of cases, control cerebral angiogram was not performed (it was refused by the patients).

There were the following focal neurologic complications in the late postoperative period: cranial nerve dysfunction – 6.9% (12/172), motor disorders – 5.2% (9/172), impaired sensitivity – 2.3% (4/172), impaired coordination – 2.9% (5/172), speech disorders – 2.3% (4/172), visual disturbances – 3.5% (6/172), cognitive impairment – 10.5% (18/172), symptomatic epilepsy – 6.4% (11/172). Focal neurological signs resulted from the experienced SAH, the pseudotumor cerebri, and intraoperative complications. Assessed dynamics of activities of daily living according to the modified Rankin scale before surgery and in the late postoperative period is presented in Fig. 1.

In the late postoperative period, the functional status of patients according to the Rankin scale was worsened in those who had past *SAH* ($p = 0.04$) and complications of surgical interventions ($p = 0.001$).

QoL scored on SF-36 subscales in the late postoperative period is presented in Table 1.

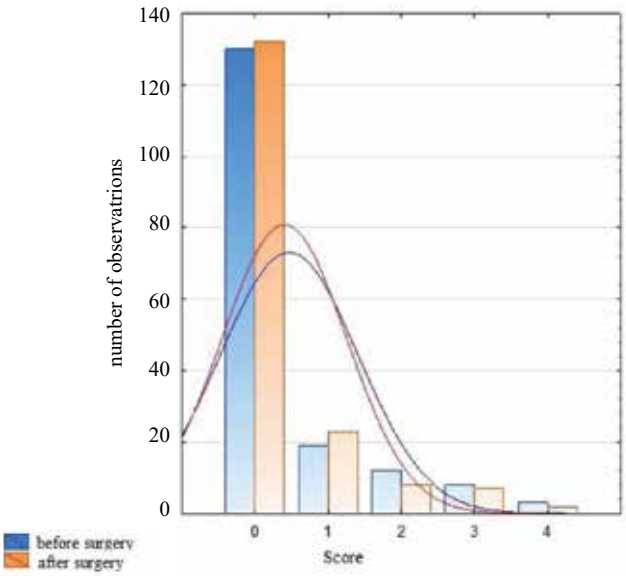

Fig. 1. Dynamics of daily activity assessment on the Rankin scale before surgery and in the late postoperative period

Table 1

Quality of life on SF-36 subscales in the late postoperative period

Subscales	*M* ± *SD*	*Min–Max*	Median, 25 and 75% quartiles (*Me (LQ;UQ)*)
PH (Physical Health)	44.91 ± 10.16	20.26–0.96	44.81 (38.64–55.05)
MH (Mental Health)	49.78 ± 9.76	20.54–61.53	51.74 (44.82–57.35)
PF (Physical Functioning)	75.15 ± 25.86	0–100	80 (67.5–95)
RP (Role-Physical)	66.72 ± 32.56	0–100	75 (50–100)
BP (Bodily Pain)	66.2 ± 26.17	0–100	62 (41–100)
GH (General Health)	61.78 ± 19.13	12–95	67 (45–77)
VT (Vitality)	60.93 ± 14.88	10–80	60 (60–70)
SF (Social Functioning)	77.33 ± 26.77	0–100	75 (75–100)
RE (Role-Emotional)	74.42 ± 29.8	0–100	66.67 (66.67–100)
MH (Mental Health)	73.07 ± 18.13	0–100	76 (66–86)

In the analysis of the QoL of different age groups, a decrease in QoL on the subscales "Physical functioning" (PF) ($p = 0.005$), and "General health" (GH) ($p < 0.045$) was found in elderly patients and patients with dementia compared to the other patients. Gender and number of AA have little impact on the QoL of patients in the late postoperative period ($p > 0.05$). A decrease in QoL of patients with the existing *SAH* on the "Social functioning" (SF) subscale ($p = 0.03$) was found. An analysis of QoL on subscales SF-36 was carried out regarding the manifestations of AA in the late preoperative period (SAH, pseudotumor cerebri, showing no symptoms AA). It has been found that the indicators were worse according to the subscale "Role-physical functioning" (RP) ($p = 0.004$), "General health" (GH) ($p = 0.049$), "Social functioning" (SF) ($p = 0.005$), and "Mental health" (MH) ($p = 0.009$) in the group of patients with pseudotumor cerebri. The presence of cephalgic disorder lowered the QoL according to the subscales "Bodily Pain" (BP) ($p = 0.004$) and "Mental health" (MH) ($p = 0.04$). Speech disturbances existing in the late postoperative period significantly reduced QOL in the subscale "Role-Physical Functioning" (RP) ($p = 0.001$), "General health" (GH) and "Mental health" (MH) ($p = 0.04$). Visual disturbances reduced QoL on the subscales "General Health" (GH) and "Mental Health" (MH) ($p = 0.03$). The presence of a disorder of the cranial nerve function decreased the indicators "Role-Physical Functioning" (RP) ($p < 0.05$), "General health" (GH), "Vitality" (VT) and "Mental health" (MH) ($p = 0.005$). The presence of motor impairment reduced the indicators "Physical Functioning" (PF), "Role-Physical Functioning" (RP) ($p = 0.0001$), "General health" (GH) ($p = 0.01$), "Role-Emotional" (RE) and "Mental health" (MH) ($p < 0.05$); in addition, the indicator "Physical component of health" was also reduced ($p = 0.001$). Sleep impairment reduced QoL on the subscales "Physical functioning" (PF) and "Role-Physical Functioning" ($p = 0.01$). It has been found that the number of stages of surgical interventions is also associated with QoL of patients. In the monitored group with 2 or more surgical interventions compared with the group with 1 surgical intervention, a reduced QoL was found according to the "Role-Physical Functioning" (RP), "Social functioning" (SF), "Role-Emotional" (RE), "Mental health" (MH) ($p = 0.04$) subscales and "Psychological Component of Health" ($p = 0.001$). The existence of complications related to the surgical treatment worsened the quality of life of patients in "Vitality" (VT) and "Psychological Component of Health" ($p < 0.05$).

The assessment of the impairments in patients in the late postoperative period according to the ICF classification was conducted using the domains of activity and participation (d4500 – walking short distance, d4501 – walking long distance, d4600 – moving around within the home, d4602 – moving around outside the home and other buildings, d5101 – washing oneself, d5400 – dressing, d630 – preparing meals, d640 – doing housework). Evaluation on the ICF components of activity and participation is presented in Fig. 2 (implementation) and Fig. 3 (capacity). Patients' adaptation to the activity impairments in the late postoperative period is visible in the difference between "implementation" and "capacity" indicators.

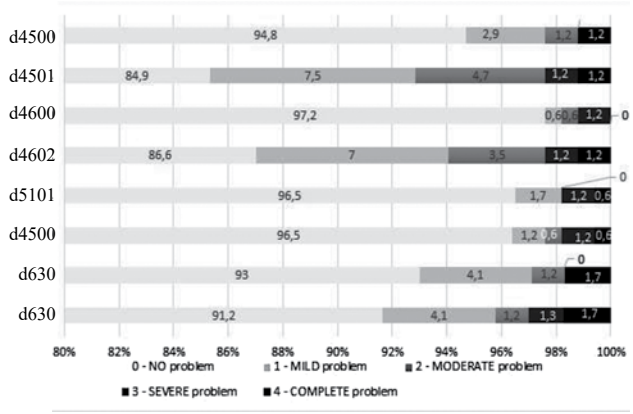

Fig. 2. Evaluation of activity and participation based on the ICF in the late postoperative period (performance)

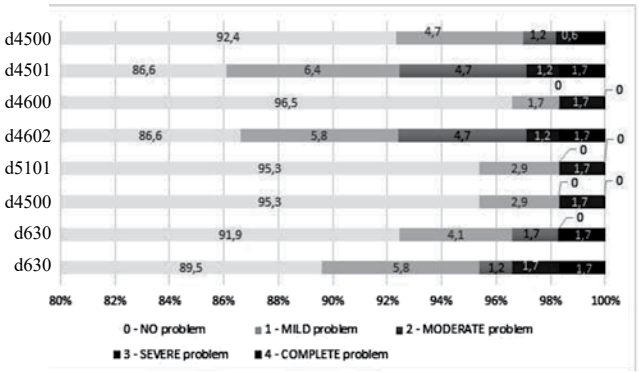

Fig. 3. Evaluation of activity and participation based on the ICF in the late postoperative period (capacity)

These indicators allow evaluating the patient's skills in the use of equipment and involvement of other people in helping the patient. The evidence of existing impairment on the d4501 domain – walking short distance, depended on the existing SAH ($p < 0.05$). Endovascular treatment complications affected the activity of patients on domains d4501 – long-distan-

ces walking ($p = 0.03$), and d640 – doing housework ($p = 0.01$).

DISCUSSION

QoL is an important indicator which allows more complete characterization of patients' functional and psychological status after suffering SAH and complications of surgical MIA repair. The research specifies the factors that cause negative subjective perception of patients after MIA endovascular treatment. The experienced SAH had an impact only on the "Social functioning" (SF) subscale, due to the long time interval after *SAH* and successful rehabilitation measures. Similar data were derived on the effect of complications of surgical interventions on the psychological component of health [17]. Unlike other studies [16–17], we did not identify an impact of the number of aneurysms on QoL. However, it was identified that QoL is associated with the number of surgical interventions performed on MIA. This indicates that the necessity of repeated surgical interventions, associated with AA recurrence or recanalization, affects QoL. Neurologic impairment worsened QoL, as in other studies [18]. In addition, we have found that the use of traditional scales for QoL assessments does not always allow understanding of how the patient functions in real life. Using ICF assessment allows the obtainment of information on the patient's activity and their participation in public life, as well as a depiction of the connection between function and structural impairments in the body. Additional information on the patients' subjective perceptions of their condition can be obtained within the SF-36 questionnaire. All these data allow forming the most complete picture of QoL of patients in the late postoperative period.

CONCLUSION

Good results of endovascular treatment for multiple aneurysms have been achieved. QoL in patients with MA depends on the past SAH, the number of surgical interventions, complications of endovascular treatment, and having a pseudotumor cerebri.

At the same time, there is a need for further rehabilitation measures, as well as for the improvement of surgical treatment methods, and for follow-up *c*erebral angiogram. Given that QoL is a comprehensive assessment of the rehabilitation measures effectiveness in patients with MA, the assessment of this indicator before and after surgery, and in the late postoperative period, is a necessary criterion for patients' functional status assessment. It is necessary to conduct a structured screening of cognitive complaints, neurologic impairment, emotional problems and clarification of personal factors. Identification of these problems is necessary for adapting rehabilitation programs to the individual needs of patients.

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Authors contribution

Oleinik A.A. – analysis and interpretation of data. Ivanova N.E. – final approval of the manuscript for publication. Goroshchenko S.A. – conception and design of the manuscript. Oleinik E.A. – conception and design of the manuscript. Ivanov A.Yu. – critical revision for important intellectual content, final approval of the manuscript for publication.

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Changes in the blood coagulation system and non-specific plasma proteinases in ischemia-reperfusion injury

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ABSTRACT

The aim of this study was to determine the general patterns of pathogenetic changes in the blood coagulation system and in non-specific proteinases and their inhibitors during the development of experimental ischemia-reperfusion injury.

Materials and methods. The study was conducted on 48 male Wistar rats (180–200 g). We used a model of ischemia-reperfusion injury achieved by applying rubber tourniquets to both hind limbs at the inguinal fold level for 6 hours. Revascularization was performed for 6, 12, or 24 hours following the application of tourniquets, after which we examined the state of the internal and external blood coagulation pathways and the activity of non-specific proteinases and their inhibitors.

Results. Indicators of blood coagulation system change show the development of blood hypocoagulation changes as the reperfusion time increases. By the 6th hour of reperfusion, the prothrombin time (PT) was lengthened by 112.0% ($p = 0.0142$) and the activated partial thromboplastin time (APTT) by 170.0% ($p = 0.0147$) compared with values in the control group. By the 12th reperfusion hour, the PT was lengthened by 174.2% ($p = 0.0389$), and the APTT increased 4.9-fold ($p = 0.0002$). When the reperfusion period was increased to 24 hours, it was characterized by lengthened PT and APTT, accompanied by an increase in antithrombin III by 11.5% ($p = 0.0371$) and a decrease in protein C by 71.4% ($p = 0.0071$). Changes in the non-specific proteinases and their inhibitors were characterized by a 2.8-fold increase in the trypsin-like proteinase activity ($p < 0.001$) relative to the control, as well as a 2.2-fold decrease in antitrypsin activity and acid-stable inhibitors ($p < 0.001$), which reached a maximum after 24 hours of reperfusion. A direct correlation was found between indicators characterizing the deficiency of coagulation system factors and a decrease in antiproteinase potential.

Conclusion. Hemostatic system disorders are characterized by the development of hypocoagulation during ischemia-reperfusion injury as the result of an increase in the trypsin-like proteinase activity and a decrease in the levels of inhibitors. The established changes may be associated with the deficiency of coagulation factors and proteinase inhibitors and share common pathogenic mechanisms.

Key words: blood coagulation, ischemia-reperfusion injury, non-specific proteinases.

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Изменение показателей свертывающей системы крови и неспецифических плазменных протеиназ при развитии синдрома ишемии-реперфузии

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РЕЗЮМЕ

Цель. Определить общие закономерности патогенетических изменений в свертывающей системе крови, неспецифических протеиназ и их ингибиторов при развитии экспериментального синдрома ишемии-реперфузии.

Материалы и методы. Исследование проведено на 48 половозрелых самцах крыс линии Вистар массой 180–200 г. Модель синдрома ишемии-реперфузии создавали наложением резиновых жгутов на обе задние конечности на уровне паховой складки сроком на 6 ч. Реваскуляризацию производили через 6, 12 и 24 ч после наложения жгутов. Оценивали состояние внутреннего и внешнего путей свертывания крови, активность неспецифических протеиназ и их ингибиторов.

Результаты. Показатели свертывающей системы крови свидетельствуют о развитии гипокоагуляционных изменений по мере удлинения времени реперфузии. Выявлено повышение значения протромбинового времени (ПВ) на 112,0% ($p = 0,0142$) и увеличение активированного частичного тромбопластинового времени (АЧТВ) на 170,0% ($p = 0,0147$) к 6-му ч реперфузии по сравнению с группой контроля. К 12-м ч реперфузии протромбиновое время возрастало до 174,2% ($p = 0,0389$), АЧТВ – в 4,95 раз ($p = 0,0002$), а растворимых фибрин-мономерных комплексов (РФМК) – на 121,3% ($p = 0,0300$). Длительность реперфузионного периода до 24 ч характеризовалась сохранением высоких значений ПВ и АЧТВ, РФМК с повышением содержания антитромбина III – на 11,4% ($p = 0,0371$) и снижением протеина С на 71,4% ($p = 0,0071$). Изменение показателей неспецифических протеиназ и их ингибиторов характеризовалось ростом активности трипсиноподобных протеназ в 2,8 раза ($p < 0,001$) по отношению к контролю, а также снижением антитриптической активности и уровня кислотостабильных ингибиторов в 2,2 раза ($p < 0,001$) с максимумом через 24 ч реперфузии. Выявлена прямая корреляционная связь между показателями, характеризующими дефицит факторов системы свертывания, и снижением антипротеиназного потенциала.

Заключение. На основании результатов исследования показателей системы свертывания крови и неспецифических протеиназ при развитии синдрома ишемии-реперфузии установлено, что нарушения в системе гемостаза характеризуются развитием гипокоагуляции на фоне роста активности трипсиноподобных протеиназ и снижения уровня их ингибиторов. Установленные изменения могут быть связаны с развитием дефицита факторов свертывания и ингибиторов протеиназ и иметь общие механизмы развития.

Ключевые слова: свертывающая система крови, синдром ишемии-реперфузии, неспецифические протеиназы.

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

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INTRODUCTION

Disorders of the blood coagulation system complicate the course of various critical conditions. Coagulation disorders, along with multiple organ dysfunction syndrome and systemic inflammatory response syndrome, which can cause the development of disseminated intravascular coagulation (DIC) [1], significantly affect the course of any underlying, concomitant, or background diseases, as well as the efficacy of treatment and patient mortality. It has become more common for doctors from a variety of specialties to encounter ischemia-reperfusion injury, which is important for disciplines including angio-surgery, transplantology, traumatology, various fields of internal medicine (myocardial infarction, acute cerebrovascular accident), and emergency medicine [1, 2]. Multiple organ dysfunction syndrome is a significant component in the pathogenesis of ischemia-reperfusion injury. The main manifestations of this syndrome are disorders of the coagulation system and non-specific proteinases [2, 3].

Tissue damage as the result of reperfusion disturbances and the associated development of an inflammatory reaction contribute significantly to the pathogenesis of coagulation and vascular-thrombocytic disorders. They tend to cause endothelial dysfunction, increased platelet activity, activation of plasma coagulation factors, hypoactivity of physiological anticoagulants, and suppression of fibrinolysis. These disorders can vary from subclinical variants (as in local venous thrombosis) to severe disturbances of hemostasis, such as disseminated intravascular coagulation (DIC), which is characterized by massive systemic thrombosis followed by episodes of bleeding due to the depletion of coagulation factors [5].

Pro-inflammatory reactions in patients with extreme conditions are accompanied by complex humoral and cellular interactions, which involve activation of numerous signaling pathways, including the generation or expression of thrombin, complement, cytokines, neutrophils, adhesion molecules, and other inflammatory mediators. Excessive inflammatory cascades coupled with the unfavorable course of the underlying disease lead to multiple organ dysfunction, which can manifest as coagulopathy, myocardial dysfunction, respiratory or renal failure, and neurocognitive defects. Coagulation and inflammation are also closely interconnected through networks of both humoral and cellular components, including coagulation factors, non-specific proteinases, and fibrinolytic cascades [6, 7]. Despite the significant number of recent studies devoted to the research of the blood coagulation system and proteolysis under critical conditions, many topics related to its pathogenesis and treatment policy remain controversial. In this regard, the aim of this work is to determine the general principles responsible for pathogenetic changes in the blood coagulation system and in non-specific proteinases and their inhibitors during the experimental development of ischemia-reperfusion injury, and to substantiate etiopathogenetic approaches to experimental correction.

MATERIALS AND METHODS

The studies were carried out on 48 white male Wistar rats weighing 180–200 g. Animals were housed under identical standard conditions in accordance with the Guide for the Care and Maintenance of Laboratory Animals. Research and euthanasia were carried out in accordance with state and international standards for the humane treatment of animals, and in compliance with the main provisions of regulatory legal acts [8–10].

Experimental studies of pathogenetic changes in the blood coagulation system and non-specific

proteinases and their inhibitors were carried out using a model of ischemia-reperfusion injury achieved by applying rubber-band tourniquets to both hind limbs at the level of the inguinal folds for a period of 6 hours [3]. The width of the tissue clamped by the tourniquet was 2–3 mm. The criteria for correct application of the tourniquet was the absence of edema in the limbs and their pale color. Revascularization was performed simultaneously by cutting the tourniquets 6 hours after they were applied [3]. Animals were placed in groups using simple randomization as follows: Control group: intact animals (n = 15); Group 2: 6 hours reperfusion group (n = 12); Group 3: 12 hours reperfusion group (n = 11); and Group 4: 24 hours reperfusion group (n = 10). Blood for analysis was obtained by cardiopuncture (4 mL) and placed in single-use Vacutainer glass tubes with 0.05 M EDTA for 10–15 s. The blood was centrifuged for 15 minutes at 1,200 g. Euthanasia of the animals was carried out by decapitation after preliminary narcotization with sodium thiopental (40 mg/kg) [11].

To assess the condition of the coagulation system, the following indicators were determined: prothrombin time (PT) (s); activated partial thromboplastin time (APTT) (s); fibrinogen concentration (FBG) (g/L); soluble fibrin-monomer complexes (SFMC) (mg/L); antithrombin III (AT III) (%); plasminogen (PG) (%); α2-antiplasmin (APL) (%); and protein C (PC) (%). Hemostasis values were measured on a CA 1500 automatic coagulometer (Sysmex, Japan) using standard commercial Siemens reagent kits (Germany). The concentrations of FBG, PT, and APTT were measured using clotting methods. The concentrations of AT III, APL, PC, and PG were determined using chromogenic methods. The content of SFMC was evaluated by the manual paracoagulation method using Technology-Standard reagent kits (Russia).

To evaluate the activity variables of non-specific proteinases and their inhibitors, trypsin-like, elastase-like, and antitryptic activities (TLA, ELA, and ATA), as well as the level of acid-stable inhibitors (ASI), were determined. The component activity of the proteinase inhibitor system was studied using enzymatic methods with a Biomat 5 spectrophotometer (UK) [12, 13]. Trypsin-like activity was determined by measuring the speed of N-benzoyl-L-arginine cleavage from the synthetic substrate of ethyl ester N-α-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) (Sigma, USA). Elastase-like activity was assessed by studying the hydrolysis rate of the Boc-L-alanine-4-nitrophenil ester synthetic substrate (Boc-Ala-ONp) (Sigma, USA). Antitryptic activity was assessed by inhibition of BAEE cleavage by trypsin. Similarly, the activity of acid-stable inhibitors was studied after preliminary preparation of serum by heating it in an acidic environment.

The data obtained during the research were analyzed statistically using the MedStat certified computer data-processing package for Windows. The main statistical variables determined were mean (M), error of mean (m), and standard deviation (s). All indicators are expressed quantitatively and the distribution did not differ from normal according to the Shapiro – Wilk test [14]. Student's t-test was used for comparison of group means in two groups. The results of statistical processing of the hemostatic system indices are represented as relative differences with the control group measured as a percentage. To assess the degree of relationship, a correlation analysis was performed, and the Pearson correlation coefficient was calculated using Microsoft Excel 2016. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The analysis of the blood coagulation system of rats with experimentally induced ischemia-reperfusion injury revealed a regular dynamic change in the hemostasis indices. Pronounced changes were observed by the 6th hour of the development of ischemia-reperfusion injury in both the external and internal blood coagulation pathways; PT increased by 112.0% (p = 0.0142) and APTT elongation by 170.0% (p = 0.0147) compared with the control group (Fig. 1). At 6 hours of ischemia-reperfusion, a marked decrease of 29.6% (p = 0.0002) was observed in the level of antithrombin III (Fig. 2), accompanied by a decrease in the plasminogen level of 29.6% (p = 0.0207) and a decrease in the level of antiplasmin of 11.7% (p = 0.0256) in comparison with the control values (Fig. 3).

After 12 hours of ischemia-reperfusion, we observed a 174.2% extension for PT (p = 0.0389) and a marked 4.9-fold extension of APTT (p = 0.0002) compared to the control indices. The level of AT III after 12-hour ischemia-reperfusion decreased by 10.4% (p = 0.0442). The content of SFMC increased by 121.3% (p = 0.0300) compared with the control level (Fig. 4).

An increase in the duration of the reperfusion period to 24 hours was characterized by an increase of 59.6% in SFMC (p = 0.0114), an increase of 11.5% in the content of AT III (p = 0.0371), a decrease of

71.4% in PC (p = 0.0071) (Fig. 3), and a 39.8% increase in Group 3 (p = 0.0494). We also observed a maximum shift in the following indicators: an PT elongation of 186.6% (p = 0. 0346) and a maximum 4.9-fold increase in APTT (p = 0.0147) compared to control values.

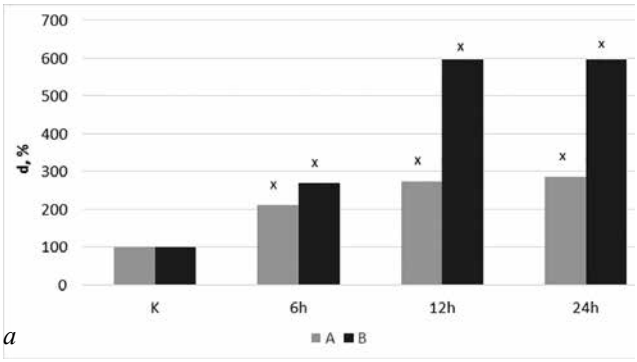

Fig. 1. Prothrombin time (*a*) and activated partial thromboplastin time (*b*) in groups with different durations of ischemia-reperfusion (6 h, 12 h, and 24 h) as a percentage (d, %) related to the control (K) group. X represents the difference from the control group (Student's t-test, $p < 0.05$)

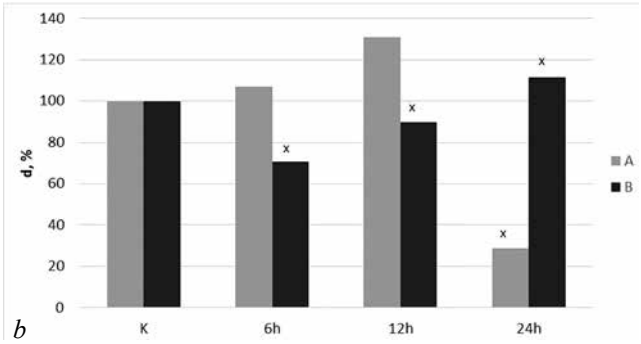

Fig. 2. Protein C (*a*) and antithrombin III (*b*) levels in groups with different durations of ischemia-reperfusion (6 h, 12 h, and 24 h) as a percentage (d, %) related to the control (K) group. X represents the difference from the control group (Student's t-test, $p < 0.05$)

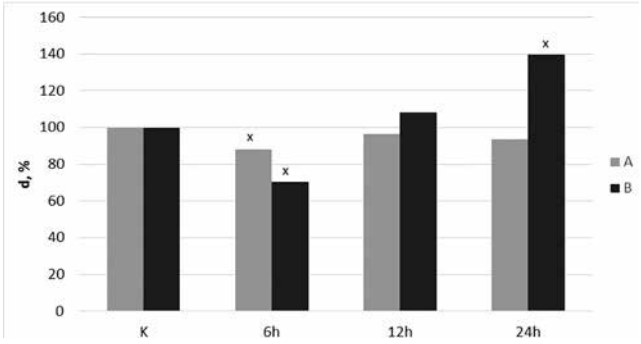

Fig. 3. Plasminogen (*a*) and antiplasmin (*b*) levels in groups with different durations of ischemia-reperfusion (6 h, 12 h, and 24 h) as a percentage (d, %) related to the control (K) group. X represents the difference from the control group (Student's t-test, $p < 0.05$)

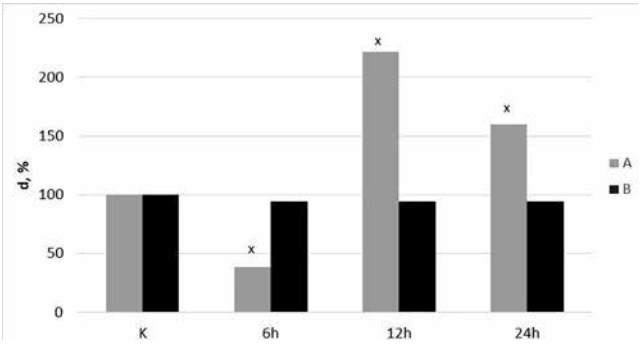

Fig. 4. SFMC (*a*) and fibrinogen (*b*) levels in groups with different durations of ischemia-reperfusion (6 h, 12 h, and 24 h) as a percentage (d, %) related to the control (K) group. X represents the difference from the control group (Student's t-test, $p < 0.05$)

In the first 6 hours, a decrease in PG activity was observed. In the subsequent periods, plasmin inactivation probably occurs in response to fibrinolysis products and/or PG activation does not occur. Free APL is also used for binding to plasmin, and takes part in the inhibition of non-specific proteinases [15]. PG and APL are acute phase proteins, and their levels increased during the 24 hours of the experiment as a result of a relatively reduced due to hypocoagulation consumption. The level of AT III decreased in the first 6 hours in response to the coagulation process activation. However, as thrombin and other AT III cofactors (Xa, XIa, IXa) are consumed, the plasma level of free AT III begins to increase.

In general, it can be assumed that during the 24-hour reperfusion period, systemic tissue damage occurs. It is accompanied by an acute-phase response that is a reaction to stress exposure. Distinct signs of the consumption coagulopathy development appear in these conditions.

During the experiment, somewhat different dynamics were observed in the PC level changes. Unlike AT and APL, cofactor proteins whose levels decrease immediately after binding to targets, protein C is an enzyme. The thrombin-AT complex is destroyed by proteolytic systems in the liver within a few minutes; the half-life of PC in the circulatory system is about 6 hours. Thus, during the course of ischemia-reperfusion, in all likelihood, there is a rapid depletion of functional reserves and hypocoagulation development within 6 hours of tissue damage.

Along with the experimental dynamics of blood coagulation indices, the nature of the shifts in the activity of non-specific proteinases and their inhibitors in

the blood serum of experimental animals was studied in relation to the timing needed for ischemia-reperfusion injury development.

Analysis of the obtained data has shown the following dynamics in non-specific proteinases and their inhibitors' activity changes (Table 1). Six hours after revascularization of the limbs, the elastase-like activity of blood serum was 3.8 times lower than that of the control values (p = 0.0012). After 12 hours, an even greater decrease, up to 19.0% of the control index (p = 0.0008), was observed. The pronounced decrease in ELA likely indicates the activation of natural proteinase inhibitors that neutralize elastase, reducing its serum activity severalfold. Twenty-four hours after the development of ischemia-reperfusion, ELA showed a tendency to increase, although the activity remained 2-fold below the control parameter (p = 0.0011). Apparently, after 24 hours of the ischemia-reperfusion development, the inhibitory control weakened, which led to an increase in the proteolytic activity of blood serum.

Table 1

Changes in the proteolytic activity and inhibitory potential of rat blood using a model of ischemia-reperfusion injury at different observation times, $M \pm m$

Experimental group	ELA, nMol/mL • min	TLA, nMol/mL • min	ATA, IU/mL	ACI, IU/mL
Control group, n = 10	2.19 ± 0.14	0.26 ± 0.02	34.67 ± 1.57	6.83 ± 0.30
Ischemia 6 h, n = 10	2.31 ± 0.09*	0.20 ± 0.01*	38.03 ± 1.33*	3.81 ± 0.36*
Ischemic-reperfusion syndrome 6 h, n = 10	0.57 ± 0.05*	0.50 ± 0.06*	20.61 ± 1.16*	3.18 ± 0.31*
Ischemic-reperfusion syndrome 12 h, n = 10	0.41 ± 0.02*	0.73 ± 0.06*	25.76 ± 1.76*	3.39 ± 0.30*
Ischemic-reperfusion syndrome P 24 h, n = 10	1.10 ± 0.09*	0.46 ± 0.12	16.02 ± 0.79 *	3.08 ± 0.23*

* indicates the reliability of differences in the data (p) in comparison with those of the control group (*$p \leq 0.05$ is statistically significant).

The dynamics of the trypsin-like activity of blood serum during the development of reperfusion injury were characterized by other changes. Six hours after revascularization of the limbs, the TLA index was 48.0% greater than that of the control (p = 0.0006). After 12 hours, the index, having reached the maximum level, exceeded the control indices 2.8-fold (p = 0.0011), which is apparently associated with the influx of a large number of proteinases into the systemic circulation from previously ischemic tissues. Later, a downward trend in the studied index was observed. Thus, 24 hours after reperfusion, TLA, having decreased by 37.0%, remained 43.0% higher than values in the control group (p = 0.0472), which indicates the onset of compensatory mechanisms and a timely increase in inhibitory activity. Interesting data confirming our previous assumptions were obtained in the study of antitryptic activity: 6 hours after reperfusion, a 1.7-fold decrease in ATA was noted (p = 0.0009), which fell to 1.4-fold after 12 hours. The decrease in this parameter progressed, and by 24 hours after reperfusion, the ATA activity had fallen 2.2 times lower than that of the control value (p = 0.0004).

The level of acid-stable inhibitors also largely depended on the duration of the reperfusion period. As a result, 6 hours after revascularization, the level of ASI was 2.2-fold lower than that of the control value (p = 0.0009); after 12 hours, 2-fold lower (p = 0.0012), and after 24 hours, 2. 2-fold lower (p = 0.0007). The described dynamics of the ASI levels is associated with their increased consumption as a result of increased protease activity.

Thus, an increase in the activity of non-specific blood proteinases during the development of reperfusion injury at its early stages should be noted, along with the concomitant increase in an inhibitory activity. With continued reperfusion, the inhibitory capacity tends to decrease and the activity of non-specific blood proteinases in the experimental animals tends to increase.

To clarify the relationship between the coagulogram indices and the state of the non-specific proteinase system and their inhibitors in the experimental ischemia-reperfusion injury, we performed a correlation analysis (Table 2).

A significant positive correlation was found between changes in TLA and PT, APTT, and SFMC in the blood during the process of modeling ischemia-reperfusion injury for 6, 12, and 24 hours (r = 0.78, 0.82 and 0.59, respectively). The more the activity of this proteinase increased, the more the values of blood coagulation indices increased along the external (PT) and internal pathways (APTT). The concentration of SFMC in the blood of experimental animals increased as well. At the same time, for the ATA

and PT indices, a negative correlation with APTT was found (correlation coefficients of –0.82 and –0.83, respectively). Thus, a higher level of antitryptic activity, mainly of α-1-proteinase inhibitor, corresponded to a lower level of the coagulogram indices described above. In addition, it was found that antitryptic activity had a direct proportional correlation with the values of FBG, APL, and PC (correlation coefficients of 0.86, 0.75, and 0.58, respectively). The greater the proteinase system inhibitory potential in the form of increased ATA was, the higher values of these laboratory indices were observed.

Table 2

Results of correlation analysis (r) of coagulogram indices and the activity of non-specific proteinases and their inhibitors

Experimental group	PT	APTT	FBG	SFMC	APL	AT III	PC	PG
ELA	–0.799 $p = 0.002$	–0.869 $p = 0.002$	+0.930 $p = 0.001$	–0.248	+0.670 $p = 0.032$	+0.521 $p = 0.048$	–0.232	+0.153
TLA	+0.781 $p = .003$	+0.820 $p = 0.002$	–0.786 $p = 0.003$	0.594 $p = 0.043$	–0.296	–0.318	+0.358	+0.031
ATA	–0.826 $p = 0.002$	–0.833 $p = 0.002$	+0.867 $p = 0.002$	–0.045	+0.755 $p = 0.006$	–0.003	+0.585 $p = 0.046$	–0.304
ASI	–0.921 $p = 0.001$	–0.959 $p = 0.001$	+0.997 $p = 0.001$	–0.217	+0.752 $p = 0.005$	+0.248	+0.201	–0.127

Note. The *p* value denotes a statistically significant correlation. The – sign indicates an inverse correlation.

A negative correlation between ASI and the PT and APTT indices (correlation coefficients of –0.92 and –0.95, respectively), and a positive correlation between the ASI indices and the FBG and APL levels (correlation coefficients of 0.99 and 0.75, respectively) were noted.

When analyzing the ELA values for ischemia-reperfusion injury and coagulogram indices (PT and APTT), a negative correlation was revealed (correlation coefficients of –0.79 and –0.86, respectively), but when studying the values of FBG, APL, and AT III, a positive correlation was found (correlation coefficients of 0.93, 0.67, and 0.52, respectively) between them.

DISCUSSION

Our analysis of the dynamics of coagulation system indices shows significant differences and stages in the reaction of the hemostatic system during different periods of ischemia-reperfusion injury. Thus, even in short-term, 6-hour ischemia-reperfusion, hypocoagulation predominates, which can be explained by systemic activation of plasmatic systems in response to the acute damage. This is indicated by an increase in SFMC, which is a marker of the consequences of developing thrombinemia in response to acute damage. Increase in the duration of ischemia-reperfusion injury (up to 12 h and, especially, 24 h) leads to further changes, indicative of hypocoagulation disorders aggravation. It seems likely that these changes are associated with depletion of coagulation factors and their inadequate entry into the systemic circulation because of impaired synthetic liver function under conditions of prolonged stimulation and severe functional stress.

Similar changes are observed in the system of non-specific proteinases and their inhibitors. Dynamics analysis of the proteinase and their inhibitors' indices taken in different time periods of reperfusion syndrome indicates a pronounced activation of non-specific proteinases (TLA) in the blood serum of experimental animals with modeled ischemia-reperfusion. It is associated with the direct participation of proteolytic enzymes in the hemocoagulation cascade, and in the body's systemic adaptive response to massive endothelial damage. Thus, during 6-hour ischemia-reperfusion, there is a compensatory increase in proteinase inhibitors with an associated decrease in proteinase activity. With an increase in the duration of ischemia-reperfusion injury (12 and 24 h), there is a depletion of inhibitory potential with an increase in proteinase activity.

The most pronounced changes both in the hemostatic system and in the system of non-specific proteinases and their inhibitors were observed in the 24-hour ischemia-reperfusion group. A disruption of the body's adaptive capabilities represented by depletion of blood coagulation factors was observed in this group. As a result, there was a shift in the hemostasis system indices towards hypocoagulation, and an increase in proteinase activity as the result of an

inhibitory potential deficiency. This may be a predisposing factor for the development of DIC. This assumption corresponds with literature data confirming the development of DIC in response to systemic activation of the coagulation pathway [16]. The intravascular formation of fibrin is enhanced by dysfunction of natural anticoagulant systems, such as antithrombin (antithrombin III) and protein systems (protein C), during the active development of DIC. Subsequently, all components of the coagulation and anticoagulation cascade are depleted, which leads to complete non-coagulation of blood [17].

Statistically significant data from the correlation analysis confirm the assumptions mentioned above and indicate a close relationship between changes in the blood clotting system parameters and proteinase-inhibitor system indices. Apparently, the changes seen in the blood clotting system status during ischemia-reperfusion injury, namely initial hypocoagulation with subsequent further increases in the duration of blood clotting time at 12 and 24 hours (indices of the external and internal pathways), are closely interrelated. This may occur due to the changes in the proteinase-inhibitory system demonstrated by an increase in proteolytic activity and a decrease in inhibitory potential. Possibly, it is the deficiency of inhibitors, together with the lack of blood coagulation factors due to the decreased synthetic liver function at a later stages of ischemia-reperfusion injury, that are the key links in the pathogenesis. For this reason, they are possible therapeutic targets, which can be corrected, not only in response to reperfusion injury, but also during DIC syndrome, acquired thrombophilic conditions, and other blood coagulation disorders.

CONCLUSION

It has been found that, during the development of ischemia-reperfusion injury for 6, 12, and 24 hours, the disturbances in the hemostatic system are characterized by the development of hypocoagulation as the result of an increase in the trypsin-like proteinases activity and a decrease in their inhibitors level. The changes observed are likely to be directly associated with the development of coagulation factors and proteinase inhibitors deficiencies. These changes share common development mechanisms associated with excessive coagulation factors depletion during prolonged activation of the coagulation cascade, and, possibly, with a decrease in the synthetic liver function under a long-term functional load of hepatocytes, as well as their hypoxic and reperfusion injury.

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The role of galectin-1 and galectin-3 in the mechanisms of T-cell immune response dysregulation in colon cancer

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ABSTRACT

The aim of the study was to characterize the features of the subpopulation composition and cytokine-secretory activity of T lymphocytes (Th1, Th17 and Treg) in relation to the concentration of galectin-1 and galectin-3 in the blood of patients with colon cancer.

Materials and methods. A total of 26 patients diagnosed with colon cancer were examined. The study material included whole peripheral blood, blood plasma, and supernatants of suspension cultures of mononuclear leukocytes. Lymphocytes isolated from blood were typed by flow cytometry using monoclonal antibodies. The content of galectin-1 and galectin-3 (in blood plasma) and IFNγ, IL-17A, and TGFβ (in supernatants of mononuclear leukocyte culture *in vitro*) were determined by enzyme-linked immunosorbent assay. The results obtained were analyzed by statistical methods.

Results. In patients with colon cancer, a significant increase in the concentration of galectin-1 and galectin-3 in the blood plasma was found, which was associated with a decrease in the content of CD4+T-bet+ Th1 lymphocytes, CD4+RORC2+ Th17 lymphocytes in the blood and *in vitro* hyposecretion of IL-17. At the same time, positive correlations were revealed between the concentration of galectin-1 and galectin-3, the content of CD4+FoxP3+ Treg cells in the blood, and the secretion of TGFβ by mononuclear leukocytes *in vitro*.

Conclusion. In colon cancer, increased levels of galectin-1 and galectin-3 in the blood are associated with quantitative deficiency and inhibited secretory activity of effector T-lymphocytes and activation of the immunosuppressive functions of regulatory T cells. These results suggest a negative role of galectin 1 and galectin 3 in the mechanisms of regulation of the T cell immune response in colon cancer.

Key words: galectins, T-lymphocytes, Th17, Treg, cytokines, immunosuppression, colon cancer.

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Роль галектина-1, -3 в механизмах дисрегуляции Т-клеточного звена иммунного ответа при раке толстого кишечника

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РЕЗЮМЕ

Цель исследования – охарактеризовать особенности субпопуляционного состава и цитокин-секреторной активности Т-лимфоцитов (Th1, Th17 и Treg) во взаимосвязи с концентрацией галектина-1 и галектина-3 в крови у больных раком толстого кишечника.

Материалы и методы. Обследованы 26 пациентов (14 мужчин и 12 женщин, средний возраст (62,9 ± 6,7) лет) с диагнозом рака толстого кишечника. В группу контроля вошли 17 здоровых доноров (11 мужчин и 6 женщин, средний возраст (58,2 ± 3,1) лет). Материалом исследования служила цельная периферическая кровь, плазма крови и супернатанты суспензионной культуры мононуклеарных лейкоцитов. Выделенные из крови лимфоциты типировали методом проточной лазерной цитофлуориметрии с использованием моноклональных антител. Методом иммуноферментного анализа определяли содержание галектина-1 и галектина-3 (в плазме крови) и IFNγ, IL-17A и TGFβ (в супернатантах культуры мононуклеарных лейкоцитов *in vitro*). Полученные результаты анализировали статистическими методами.

Результаты. У больных раком толстого кишечника установлено значимое увеличение концентрации галектина-1 и галектина-3 в плазме крови, ассоциированное со снижением содержания CD4+T-bet+ Th1-лимфоцитов, CD4+RORC2+ Th17-лимфоцитов в крови и гипосекрецией IL-17 лимфоцитами *in vitro.* Напротив, выявлена положительная корреляция между концентрацией галектинов 1 и 3, содержанием CD4+FoxP3+Treg клеток в крови и секрецией TGFβ мононуклеарными лейкоцитами *in vitro.*

Заключение. При раке толстого кишечника повышенный уровень галектинов 1 и 3 в крови сопряжен с количественным дефицитом и угнетением секреторной активности эффекторных Т-лимфоцитов, и, напротив, активацией иммуносупрессорных функций регуляторных Т-клеток. Полученные результаты указывают на негативную роль галектина-1 и галектина-3 в механизмах регуляции Т-клеточного звена иммунного ответа при раке толстого кишечника.

Ключевые слова: галектины, Т-лимфоциты, Th17, Treg, цитокины, иммуносупрессия, рак толстого кишечника.

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INTRODUCTION

Dysregulation of the antitumor immune response plays a pivotal role in the pathogenesis of malignancies and is typically represented by an imbalance of effector and regulatory T cells [1–3]. Throughout tumor progression, cancer cells obtain a variety of mechanisms that allow them to "program" their microenvironment and induce immunosuppression [4]. One of such mechanisms is tumor-associated production of galectins, galactoside-binding proteins with a wide spectrum of extra- and intracellular functions. [5, 6]. Among the galectin family, galectin-1 and galectin-3 were involved in the key stages of the tumor development, including malignant transformation, neoangiogenesis, invasion, metastasis, and modulation of the immune microenvironment [7, 8].

A number of *in vitro* studies have demonstrated that galectin-1 and galectin-3 are able to influence cell-mediated immune response by regulating differentiation and survival of type 1 and type 17 effector T-helper (Th) lymphocytes, as well as regulatory T-cells (Treg) with immunosuppressive phenotype [9–12]. Galectin-1 and galectin-3 expression by malignant cells and elements of the tumor microenvironment is considered to be one of the strategies to suppress antitumor immunity wielded by cancer cells [13, 14]. However, the features of the immunotropic effects of these galectins in tumor diseases remain understudied.

The aim of the study was to investigate the characteristics of subpopulation constitution and cytokine-secretory activity of blood T-lymphocytes (Th1, Th17, and Treg) in connection with the plasma concentration of galectin-1 and galectin-3 in patients with colon cancer.

MATERIALS AND METHODS

The study was carried out in the laboratory of clinical and experimental pathophysiology in the Pathophysiology Department, Siberian State Medical University (head – O.I. Urazova, Dr. Si. (Med.), Professor, Corresponding member of the RAS), and the Pathoanatomical Department of Tomsk Regional Oncological Dispensary (TROD) (head – I.L. Purlik, Dr. Si. (Med.)). The study included 26 patients diagnosed with colon cancer (14 men and 12 women, average age was 62.9 ± 6.7 years), who underwent treatment in the TROD (acting chief physician – M.Yu. Grishenko). The control group included 17 healthy donors consisting of 11 men and 6 women (average age was 58.2 ± 3.1 years). The criteria for exclusion of patients from the study were preoperative therapy, other malignancies, exacerbation of chronic diseases of an allergic, autoimmune, and infectious nature, and refusal to participate in the study. All patients were examined and operated on before the start of specific radiation and drug therapy.

The study material included whole peripheral blood collected from the median cubital vein on an empty stomach, blood plasma, as well as supernatants of the suspension cultures of mononuclear leukocytes. Isolation of mononuclear leukocytes from the whole blood was performed in the Ficoll-Paque density gradient centrifugation medium (p = 1,077 g/ml). The cultivation of mononuclear leukocytes was carried out in a complete nutrient medium RPMI-1640 in a CO2 incubator in a gas mixture containing 5% carbon dioxide at a temperature of 37 degrees Celsius for 48 hours. Measurement of the concentration of interferon (IFN) γ, transforming growth factor (TGF) β1, interleukin (IL) 17 in the supernatants of culture suspensions of mononuclear leukocytes, as well as levels of galectin-1 and galectin-3 in blood plasma was performed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (BosterBio, USA; Vector-Best, Russia). Optical density was recorded on a Multiscan EX photometer-analyzer (Finland) at a wavelength of 450 nm.

To evaluate the content of CD4+T-bet+ (Th1), CD4+RORC2+ (Th17), and CD4+FoxP3+ (Treg) lymphocytes in the peripheral blood, expression of the surface receptor CD4 and intracellular transcription factors T-bet, RORC2, and FoxP3 in the whole blood was assessed by flow cytometry using monoclonal antibodies (PerCP-Cy5.5, Alexa Fluor 488, PE, APC; BD Biosciences, USA; RnD Systems, USA). Red blood cell lysis was performed using a BD Pharm Lyse lysing solution (BD Biosciences, USA). For fixation and permeabilization of cells for intranuclear staining, the Human FoxP3 Buffer Set (BD Biosciences, USA) was used. The stain Buffer (BD Biosciences, USA) was utilized to wash and resuspend cells.

Statistical processing of the results was carried out using Statistica 12.0 for Windows (StatSoft Inc., USA). Quantitative traits in the comparison groups were represented as the median (Me), upper (Q_1) and lower (Q_3) quartiles. The significance of differences in independent samples was evaluated with the nonparametric Mann–Whitney U-test. Correlation analysis was carried out us-

ing the Spearman rank correlation test. Differences were considered significant at $p < 0.05$.

RESULTS AND DICUSSION

The imbalance of the galectin expression in the tumor tissue and their concentration in the peripheral blood is characteristic of many malignant neoplasms and often correlates with the degree of tumor progression [15–17]. According to the literature, a high plasma level of galectin-1 in patients with colorectal cancer is associated with high aggressiveness of the tumor, advanced stages of the tumor process, and poor prognosis [18]. Some authors have noted a positive correlation of the level of galectin-3 expression by tumor cells with the stage of the disease and the presence of metastases [19, 20], while other authors, on the contrary, have reported a decrease in the expression of galectin-3 at the later stages of the tumor process [21, 22].

According to the results of ELISA, we found a significant increase in the concentration of galectin-1 and galectin-3 in blood plasma in patients with colon cancer compared with the corresponding values in healthy donors (Table 1).

Table 1

The content of galectin-1 and galectin-3 (ng/ml) in blood plasma in patients with colon cancer, *Me* (Q_1–Q_3)

Parameter	Patients with colon cancer	Healthy donors
Galectin-1	16.17 (15.31–17.10) $p = 0.0031$	13.74 (12.23–14.79)
Galectin-3	3.28 (2.30–5.71) $p = 0.0055$	1.56 (1.19-2.17)

Note. Level of significance of differences compared with corresponding indicators in healthy donors – p (here and Table 2,3).

High plasma levels of galectin-1 and glectin-3 are apparently the result of their over-expression by tumor cells and elements of the tumor microenvironment, which could in turn initiate an imbalance of individual subpopulations of T-lymphocytes in the development of antitumor immunity in colon cancer.

The key cells of antitumor resistance are CD4+ Th1 lymphocytes, which, via IFNγ secretion, activate cytotoxic CD8+ cells and stimulate the presentation of tumor-associated antigens by macrophages [23, 24]. CD4+ Th17 lymphocytes producing the IL-17A cytokine, on the one hand, increase the recruitment of cytotoxic lymphocytes and neutrophils in the tumor site and, on the other hand, induce tumor neoangiogenesis and formation of metastases [25, 26]. Regulatory T lymphocytes, characterized by secretion of the immunosuppressive cytokines IL-10 and TGFβ, are capable of inhibiting the antitumor immune response [27, 2].

An assessment of the subpopulation composition of peripheral blood T-lymphocytes in patients with colon cancer revealed a significant decrease in the relative content of CD4+T-bet+ Th1- and CD4+RORC2+ Th17 lymphocytes in comparison with corresponding parameters in healthy donors (Table 2). The percentage of CD4+FoxP+ Treg lymphocytes in blood, in contrast, exceeded the corresponding indicator in the control group (Table 2).

Table 2

Relative content of peripheral blood Th1, Th17, and Treg lymphocytes (% from the total lymphocytes population) in patients with colon cancer, *Me* (Q_1–Q_3)

Parameter	Patients with colon cancer	Healthy donors
Th1 (CD4+ T-bet+)	0.82 (0.24–0.94) $p = 0.0454$	1.24 (0.48–2.43)
Th17 (CD4+ RORC2+)	1.44 (0.19–2.13) $p = 0.0051$	3.51 (1.56–4.79)
Treg (CD4+ FoxP3+)	1.19 (0.8–1.48) $p = 0.0114$	0.55 (0.23–1.20)

The effect of galectin-1 and galectin-3 on individual subpopulations of helper T lymphocytes could be related to the heterogeneity of surface glycans, which are responsible for the binding of individual galectins, as well as the expression of cell surface glycoproteins that mediate lectin resistance [28, 29]. It is worth noting that galectin-1 and galectin-3 are able to exert a modulating effect not only on the proliferation and apoptosis of individual subpopulations of T-lymphocytes, but also on their cytokine-secretory activity.

According to our results, patients with colon cancer display a significant decrease in basal secretion of IL-17 by blood lymphocytes *in vitro* compared to healthy donors (Table 3). *In vitro* secretion of TGFβ1 by blood lymphocytes in the examined patients, in contrast, was 1.3 times higher than that in the control group. As for IFNγ, we did not find a significant difference in its basal secretion in patients with colon cancer relative to control values (Table 3).

Table 3

Secretion of cytokines in an in vitro culture of mononuclear leukocytes (pg/ml) in patients with colon cancer, *Me* (Q_1–Q_3)

Parameter	Patients with colon cancer	Healthy donors
IFNγ	1.286 (0.100–3.571)	1.429 (0.100–2.857)
IL-17	0.116 (0.100–0.425) $p = 0.0058$	0.657 (0.108–0.889)
TGFβ1	835.8 (534.3–1,949.0) $p = 0.0484$	628.6 (471.4–777.2)

To identify the relationship between the concentration of galectin-1 and galectin-3 in plasma and the identified structural and functional imbalance of CD4+ T lymphocytes, a correlation analysis was performed. In patients with colon cancer, negative correlations between the plasma concentration of galectin-1 and the relative content of CD4+T-bet+ Th1 lymphocytes (r =-0.56, p = 0.0353), CD4+RORC2+ Th17 lymphocytes (r = -0.59, p = 0.0334) and *in vitro* secretion of IL-17 (r = -0.63, p = 0.0013) were found. At the same time, a positive correlation was found between the plasma level of galectin-1 and the content of CD4+FoxP3+ Treg cells (r = 0.55, p = 0.0346) and basal secretion of TGFβ1 (r = 0.48, p = 0.0198). Similar results were obtained in an experimental *in vitro* study conducted by O.A.Vasilieva et al. (2015). Using the lymphocytes from healthy donors, the authors demonstrated the negative effect of recombinant galectin-1 on Th1- and Th17-mediated immune reactions with concomitant increase in Treg lymphocytes [28]. In turn, F. Cedeno-Laurent et al. (2012) demonstrated the ability of galectin-1, produced by malignant blood T-lymphocytes, to induce apoptosis of Th1 cells and, as a result, shift the Th1/Th2 balance towards Th2-dependent immune reactions and lower the effectiveness of antitumor resistance mechanisms in patients with skin T cell lymphoma [10].

According to *in vitro* studies, galectin-3 exerts dose-dependent stimulating effect on the differentiation and functional activity of Th17 lymphocytes, while inhibiting maturation and functions of Th1 and Treg cells [30]. This thesis is partially consistent with the results of our study, which established a negative correlation between the concentration of galectin-3 and the relative number of CD4+T-bet+ Th1 lymphocytes in the blood (r = –0.81, p = 0.0004). At the same time, we found a positive relationship between the plasma level of galectin-3 and basal secretion of TGFβ1 by peripheral blood lymphocytes (r = 0.70, p = 0.0001). The ability of galectin-3 to participate in the regulation of TGFβ1-associated signaling pathways was demonstrated by A.C. MacKinnon et al. (2012), who showed that specific inhibition of galectin-3 suppresses TGFβ1-dependent activation of β-catenin *in vitro* and *in vivo* [31].

Taken together, the results of the present study indicate the ability of galectin-1 and galectin-3 to modulate the functional activity of effector and regulatory blood T-lymphocytes in malignant neoplasms of the colon.

CONCLUSION

In patients with colon cancer, an increase in the concentration of galectin-1 and galectin-3 is associated with an imbalance of subpopulations of helper T-lymphocytes in the blood, inhibition of Th1- and Th17-dependent immune reactions, and activation of Treg lymphocytes with immunosuppressive properties. Tumor-associated production of galectin-1 and galectin-3 in colon cancer may represent one of the mechanisms by which tumor cells escape from immunological surveillance. The above said indicates the negative role of galectin-1 and galectin-3 in the mechanisms of regulation of the T cell immune response in colon cancer.

Further studies of the immunotropic effects of galectin-1 and galectin-3 on individual subpopulations of T lymphocytes will help to establish the relevance of these lectins as prognostic markers and advocate for the modulation of their activity in colon cancer.

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Changes in the activity of lysosomal cysteine proteases of plasma mononuclear and polymorphonuclear blood leukocytes in Alzheimer's disease

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ABSTRACT

Aim. To study the level of activity of lysosomal cysteine proteases (cathepsins H, B, L) in blood plasma and fractionated leukocytes (polymorphonuclear and mononuclear) in patients with Alzheimer's disease in comparison with similar indicators in persons without signs of neurodegeneration as a possible marker of Alzheimer's disease development and diagnosis.

Materials and methods. The spectrofluorimetric study of cathepsins B, L, H activity level in plasma and fractionated leukocytes was conducted in 22 patients diagnosed with Alzheimer's disease in comparison with the same indicators in 22 patients matched by sex, age and associated diseases with patients of the observation group, but having no signs of neurodegeneration.

Results. The activity of all three enzymes, and especially cathepsin H, increased significantly in blood plasma. A significant increase is also noted in the activity of cathepsins H, B, and L in homogenates of fractionated leukocytes. At the same time, in both polymorphonuclear and mononuclear leukocytes the greatest degree of changes is demonstrated by the activity of cathepsin B, and the least is the activity of cathepsin L. Given the available data on an increased cathepsin B activity in the cerebrospinal fluid of patients with Alzheimer's disease, we can assume a correlation between the state of lysosomal proteases activity in the Central nervous system and in the peripheral blood cells.

Conclusion. Alzheimer's disease is associated with increased activity of cysteine cathepsins in plasma, polymorphonuclear and mononuclear leukocytes of peripheral blood, which can be considered as one of the possible markers of development and diagnosis of the disease.

Key words: Alzheimer's disease, neurodegeneration, proteolysis, cysteine cathepsins, blood plasma, polymorphonuclear leukocytes, mononuclear leukocytes.

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Conformity with the principles of ethics. The study complies with ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association "Ethical Principles for the Conducting of Scientific Medical Research with Human Participation" as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation of June 19, 2003 No. 266. All patients signed an informed consent to participate in the study. The study protocol was approved by the local ethics committee of Ryazan State Medical University (Protocol No. 6 of 6.11.2018).

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Изменение активности лизосомальных цистеиновых протеаз плазмы, моноядерных и полиморфноядерных лейкоцитов крови при болезни Альцгеймера

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РЕЗЮМЕ

Цель. Изучить уровень активности лизосомальных цистеиновых протеиназ (катепсинов H, B, L) в плазме крови и фракционированных лейкоцитах (полиморфноядерных и моноядерных) пациентов с болезнью Альцгеймера в сравнении с аналогичными показателями у лиц, не имеющих признаков нейродегенерации, как возможный маркер развития и диагностики болезни Альцгеймера.

Материалы и методы. Проведено спектрофлуориметрическое исследование уровня активности катепсинов B, L, H в плазме крови и фракционированных лейкоцитах 22 пациентов с диагнозом «Болезнь Альцгеймера» в сравнении с аналогичными показателями 22 пациентов, сопоставимых по полу, возрасту и сопутствующим заболеваниям с пациентами группы наблюдения, но не имеющих признаков нейродегенерации.

Результаты. В плазме крови статистически значимо повышена активность всех трех ферментов, в наибольшей степени – активности катепсина H. В гомогенатах фракционированных лейкоцитов также отмечается статистически значимое повышение активности катепсинов H, B, L, при этом как в полиморфноядерных, так и в моноядерных лейкоцитах в наибольшей степени изменяется активность катепсина B, наименьшей –катепсина L. Учитывая имеющиеся данные о повышении активности катепсина B в цереброспинальной жидкости пациентов с болезнью Альцгеймера, можно предположить взаимосвязь между состоянием активности лизосомальных протеиназ в центральной нервной системе и периферических клетках крови.

Заключение. Болезнь Альцгеймера ассоциирована с нарастанием активности цистеиновых катепсинов в плазме, полиморфноядерных и моноядерных лейкоцитах периферической крови, что может рассматриваться как один из возможных маркеров развития и диагностики заболевания.

Ключевые слова: болезнь Альцгеймера, нейродегенерация, протеолиз, цистеиновые катепсины, плазма крови, полиморфноядерные лейкоциты, моноядерные лейкоциты.

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

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INTRODUCTION

Every year, the world's population of elderly people is growing, which leads to an increase in the number of patients with neurodegenerative diseases. Alzheimer's disease (AD) occupies a leading position among them in both prevalence and economic expenses. An important and unresolved problem is the late diagnosis and, accordingly, the late start of treatment, which only begins at the stages of clinically apparent dementia. Therefore, the issue of finding diagnostic methods for AD at the earliest (pre-demented and asymptomatic) stages of the development of the neurodegenerative process, which, according to a number of studies, is 15–20 years ahead of the formation of clinically apparent dementia, remains

relevant. To apply such preventive strategies in relation to AD, it is necessary to search for peripheral biomarkers in environments easily accessible for research (blood serum, saliva, urine). Given the multifactorial nature of neurodegeneration in AD, the creation of a multimodal diagnostic panel is considered more justified [1–3]. One of the possible biomarkers of AD may be a change in the activity of lysosomal enzymes of various cells.

Correct functioning of lysosomes is especially important for neurons, since they cannot reduce the content of accumulated toxic molecules and aggregates by cell destruction [2]. Disruption of lysosomal function plays an important role in the degeneration of neurons and in the pathogenesis of numerous neurodegenerative diseases [4]. In recent years, information indicating the participation of lysosomal proteases in the pathogenesis of AD has appeared [2, 3], although the data are very fragmented and not always unambiguous. However, there is no doubt that proteolytic enzymes are a very sensitive marker of cellular "trouble", and information about the level of activity of these enzymes can be used for early diagnosis and determination of the severity of a number of pathological conditions [5].

It has been proven that the amyloid precursor protein (APP) plays a key role in the pathogenesis of AD. The full-sized APP belongs to the type I transmembrane protein family and is thought to be involved in the regulation of protein transport [6]. The most studied extracellular region consists of several domains: E1, consisting of two subdomains (growth factor-like domain and copper-binding domain), and E2, linked by an acid domain (AcD). APP can undergo various types of proteolytic treatment [4, 5]. Successive cleavage of the protein by alpha and gamma secretase leads to the formation of p3 peptides (non-amyloidogenic pathway). In the case of alternative processing with the help of beta and gamma secretase, a polypeptide consisting of 40–43 amino acid residues (β-amyloid), insoluble in water, aggregating with the formation of polymers deposited in the form of plaques, is formed [7].

APP is the subject of extensive proteolytic treatment; therefore, theories about the effect of lysosomal proteases on the occurrence of AD, the possibility of a diagnostic study of cathepsin activity as a marker, and the use of their inhibitors or inducers in the treatment of the disease have been developed for a long time [8]. Cathepsin B is one of the proteins involved in the regulation of the number of Aβ peptides, but its role in the pathogenesis of AD requires further research. Paradoxically, on the one hand, since it possesses β-secretase activity, it can participate in the formation of Aβ peptides, and on the other hand, it can also participate in the processes of their degradation [6].

It was proved that the cathepsin B sulfhydryl group of cysteine (Cys32) cleaves the Aβ peptide from the carboxyl end at the location of the glutamic acid residue (Glu11), and a decrease in the production and activity of cathepsin B initiates the accumulation of Aβ peptides [9].

It is also known that cysteine cathepsins B and L are involved in the degradation of not only amyloid peptides, but also C-terminal fragments of APP and β-secretase (BACE1), and affect cholesterol metabolism in neurons. A decrease in the activity of these cathepsins or their inhibition leads to lysosomal deficiency, impaired synthesis of NPC1 and ABCA1 proteins that are involved in the release of cholesterol, and impaired degradation of key AD proteins [4].

A recent study showed that cathepsin B can accelerate the metabolism of Aβ peptides via lysosomal pathways and reduce memory deficit associated with AD. Hippocampal injections of the adeno-associated virus expressing cathepsin B decreased Aβ levels, increased Lamp1 and improved learning and memory [10].

At the same time, it is known that pyroglutamate-amyloid-β-peptides (pGlu-Aβ), which are especially harmful forms of amyloid-β-peptides present in the brain in AD, exist. PGlu-Aβ peptides are N-terminal truncated forms of full-sized Aβ peptides in which the N-terminal glutamate is cyclized to pyroglutamate to form pGlu-Aβ (3-40/42). Cathepsin B gene switching off the leads to a decrease in the level of pGlu-Aβ, and the use of an inhibitor of this enzyme (E64d experimental drug) demonstrated a decrease in memory deficit in experimental animals [11].

There is extensive evidence that the accumulation of mononuclear phagocytes, including microglial cells, monocytes and macrophages at the sites of β-amyloid deposition in the brain, is an important pathological characteristic of AD, and the concentration of these cells grouped around Aβ deposits is several times higher than in neighboring brain regions [12]. Since the blood-brain barrier is permeable to mononuclear and polymorphonuclear leukocytes, it can be assumed that changes in the metabolism of these cells may indirectly indicate pathological changes in brain tissue and be a peripheral marker of the neurodegenerative process. The aim of this study is to study the level of activity of lysosomal cysteine proteases (cathepsins H, B, and L) in blood plasma and fractionated leukocytes (polymorphonuclear (PMNL) and mononuclear leukocytes (MNL)) in patients with Alzheimer's disease and to compare with similar indicators in individuals without signs neurodegeneration as a possible marker of Alzheimer's disease development and diagnosis.

MATERIALS AND METHODS

Clinical material for the study were blood plasma and fractionated leukocytes (PMNL and MNL) obtained from 22 patients with Alzheimer's disease who underwent in-patient treatment and dispensary observation at the N.N. Bazhenova Regional Clinical Psychiatric Hospital. All patients included in the observation group have a diagnosis confirmed by instrumental laboratory methods according to modern diagnostic criteria. As a comparison group, we used blood plasma and fractionated leukocytes obtained from 22 patients of the same hospital, comparable in age and gender to patients of the observation group, but who did not have clinical signs of dementia and neurodegeneration. The study complies with ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association "Ethical Principles for the Conducting of Scientific Medical Research with Human Participation" as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation of June 19, 2003 No. 266.

Blood sampling was performed once on an empty stomach from the ulnar vein in an amount of 10 ml, heparin was used as an anticoagulant. Separation of leukocytes into fractions was carried out by the isopycnic centrifugation method [13]. Counting of leukocytes isolated from samples was carried out in the Goryaev chamber using a R-15 "Biolam" binocular microscope (Russia).

The resulting precipitation of washed leukocytes was brought to a concentration of 106 cells/ml with distilled water containing 0.1% X-100 triton solution and subjected to freezing and thawing three times to destroy plasma and lysosomal membranes. The resulting lysates were used to determine the activity of the studied enzymes [14–16].

The activity of cathepsins L, B, and H was studied by the spectrofluorimetric method of Barrett and Kirschke [17] with the measurement of the fluorescent reaction product 7-amido-4-methylcoumarin, which is formed upon cleavage of specific fluorogenic substrates: Na-carbobenzoxy-L-phenylalanyl-arginine-7-amido-4-methylcoumarin (N-CBZ-Phe-Arg-7-amido-4-methylcoumarin, Sigma, USA) for cathepsin L, arginine-7-amido-4-methylcoumarin (Arg-7-amido-4-methylcoumarin, Sigma, USA) for cathepsin H, and Na-carbobenzoxy-arginine-arginine-7-amido-4-methicoumarin (NaCBZ-Arg-Arg-7-amido-4-methylcoumarin, "Sig ma ", USA).

The activity of cathepsins in blood plasma was calculated in ncat/ml, and the activity of leukocytes in ncat/10^6 cells. For statistical processing of the results, Microsoft Excel and Statistica 10 programs were used. The normality of the distribution of the sample was evaluated by the Shapiro – Wilk test. The groups were compared using the nonparametric Mann – Whitney *U*-test. The result was statistically significant at $p < 0.05$. The results are presented as median, upper and lower quartiles Me (Q_1–Q_3).

RESULTS AND DISCUSSION

In the blood plasma of patients with AD, the activity of cathepsins H, B, L was statistically significantly increased compared to patients who did not have signs of neurodegeneration. Among the studied enzymes, the most significant increase was in the activity of cathepsin H (22-fold increase relative to the comparison group), while the activity of cathepsin B increased 2.8 times, and cathepsin L was 1.9 times (Fig. 1).

In PMNL, the activity of cathepsins B and H was increased 5 and 5.4 times, respectively, and the cathepsin L activity was 2 times (Fig. 2).

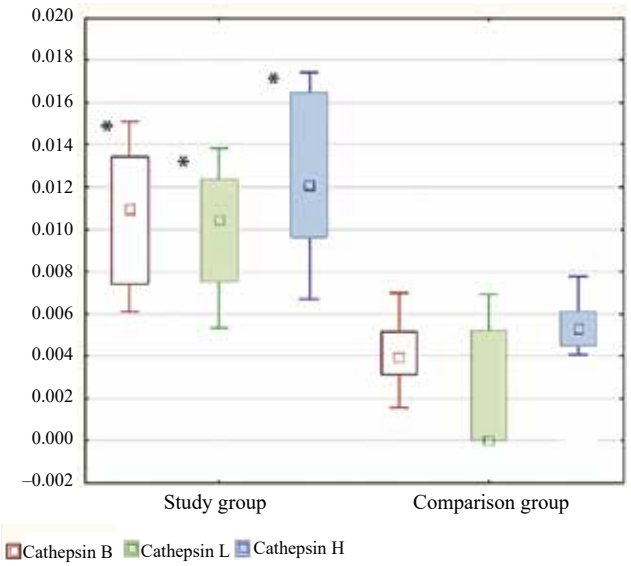

Fig. 1. Activity of plasma cathepsins in patients with Alzheimer's disease (observation group) and patients without signs of neurodegeneration (comparison group), ncat/ml

* marked statistically significant data.

The following pattern is noted in MNL: the most pronounced change in the cathepsin B activity is a 5 times increase relative to the comparison group, the cathepsin H activity is increased by 3.5 times, and cathepsin L increased by 1.7 times (Fig. 3).

A significant increase in the activity of cathepsin H in the blood plasma of patients with AD is of interest. Cathepsin H is an aminopeptidase with endopeptidase activity. It is possible that the increase in activity is relative, since the decrease in the activity of cathepsins B and L correlates with the accumulation of Aβ [10].

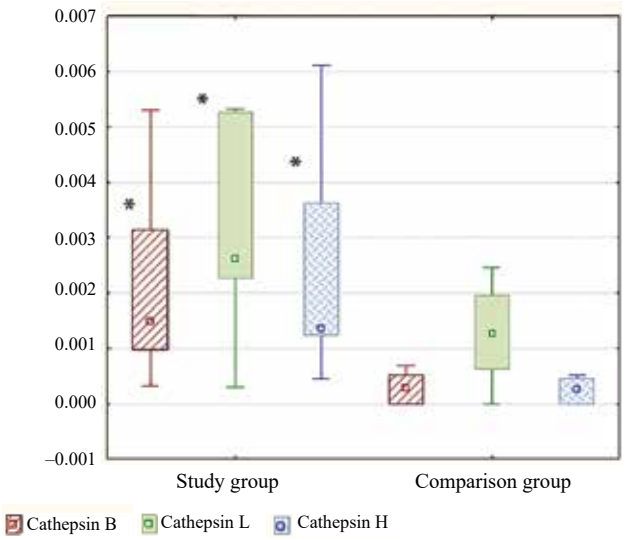

Fig. 2. Activity of polymorphonuclear leukocyte cathepsins in patients with Alzheimer's disease (observation group) and patients without signs of neurodegeneration (comparison group), nkat/10^6 cells

* marked statistically significant data

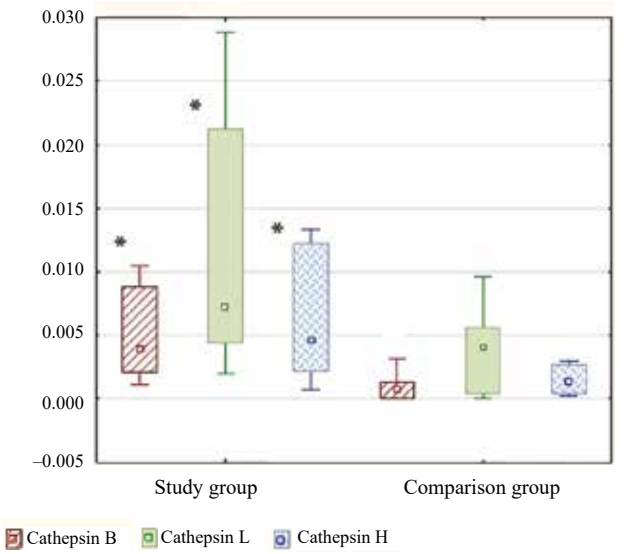

Fig. 3. Activity of cathepsin mononuclear leukocytes in patients with Alzheimer's disease (observation group) and patients without signs of neurodegeneration (comparison group), nkat/10^6 cells

* marked statistically significant data

However, on the other hand, an increase in the activity of cathepsin H can be explained by the participation of this enzyme in the metabolism of modified low-density lipoproteins, which include aprotein E (ApoE). The participation of ApoE in both the formation of amyloid plaques and the metabolism of APP has been proven. The ApoE4 isoform is the most susceptible to protease cleavage, and the resulting C-terminal fragment of the molecule has pronounced neurotoxic properties [6]. The increased activity of cathepsin B can be explained by its ability to penetrate the blood-brain barrier [8], as well as the active participation of this enzyme in the metabolism of APP.

The obtained results suggest the involvement of lysosomal leukocyte proteases in the neurodegenerative process, which is consistent with literature data on the participation of these blood cells in this pathology.

In fractionated leukocytes, the following unidirectional tendency is observed: a predominant increase in the cathepsins B and H activity against the background of a slight increase in the cathepsin L activity. Specific neutrophil granules contain more than 20 different types of proteases, and a huge number of receptors that determine their functional activity (for various interleukins, complement system factors and other biologically active molecules) are located on cell membranes. PMNLs contain a large number of lysosomes; various factors, including hypoxia, oxidative stress, and decreased insulin synthesis, can lead to a violation of the integrity of the lysosomal membrane [18]. Numerous studies in recent years prove the correlation between insulin resistance in brain tissue and neurodegenerative processes [19]. The destruction of PMNL lysosomal membranes and the release of cathepsins B and H into the cytoplasm can be considered a pathogenetically significant factor in AD and the development of neurodegeneration. Given the increased concentration of these cells around Aβ and the ability of leukocytes to penetrate the blood-brain barrier, a change in the activity level of blood leukocyte cathepsins is an important biomarker of the neurodegenerative process.

In literature there are references to an increase in the cathepsin B activity in the cerebrospinal fluid of patients with AD [20], which suggests a correlation between the state of lysosomal cysteine proteases activity in the central nervous system and peripheral blood cells. This means the studied parameter can be considered as a possible marker for the AD diagnosis.

CONCLUSION

Alzheimer's disease is associated with increased activity of plasma cysteine cathepsins, and polymorphonuclear and mononuclear peripheral blood leukocytes, which can be considered as one of the possible markers of the development and diagnosis of the disease.

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Modified method of vacuum therapy in the treatment of infected post-sternotomy wounds

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ABSTRACT

Sternal wound infections are a terrible complication that require long and complex treatment.

The aim of the study was to evaluate the results of using the modified method of vacuum therapy to treat purulent-septic complications of post-sternotomy wounds in clinical practice.

Materials and methods. According to the applied method of vacuum therapy, all patients with infectious complications of post-sternotomy wounds were divided into two groups (n = 25, average age 56.6 years). The classical vacuum therapy was used in the first group consisting of 12 patients. In the second group, 13 patients were treated with the help of the modified method of vacuum therapy.

Results. In the first group, 1 patient (8.3%) experienced osteomyelitis of the sternum, following a partial resection of bone plates; 1 patient (8.3%) developed sternal fistulas, which required long-term treatment; 1 patient (8.3%) had bleeding due to the injury of the left brachiocephalic venous trunk because of the direct contact of the polyurethane pad with the blood vessel wall. The bleeding was eliminated by fixing the damaged area of the vascular wall with U-shaped sutures using polytetrafluoroethylene pads. In the second group, no complications of this nature were observed. The modified method of vacuum therapy allows for the effective evacuation of the hemorrhagic discharge of the wound surface, the reduction of the degree of pathogen contamination in the adjacent tissues, and the elimination of bleeding risk.

Conclusion. The modified method of vacuum therapy in combination with effective algorithms for treating purulent-septic complications of post-sternotomy wounds allows physicians to avoid fatal complications and achieve good clinical results.

Key words: median sternotomy, sternal infection, postoperative mediastinitis, vacuum therapy, reosteosynthesis of the sternum.

Conflict of interest. The authors declare no obvious or potential conflict of interest related to publication of this manuscript.

Source of financing. The authors state that there is no funding for the study.

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 Ulyanovsk State University.

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Применение модифицированного метода вакуум-терапии при лечении инфицированных постстернотомных ран

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РЕЗЮМЕ

Актуальность. Раневая стернальная инфекция является грозным осложнением, требующим длительного и сложного лечения.

Цель исследования. Оценка результатов применения модифицированного метода вакуум-терапии при лечении гнойно-септических осложнений постстернотомных ран.

Материалы и методы. Все пациенты с инфекционными осложнениями постстернотомных ран (n = 25, средний возраст 56,6 лет) разделены на две группы. В 1-ю группу вошли 12 пациентов, у которых использовался классический метод вакуум-терапии. Во 2-й группе для лечения 13 пациентов применялся модифицированный метод вакуум-терапии.

Результаты. В 1-й группе у 1 (8,3%) больного наблюдался остеомиелит грудины, выполнена частичная резекция костных пластин, у 1 (8,3%) возникли стерно-кутальные свищи, что потребовало длительного лечения, у 1 (8,3%) вследствие травматизации левого венозного брахиоцефального ствола на фоне прямого контакта полиуретанового наполнителя со стенкой сосуда – кровотечение. Кровотечение удалось ликвидировать, ушив поврежденный участок сосудистой стенки п-образными швами с использованием прокладок из политетрафторэтилена. Во 2-й группе осложнений подобного характера не наблюдалось. Применение модифицированного метода вакуум-терапии позволяет эффективно эвакуировать геморрагическое отделяемое раневой поверхности, уменьшить степень контаминации патогеном прилежащих тканей, исключает риск возникновения кровотечения.

Заключение. Применение модифицированного метода вакуум-терапии в сочетании с эффективными алгоритмами лечения гнойно-септических осложнений постстернотомных ран позволяют избежать фатальных осложнений и добиться хороших клинических результатов.

Ключевые слова: срединная стернотомия, стернальная инфекция, послеоперационный медиастинит, вакуум-терапия, реостеосинтез грудины.

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 дали информированное письменное согласие. Исследование одобрено решением локального этического комитета Ульяновского государственного университета.

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INTRODUCTION

More than 57,000 open heart operations to treat cardiac pathology are performed annually in Russia [1]. A tendency to increase the total number of cardiac surgery interventions remains due to the development of large medical centers and the establishment of new cardiac surgery departments. Despite the proliferation of minimally invasive and interventional technologies, open heart surgery using median sternotomy as a surgical access remains one of the main treatment methods.

The active use of sternotomy as an access is due to this method being relatively simple and effective, as it provides visualization of all the main cardiac structures and vessels. However, despite the advantages of median sternotomy, the main disadvantages are the degree of traumatization and risks of infectious complications.

In order to prevent purulent-septic complications after sternotomy, guidelines for the elimination of sternal infection were developed and introduced into clinical practice [2]. Despite this fact, the incidence of post-sternotomy infectious complications remains quite high and ranges from 0.25 to 10% [3].

Vacuum therapy is among the most effective treatment methods of postoperative sternomediastinitis. However, the complex architectonics of post-sternotomy wounds, heterogeneity of tissues, and spread of bacterial strains resistant to antibiotics and antiseptics do not allow for complete cleansing of the wound cavity in 100% of cases.

The aim of the research was to evaluate the results of using the modified method of vacuum therapy in clinical practice to treat purulent-septic complications of post-sternotomy wounds.

MATERIALS AND METHODS

From January 2015 to December 2018, 379 open heart surgeries were performed using median sternotomy as a surgical access in the Department of Cardiac Surgery and Heart Rhythm Disorders of the Ulyanovsk Regional Clinical Hospital. In 10 cases (2.65%) the postoperative period was complicated by bleeding due to coagulopathy. In 3 cases (0.8%) the postoperative period was complicated by acute myocardial infarction. Purulent-septic complications of the wound surface of various severity developed in 25 cases, which is 6.6% of the total number of patients operated on.

Patients with infectious complications of the sternal wound were divided into two groups according to the method of vacuum therapy used for treatment. Patients were comparable by gender and age in both groups. The average age was 56.6 years old. The number of elderly patients (over 60 years old) was 10 people (40%) (Table 1). The first group (comparison group) included 12 patients whose purulent-septic complications of the sternal wound were treated with the standard method of vacuum therapy. The second group (main group) included 13 patients who underwent a course of the modified vacuum therapy (RF patent No. 183866, authors A. L. Charyshkin, A. A. Guryanov) (Fig. 1).

Table 1

The distribution of patients by age

Age (years old)	The 1st group (n = 12)	The 2nd group (n = 13)
18–39	1 (8.3%)	0 (0%)
40–59	7 (58.4%)	7 (53.8%)
60–74	4 (33.3%)	6 (46.2%)

Note. The number of patients – n.

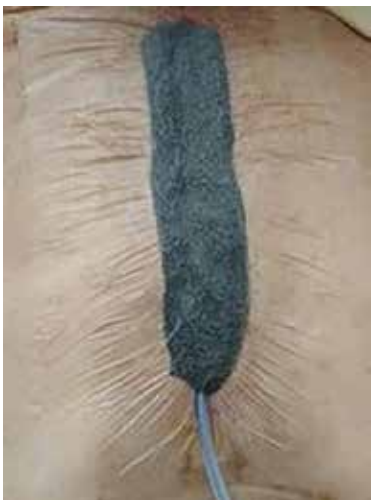

Figure 1. General view of the modified system

The developed vacuum system provides effective vacuum drainage of wounds using a device that consists of an air-tight dressing connected to a container for collecting wound discharge and a vacuum source (creating alternating negative pressure) though a vacuum-wire port. The vacuum-wire is placed in the center of the porous pad along its entire length beforehand (Fig. 2, *a*, *b*). In places where the pad comes into contact with potentially dangerous wound sites (the aortic wall, myocardium, coronary bypass grafts, left brachiocephalic vein, etc.), the polyurethane pad is covered with a film of synthetic material that is perforated with a 21G injection needle, 6 to 9 holes per 1 cm^2 are made throughout the film (Fig. 3).

The vacuum-wire located in the middle of the porous pad, as well as a special structure of the tube provides a uniform, more effective wound drainage. The synthetic film protects the porous pad from the granulation tissue germination. Moreover, the risk of bleeding during the

pad removal is eliminated. The film also prevents vulnerable sections of the wound from direct vacuum exposure. The perforated surface of the film retains adhesive properties of the pad.

To determine nature, extent and localization of the pathological process in the postoperative period, patients underwent chest CT with 3D reconstruction, ultrasound of soft tissues, wound culture test, and antibiotic sensitivity test.

a

b

Fig. 2. Installation of the drainage in the pad

Fig. 3. Perforated protective film

RESULTS

In both groups of patients, the same phasing of treatment was observed. Initially, the prevalence of infection was determined. When the infection was localized within the skin and subcutaneous tissue (8 patients (66.6%) in the first group; 8 patients (61.5%) in the second group), soft tissues were expanded to the sternum. Ligatures from the skin and subcutaneous tissue were removed, consistency of sternal sutures and their involvement in the inflammatory process of the sternum and retrosternal space were evaluated. If the sternal sutures were consistent, and the sternum and retrosternal space were intact, the wound was debrided with antiseptic solutions and a vacuum system was installed.

If sternal sutures were inconsistent, meaning that there was severe diastasis of the sternal edges or presence of an infection on the anterior mediastinum (4 patients (33.4%) in the first group; 5 patients (38.5%) in the second group), wire ligatures were removed and the sternal edges were spread. After that, the wound was debrided with antiseptic solutions and a vacuum system was installed. Removing sternal sutures in these conditions is a mandatory procedure, since otherwise they contribute to the destruction of the sternal edges during breathing and coughing. In addition, fragments of a wire suture can damage nearby structures, such as the lungs, heart, and great vessels [4].

When using vacuum systems in the Department of Cardiac Surgery of the Ulyanovsk Regional Clinical Hospital, both standard and modified methods were employed. In the modified system, a four-channel silicone tube, located in the center of the polyurethane pad throughout the wound, was used for drainage. Given the size of the wound surface, the complex structure of the wound and the heterogeneity of tissues, this arrangement of drainage made it possible to evacuate wound discharge as efficiently as possible and create a uniform rarefaction in all areas of the wound cavity. Considering that the polyurethane pad quite often comes into contact with such surfaces as the aortic wall, myocardium, suture area, mammary-coronary and aortocoronary bypass grafts, as well as their anastomoses, there is a risk of damage to these structures from direct vacuum exposure. It is also important that the polyurethane pad can tightly adhere to tissues after a prolonged contact, making further removal of the pad from the wound extremely dangerous (Fig. 4), since it can cause damage to the above structures.

In both groups the duration of vacuum therapy ranged from 48 to 72 hours, after that the system was removed, and the wound cavity was carefully debrided with antiseptic solutions before the vacuum system was reinstalled. This algorithm was followed until the wound cavity was cleansed and the level of contamination by the infectious agent reduced to acceptable parameters, less than 10^3 CFU [5]. These drugs were used as antiseptics in both groups: 3% hydrogen peroxide solution; 10% betadine solution; 1% dioxidine solution; Baneocin.

When the wound reached the optimal condition, further tactics were determined according to the size and location of the defect. If infection was limited to soft tissues, in both groups the wound was closed with vicryl No. 1 thread through all layers, using MacMillan – Donati sutures. In the case of retrosternal space involvement in the pathological process and the need for reosteosyn-

thesis of the sternum, the strategies of defect closure in the first and second groups were slightly different.

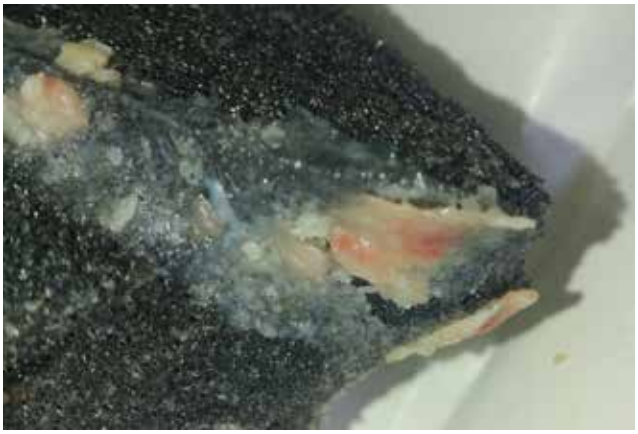

Fig. 4. Germination of the pad into adjacent tissues

Good revascularization of granulation tissue and sternal edges facilitate bleeding of different severity when sternum reosteosynthesis and re-closure of a postoperative wound are being performed. To reduce the risk of re-development of purulent-septic complications after sternum reosteosynthesis and soft tissue approximation in the first group of patients with deep sternal infection, the method of flow-washing drainage was used, and in the second group the method of gradual closure of the wound using vacuum therapy was employed.

During gradual closure of the wound, the first stage was reosteosynthesis of the sternum and suturing of the soft tissues of the upper half of the postoperative wound. A negative pressure system was installed at the lower part of the wound. Vacuum therapy was carried out for 3 days, after that the defect of the lower wound segment was closed.

This method made it possible to effectively evacuate the hemorrhagic discharge of the wound surface, preventing the formation of extensive hematomas, which subsequently lead to a relapse of infectious complications. In addition, the modified vacuum therapy allows the reduction of pathogen contamination of the adjacent tissues, diminishing risks of a relapse. While using this method in our department, we did not register a single case that required additional surgical hemostasis.

According to the results of treatment, in the first group, one patient (8.3%) suffered from sternal osteomyelitis that subsequently required a partial resection of the bone plates. One patient (8.3%) developed sternal fistulas in the long-term postoperative period, which required further prolonged treatment. One patient (8.3%) had bleeding due to the injured left venous brachiocephalic trunk because of the direct contact of the polyurethane pad with the vessel wall. The bleeding was eliminated by suturing the damaged part of the vascular wall with P-shaped sutures using polytetrafluorethylene pads. In the second group, complications of this nature were not observed. Mortality was absent in both groups.

DISCUSSION

According to many authors, the use of vacuum therapy in the treatment of infected post-sternotomy wounds is an effective method to achieve good results. However, experience of using such systems shows that it is not always possible to achieve complete cleansing of the wound. The large size, complex and heterogeneous structure of the wound cavity, as well as the infectious agent resistance greatly complicate the task.

To increase the operating efficiency of the vacuum system in our hospital, a non-standard way of arranging the evacuation drainage in relation to the polyurethane pad was used. We used the silicone drainage with a four-channel structure that was located in the center of the pad equidistant from all wound sections, which, in our opinion, ensured more effective elimination of the infectious agent and wound discharge.

One of the problems of using vacuum therapy in the treatment of deep sternal infection is the risk of damage to structures such as the great vessels walls, myocardium, coronary bypass grafts due to direct exposure to vacuum, or a combined effect of infection and negative pressure on tissues [6, 7, 8]. In order to prevent such problems in our hospital, we used a synthetic film to cover the polyurethane pad, while the adhesive properties of the pad due to perforation of the film were maintained.

Early detection of purulent-septic complications of post-sternotomy wounds allows to resort to active surgical tactics in a timely fashion, thereby to prevent the spread of infection and deeper structures from being involved in the pathological process [9, 10]. This contributes to effective treatment with less aggressive surgical tactics in the future. If sternal sutures become inconsistent and a patient with sternomediastinitis develops diastasis of the sternal edges, it is necessary to remove sternal sutures and expand the post-sternotomy wound completely. This allows to adequately sanitize the retrosternal space, and also to avoid further destruction of the sternal edges and damage to nearby organs by wire fragments.

CONCLUSION

The modified structure of a vacuum system allows to provide a more efficient evacuation of exudate and to create uniform rarefaction in all parts of the wound cavity. Coating the polyurethane pad with a film at the places of contact with the most vulnerable areas allows the avoidance of such terrible complications as damage

to the myocardium, walls of large vessels, and coronary bypass grafts. The gradual closure of the wound using vacuum therapy reduces the risk of septic complications relapse, because it prevents hematoma formation in soft tissues and further reduces the contamination degree of the wound until it is completely closed.

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Some aspects of complex rehabilitation of patients with acquired defects and deformities of the oropharyngeal area

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ABSTRACT

The aim of this study was to assess the features of disturbed food intake and find ways to optimize rehabilitation and resocialization processes for patients with acquired defects and deformities of the oropharyngeal zone.

Materials and methods. The study included 86 patients of a surgical hospital with defects and deformities of the oropharyngeal zone: 59 men and 27 women. The degree of dysphagia was assessed using clinical scales: volume-viscosity swallow test (V-VST) [7] and swallowing disability scale (SDS) [7]. Rehabilitation measures to normalize swallowing were performed in the experimental group (I), which consisted of 42 patients. The control group (II) consisted of 40 patients and was not included in the restorative effect. The groups were balanced according to the severity of the disorder, sex and age. Comparative analysis of the severity of impaired swallowing before and after rehabilitation and evaluation of its effectiveness were conducted.

Results. Data from the study of the dysphagia degree on the SDS scale for the whole sample ($n = 82$) suggest that the degree of disorder manifestation depends on the location and extent of anatomical defect. Moreover, comparative analysis suggests that the presence of a combined defect exacerbates the severity of dysphagia. Step-by-step speech therapy in the control group aimed at overcoming swallowing disorders included adaptive, compensatory and restorative strategies used in various combinations depending on the location of the defect and the severity of dysphagia. The comparison of the repeated assessment data on dysphagia severity in two groups of patients (I and II) showed that the rehabilitation measures had a positive impact.

Conclusion. We can state that speech therapy, which is a non-drug and non-invasive rehabilitation method, allows patients to successfully normalize eating process and helps in preventing cachexia-anorexia and dehydration, which is important for a successful postoperative period, as well as for improving the life quality of patients.

Key words. Dysphagia, swallowing disorders, oropharyngeal zone, speech therapy, rehabilitation, postoperative defects and deformities of the oropharyngeal region.

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

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Conformity with the principles of ethics. An initial conversation was conducted with each patient, informing them about the purpose, tasks, methods and techniques of pedagogical impact on the eating process normalization. All patients signed an informed consent to participate in the study.

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Некоторые аспекты комплексной реабилитации пациентов с приобретенными дефектами и деформациями орофарингеальной зоны

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РЕЗЮМЕ

Цель: оценка особенностей нарушений процесса приема пищи и поиск путей оптимизации процесса реабилитации пациентов с приобретенными дефектами и деформациями орофарингеальной зоны.

Материалы и методы. В исследование были включены 86 пациентов хирургического стационара с дефектами и деформациями орофарингеальной зоны: 59 мужчин и 27 женщин. Степень дисфагии оценивалась с помощью клинических шкал: Volume Viscosity Swallow Test (V-VST), Swallowing Disability Scale (SDS). Реабилитационные мероприятия по нормализации глотания проводились в экспериментальной группе (I), которую составили 42 пациента. Группа контроля (II), не включенная в восстановительное воздействие, состояла из 40 пациентов. Группы были уравновешены по тяжести дефекта, полу и возрасту. Проведен сравнительный анализ выраженности нарушений акта глотания до и после восстановительного воздействия и оценка его эффективности.

Результаты. Данные исследования степени дисфагии по шкале SDS по выборке в целом ($n = 82$) позволяют говорить о наличии зависимости степени проявления нарушения от места и объема анатомического дефекта. Причем сопоставительный анализ позволяет утверждать, именно наличие комбинированного дефекта усугубляет тяжесть дисфагии. Поэтапно проводимое в контрольной группе логопедическое воздействие, нацеленное на преодоление расстройств глотания, включало в себя адаптивные, компенсаторные и восстановительные стратегии, применяемые в различных комбинациях в зависимости от локализации дефекта и тяжести дисфагии. Результаты сопоставления данных повторной оценки тяжести дисфагии у двух групп пациентов (I и II) показали, что проведенные реабилитационные мероприятия оказали положительное влияние.

Заключение. Таким образом, можно утверждать, что логопедическое воздействие, которое относится к немедикаментозным, неинвазивным методам реабилитации, позволяет успешно нормализовать процесс приема пищи, способствует профилактике кахексии-анорексии и дегидратации, что важно для успешного течения послеоперационного периода, а также улучшения качества жизни пациентов.

Ключевые слова: дисфагия, нарушения глотания, орофарингеальная зона, логопедическое воздействие, реабилитация, послеоперационные дефекты и деформации орофарингеальной области.

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

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

Соответствие принципам этики. С каждым из пациентов была проведена первичная беседа, информирующая о целях, задачах, методах и приемах педагогического воздействия по нормализации процесса приема пищи. Все пациенты подписали информированное согласие.

Для цитирования: Уклонская Д.В., Косова Е.В., Неклюдова М.В., Решетов Д.Н., Уклонская А.А. Некоторые аспекты комплексной реабилитации пациентов с приобретенными дефектами и деформациями орофарингеальной зоны. *Бюллетень сибирской медицины.* 2020; 19 (3): 95–100. https://doi.org: 10.20538/1682-0363-2020-3-95-100.

INTRODUCTION

Currently, most experts support the view that eating disorders increase the risk of postoperative complications, and adequate and timely correction makes it possible to reduce their frequency after surgery, as well as to increase the treatment tolerance and life quality of patients [1]. In patients with cachexia-anorexia syndrome, the overall results of treatment deteriorate in proportion to the degree of body exhaustion. Today, it is obvious for specialists that nutritional support is a necessary part of accompanying therapy in the treatment of patients, including those with a surgical profile [2–5]. Within multidisciplinary approach, it is a convincing proof that a dietitian should participate in a rehabilitation process [6]. However, there are categories of patients with various types of eating disorders, for example, caused by structural or neurogenic disorders in head and neck [7–9]. In these cases, a rehabilitation process is more complicated, and it requires special approaches to recovering of impaired functions and inclusion of a dysphagia rehabilitation specialist into a multidisciplinary team.

Eating process is important not only for life quality, but also for social integration. In this context, it is important to mention an extremely difficult contingent of children with congenital cleft lip and palate, as well as patients with acquired defects and deformities of the oropharyngeal area because of various injuries or anti-neoplastic treatment. These anatomical areas are important for chewing, swallowing, breathing, and speech production. Therefore, inevitably occurring disorders of the above functions significantly worsen the body exhaustion and complicate treatment and rehabilitation [10].

This issue is most acute after surgical removal of neoplasms of the oropharyngeal area. According to G. Nitenberg and B. Raynard, malnutrition is detected in 40–80% of patients with head and neck tumors [1]. Alimentary disorders are the main cause of developing complications or increase them. Our observations suggest that 64.7% of patients after oropharyngeal surgeries reported significant weight loss (more than 10 kg within 3 months after surgery).

According to J. Logemann, the act of swallowing involves several successive stages: placing food in the oral cavity (oral-preparatory phase), passing it through the oral cavity (oral-transfer phase), transporting through the pharynx to the cricopharyngeal sphincter (pharyngeal phase), the process of overcoming the pharyngeal-esophageal junction and getting food into the esophagus (esophageal phase) [12]. Thus, anatomical defects of the oropharyngeal area with high probability lead to so-called “pre-swallowing” disorders that occur before the swallowing reflex is triggered. The result of such swallowing disorders will be the development of malnutrition and dehydration. Thus, according to S.A. Kravtsov, N.V. Kirillov and T.V. Korshunova, one of the main reasons of nutritional insufficiency development is post-resection defects of the oropharyngeal area [2].

Unfortunately, there is not enough information on the pathogenesis and correction of these disorders in specialized literature. This explains the lack of awareness in specifics of working with this pathology. Traditionally, it is a speech pathologist who treats swallowing and speech disorders, but this issue is not clearly defined within a multidisciplinary rehabilitation program yet.

The aim of our research is to assess the features of disturbed food intake and find ways to optimize rehabilitation for patients with acquired defects and deformities of the oropharyngeal zone.

MATERIALS AND METHODS

This multicenter parallel study was conducted from 2017 to 2019 at FSAI “LRC” of Health Ministry of the Russian Federation and PHI CCH “RZD-Medicine”. The study was open, non-randomized, longitudinal, panel, and controlled.

86 patients of the surgical hospital with postoperative defects and deformities of the oropharyngeal area were examined: 59 men and 27 women. The entry criterion was the disturbed pre-swallowing phase of eating. Later 4 patients were excluded from the study due to the main disease progression.

The experimental group (I) included 42 patients, 26 men and 16 women. These patients underwent a full course of speech therapy for correcting dysphagia during 2.5–3 months. The control group (II) consisted of 40 patients (29 men and 11 women) with similar swallowing disorders who, due to various objective and subjective circumstances, were unable to complete a full rehabilitation course aimed at dysphagia treatment. The groups were balanced according to the severity of the defect, sex and age. The control group was examined for swallowing act problems and consulted. A repeated examination was performed in both groups of patients during their planned hospitalization after 3 months.

Examination and case management of patients were carried out by the multidisciplinary team of specialists. After removal of nasogastric tubes, all

observed patients underwent a postoperative speech examination. With the help of a surgeon, their swallowing process was tested by asking them to swallow food and liquids of various consistencies (the volume viscosity swallow test (V-VST) [7]. To determine a diet type for each patient, the degree of dysphagia was also assessed (swallowing disability scale (SDS)) [7]. According to this scale, a score of 0 points was considered as no dysphagia, 1 point – light dysphagia, 2 points – medium-light dysphagia, 3 points – medium dysphagia, 4 points – medium-severe dysphagia, 5 points – severe dysphagia, 6 points – aphagia, lack of ability to take food perorally and the need for a nasogastric tube or gastrostomy. It was also visually assessed which swallowing phase demonstrated most severe disorders.

After detecting dysphagia, speech therapy was used to eliminate swallowing disorders, differentiated by stage and the degree of severity. In the course of speech therapy, compensatory, adaptive and restorative strategies were employed.

The IBM SPSS Statistics 22.0 statistical package was used to process the received data.

RESULTS AND DISCUSSION

Among patients with acquired food intake disorders caused by "pre-swallowing" disorders, difficulties are observed in the oral-preparatory and oral-transfer phase of the swallowing act or in both phases. The problematic phase is determined by the location and extent of anatomical defect. The number of patients with one or another problem in the "pre-swallowing" phase is shown in Table 1, which demonstrates that groups I and II are comparable in frequency of occurrence of various disorders.

Table 1

The localization of violations in eating acts (phases of the "pre-swallowing" cycle)

Disturbed phase before swallowing cycle	Group I (n = 42)	Group II (n = 40)	The sample as a whole (n = 82)
Oral-preparatory (subgroup 1)	7 (16.7%)	9 (22.5%)	16 (20%)
Oral-transfer (subgroup 2)	20 (47.6%)	18 (45%)	38 (46%)
Combined (subgroup 3)	15 (35.7%)	13 (32.5%)	28 (34%)

Note. Number of patients – n.

Data on the degree of dysphagia on the SDS scale [7] for the sample as a whole (n = 82) allow to claim that the degree of disorder manifestation depends on the location and extent of anatomical defect. The Mann – Whitney U-test for independent samples is significant when comparing the subgroups 1 and 3 ($p < 0.0001$), as well as the subgroups 2 and 3 ($p < 0.0001$), and is not significant when comparing subgroups 1 and 2 ($p < 0.249$), which suggests that it is the combined defect that aggravates the severity of dysphagia. Figure 1 shows differences in the frequency of occurrence for different degrees of dysphagia in patients with different problematic phases in the pre-swallowing cycle.

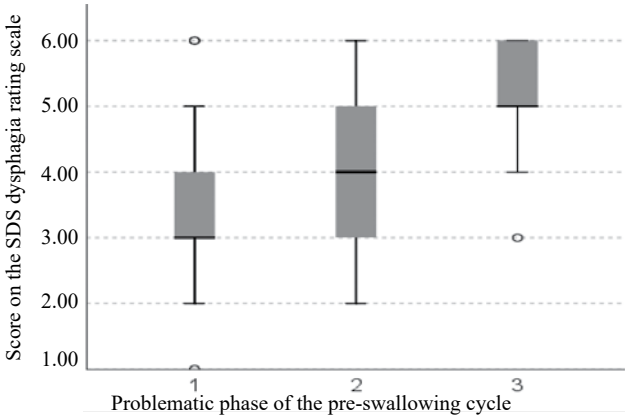

Fig. 1. Severity of dysphagia for various phases of the pre-swallowing cycle

These data were taken into account when defining the strategy of rehabilitation measures.

Then, patients in the group I underwent a course of speech therapy aimed at normalizing the eating process. Speech therapy began in a hospital, where classes were held daily for 7–10 days, and then continued on an outpatient basis, on average 2–3 times a week for 2 months. After completing the course on normalization of the swallowing act, the food intake process was evaluated again in 3 months.

The content of speech therapy for dysphagia correction is correctional-pedagogical work in the following ways: teaching hygienic care of the oral cavity; static and dynamic (active and passive) gymnastics aimed at normalization of swallowing, recovery of the functional activity of the preserved muscles involved in the swallowing act (chewing, mimic muscles, tongue muscles), as well as stimulation of the oral mucosa sensitivity; if necessary, "disinhibition" of the swallowing act; differentiated (activating/relaxing) massage of the face and cheeks from the outside and inside, massage of the tongue and soft palate, neck and shoulder girdle (with caution, after discussing it with a surgical oncologist); recovery of coordination between swallowing, breathing and phonation.

When treating swallowing disorders of a peripheral genesis by pedagogical methods, we followed the certain steps:

1. Preparatory – establishing contact, assessing the manifestation degree of swallowing disorders, physiologically conditioned phonation breathing, passive and active articulatory gymnastics, recommendations on nutrition (food consistency) and conversations aimed at supporting and motivating the patient to recover.

2. Active training – restoration of the functional activity of the muscles involved in the swallowing act with the help of static and dynamic articulatory gymnastics, the formation of the skill of "safe swallowing" using adaptive and compensatory technologies.

3. Consolidation of the restored skills and the formation of a stable stereotype of "safe" swallowing (coordination of swallowing, phonation and breathing).

From the viewpoint of corrective action, the restoration of swallowing function is possible through restoring the motor function of intact anatomical structures. Because of a significant anatomical defect in the tongue tissues and floor of the oral cavity, there can arise severe difficulties. An effective movement (migration) of the bolus to the pharynx (into the oropharynx) for a subsequent swallow can be even impossible. In this case, during the postoperative period patients often complain about the accumulation of food in the mouth or their inability to swallow it. In this regard, the use of postural techniques and the selection of textures for swallowing is particularly relevant in rehabilitation of the swallowing function.

On the one hand, the texture of the bolus should be moderately viscous (solid) so that the intact neuromuscular zones "have time" to react to the bolus and activate for the act of swallowing. On the other hand, this texture must be sufficiently liquid (fluid) so that when the head is thrown back, the bolus can automatically move to the pharynx for swallowing, which becomes especially relevant in the case of extensive tongue resections. The optimal (to be transported through the oral cavity) food consistency was selected empirically, individually for each patient.

Since the wound healing process in this case can be time-consuming, and there are often a number of objective reasons that impede eating through natural ways, an important component of speech therapy is to inform not only medical personnel, but also a patient and his family about the rules and peculiarities of food intake in the postoperative period. These rules must be followed in case of such swallowing disorders. After removal of the nasogastric tube, food should be eaten in a sitting, half-sitting position, or lying on the side with the chin raised as in this case aspiration is less likely. In severe cases, when swallowing food is completely impossible, the patient continues to use a nasogastric tube, and then feeding can be carried out through a gastrostomy tube.

At the end of the course aimed at normalizing the act of swallowing, the food intake process was evaluated again.

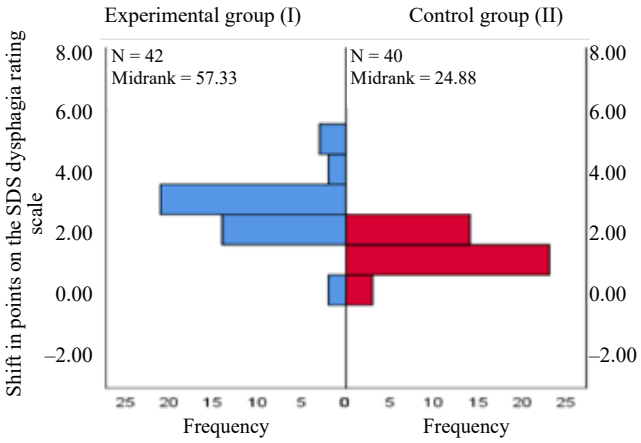

Fig. 2. SDS scale shift in two groups of patients (points)

The repeated assessment of the dysphagia severity was carried out, the results of two groups of patients (I and II) were compared. They showed that the rehabilitation measures carried out in the group I had a positive impact. Differences in the shift on the SDS scale [7] (the difference in points between the first and the second assessment of the dysphagia degree) between the groups were significant (Mann – Whitney *U*-test, $p < 0.0001$). Figure 2 demonstrates the frequency of improvement degree (in 0, 1, 2, 3, 4, 5 points) in two groups (I – took the rehabilitation course; II – did not take the rehabilitation course).

CONCLUSION

Among patients with acquired anatomical defects and deformities of the oropharyngeal area, disturbances of the food intake act at the "pre-swallowing" stage are observed. These disorders manifest in the oral-preparatory or oral-transfer phase or in both phases, and require immediate normalization. Moreover, the presence of a combined defect aggravates the severity of dysphagia. Eating process is a vital function, so normalizing food intake in the postoperative period is an integral part of speech therapy of acquired swallowing disorders. Corrective action, which refers to non-drug and non-invasive rehabilitation methods, includes compensatory, adaptive and restorative strategies. This allows successful normalization of the eating process and helps to prevent cachexia-anorexia and dehydration, which is important for a successful postoperative period, as well as for improving the life quality of patients.

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Uklonskaya D.V., Kosova E.V. – conception and design, carrying out of research, analysis and interpretation of data, drafting and justification of the manuscript. Neklyudova M.V., Reshetov D.N. – critical revision of the manuscript for important intellectual content. Uklonskaya A.A. – analysis of modern foreign sources on the research topic, compilation of databases, statistical processing.

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Features of functional annotation of rheumatoid arthritis susceptibility genes by Cytoscape

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ABSTRACT

Aim. To evaluate the functional annotation of genes associated with rheumatoid arthritis with different parameters of the ClueGO Cytoscape tool.

Materials and methods. Genes of susceptibility to rheumatoid arthritis were extracted from publicly available database GWAS (catalog of associations of single nucleotide polymorphisms with diseases). The Gene Ontology (GO), the functional annotation of genes, was performed using Cytoscape ClueGO. The features of the functional annotation using the plugin ClueGO Cytoscape were analyzed.

Results. Depending on the initial parameters specified in the plugin, the grouping of terms according to the gene ontology was carried out with a different degree of generalization. A smaller minimum number of genes in a group allows to form a larger number of groups, which makes it possible to obtain more detailed functional characteristics.

Conclusion. The results obtained with different grouping options can be useful for further studies of genetic mechanisms of rheumatoid arthritis.

Key words: functional annotation of genes, rheumatoid arthritis, GWAS, Cytoscape.

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Особенности функциональной аннотации генов предрасположенности к ревматоидному артриту при использовании Cytoscape

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РЕЗЮМЕ

Цель. Оценить функциональную аннотацию генов, ассоциированных с ревматоидным артритом, при разных параметрах инструмента ClueGO Cytoscape.

Материалы и методы. Гены предрасположенности к ревматоидному артриту были извлечены из публичной базы данных GWAS (каталог ассоциаций однонуклеотидных полиморфизмов с заболеваниями). Генная онтология (Gene Ontology, GO) – функциональная аннотация генов была произведена с помощью алгоритма, реализованного в Cytoscape ClueGO.

Результаты. Рассмотрены особенности выполнения функциональной аннотации с помощью плагина ClueGO при разных условиях формирования функциональных групп. В зависимости от исходных пара-

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метров, задаваемых в плагине, группировка терминов согласно генной онтологии осуществляется с различной долей обобщения. Меньшее минимальное число генов в группе позволяет сформировать большее число групп, что дает возможность получить более детальную функциональную характеристику.

Заключение. Полученные варианты результатов функциональной аннотации могут быть востребованы для дальнейших исследований генетических механизмов ревматоидного артрита.

Ключевые слова: функциональная аннотация генов, ревматоидный артрит, GWAS, Cytoscape.

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

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INTRODUCTION

The increase in the volume of genomic and proteomic research has led to the need to accumulate its results in specialized databases, including publicly available online resources. A huge role in the systematization and description of such data is played by gene ontology (GO, GeneOntology), a unified universal hierarchical terminology system [1]. It allows for the characterization of data into such sections as biological processes, molecular functions, and cellular components [2], in accordance with which the annotation of genes (proteins) is performed. For GO capabilities to be applicable to a specific data set, specialized tools exist, such as ClueGO Cytoscape [3], which allows simultaneous work with multiple data lists and networks.

The ability to describe the results of studies in terms of gene ontology plays an important role in the implementation of the functional characteristics of thousands of genes (for example, in microchipping). Grouping them in a certain way allows researchers to evaluate the possible contribution of the studied genes to the implementation of the physiological response or etiopathogenesis of diseases. This approach is relevant for studying the genetic factors of multifactorial (complex) pathologies [4], in particular, rheumatoid arthritis, regarding which no similar studies (based on the results of a genome-wide search) have been conducted.

The aim of this study was to evaluate the functional annotation of genes which are associated with rheumatoid arthritis with different parameters of the ClueGO Cytoscape tool.

MATERIALS AND METHODS

Rheumatoid arthritis susceptibility genes were obtained by analyzing data from an information source containing the results of genome-wide association studies (GWAS) [5], namely, associations between single nucleotide polymorphisms and diseases. To carry out functional analysis, ontology types corresponding to the GeneOntology service were selected in the ClueGO Cytoscape version 3.2.1 plugin. ClueGO allows for updates of this information, since GeneOntology is constantly updated [6].

The functional similarity of the genes was evaluated using a hypergeometric test, and the genes belonged to specific functions in terms of gene ontology. Additionally, the plugin allows users to adjust the minimum number (percentage) of genes used to form groups, by default, 3 and 4, respectively. On a positive scale from 0 to 1, the level of Cohen's kappa coefficient was established, reflecting the functional relationships between genes (0.4). In case of testing a large number of hypotheses, ClueGO allows for correction for *P* (probability of committing a type I error) using the Bonferroni and Benyamini – Hochberg methods [7]. At the same time, both *P* values for each term are presented in the Table.

Functional groups were created by iteratively merging initially defined groups based on a predetermined kappa threshold value. The program suggests choosing a "leading" term in each group according to their statistical significance and number or percentage of genes.

RESULTS AND DISCUSSION

A list of 94 genes associated with rheumatoid arthritis was formed using the GWAS resource: *ACOXL, AIRE, ALS2CR12, ANAPC4, ANO8, ANXA3, APOM, ARHGEF3, ARID5B, BAG6, BLK, BTNL2, C1orf159, C2,C5orf30, CBFB, CCL21, CCR6, CD226, CD247, CD40, CDK5RAP2, CDK6, CLYBL, CMKLR1, CTTNBP2, DDA1, DPP4, EOMES, FADS2, FAM107A, FAM98B, GATA3, GATSL3, GCH1, GRM5, GUCY1B2, HNRNPA1, IFI16, IL2RA, IL2RB, IL6R, IRF5, JAZF1, KIAA1109, KIF5A, LLGL1, MECP2, MMEL1, MOV10L1, MTF1, MTG1, MTM1, NFKBIE,*

OS9, PDE2A, PDGFA, PHF19, PHTF1, PLCL2, PLD4, PPIL4, PPM1D, PPME1, PRKCH, PTPN2, PTPN22, RABEP1, RAD51B, RASGRP1, REL, RPP14, RTKN2, SFTPD, SPTBN2, STAG1, STAT4, SUOX, SYNGR1, TEC, TIMMDC1, TNFAIP3, TNPO3, TRHDE, TYK2, UBASH3A, UBE2F, VTCN1, WDFY4, WNT10B, YDJC, ZBTB12, ZNF133, ZNF774.

The following types of ontologies were used to classify the genes: GO_ImmuneSystemProcess (immune system process), GO_Molecular Function (molecular function), GO_CellularComponent (cellular component), and GO_Biological Process (biological process). The minimum GO Level was 3, the maximum GO Level was 8.

Functional analysis of genes (for a predetermined minimum number of genes in groups 2, $p < 0.05$) revealed 8 groups of genes in accordance with the terms of gene ontology:

1) regulation of the production of interleukin (IL)-2 (includes 22 functions);

2) the signaling pathway of IL-2 (includes 15 functions);

3) antigen receptor-mediated signaling pathway (includes 12 functions);

4) production of IL-12 (includes 5 functions);

5) positive regulation of the G2 /M transition of the mitotic cell cycle (includes 2 functions);

6) regulation of neuronal synaptic plasticity (includes 2 functions);

7) positive regulation of cytotoxicity associated with natural killer cells (includes 2 functions);

8) regulation of the respiratory processes (includes 2 functions).

In addition, 6 functions that were not merged were identified:

1) the signaling pathway of IL-6;

2) chemotaxis of dendritic cells;

3) neuromuscular control of body position;

4) negative regulation of the innate immune response;

5) response to muramyl dipeptide;

6) regulation of platelet activation.

In order to determine the most informative results in terms of biological interpretation, a functional analysis of the genes associated with rheumatoid arthritis was carried out with a different minimum number of genes in groups (3 genes), $p < 0.05$.

As a result of gene grouping, 5 groups were obtained:

1) the signaling pathway of IL-2 (includes 16 functions);

2) antigen receptor-mediated signaling pathway (includes 12 functions);

3) negative regulation of cell activation (includes 11 functions);

4) production of IL-12 (includes 3 functions);

5) regulation of neuronal synaptic plasticity (includes 2 functions).

Four functions were represented in separate terms (without association):

1) negative regulation of the innate immune response;

2) respiratory gas exchange;

3) regulation of histone methylation;

4) platelet-derived growth factor receptor signaling pathway.

The results obtained indicate that depending on the initial parameters specified in the ClueGO Cytoscape plugin, the grouping of gene ontology terms associated with genes is carried out with a different degree of generalization.

In the first case (with the minimum number of genes in group 2), a larger number of groups was formed compared to those in the second case (with a minimum number of genes in group 3), which made it possible to obtain a more detailed functional characteristic.

Moreover, for some functions identified in both research options, the number of genes in the groups was smaller in the first case compared to the second one. So, for the production of IL-12, the corresponding number of genes was 5 (*CD40, CMKLR1, IRF5, NFKB1, REL*) and 6 (*CD40, CMKLR1, IRF5, NFKB1, REL, TRAF3*), respectively. For the IL-2 signaling pathway, the number of genes was 14 (*BPI, BTNL2, CCL21, CCR6, CMKLR1, GATA3, IL2RA, IL2RB, IL6R, NFKB1, PDGFA, PTGIS, PTPN2, PTPN22, RORA, SFTPD, TNFAIP3, UBASH3A)* and 19 *(BPI, BTNL2, CCL21, CCR6, CMKLR1, GATA3, IL2RA, IL2RB, IL6R, NFKB1, PDGFA, PTGIS, PTPN2, PTPN22, RORA, SFTPD, TNFAIP3, UBASH3A, VTCN1*), respectively. At the same time, for the functions of the antigen receptor-mediated signaling pathway and regulation of neuronal synaptic plasticity, the number of genes remained the same in both variants of the study.

With detailed functional annotation in the first version of the study, the presence of rheumatoid arthritis susceptibility genes in the following functions of the immune response regulation was revealed: the signaling pathway of IL-6, which is the key cytokine responsible for autoimmune inflammation [8, 9]; regulation of chemotaxis of dendritic cells; response to muramyl dipeptide (an element of the bacterial cell wall that activates both innate and acquired immunity). In addition, the affiliation of genes to the functions of positive regulation of the G2 /M transition of the mitotic cell cycle and neuromuscular control of body position was determined.

The enlarged functional groups obtained in the second case reflect the general pattern characteristic of the previous result: the participation of genes in the signa-

ling pathways of IL-2 and IL-12 was revealed. IL-2 is known to play an essential role in the development of the immune response, as it stimulates killer cells [10]. IL-12 has pronounced pro-inflammatory properties and increases the activity of natural killer cells and dendritic cells, linking the innate and acquired immunity through the combined effect [10–12]. In addition, the functional group of regulation of neuronal synaptic plasticity indicates a possible effect of genes on the process of neuronal processing of the synaptic signal.

CONCLUSION

The results obtained indicate that with rheumatoid arthritis, susceptibility genes affect not only the implementation of the immune response mediated by signaling of pro-inflammatory cytokines (interleukin-2, -6, -12) and regulation of immunocytes, but also the functions of the nervous system; in particular, synaptic signal processing and neuromuscular body position control.

To study the possible mechanisms of diseases or physiological processes, details regarding the involvement of individual signaling pathways and cellular responses may be important. To do this, it is advisable to change the minimum number of genes that are combined into a functional group towards reduction (compared to the default value in the plugin). At the same time, enlarged groups of functional gene characteristics demonstrate greater clarity when identifying general trends in biological processes.

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The content of hypoxia-inducible factors and mediators of immunosuppression in the blood in diseases associated with hypoxia

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ABSTRACT

The aim of the study was to identify general patterns and features of changes in the content of hypoxia-inducible factors-1 and -2 in association with an imbalance of cytokines (IL-10, IL-13, galectin-2 and -9, IFN-gamma) in the blood in diseases associated with hypoxia.

Materials and methods. We examined 25 patients with coronary heart disease (CHD) with heart failure II-III according to NYHA, 16 patients with chronic obstructive pulmonary disease (COPD) without exacerbation, 16 patients with infiltrative pulmonary tuberculosis (TB) before anti-TB therapy, and 18 relatively healthy donors. Plasma concentrations of HIF-1alpha, HIF-2alpha, IL-10, IL-13, galectins-2 and -9, and IFN-gamma were determined by enzyme-linked immunosorbent assay (ELISA).

Results. Positive outcomes of quantity determination of HIF-2alpha in the blood (24.00 ± 8.54 %, 75.00 ± 10.83%, 43.75 ± 12.40% of patients, respectively, against «zero» values in healthy donors) and also signs of immunosuppression at normal plasma concentrations of HIF-1alpha were determined in diseases associated with chronic hypoxia (in patients with CHD, COPD, TB). Immunological insufficiency in CHD and TB is caused by a deficiency of IFN-gamma and galectin-2 in association with an excess of galectin-9 (in patients with CHD 1.10 [0.52; 2.60] pg/ml $p = 0.038$) or IL-13 (in patients with TB 0.81 [0.79; 1.40] pg/ml, $p = 0.043$), and in patients with COPD it is caused by a surplus of galectin-9 and IL-13 (8.50 [3.96; 15.00] pg/ml, $p = 0.001$ and 2.62 [1.20; 7.58] pg/ml, $p = 0.002$, respectively) at normal concentrations of IFN-gamma and galectin-2. The content of IL-10 in the blood tends to increase in CHD and COPD.

Conclusion. In patients with CHD, COPD and TB, chronic hypoxia is associated with immunosuppression mediated by an imbalance of IL-10, IL-13, IFN-gamma, galectins (2 and 9) in the blood and the secretion of HIF-2alpha, which has the property to stimulate the differentiation of M2-macrophages synthesizing anti-inflammatory cytokines.

Key words: hypoxia, hypoxia-inducible factor (HIF), coronary heart disease, chronic obstructive pulmonary disease, tuberculosis, interleukin, galectin, immunosuppression.

Conflict of interest. The authors declare no obvious or potential conflicts of interests 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 of the Siberian State Medical University (Protocol No. 5046 of 28.11.2016).

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Содержание гипоксия-индуцируемых факторов и медиаторов иммуносупрессии в крови при заболеваниях, ассоциированных с гипоксией

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РЕЗЮМЕ

Цель. Выявить общие закономерности и особенности изменений содержания гипоксия-индуцируемых факторов-1 и -2 в ассоциации с дисбалансом цитокинов (интерлейкина (IL)-10, IL-13, галектина-2 и -9, интерферона γ (IFNγ)) в крови при заболеваниях, ассоциированных с гипоксией.

Материалы и методы. Обследованы 25 пациентов с ишемической болезнью сердца (ИБС) с сердечной недостаточностью II–III по NYHA; 16 пациентов с хронической обструктивной болезнью легких (ХОБЛ) вне обострения; 16 больных с инфильтративным туберкулезом легких (ТЛ) до проведения противотуберкулезной терапии; 18 относительно здоровых доноров. В плазме крови определяли концентрацию HIF-1α, HIF-2α, IL-10 и -13, галектина-2 и -9, IFNγ методом иммуноферментного анализа.

Результаты. При заболеваниях, ассоциированных с хронической гипоксией (у больных ИБС, ХОБЛ, ТЛ), обнаруживаются положительные результаты определения HIF-1α в крови (у 24,00 ± 8,54; 75,00 ± 10,83; 43,75 ± 12,40% больных соответственно при «нулевых» значениях показателя у здоровых доноров) на фоне нормальной плазменной концентрации HIF-1α, а также признаки иммуносупрессии. Иммунологическая недостаточность при ИБС и ТЛ обусловлена дефицитом IFNγ и галектина-2 в ассоциации с избытком галектина-9 (у больных ИБС 1,10 [0,52; 2,60] пг/мл; p = 0,038) или IL-13 (у пациентов с ТЛ 0,81 [0,79; 1,40] пг/мл; p = 0,043), а у больных ХОБЛ – профицитом галектина-9 и IL-13 (8,50 [3,96; 15,00] пг/мл; p = 0,001 и 2,62 [1,20; 7,58] пг/мл; p = 0,002 соответственно) при нормальной концентрации IFNγ и галектина-2. Содержание IL-10 в крови проявляет тенденцию к увеличению при ИБС и ХОБЛ.

Заключение. У больных ИБС, ХОБЛ и ТЛ хроническая гипоксия ассоциирована с иммуносупрессией, опосредованной дисбалансом IL-10, IL-13, IFNγ, галектина-2 и -9, в крови и секрецией HIF-2α, который обладает свойством стимулировать дифференциацию М2-макрофагов, синтезирующих противовоспалительные цитокины.

Ключевые слова: гипоксия, гипоксия-индуцируемый фактор (HIF), ишемическая болезнь сердца, хроническая обструктивная болезнь легких, туберкулез, интерлейкины, галектины, иммуносупрессия.

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

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INTRODUCTION

In the last decade, the number of publications devoted to the study of features of intracellular signaling and processes of intercellular cooperation in cell adaptation to hypoxia has increased. Moreover, the majority of literature concerning this topic contains information on the formation of hypoxia-induced factor-1 (HIF-1) increasing under oxygen deficiency condition [1–4]. Scientists' interest in assessment of this factor in hypoxia results from the universality of this response (hypoxia refers to typical pathological processes) and, on the other hand, is due to the fact that the oxygen dependent HIF-1alpha subunit is synthesized in any nucleated cell of the body. After its interaction with the constitutive HIF-beta subunit, the transcription factor HIF-1 is formed. Then it translocates to the cell nucleus and activates the expression of glycolysis enzymes genes, anti-apoptotic factors, and proinflammatory cytokines, realizing a stress reaction, which includes a quick cell adaptation to oxygen deficiency conditions, cell protection from death, and an inflammatory response [1]. Thereby, the notion that hypoxia induces inflammation and activation of immune cells is naturally formed.

However, despite an increase in the proportion of CD4+ T-lymphocytes in chronic heart failure, of both ischemic and non-ischemic origin, these patients have interferon-gamma (IFN-gamma) deficiency [5], which suggests a qualitative inferiority of cell-mediated immune response in hypoxia. In addition, in diseases associated with hypoxia (in particular, in patients with coronary heart disease (CHD), pulmonary tuberculosis (TB) and chronic obstructive pulmonary disease (COPD)), an increase in the production of the main immunosuppressive cytokine, transforming growth factor beta (TGF-beta), has been observed. This cytokine inhibits Th1-pathway of the immune response, the synthesis of interleukin (IL)-2, as well as IL-1 and other proinflammatory cytokines, suppresses IL-2-depended proliferation of T-lymphocytes, activity of natural killers and B-lymphocytes, and production of reactive oxygen species, but it stimulates the differentiation of immunosuppressive regulatory T-lymphocytes, fibroblasts, tissue regeneration and organ fibrosis [7, 8]. Since the presence of hypoxia in CHD, COPD and TB is positive, and the TGF-beta surplus and immunosuppression and anti-inflammatory response mediated by it obviously do not correspond to the effects of HIF-1, this may be provided by the change in the production of HIF-2 also having two subunits HIF-beta and HIF-2alpha. At the same time, little is known about the modulation of the synthesis of the latter in various diseases associated with hypoxia, as well as about the production of other immunosuppressive and anti-inflammatory mediators, such as IL-10, IL-13, galectin-9, especially in combination with an imbalance of cytokines stimulating the immune system (IFN-gamma, galectin-2, etc.).

The aim of the study is to identify general patterns and features of changes in the content of hypoxia-inducible factors-1 and -2 in association with an imbalance of immunoregulatory cytokines (IL-10, IL-13, galectin-9, IFN-gamma) in the blood in diseases associated with hypoxia (coronary heart disease, chronic obstructive pulmonary disease, pulmonary tuberculosis).

MATERIAL AND METHODS

The study involved 75 people aged 45–65 years: 25 patients with CHD and postinfarction cardiosclerosis (19 men and 6 women, average age 52.18 ± 4.37 years) and 18 relatively healthy donors (12 men and 6 women, average age 49.82 ± 5.9 years) comparable in terms of gender and age with patient groups.

The criteria for including patients in the study were the presence of cyanosis, dyspnea, weakness according

to medical records analysis, as well as a decrease in the degree of saturation of hemoglobin with oxygen less than 60% and partial pressure of oxygen (p_{O_2}) in venous blood less than 37 mm Hg for patients with coronary heart disease [9], a decrease in forced expiratory volume in 1 sec (FEV_1) and Index Tiffeneau less than 75% for patients with COPD, and a decrease in FEV_1 less than 75% and lung capacity less than 85% for patients with TB, which corresponds to hypoxemia in patients with COPD and TB [9, 10]. The criteria for exclusion of patients with CHD, COPD, and TB from the study were age over 65 or younger than 45 years, the presence of hematological, tumor or autoimmune diseases, HIV infection, viral hepatitis, megaloblastic or hypoplastic anemia, acute inflammatory process at the time of the study or in 3 previous weeks before it, treatment with anti-inflammatory (steroidal and non-steroidal), immunomodulating or antibacterial agents, refusal of the study, as well as the presence of COPD or TB in patients with CHD, and the presence of CHD in patients with COPD or TB.

The material for the study was 5 ml of heparinized venous blood (25 U/ml) taken in the morning in the fasted state under aseptic conditions. The concentrations of the following cytokines IL-10, IL-13, IFN-gamma, galectin-2 and -9, HIF-1alpha and HIF-2alpha in blood plasma were determined by enzyme-linked immunosorbent assay by the aid of the following commercial kits: "IL-10-ELISA-BEST", "gamma-IFN-ELISA-BEST" produced by Vector-BEST JSC (Novosibirsk); "Human IL-13 Platinum ELISA" (eBioscience, Austria), "Human Galectin-2 ELISA Kit", "Human Galectin-9 ELISA Kit", "Human HIF-1alpha ELISA Kit" and "Human HIF-2alpha ELISA Kit" (Cloud-Clone-Corp., USA).

Statistical analysis of the results was carried out using the program Statistica 10.0 and Microsoft Excel. The median (*Me*), and the 1st and the 3rd quartiles (Q_1 and Q_3) were calculated for a statistical description; the sample fraction of the occurrence of the feature (the presence of HIF-2alpha in the blood) and its error were calculated to assess the HIF-2alpha content in the blood. The nonparametric Mann – Whitney test was used for the purpose of comparative analysis. The results were considered reliable at a statistical significance level $p < 0.05$.

RESULTS

It was shown that the content of HIF-1alpha in the blood of all examined patients corresponds to the norm (Table 1). The absence of HIF-1alpha surplus in the blood in CHD, COPD, and TB in the setting of hypoxia (see criteria for inclusion of patients in the study) can be explained by the formation of chronic hypoxia in these diseases rather than an acute one. It was shown that the content of HIF-1alpha in cells and in the blood increases in response to a rapid decrease in its oxygenation, and it normalizes after several episodes of hypoxic preconditioning of tissues [2]. HIF synthesis switches from HIF-1 to HIF-2 in chronic hypoxia: HIF-1alpha mediates a proinflammatory response and a rapid cell adaptation to acute hypoxia, and HIF-2alpha initiates a prolonged adaptive tissue response to oxygen deficiency, inducing neoangiogenesis, fibrosis, and tissue remodeling, as a consequence [1, 4].

A comparative analysis of the concentration of HIF-2alpha in the blood of patients with CHD, COPD, TB and healthy individuals was difficult because there were a large number of variants with zero value. According to the manufacturer's data (Cloud-Clone-Corp., USA) set forth in the instructions for the reagent kit for HIF-2alpha evaluation, this molecule is not determined in the blood of healthy individuals or its content is below the sensitivity limit of the kit. Therefore, a comparative analysis of the frequencies of occurrence of values other than zero in the groups of examined individuals was carried out. The study showed that positive results of HIF-2alpha determination in the blood were positive in patients with all the three types of pathology associated with hypoxia by contrast with healthy donors (Table 1). The highest number of positive results was in patients with COPD, apparently due to bronchial wall fibrosis resulting from productive inflammation, airway obstruction, and destructive changes in the lung parenchyma. The frequency of determination of HIF-2alpha in blood plasma of patients with TB was slightly lower, probably, due to the ability of intact sections of the lungs to make compensation for ventilation disorders. The lowest frequency of positive results of HIF-2alpha determination was observed in patients with CHD, which may be explained by the category of examined individuals in whom the left ventricular ejection fraction was intact, and therefore hypoxia, obviously, was of a recurring, episodic nature (increases with moderate physical exertion and practically absent at rest).

The balance between HIF-1 and HIF-2 has a significant effect on the state of the immune system. Thus, the accumulation of HIF-1 in myeloid cells promotes the synthesis of proinflammatory cytokines, while its accumulation in lymphocytes inhibits the maturation of $Foxp3^+$ T-cells (Treg) with an immunosuppressive

function [11]. An increase in the HIF-1 concentration in the medium promotes the maturation of macrophages into proinflammatory M1-cells; and an excess of HIF-2 promotes the polarization of differentiation of macrophages into anti-inflammatory M2-cells [4], which synthesize a spectrum of anti-inflammatory cytokines capable of inducing immunosuppression [12]. In view of the above mentioned, the formation of chronic hypoxia with the accumulation of HIF-2alpha in patients with CHD, COPD or TB should naturally lead to immunosuppression, which explains the excessive secretion of TGF-beta by various cells in these diseases [6, 7].

Analysis of the cytokine spectrum, namely a different combination of mediators in the blood depending on the nature of the disease, confirmed signs of immunosuppression in patients of all the three groups of study. In such a way, a high level of galectin-9 was observed in patients with CHD; an excess of IL-13 was detected in patients with TB; and both factors were presented simultaneously in individuals suffering from COPD (Table 1). Moreover, a deficiency of such immuno-stimulating factors, as IFN-gamma and galectin-2, was observed in patients with CHD and patients with TB, which was not observed in patients with COPD. The content of IL-10 in the blood in TB corresponded to norm, but it tended to elevated IL-10 in the blood in patients with CHD and patients with COPD (Table 1).

An increase in the concentration of IL-10 in the blood in CHD and COPD did not reach statistical significance of the differences compared with the group of healthy donors ($p < 0.05$), possibly, due to the small number of examined patients with COPD and the low degree of intracardiac hemodynamics disorder in CHD. In particular, a significant excess of IL-10 in the blood of patients with CHD suffering from ischemic cardiomyopathy with reduced ejection fraction of left ventricle was observed [13], as we have previously shown, which confirms the significance of the trend identified in this study. Therefore, it is possible, that IL-10 in CHD and COPD may exert its immunosuppressive effect, which involves inhibition of the cell-mediated immune response, reducing in the synthesis of IL-2, IL-3, IFN-gamma, tumor necrosis factor alpha (TNF-alpha), CD4+ T-cell migration and antigen presenting properties of macrophages and B-lymphocytes [3, 8]. The relationship between IL-10 production and hypoxia is confirmed by the presence of several HIF-1alpha motifs in the structure of its gene and stimulation of IL-10 synthesis under prolonged (22 weeks) experimental hypoxia conditions [3]. The prolonged nature of hypoxia in this experiment and data on 48% homology of HIF-1alpha and HIF-2alpha [1] suggest IL-10-stimulative effect of HIF-2alpha too, the presence of which was observed in the blood of the patients with CHD and COPD (Table 1).

Table 1

Concentration of hypoxia-inducible factors, cytokines and galectins in the blood of patients with CHD, COPD and pulmonary tuberculosis, *Me* [Q_1; Q_3]

Content of mediators in the blood	Groups of examined persons			
	CHD	COPD	PTB	Healthy donors
HIF-1alfa, ng/ml	0.052 [0.041; 0.140] $p = 0.187$	0.078 [0.026; 0.986] $p = 0.912$	0.050 [0.027; 0.092] $p = 0.203$	0.080 [0.052; 0.096]
Percentage of patients with a positive result of HIF-2alfa determination in the blood, %	24.00 ± 8.54 $p < 0.05$	75.00 ± 10.83 $p < 0.001$	43.75 ± 12.40 $p < 0.01$	0
IFN-gamma, pg/ml	0	0.60 [0; 0.87] $p = 0.364$	0	3.00 [0.50; 5.40]
IL-10, pg/ml	25.00 [23.00; 29.50] $p = 0.113$	27.50 [23.50; 31.00] $p = 0.094$	20.50 [18.50; 22.50] $p = 0.871$	19.50 [18.00; 24.00]
IL-13, pg/ml	0.62 [0.41; 0.84] $p = 0.720$	2.62 [1.20; 7.58] $p = 0.002$	0.81 [0.79; 1.40] $p = 0.043$	0.50 [0.40; 0.75]
Galectin-2, pg/ml	3.18 [2.00; 3.96] $p < 0.001$	11.00 [7.05; 12.10] $p = 0.518$	3.85 [1.55; 10.88] $p = 0.047$	13.50 [11.50; 17.00]
Galectin-9, pg/ml	1.10 [0.52; 2.60] $p = 0.038$	8.50 [3.96; 15.00] $p = 0.001$	0.50 [0; 1.00] $p = 0.419$	0.16 [0; 0.50]

Notes. CHD – coronary heart disease, COPD – chronic obstructive pulmonary disease, PTB – pulmonary tuberculosis, p – level of statistical significance of differences in indicators compared with healthy donors.

The immunosuppressive effects of galectin-9 are described in sufficient detail in the literature. In high concentrations, it induces apoptosis of activated CD4+ and CD8+ T-lymphocytes (but not Th2-cells), B-lymphocytes, monocytes, endotheliocytes; it promotes the maturation of $Foxp3^+$ T-cells in the presence of TGF-beta, and inhibits the maturation of Th17 lymphocytes in combination with IL-6; in moderate concentrations, it promotes the secretion of Th2-profile cytokines and shifts the balance of CD4+/CD8+ lymphocytes in favor of predominant subpopulation of CD4+ cells, and also activates cell adhesion, migration of neutrophils and eosinophils, dendritic cells differentiation, angiogenesis [14–17]. Galectin-9 is widespread in the human body: it is expressed in muscle, bone, lymphoid, and nervous tissues; it is found in the organs of cardiovascular, respiratory, digestive and many other systems; it is synthesized by endothelial cells, fibroblasts, macrophages, astrocytes, and also in excess by tumor cells [15]. Since fibroblasts are determined among the cells producing galectin-9, the excessive presence of this molecule in the blood in patients with CHD and COPD is probably explained by the presence of foci of fibrosis in the heart and bronchopulmonary system, respectively. In infiltrative TB, inflammation develops in an exudative manner with phenomena of destruction in the lungs. Signs of significant activation of fibroblasts and fibrosis, as a consequence, are detected only at the regenerations during the regression of infiltrative changes. This and the fact that patients with infiltrative TB were examined by us at the height of the disease explain the fact that the content of galectin-9 in their blood remained within normal limits (Table 1).

IL-13 is an anti-inflammatory and profibrotic mediator. It is synthesized by activated Th2 cells and CD8+ T-lymphocytes, according to some reports; it induces the production of TGF-beta, eotaxin mucin, activation of calcium-depended chlorine channel 1 (hCLCA1) in bronchial epithelial cells, increases the contractility of their smooth muscle cells, stimulates fibroblasts both directly and indirectly through polarization of macrophages maturation in M2 cells synthesizing TGF-beta [18]. At the same time, IL-13 activates the proliferation and differentiation of B-cells, the antigen-presenting function of macrophages, inhibits their secretion of cytokines, the synthesis of IFN-gamma by natural killer cells, and antibody-dependent cellular cytotoxicity [8]. In view of the profibrotic role of IL-13, an increase in its concentration in patients with COPD appears to be natural, since progressive fibrosis takes place in the bronchi in this pathology. In patients with CHD, fibrosis of the necrosis zone after myocardial infarction at the time of the study had already completed (a heart attack in past medical history), which likely explains the normal concentration of IL-13 in their blood.

In addition to an immunosuppressive cytokines excess in the blood, a deficiency of galectin-2 and IFN-gamma was detected in patients with CHD and TB (Table 1). The latter demonstrates the suppression of the Th1 immune response and the shift of the Th1/Th2 balance in the direction of the Th2 pathway, which is due to the biology of the pathogens in TB, mycobacteria (in order to escape from immunological surveillance of intracellular pathogens) [6], and the formation of soluble oxidized low density lipoproteins acquiring the properties of autoantigens in CHD [19]. At the same time, the negative effects of IFN-gamma deficiency for the immune system are quite obvious, since this cytokine is crucial in the implementation of the Th1 immune response, activates CD4+ and CD8+ T-lymphocytes, natural killer cells, increases the antigen-presenting properties of macrophages and their cytotoxicity, stimulates the synthesis of IL-6, IL-8, IL-15, the expression of adhesive molecules, etc. [8, 20]. The role of galectin-2 in immunity cannot be interpreted unambiguously. On the one hand, it is proinflammatory; it promotes the differentiation of M1 macrophages and inhibits the formation of M2 cells; stimulates monocytes synthesis of the proinflammatory cytokines, such as IL-6, TNFα, IL-12p40, IFNβ, and inhibits their production of TGF-beta, matrix metalloproteinases-2 and -9, vascular growth factor A (VEGF-A), and arteriogenesis, as a consequence [21]. On the other hand, galectin-2 inhibits the migration of monocytes/macrophages and the ability of the latter to activate T cells [21, 22]. In general, IFN-gamma can be characterized as a proinflammatory cytokine that activates the Th1 pathway of the adaptive immune response and cell-mediated mechanisms of the antigen (pathogen) destruction and galectin-2 can be characterized predominantly as a mediator of innate immunity. Deficiency of IFN-gamma and galectin-2 in the blood of patients with CHD and TB may be considered as a manifestation of secondary immunological deficiency, one of the mechanisms of which is the depression of maturation and the function of proinflammatory M1 macrophages with a predominance of the effects of M2 cells. It is known, that both mediators serve as triggers for the differentiation of

M1 macrophages [12, 21], and IL-13 and galectin-9 (their concentrations increased in patients with TB and CHD, respectively) serve as inducers of the formation of M2 macrophages [12, 14]. However, there was no deficiency of IFN-gamma and galectin-9 in patients with COPD, but the combined and significant accumulation of IL-13 and galectin-9 in the blood was revealed (Table 1), which is a sufficient condition for the differentiation of M2 cells. Moreover, the spectrum of cytokines secreted by M2 macrophages includes TGF-beta, IL-10, IL-13, etc. [12], an excess of which in patients with CHD, COPD, and TB was registered by us and described in the literature [6, 7]. The crucial factor in the formation of such an imbalance of cytokines in these diseases is, probably, chronic hypoxia, in which the accumulation of HIF-2 polarizes the differentiation of macrophages into M2 cells [4] and causes cytokine-mediated immunosuppression.

CONCLUSION

In diseases associated with chronic hypoxia (CHD, COPD, and TB), prolonged oxygen deficiency in the body is followed by the accumulation of HIF-2alpha in the blood at normal HIF-1alpha content. It seems that chronic hypoxia is associated with HIF-2alpha-mediated immunosuppression despite the proinflammatory effect of short-term oxygen starvation of organs and tissues and the inflammatory nature of these diseases. The latter stimulates the differentiation of macrophages into M2 cells synthesizing anti-inflammatory cytokines. Moreover, immunosuppression in CHD, COPD, and TB is realized through various combinations of mediators; in CHD and TB, it is caused by a deficiency of IFN-gamma and galectin-2 in association with an excess of galectin-9 (in patients with CHD) or IL-13 (in patients with TB); in COPD, it is caused by a combined surplus of both mediators under the conditions of normal IFN-gamma and galectin-2 levels in blood plasma. The content of IL-10 in the blood in CHD and COPD tends to increase and may be an additional factor of immunosuppression of these diseases, which requires further research.

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Authors contribution

Chumakova S.P. – research design, analysis of literature, statistical processing of research results and their interpretation, drafting of the manuscript. Urazova O.I. – research design, material and technical support of the laboratory research, interpretation of the results, drafting of the manuscript. Vins M.V. – preparation of the biomaterial, implementation of the enzyme immunoassay method, analysisof literature . Shipulin V.M. – consulting on the research planning and interpretation of the results. Pryakhin A.S. – interaction with cardiac patients, provision of the clinical material, literature search. Bukreeva E.B. – ensuring interaction with patients, consulting on the interpretation of the results. Bulanova A.A. – interaction with patients, participation in the collection and preparation of the biomaterial, literature search. Koshel A.P. – provision of the clinical material, consulting on research planning. Novitsky V.V. – consulting on the interpretation of the results, editing of the text of the manuscript.

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Ways to improve the diagnosis and treatment of interstitial lung disease associated with systemic sclerosis in the Siberian Federal District (materials of the advisory board of rheumatologists and pulmonologists from December 08, 2019)

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ABSTRACT

The aim of the study was to develop ways to improve the diagnosis and treatment of systemic sclerosis (SSc)-ILD. Interstitial lung disease (ILD) is a common manifestation of SSc. In the territory of the Siberian Federal District (SFD), the number of patients with the progressive phenotype of SSc-ILD is approximately 750 people. When immunosuppressive therapy is ineffective and pulmonary fibrosis progresses, lung transplantation is indicated. The emergence of new possibilities of pathogenetic therapy currently requires studying the possibilities of their applications in real clinical practice on the territory of the SFD.

Discussion. The results of a discussion of diagnostics, therapy, and routing of a rheumatology patient during the interdisciplinary observation of SSc-ILD in the SFD are presented. The reason for this discussion was the new data on the use of nintedanib in this category of patients

Conclusion. To improve the efficiency of diagnosis and treatment of patients with SSc in the SFD, it is necessary to implement the principle of a multidisciplinary approach with the obligatory involvement of a pulmonologist and a radiologist (a specialist in CT diagnostics), and, if differential diagnosis is necessary in difficult clinical situations, of a pathomorphologist. An urgent task is the introduction of an algorithm for examining patients with SSc for the timely diagnosis of ILD in the territory of the Siberian Federal District. To improve the quality of medical care in the territory of the SFD for patients with ILD-SSc it is necessary to create a reference center in the city of Novosibirsk with the possibility of initiating anti-fibrosis therapy.

Key words: systemic sclerosis, interstitial lung diseases, nintedanib, anti-fibrotic therapy.

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Пути улучшения диагностики и лечения поражения легких при системной склеродермии на территории Сибирского федерального округа (материалы совместного совета экспертов ревматологов и пульмонологов от 8.12.2019)

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РЕЗЮМЕ

Цель. Разработать пути улучшения диагностики и лечения поражений легких при системной склеродермии (ССД).

Введение. Поражение легких у больных ССД является одним из наиболее частых проявлений висцеральной патологии и рассматривается как вариант фиброзирующих диффузных интерстициальных заболеваний легких (ИЗЛ). Несмотря на продемонстрированную эффективность патогенетической иммуносупрессивной терапии, у ряда пациентов фиброзные изменения в легочной ткани имеют прогрессирующее течение, что негативно сказывается на качестве и продолжительности жизни пациента. На территории Сибирского федерального округа (СФО) количество пациентов с ССД, имеющих прогрессирующее поражение легких, составляет приблизительно 750 человек. Таким образом, проблема своевременной диагностики и лечения поражения легких при ССД оказывается весьма актуальной и для СФО. Имеющиеся в настоящее время данные об эффективности современной противофиброзной терапии ИЗЛ при ССД требуют изучения возможности ее применения в реальной клинической практике на территории СФО.

Обсуждение. Представлены результаты междисциплинарного обсуждения вопросов диагностики, терапии, маршрутизации пациентов с ССД и ИЗЛ на территории СФО. Причиной данного обсуждения явилось появление сведений об эффективности нинтеданиба у данной категории пациентов.

Заключение. Для повышения эффективности диагностики и лечения больных ССД с поражением легких необходимо реализовать принцип мультидисциплинарного подхода с обязательным привлечением пульмонолога и рентгенолога (специалиста по КТ-диагностике), а при необходимости дифференциальной диагностики в сложных клинических ситуациях – патоморфолога. Актуальной задачей оказывается внедрение на территории СФО алгоритма обследования пациентов с ССД для своевременной диагностики ИЗЛ. Для повышения качества оказания медицинской помощи на территории СФО пациентам с ИЗЛ при ССД необходимо создать референсный центр в г. Новосибирске с возможностью инициации специалистами этого центра антифибротической терапии.

Ключевые слова: системная склеродермия, интерстициальные заболевания легких, нинтеданиб, антифибротическая терапия.

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

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INTRODUCTION

Systemic sclerosis (SSc) is a rare autoimmune disease characterized by dysregulation of the immune system, microvascular damage, and the development of fibrosis of internal organs. One of the frequent and potentially life-threatening manifestations of visceral pathology in patients with SSc is the development of a lesion of the pulmonary parenchyma, which occurs in 80% of patients, and is currently considered as one of the variants of fibrosing diffuse interstitial lung diseases (ILD). Lung involvement in SSc is more common than in other systemic connective tissue diseases. Obvious changes in lung tissue are found in 25–65% of patients according to chest X-ray data, and in 90% of patients according to the results of high-resolution computed tomography (HRCT). At autopsy, pulmonary fibrosis with SSc is verified in 75–100% of cases [1]. Approximately one third of patients with SSc-ILD show progression of fibrosis in the lungs. Progressive lung damage in SSc is considered one of the leading causes of death and is a significant burden on the health care system [2, 3]. Data from a preliminary estimate of the prevalence of this pathology in the Siberian Federal District (SFD) indicate that the number of patients with progressive course of SSc-ILD is approximately 750 people.

Among the functional parameters used to control the effectiveness of the therapy for ILD, the most commonly used function of external respiration (or spirometry) with the assessment of forced vital capacity (FVC). The effectiveness of therapy is indicated by a slowdown in the rate of decrease in FVC or its stabilization. The severity of respiratory dysfunction and the rate of progression of SSc-ILD vary significantly. The initial state and the rate of progression of ILD are of primary importance in the tactics of patient management.

To date, the basis of the treatment of SSc-ILD is drugs with immunosuppressive action, most often with cyclophosphamide and mycophenolate mofetil, the effectiveness of which was studied in two randomized, double-blind studies (studies of systemic sclerosis I and II (SLS-I and SLS-II)) [4, 5]. If this therapy is ineffective or intolerant, it is possible to use azathioprine or cyclosporine A. If immunosuppressive therapy is ineffective and pulmonary fibrosis progresses, lung or hematopoietic stem cell transplantation is indicated. The emergence of a new drug of pathogenetic action at present requires studying the possibility of its use in real clinical practice in the territory of the Siberian Federal District.

The aim of this study is to develop ways to improve the diagnosis and treatment of lung lesions in SSc.

To achieve this goal, the following tasks were formulated:

1. Analyze new possibilities of pathogenetic therapy of patients with SSc-ILD and assess the need for their use in the Siberian Federal District.

2. Consider the possibility of using a multidisciplinary approach to the diagnosis, differential diagnosis and treatment of SSc-ILD.

3. Develop a regulation on the routing of patients with SSc-ILD in the Siberian Federal District and the procedure for obtaining high-tech medical care, including a regional subsidy.

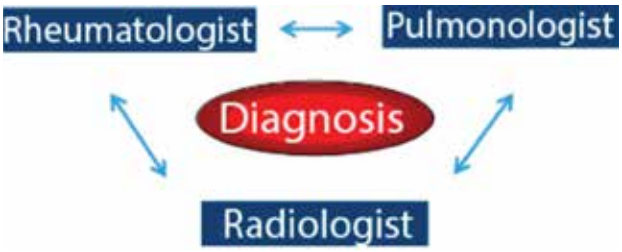
4. Assess the need to create a reference center for patients with SSc-ILD in the Siberian Federal District with the possibility of initiating antifibrotic therapy.

DISCUSSION

One of the topical issues is the routing of patients with SSc-ILD. The management of such patients should be based on the interdisciplinary interaction of various specialists: rheumatologist, pulmonologist, radiologist and pathomorphologist. By analogy with the algorithm for verifying the diagnosis in idiopathic pulmonary fibrosis, an algorithm for the diagnosis of SSc-ILD is proposed (Fig. 1).

Verification of pulmonary involvement in SSc requires a comprehensive examination of the patient, which includes an assessment of the clinical manifestations of ILD, pulmonary function tests (PFT), an examination of the diffusion lung capacity (DLC) and mandatory HRCT. It is extremely rare, especially in recent years, to resort to lung biopsy and morphological examination of the biopsy material.

PFT, DLC and HRCT should be performed when determining the diagnosis and subsequently at least once a year provided that the patient is in a stable condition. When respiratory symptoms progress, HRCT must be performed or repeated to assess the progression of ILD.



Diagnostics of SSc-ILD requires a multidisciplinary approach and experience

Fig. 1. Multidisciplinary approach to verification of the diagnosis of SSc-ILD

Most often, with SSc, the main manifestation of IDL is the so-called nonspecific interstitial pneumonia, which can be diagnosed in the presence of appropriate computed tomographic or morphological patterns.

New in-depth understanding of the mechanisms of connective tissue damage in SSc and the formation of pulmonary fibrosis in this pathology has opened a new stage in the study of the pathogenesis of SSc and the use of anti-fibrotic therapy. The data from the SENSCIS study showed that the tyrosine kinase inhibitor nintedanib was effective in patients with SSc with pulmonary involvement, as it reduced the rate of progression of pulmonary pathology. This study included 576 patients who received at least one dose of nintedanib or placebo. In the analysis of the primary endpoint, the annual rate of change in FVC was – 52.4 ml per year in the nintedanib group and – 93.3 ml per year in the placebo group (difference 41.0 ml per year; 95% confidence interval, 2.9–79.0; $p = 0.04$) [6].

The anti-fibrotic effect of nintedanib is realized by blocking the intracellular signaling pathway and inhibiting the proliferation and transformation of fibroblasts. Previously, the effectiveness of nintedanib has been proven in numerous studies involving patients with idiopathic pulmonary fibrosis, and today the drug has found wide application in the treatment of this pathology [7–9].

Currently, nintedanib has been registered and approved by the Food and Drug Administration and the Ministry of Health of the Russian Federation as the only anti-fibrotic drug for the treatment of SSc-ILD.

Anti-fibrotic therapy for patients with SSc with pulmonary involvement requires an assessment of the initial severity of the disease, as well as the risk of its progression. According to modern ideas about the mechanisms of the pathogenesis of the disease and the data of clinical trials, nintedanib therapy should be initiated in the following cases:

1. Patients with verified ILD according to HRCT with clinical manifestations of lung lesions (dyspnea, cough) and FVC ≤ 70% and (or) DLC ≤ 60% at the time of diagnosis.

2. When signs of SSc-ILD progression are detected, which is determined based on the presence of one or more criteria:

– decrease in FVC by 10% or more from the previous examination;

– decrease in FVC by 5–10% from the previous examination with worsening respiratory symptoms;

– decrease in FVC by 5–10% from the previous examination with the presence of negative dynamics according to HRCT associated with the underlying disease;

– progression of respiratory symptoms and an increase in the spread of pulmonary fibrosis according to HRCT data.

These criteria are relevant only in cases of exclusion of pulmonary infection and other causes of respiratory symptoms, as well as changes according to HRCT data characteristic of other interstitial lung lesions, pulmonary lesions caused by cardiac pathology (acute left ventricular heart failure, pulmonary embolism). The algorithm for prescribing anti-fibrotic therapy was determined based on the initial severity of ILD and in the presence of an obvious risk of disease progression (Fig. 2).

The appointment of anti-fibrotic therapy should be carried out by the decision of the medical commission, taking into account the existence of vital indications in patients of this group. It should also be borne in mind that nintedanib is currently the only drug with an appropriate approved indication for this. Based on the results of the examination, the patient should be sent for a medical and social examination at the place of residence to establish a disability group. Based on the status of a disabled person, the patient will be entitled to free drug provision for outpatient treatment on a regular basis at the expense of the regional or federal budget. Before obtaining the status of a disabled person, drug provision is carried out at the expense of monthly individual purchases based on the decision of the medical commission.

CONCLUSION

Thus, based on the above, the members of the Advisory Board consider it necessary to:

– actualize the problem of diagnostics and therapy of SSc-ILD on the territory of the Siberian Federal District;

– introduce an algorithm for examining patients with SSc-ILD;

– be guided by a multidisciplinary approach to the diagnosis and treatment of SSc-ILD with the obligatory involvement of a pulmonologist and a radiologist (a specialist in CT diagnostics), and a pathomorphologist if differential diagnosis is necessary in difficult clinical situations;

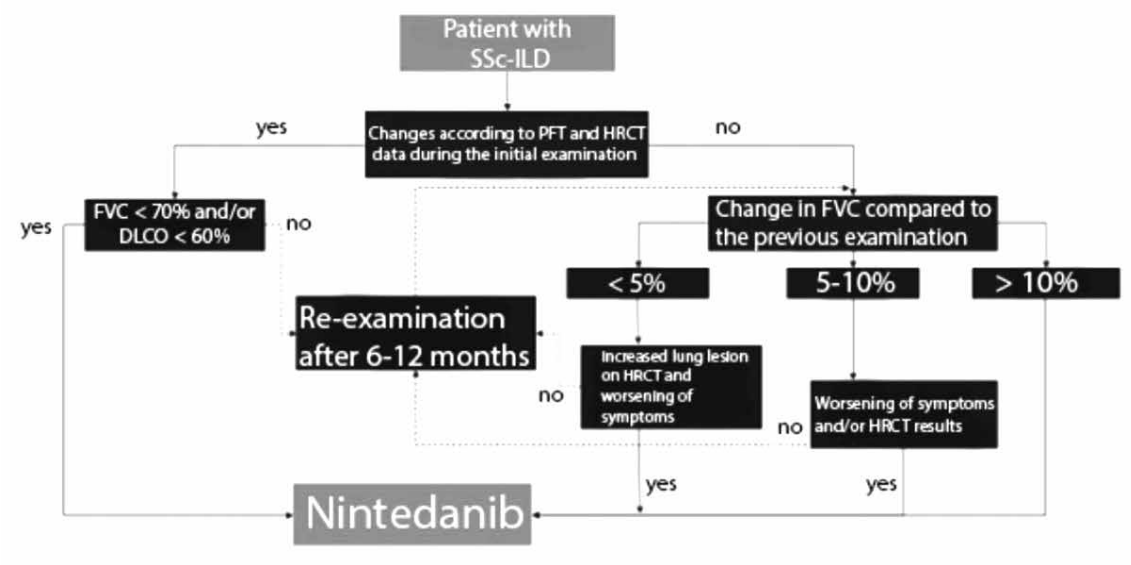


Fig. 2. Algorithm for prescribing anti-fibrotic therapy for patients with SSc-ILD

– develop a regulation on the routing of patients with SSc-ILD and the procedure for receiving high-tech medical care, including receiving anti-fibrotic drug therapy as a regional subsidy, in each region of the Siberian Federal District;

– create a reference center in the city of Novosibirsk with the possibility of initiating anti-fibrotic therapy to improve the quality of medical care in the territory of the Siberian Federal District for patients with SSc-ILD;

– initiate work with public organizations.

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Chronic pelvic pain in women

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ABSTRACT

Chronic pelvic pain resulting from varicose veins of the small pelvis is a multidisciplinary problem. A key cause of pelvic congestion is congenital or acquired gonadal valve failure. Ultrasound and Doppler examination for chronic pelvic pain allows in most cases to diagnose pelvic varicose veins. Multispiral computed tomography or magnetic resonance imaging details the nature and extent of the pathology. Selective phlebography is considered the gold standard for diagnosing varicose veins of the small pelvis. Conservative treatment with phlebotropic drugs is prescribed for limited pelvic varicose veins. Surgical treatments include open resection and retroperitoneal and transperitoneal laparoscopic gonadal vein excision or clipping. The most effective is minimally invasive endovascular occlusion of reflux veins using spiral technologies and sclerosants. The left ovarian vein is reduced more often. The decision on bilateral embolization of blood vessels depends on the severity of changes in veins and the intensity of blood reflux. A decrease in the intensity or disappearance of pain in the pelvic area is achieved in 80-100% of cases after the procedure. Diagnosis of this condition is difficult due to the fact that the appearance of pelvic varicose veins is nonspecific and includes symptoms of surgical, urological, gynecological and other diseases of the pelvic organs.

Key words: pelvic vein varicosity, pelvic vein congestion, radiologic diagnostics, endovascular occlusion, surgical treatment.

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Хроническая тазовая боль у женщин

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РЕЗЮМЕ

Хроническая тазовая боль, возникающая в результате варикозной болезни вен малого таза, – мультидисциплинарная проблема. Ключевая причина тазовой конгестии – врожденная или приобретенная несостоятельность клапанов гонадных вен. Ультразвуковое и допплерографическое исследование при хронической тазовой боли позволяет в большинстве наблюдений диагностировать тазовый варикоз. Мультиспиральная компьютерная или магнитно-резонансная томография детализирует характер и распространенность патологии. Золотым стандартом диагностики варикозной болезни вен малого таза считается селективная флебография. Консервативное лечение флеботропными препаратами назначается при ограниченном тазовом варикозе. Хирургические методы лечения включают открытые резекционные вмешательства, ретроперитонеальное и трансперитонеальное лапароскопическое иссечение гонадных вен или их клипирование. Наиболее эффективна мининвазивная эндоваскулярная окклюзия рефлюксных вен с использованием спиральных технологий и склерозантов. Чаще редуцируется левая яичниковая вена. Решение о билатеральной эмболизации сосудов зависит от выраженности изменения вен и интенсивности рефлюкса крови. Уменьшение интенсивности или исчезновение боли в области малого таза достигается в 80–100% наблюдений после процедуры. Диагностика этого состояния затруднена в связи с тем, что проявления тазового варикоза неспецифичны и включают симптомы хирургических, урологических, гинекологических и других заболеваний органов малого таза.

Ключевые слова: варикозная болезнь вен малого таза, тазовая венозная конгестия, лучевая диагностика, эндоваскулярная окклюзия, хирургическое лечение.

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INTRODUCTION

Varicose veins of the small pelvis (VVSP) is an insufficiently studied problem. There is no universal terminology in the Russian and English-language literature; there are no common approaches to the diagnosis and provision of specialized care for patients with this disease.

The development of the syndrome of pelvic congestion as a result of dilatation of the gonadal and intrapelvic veins due to retrograde turbulent blood flow is the cornerstone of VVSP. The most typical manifestations are chronic pelvic pain (CPP) not associated with the menstrual cycle lasting more than 6 months, dyspareunia, and dyschezia [1].

In 1857 M.A. Richet first described ovaricocele [2]. Later, V.F. Snegirev [3] formulated a hypothesis about the role of the venous system in the pathogenesis of chronic ("plethoric") pain in the small pelvis in women. On bimanual examination, the pelvic plexus distended with blood was identified in the form of dense, painful tumors, which, in his opinion, could be analogous to varicocele in men. In 1949, N.S. Taylor established a relationship between varicose veins and chronic pelvic pain [4]. To CPP of mechanical nature A.E. Mandelstam (1956) also attributed plethoric pain [5]. In 1975, O. Craig and J. Hobbs first described in detail VVSP and gave this disease the name adopted in the English language literature, pelvic congestion syndrome [6].

EPIDEMIOLOGY

P. Latthe et al. [7] noted that pelvic congestion syndrome is diagnosed in 24% of women of reproductive age, but the real prevalence of this disease is higher, since many for various reasons do not seek medical help. In the USA, in patients with CPP, one third of women are diagnosed with pelvic varicose veins [8]. In the UK, among patients 12–70 years old, the incidence is 38 per 1,000 per year [9]. CPP significantly reduces the quality of life, leads to psycho-emotional problems, family conflicts, and social maladjustment. Pathology is of socioeconomic importance, as it causes temporary disability in 10–12% of women [10]. They

are being observed for a long time by gynecologists, surgeons and other specialists without receiving adequate treatment. The annual pan-European costs of treatment are 3.8 billion euros [9].

ETIOPATHOGENESIS

There are primary (idiopathic) varicose veins (VV) of the pelvic veins and secondary, caused by obstruction of the pelvic veins from the outside against the background of gynecological, urological, or surgical organ pathology [11].

Two main causes of primary pelvic vein insufficiency and CPP have been described. First, it is congenital or acquired valvular failure of the gonadal, parametric and uterine veins (in 15% of cases on the left, on the right in 6%) [11]. Secondly, during pregnancy, the capacity of the pelvic veins increases 60 times compared to their normal value [12]. Veins do not have the elasticity of arteries, and after childbirth, their diameter does not return to its original diameter, which ultimately can contribute to permanent venous reflux. It is not excluded that the hormonal influence on the formation of VVSP during pregnancy is also possible.

Secondary VVSP includes the features of the architectonics of the abdominal vessels: compression of the left internal iliac vein by the right common iliac artery (May – Thurner syndrome) and compression of the left renal vein by the superior mesenteric artery (Nutcracker syndrome), extending from the aorta at an acute angle, with the development of left-sided renal phlebohypotension. As a result, excessive reflux of blood develops into the venous network of the small pelvis.

Some authors classify VVSP as a type of chronic venous insufficiency, considering it a problem in vascular surgery. This is indicated by the presence of varicose veins of the lower extremities in half of women with VVSP, which does not exclude the version of the unity of causes and pathogenetic mechanisms in these diseases [13, 14].

In the pathogenesis of phlebohypertension, the prevalence of varicose transformation of the pelvic veins and the formation of varicose veins of the external genital organs as a result of drainage of blood from the vessels of the small pelvis into the saphenous veins of the perineum are important [15], which probably reduces pelvic congestion. The formation of CPP in varicose disease of the pelvic veins remains unclear. It is possible that turbulent blood flow affects the receptors of the vascular wall, disrupts the production of neurotransmitters and contributes to the appearance of pain syndrome [16]. S.G. Gavrilov et al. observed patients with VVSP and dilation of the gonadal and parametric veins up to 10 mm or more in the absence of CPP [15]. Therefore, the dilation of the ovarian veins should not be considered an objective indicator of pathological blood reflux [17].

Risk factors for the development of the pathology under discussion coincide with those in varicose veins of the lower extremities: heredity, gender, age, and sedentary lifestyle, nature of work, two or more pregnancies, and hormonal imbalance.

CLASSIFICATION

A.E. Volkov (2000) proposed a classification of varicose veins of the small pelvis depending on the diameter of the vessels and their localization:

– first degree: vein diameter up to 5 mm (of any venous plexus of the small pelvis), "corkscrew" course of the vessel;

– second degree: vein diameter 6-10 mm with the total type of varicose veins, diffuse ectasia of the ovarian plexus (*pl. pampiniformis ovarii*), varicose parametric veins (*pl. uterovaginalis*), VV of the arcuate plexus of the uterus;

– third degree: vein diameter > 10 mm with the total or main type of VV of parametric localization [18].

In 2004, the Japanese radiologist T. Hiromura proposed a classification of blood reflux in the left ovarian vein [19]:

– reno-gonadal reflux of blood without varicose changes in the pelvic veins;

– reflux into the gonadal vein with limited left-sided varicose veins of the pelvic veins;

– reflux of blood into the gonadal vein with total varicose veins and drainage of blood into the right ovarian vein.

G. Asciutto [20] diagnosed VVSP on the following grounds:

– varicose reflux towards the ipsilateral or contralateral proximal thigh;

– visualization of reflux along the entire course of the ovarian vein;

– retrograde filling of the main trunk of the internal iliac vein and at least one lateral branch (gluteal, sciatic or obturator veins);

– retrograde filling of contrast medium along the midline.

The authors of the Guidelines of the Russian Phlebological Association for the Diagnosis and Treatment of Chronic Venous Diseases (2018) classify the following forms:

1. According to the clinical manifestations:

– varicose veins of the pelvis – a disease characterized by the dilation of the ovarian veins and intrapelvic venous plexuses;

– varicose veins of the vulva (vulvar varicose veins) - dilation of the veins of the external genital organs;

– varicose veins of the perineum (perineal varicose veins) – dilation of the veins of the perineum outside the external genital organs;

– varicose veins of the gluteal region (gluteal varicose veins);

– secondary dilation of the intrapelvic veins – dilation and reflux of blood through the intrapelvic veins against the background of postthrombotic occlusions of the iliac and (or) inferior vena cava.

2. According to the clinical course:

– painful form;

– painless form;

– latent form (asymptomatic).

3. According to the prevalence of the pelvic vein lesions:

– isolated dilation of the pelvic venous plexus;

– combined dilation of the gonadal veins and pelvic venous plexuses;

– unilateral or bilateral dilation of the gonadal veins;

– dilation of the trunk or branches of the internal iliac veins [1].

S.G. Gavrilov et al. argue that since the symptoms of plethora are not due to the degree of dilation of the pelvic veins, pathology should be classified depending on the intensity of gonadal reflux of blood, which will allow formulating an algorithm for further examination and treatment [15].

The relationship between the severity of patients' complaints and the degree of dilation of the pelvic veins and the area of their lesions is subject for further study.

CLINICAL PICTURE

The manifestations of VVSP are nonspecific and include signs symptoms found in various gynecological, surgical, urological and neurological diseases. The pathognomonic clinical equivalent is chronic pelvic pain (72%), which increases during the day with prolonged standing and decreases in the supine position. A decrease in the frequency of pain during pregnancy was noted, possibly due to a change in hormonal levels [21]. Increased pain may be associated with overwork, emotional stress, hypothermia, exacerbation of chronic diseases of the genitals [22].

The syndrome of pelvic venous congestion is accompanied by discomfort and pain in the hypogastric zone of the abdomen during exercise in 67% of cases, dyspareunia in 57.5%, fear of sexual intercourse, and less often, urinary incontinence and frequent painful urination as a result of congestion in the venous bladder plexus [23]. Varicose veins of the perineum, pubic and groin areas occur in 25% of cases. Dysmenorrhea develops in 22.5% of patients [13]. An increased secretion from the vagina has been described, especially in the second half of the cycle [24]. In addition, there is a direct link between pelvic congestive syndrome and the development of obstetric problems in 6% of cases: infertility, miscarriage and termination of pregnancy, secondary ovarian dysfunction [25].

With CPP, psychoemotional disorders (anxiety, depression) of varying severity are observed, and a pathogenetic nociceptive vicious circle is formed. Sometimes the patient's interpretation of the pain sensation, her emotional reaction and behavior may not correspond to the severity of pelvic venous congestion, which must be taken into account when choosing a treatment strategy [26].

DIAGNOSIS

In the 1950s P. Guilhem et al. [27, 28] diagnosed venous pelvic varicose veins based on X-ray examinations without describing the clinical equivalent of the detected changes.

Congestive syndrome should be suspected already in the presence of CPP. To confirm this condition, methods of visualization of the pelvic venous system using ultrasound and Doppler examinations are used [29]. As a result, it is possible to diagnose pathology in most cases [30]. The examination is carried out in the Fowler position, transabdominally and transvaginally. In some cases, proximal compression techniques are used to stimulate reflux through the gonadal veins.

Varicose veins of the small pelvis are often an accidental finding when performing multislice computed tomography of the vessels (MSCT) or magnetic resonance imaging (MRI) for another reason. These methods make it possible to detect not only varicose intrapelvic venous plexuses, but also compression from the outside of the left renal and internal iliac veins (Nutcracker and May – Thurner syndromes).

It is advisable to perform MSCT phlebography or MR phlebography with the creation of a three-dimensional image of the topographic and anatomical picture.

S.G. Gavrilov et al. in their study used emission computed tomography with "*in vivo* labeled erythrocytes before and after conservative therapy or surgery to evaluate the effectiveness of treatment" [31].

The disadvantages of these methods are the difficulties in examining patients in the Fowler position and performing proximal compression to improve visualization of dilated gonadal and intrapelvic veins.

Invasive selective phlebography proposed in 1965 by J. Tavernier and D. Lange [32], which allows detailing pathological changes in the venous system of the small pelvis [30], is considered the gold standard for diagnosing VVSP. The femoral vein is more often used for access. The English-language literature describes catheterization under the control of ultrasound sonography of the jugular, subclavian, brachial veins, which provides reliable access to intrapelvic communications. Selective left-sided renal phlebography is performed against the background of the Valsalva maneuver. With reflux of blood into the ovarian vein, it is superselectively contrasted. After that, ovarycography from the opposite side is performed. In some cases, the internal iliac veins are examined. This reveals isolated refluxes and dilatation of the perineal veins, which can also cause pelvic pain [33].

Diagnostic laparoscopy is more often used in gynecological practice as the final stage of a comprehensive examination of patients to identify competitive pelvic pathology (endometriosis, adhesions, Allen – Masters syndrome, etc.). Pathological reflux of blood, causing congestion in the veins of the small pelvis, was found in only 20% of patients with visually detectable varicose veins. The dilated veins of the small pelvis without pathological reflux of blood, revealed during the examination, cannot be the cause of venous pelvic pain. Such findings should be considered as a common condition in multiparous women [31]. The informative value of laparoscopic examination of vascular communications of the small pelvis is significantly inferior to the results of radiation diagnostic methods (ultrasound, MSCT, MRI, catheter phlebography).

On this basis, a diagnostic algorithm for chronic pelvic pain has been proposed by S.G. Gavrilov et al. [31].

LABORATORY DIAGNOSTICS

There are no specific biochemical signs of VVSP. Markers of collagen breakdown and connective tissue dysfunction (hydroxyproline, glycosaminoglycans, neuraminic acid metabolites and its derivatives) indicate only the presence of varicose veins, without its clear localization [8]. In 2018, a study was published on the use of pro- and antioxidant systems (diene conjugates, malondialdehyde, catalase, superoxide dismutase, and glutathione peroxidase) as additional diagnostic markers. This is due to their role in the onset and development of VVSP in women [34].

TREATMENT

Conservative treatment is prescribed for dilated gonadal veins without clinical manifestations of pelvic congestion and limited intrapelvic varicose veins [31]. Modern phlebotropic drugs occupy a leading place in the treatment of VVSP [8]. Anticoagulants, antioxidants, collagen formation stimulants, glycosaminoglycan metabolism regulators, physiotherapy and hirudotherapy are also used. In 2018, a work was published on the prophylactic use of antioxidants, taking into account their role in the pathogenesis of the disease [34].

Adnexectomy, hysterectomy and resection of the wide ligament of the uterus are the first surgical interventions that have been used to treat this pathology [35]. When analyzing the results obtained, it turned out that 33% of patients still had discomfort, and 20% of the pain remained at the same level. Later, ligation or clipping of the ovarian veins was proposed. However, due to frequent recurrences of CPP (up to 80%), this method was abandoned.

The trunk type of gonadal veins without concomitant gynecological pathology and in the absence of varicose veins of the lower extremities allows the use of endovascular occlusion of the ovarian veins for the treatment of pelvic congestion syndrome. In the diffuse type, it is advisable to use laparoscopic resection of the gonadal veins, which allows simultaneous correction of concomitant pathology. With this approach, the number of relapses can be minimized [8, 31].

Unlike conventional open or laparoscopic surgical methods, endovascular interventions use a minimally invasive approach: selective occlusion of reflux veins [36]. Transcatheter ovarian vein embolization was first described by R.D. Edward et al. in 1993 [37]. For this procedure, metal elements, adhesive compositions, polyvinyl alcohol, and liquid sclerosants are used. Further study of the problem showed that spiral technologies are effective in no more than 60% of cases, which is associated with the formation of roundabout reflux venous blood flow [38]. According to V.Yu Bogachev., it is advisable to combine spiral technologies with the introduction of sclerosants [29]. A fairly effective sclerosing agent is 96% ethyl alco-

hol [39]. The technique involves the installation of a coil and (or) the introduction of a sclerosant at the L4–L5 level into the gonadal vein [40]. Some authors use a laparoscopic approach for sclerosing, reporting a decrease in pain intensity in 61.2% of patients with concomitant varicose veins of the uterine appendages and in 90.0% of patients with isolated congestion [11].

The decision on mono- or bilateral embolization of vessels is made based on the severity of changes in veins and the intensity of blood reflux [8].

A decrease in the intensity or disappearance of chronic pain in the pelvic area was achieved in 80% of patients. In further studies on the occlusion of both ovarian and internal iliac veins, a positive result was achieved in 94% of cases [33]. In the treatment of 41 patients G. Maleux noted its effectiveness in 98% of cases [41]. In the long-term period, 59% of patients with unilateral ovarian vein embolization had no symptoms of the disease [41]. The embolization efficiency, according to different authors, is 67–89% [33]. There is evidence of 100% success and symptom improvement over a 12-month follow-up period after the procedure [33]. S.G. Gavrilov et al. report an 86% success rate [31].

Complications of the endovascular method of treatment occur in 4–22%. Migration of embolizing material into the right chambers of the heart and the pulmonary artery is the most commonly diagnosed (1.9%). It has not been confirmed that pelvic vein embolization has an adverse effect on fertility associated with decreased ovarian function [33].

It is advisable to perform open extraperitoneal excision of altered veins in patients with a trunk and (or) multilateral type of ovarian vein structure and concomitant varicose veins of the pelvis or atypically located varicose veins. The efficiency of the operation reaches 100%; meanwhile, priority is given to minimally invasive techniques [31].

With regard to endoscopic interventions, depending on the access, retroperitoneal and transperitoneal resections are distinguished [42].

The first is performed with the patient on the right or left side. The obvious benefits of the method as compared to the transperitoneal approach include half the time of the intervention, decrease of complication rates, relief of pain in the site of ports, and a decrease in the postoperative hospital stay. This technique is optimal for unilateral lesions of the gonadal veins. In addition, the use of retrocarboxyperitoneum determines the possibility of wide mobilization of the gonadal vein from the ovary to the left renal or inferior vena cava without trauma to the abdominal organs [42].

Transperitoneal endoscopic resection is performed for bilateral lesions of the gonadal veins [42].

According to S.G. Gavrilov et al., the efficiency of laparoscopic resection of the gonadal veins also reaches 100% [26].

CONCLUSION

Varicose veins of the small pelvis is a fairly common disease, its main manifestation is pelvic pain, which reduces the quality of life, leads to the formation of psychoemotional problems, and leads to social maladjustment. Often, women are observed by various specialists (obstetricians, gynecologists, surgeons, urologists, neurologists, psychiatrists, etc.) without prescribing etiopathogenetic treatment.

Since the middle of the nineteenth century, the causes of plethoric pain and, above all, the role of changes in the venous system of the pelvis in their occurrence are discussed. Varicose transformation of the pelvic veins with the formation of phlebohypertension occurs primarily as a result of reflux of blood into the gonadal veins. It is possible that pelvic congestion is a type of chronic venous insufficiency, given the theory of the unity of the etiopathogenetic mechanism of development of varicose veins of the small pelvis and lower extremities.

Difficulties in diagnosis are due to the fact that the manifestations of pelvic varicose veins are nonspecific and include symptoms of surgical, gynecological, urological and other diseases of the pelvic organs.

At the core of the instrumental diagnosis of pelvic varicose veins are radiation methods: ultrasound and Doppler examinations, MSCT phlebography, and MRI. The most accurate information can be obtained after performing invasive selective and superselective phlebography.

For the treatment of pelvic congestion, open resection and endoscopic interventions, clipping of ovarian veins, and endovascular reduction of pathological blood flow have been proposed. The latter method of correcting pelvic venous congestion is highly effective in the main type of gonadal reflux.

In the problem of pelvic venous congestion, questions and unresolved problems remain in the diagnosis and treatment of chronic pain.

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Correct diagnostic conclusion in patients with chronic heart failure: a reality or a pipe dream?

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ABSTRACT

The authors of this article have analyzed the problem of diagnostic conclusion unification in patients with chronic heart failure (CHF). The root of this problematic situation in which practitioners find themselves is that, despite the large number of different regulatory documents, there is no consensus on what is considered correct and what is wrong when formulating a diagnostic conclusion in a patient with CHF. The many-faced syndrome is designated differently: CHF, congestive heart failure, chronic circulatory failure. There are difficulties in determining the stage of CHF in patients receiving optimal drug therapy or in those who are in a state of compensation after a successful surgical correction. When assessing the functional status in a patient with CHF, a distinct subjectivity should be taken into account in determining which limited physical activity is slight or, conversely, marked, as well as what kind of physical exertion is normal for the patient. This subjectivity naturally leads to low reproducibility of the assessment results of the CHF functional class in the same patient by different doctors. CHF should also be classified according to the value of a left ventricular ejection fraction. The diagnosis should also take into account the state characteristics of a diastolic function of the left ventricle (especially in patients with CHF and preserved left ventricular ejection fraction). The authors give examples of diagnostic conclusions, including cases of comorbid pathology.

Key words: chronic heart failure, classification, stage, functional class, left ventricle, ejection fraction, diastolic function, chronic cor pulmonale, 6-minute walk test, diagnostic conclusion.

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Корректная формулировка диагноза у пациента с хронической сердечной недостаточностью: реальность или несбыточная мечта?

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РЕЗЮМЕ

Проанализирована проблема унификации формулировки диагноза у пациента с хронической сердечной недостаточностью (ХСН). Корень проблемной ситуации, в которой находятся практические врачи, кроется в том, что несмотря на большое количество различных регламентирующих документов, нет единого мнения относительного того, что считать правильным, а что неправильным при формулировке диагностического заключения у пациента с ХСН. Многоликий синдром обозначают по-разному: ХСН, застойная сердечная недостаточность, хроническая недостаточность кровообращения. Сложности есть при определении стадии ХСН у пациентов, получающих оптимальную медикаментозную терапию или находящихся в состоянии компенсации после успешной хирургической коррекции. При оценке функционального статуса у пациента с ХСН следует учитывать отчетливый субъективизм в определении того, какое ограничение физической активности является небольшим или, наоборот, значительным, а также того, какая нагрузка является привычной для больного. Данный субъективизм закономерно приводит к низкой воспроизводимости результатов оценки функционального класса ХСН у одного и того же пациента разными врачами. ХСН необходимо классифицировать и в зависимости от значения фракции выброса левого желудочка. В диагнозе следует приветствовать и характеристику состояния диастолической функции левого желудочка (особенно у пациентов с ХСН и сохраненной фракцией выброса). В лекции приведены примеры диагностических заключений, в том числе при коморбидной патологии.

Ключевые слова: хроническая сердечная недостаточность, классификация, стадия, функциональный класс, левый желудочек, фракция выброса, диастолическая функция, хроническое легочное сердце, тест 6-минутной ходьбы, диагностическое заключение.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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INTRODUCTION

Though diagnosis of chronic heart failure (CHF) is not a bedside procedure, usually it can be recognized without great difficulties. The main thing is not to be limited to the clinical assessment of signs and symptoms, as “experienced” cardiologists often do by repeating the mistakes of their teachers. They are convinced that cold hands (“cold” cyanosis) in a patient with dyspnea clearly indicate CHF, while warm hands (“warm” cyanosis) indicate lung disease. In one way or another, for example, using echocardiography, they try to study the heart structure and

function (primarily the left ventricle (LV)), and also to determine the plasma concentration of natriuretic peptides (most often brain natriuretic peptide, N-terminal pro-brain natriuretic peptide and mid-regional pro-atrial natriuretic peptide) before diagnosing a heart failure [1–6].

The authors are convinced that the clinical stage of diagnosis is very important in recognizing CHF [7]. Moreover, if the approach of constant substitution of physical examination of the patient with certain paraclinic tests is practiced for a long time, it can lead to atrophy of the doctor's skills of the so-called bedside diagnosis [8]. However, without the verification stage, the diagnosis of CHF is not always infallible, and the diagnosis itself is imperfect [9, 10], since the coincidence of opinions of different specialists on the presence or absence of symptoms and CHF clinical signs (cross-reproducibility) is not observed in every case [8].

After correct recognition of CHF, paradoxically, the doctor faces even greater difficulties, since he needs to formulate a detailed clinical diagnosis so that the latter performs all its functions (a patient with their diseases and their key mechanisms is visible behind it, it serves as an accurate justification for choosing methods of personalized treatment/prevention and rehabilitation, provides continuity of therapy; it is a tool for statistical accounting and medical forecasting, it helps to assess the ability to work and fitness for military service, as well as professional selection and medical control in sports and so on). On the other hand, it should avoid conflict situations of administrative and legal nature, related to issues of insurance medicine. Sometimes it should also help against the almost manic desire of some experts to find medical errors [11]. As leading Russian morphologists rightly point out, any diagnosis from a medical diagnosis becomes a medical-social, in fact becoming a legal, "insurance" and legal element in solving many life situations. Payment for the completed case of treatment and sometimes even the doctor's fate depend on it.

The root of the problem situation in which practitioners find themselves is that, despite a large number of different orders, regulatory documents, guidelines and manuals, as well as reference books (updated by the WHO Committee of experts international statistical classification of diseases and health problems of the tenth revision, orders of the Ministry of Health of the USSR and the Russian Federation, recommendations of the European Society of Cardiology on the diagnosis and treatment of acute and chronic heart failure, National recommendations for the diagnosis and treatment of CHF, the standard of rules for the formulation of final clinical and pathoanatomical diagnoses, approved in 2006 by the Federal service for supervision of health and social development, as well as clinical recommendations / treatment protocols approved by the Ministry of Health of the Russian Federation) [11, 13–18], the citation of which could be continued, there is no consensus on what is considered correct and what is wrong when formulating a diagnostic conclusion. And this is not only among representatives of various medical specialties, but even among specialists of the same profile, some of whom may change their views over time (for example, considering CHF first as a disease, then as a syndrome). We see nothing wrong in the latter, as it says *"Cujusvis hominis est errare, nullius, nisi insipientis, in errore perseverare".*

To those who doubt this, we suggest comparing examples of the formulation of a clinical (or pathoanatomical) diagnosis, which are given in the works by individual authors. They will include examples of lapidary (CHF IIB FC III) and detailed diagnostic conclusions, as well as those in which CHF (sometimes out of habit referred to as circulatory failure or congestive heart failure) is considered an independent nosological form and is a syndrome. There is no consensus on what is considered the main disease in the case of comorbid pathology (recall that a patient with heart failure in most cases is a middle-aged person with a "bouquet" of diseases). At the same time, clinicians, referring to these documents with different legal force, can formulate diagnoses in completely different ways.Sometimes it seems that practitioners can avoid cognitive dissonance under the avalanche of information coming from various sources, which is based on conflicting views, only if they decide to leave the profession, since the prospect of achieving harmony of their own ideas formed at the university and during medical practice, with the varying requirements of professional medical associations and expert communities, looks very vague.

The target issue of this lecture is an attempt to deal with the problem of unifying the formulation of the diagnosis for a patient with CHF.

Diagnosis (Greek. διάγνωσις, lat. diagnosis – "recognition") is a brief medical conclusion (more precisely − medical, since physician assistants in accordance with the Order of the Ministry of health and social development of the Russian Federation of 06.11.2009 No. 869 also "diagnoses typical cases of the most common diseases..") about the pathological state of health of the subject, about the diseases (in-

juries) available to him or about the cause of death, issued in accordance with the current standards and expressed in terms provided by the current classifications and nomenclature of diseases [17].

As for the terms, despite the pluralism of opinions, in accordance with the dominant views in the diagnosis, only the abbreviation of CHF should be used. Proponents of the term "chronic circulatory failure" (as a rule, they use abbreviations CF, CCF or C) rightly point out that the classification of N. D. Strazhesco and V. H. Vasilenko, approved at the XII All-Union Congress of therapists in 1935, and which is still a current tool for classifying CHF, was precisely about circulatory failure. Other experts justifiably note that severe CHF is often associated with hypervolemia (in the absence of effective therapy, including diuretics, it is almost natural), associated with retention of sodium and water, manifested by symptoms and signs of "congestion" (sometimes only in a small circle of blood circulation), for descriptions of which the term "congestive heart failure" is recommended. However, in accordance with National recommendations for the diagnosis and treatment of CHF, the terms "congestive heart fail- ure" and "chronic circulatory failure", which are essentially synonymous with CHF, should not be used to unify the terminology [13, 15].

The modern classification of heart failure was developed by experts of the Society of Heart Failure Specialists (SHFS) and approved at a meeting of the Presidium of the All-Russian Scientific Society of Cardiology on October 11, 2002 [19]. In the official comment of the SHFS [20], attention was drawn to the continuity of this edition of the classification with the classifications of N.D. Strazhesko and V.H. Vasilenko (the classification "lost" all the additions to the classic version that were made over its long history, but new concepts were introduced into it: "asymp- tomatic dysfunction of the left ventricle", "adaptive remodeling of the heart and blood vessels", "mal- adaptive remodeling of the heart and blood vessels") and the New York Heart Association (NYHA) [21]. Thus, an internist should reflect the stage of heart failure and its functional class (FC) in the diagnosis.

It is quite simple to determine the stages I and II of an untreated heart failure: Stage I − latent heart failure, II A − monoventricular (consider left ventricular), II B − biventricular (right ventricular, secondary to left ventricular). It is not easy at a physical examination to diagnose these stages in a patient with heart failure, who, during the previously prescribed optimal drug therapy, managed to achieve euvolemia, when in the situation of compensated heart failure the information content of the so-called hemodynamic changes is lost (symptoms and signs of stagnation in the pulmonary circulation and large circulation with full compensation may be absent!). With the phenotype of the treated CHF, it is possible to objectify its I or II stage by the results of an echocardiographic assessment of remodeling (the presence and severity of spherification and thinning of the walls) and function (primarily diastolic) of the left ventricle, diagnosing asymptomatic dysfunction of the left ventricle, adaptive or maladaptive remodeling of the left ventricle, which correspond to I, II A or II B stages of heart failure [15].

A more complicated situation develops with the justification of the III stage of heart failure. According to SHFS experts, the difference between CHF III stage and CHF II B stage is the presence of irreversible structural changes in target organs (heart, lungs, blood vessels, brain, kidneys) [15]. However, in the comments of the SHFS experts to their classification, nothing is said about what "irreversible" structural changes, for example, in blood vessels or brain, have a direct causal relationship with heart failure, on the one hand, and can be considered as criteria for verification of CHF III stage, on the other. We were taught at different times that the most striking manifestation of CHF III stage is congestive (cardiac) fibrosis and cirrhosis of the liver [22–24]. However, the liver is not included in the list of target organs in the SHFS classification, which, in our opinion, along with the lack of clear criteria for irreversible structural changes associated with heart failure in these organs and systems (heart, lungs, blood vessels, brain, kidneys) is a significant omission of the classification under discussion, which impedes the unification of diagnostic conclusions. For example, we had to deal with a more than controversial diagnosis when in a patient with coronary heart disease, developed on the background of type 2 diabetes mellitus, and complicated by the development of refractory nephrotic syndrome, and massive proteinuria ("severe, irreversible kidney changes"), justified the CHF III stage, despite moderate manifestations of biventricular heart failure.

Finally, another drawback of the modern classification of CHF stages, which is often criticized by specialists in heart failure [25–28], is its so-called rigidity in gradation, as the authors use a staged approach that excludes the transition from higher gradations to lower. Recall that the classification of N.D. Strazhesko and V.H. Vasilenko was approved at the XII All-Union Congress of Therapists back in 1935, when the possibilities of effective

pharmacological or surgical correction of a severe heart failure were more than modest, and when doctors actually observed a "natural" progressive course of a heart failure. Therefore, the classification did not provide for a revision of the established stage in the opposite direction. But even in our time, when no one doubts that the introduction in clinical practice advances of clinical pharmacology and cardiac surgery often provides positive dynamics for the parameters characterizing the process of heart remodeling, SHFS experts allow only ascendant revision of the stage ("the stage of heart failure may worsen despite treat- ment") [20].

A patient with anasarca hospitalized for qualified or specialized care should be discharged with a diagnosis of CHF II B stage, even if during treatment the patient at rest managed to eliminate all the symptoms and clinical signs of heart failure without exception (we discharge the patient with the diagnosis of pneumonia or acute appendicitis, when after successful treatment there are no clinical and X-ray/ tomographic signs of pneumonia, and there is no appendix at all). And in this case, everything is clear, since such a diagnosis serves as the most solid justification for active combination therapy conducted at the stationary stage [7, 25].

But at the outpatient stage, the need for revision of the CHF stage will inevitably arise, in order to be able to determine the actual disability and prescribe the appropriate treatment, looking at the clinical diagnosis and correctly assess the severity and prognosis of the disease (it is obvious that in a patient with latent heart failure, manifested only at physical exertion, less active therapeutic measures are required to control the symptoms of the disease than in a decompensated patient). It is not easy to understand why in a young man with rheumatic mitral stenosis, complicated with clinically pronounced left ventricular heart failure, and after effective surgical treatment, should have stage II A of CHF in medical documents until the end of his life.

In view of the above, no matter how important the memories of the distant past are, when the patient had decompensation, the possibility of restaging should be discussed. The actual stage of heart failure (including that established on the basis of an echocardiographic study) should be indicated in the diagnosis, along with the stage that the patient had before treatment. D.V. Preobrazhensky and B.A. Sidorenko [28] offer the following example of such flexible approach to diagnosis: Dilated cardiomyopathy. CHF stage I (stage II B in 1998), I FC (IV FC in 1999). Heart Transplantation (1999).

While there is no official decision that the stage can be changed either one way or the other, it was proposed to use the NYHA functional classification to reflect the dynamics of heart failure [21]. Since at rest the symptoms of heart failure are observed only in case of CHF IV FC (so-called manifest or clinically pronounced heart failure), the latter is fundamentally different from the heart failure corresponding to FC I, II or III, in which symptoms occur only during physical exertion (in fact, latent heart failure) – intense, ordinary or less than usual, respectively. Nevertheless, we note that in the latest recommendations of Russian cardiologists [16], clinically expressed and severe CHF correspond to FC II, III and IV, and it is proposed to describe latent heart failure using only I FC.

When assessing the functional status in a patient with CHF, a distinct subjectivity should be taken into account in determining which limited physical activity is slight or, conversely, marked, as well as what kind of physical exertion is ordinary to the patient. This subjectivity naturally leads to low reproducibility of the assessment results of the CHF functional class in the same patient by different doctors.

At the same time, a fairly free interpretation of the NYHA classification by some doctors and researchers, allowing the allocation of intermediate FC values (in fact, three additional gradations: I – II, II – III, III – IV), cannot be considered a good alternative to an attempt to objectify the assessment of FC using any approaches and make it more accurate. For this purpose, it is most often proposed to evaluate exercise tolerance in a functional test (distance traveled in 6 minutes, threshold stress test etc.) and the maximum amount of oxygen consumed, or to use the so-called clinical condition assessment scale [15, 29], as well as other approaches to stratification (when developing a decision rule, a wide range of indicators are used to characterize the condition of patients, in particular, the level of markers of inflammation in the blood serum) [30].

Unfortunately, not all medical institutions have a flat, precisely marked, corridor free of obstacles (pieces of furniture, oncoming and passing traffic of patients and medical workers, doors opening into the corridor, and so on), and even more so a system for performing spiroergometry. But even if there are all the necessary conditions for conducting stress tests, the possibility of their successful implementation in many patients is limited due to associated diseases and conditions: angina pectoris, intermittent claudication, joint diseases, paresis, paralysis and other pathology of the nervous system, which makes it dif-

ficult or precludes the possibility of movement, respiratory failure, severe anemia, morbid obesity etc. [28]. Obviously, if in this case, a stress test (say, a 6-minute walk test) is performed, a correct interpretation of its results will be impossible, since not only myocardial, but also coronary, respiratory failure and other factors will affect the distance traveled by the patient (taking into account the fact that CHF affects mainly the elderly, you cannot surprise anyone with high comorbidity) [7, 31].

Presented in the National Recommendations for the Diagnosis and Treatment of CHF and modified by V.Yu. Mareev, the clinical condition assessment scale for CHF can be a good alternative to the 6-minute walk test when objectifying FC CHF in the absence of the possibility for any reason to perform (correctly interpret) the last [15].

Note that the diagnosis does not need to mention the fact that CHF FC belongs to the NYHA recommendations, like it is often done (apparently out of habit) in clinical practice – CHF II B stage FC II (by NYHA). We get along with indicating the stage of heart failure in the diagnosis without specifying the "copyright" of N.D. Strazhesko and V.H. Vasilenko, as in the description of stable angina pectoris FC – without mentioning Canadian cardiologists [7].

In accordance with the latest recommendations of the European Society of Cardiology for the diagnosis and treatment of acute and chronic heart failure [32], CHF should also be classified depending on the value of LV ejection fraction (EF), as shown in the Clinical Recommendations approved by the Ministry of Health of the Russian Federation [18]: *Ischemic heart disease: Angina of effort, FC III, post-infarction cardiosclerosis, CHF with reduced EF (32%), stage II A, FC III.*

Taking into account the numerous experimental and clinical studies, the results of which cast doubt on the "monopoly" role of systolic dysfunction of the heart as the main and only hemodynamic cause responsible for the onset and clinical manifestations of heart failure, the characteristic of LV diastolic function should be welcomed in the diagnostic conclusion (especially in patients with CHF and preserved LV EF) [33–37].

In our opinion, the clinicians' desire to reflect the clinical situation with heart failure in the diagnostic conclusion in detail (for example, indicating the severity of LV diastolic dysfunction) in terms provided for by the classifications available, should be welcomed, as it is dictated by the desire to build the most effective differentiated therapy and accurately determine the prognosis of the disease. However, one cannot reach the point of absurdity. We had to consult patients who were diagnosed with several CHF at once (!). Most often, this occurs in patients with a combination of coronary heart disease with chronic obstructive pulmonary disease of stage (degree) IV, when in a combined diagnostic conclusion with competing diseases, stage II B CHF is first indicated as a manifestation of severe ischemic heart dysfunction, and then as a decompensated chronic cor pulmonary as a complication of chronic obstructive bronchitis. We deliberately will not give an example of such a diagnostic conclusion, since "a bad lesson is often well learned" [7].

We propose to proceed from the rule "one heart – one heart failure" and recall that, in accordance with the initial definition of the World Health Organization committee of experts (1961), the term cor pulmonary cannot be used to describe a situation in which pulmonary hypertension is associated with primary failure of the left heart or congenital and acquired heart defects (in most cases it is) [7, 38]. The following is an example of a diagnosis statement in which we tried to avoid reiteration:

The main disease: *Coronary heart disease: Post-infarction cardiosclerosis (1999, 2001): aneurysm of the posterior LV wall at the apex with parietal thrombosis, akinesia of the anterior LV myocardium segments throughout.*

Background disease*: essential arterial hypertension, stage III, 3 degrees, risk IV.*

Competing disease:

chronic obstructive pulmonary disease, stage IV, group D; severe infectious (H. influenzae, M. catarrhalis, S. pneumoniae) type I exacerbation (by N. R. Anthonisen). Respiratory failure, III degree.

Complication of competing diseases*: CHF with reduced LV FV (38%) and restrictive type of LV diastolic dysfunction, stage II B, FC IV.*

In addition to the fact that the diagnosis must be justified, timely, structured and detailed, in accordance with the rules for formulating clinical and pathological diagnostic conclusion approved in the established order [11], it is necessary to observe the nosological principle in it. CHF, being a syndrome without any reservations, cannot be considered as the main disease [39], even if direct costs are associated mainly with the treatment of heart failure. We are convinced that the correct diagnostic conclusion, which corresponds to the rules of formulation, serves as the best justification for treatment and the practitioner should not, adjusting to deviant requirements, replace the main disease in the diagnostic conclusion (for example, any form of coronary heart disease)

with its complication in order not to face a fear of a refusal to pay for a completed case of a patient's treatment in a specialized hospital (for example, in a heart failure clinic). After all, it is not clinical medicine with its scientific basis for the health insurance system, but vice versa.

CONCLUSION

In conclusion, we would like to say about the inadmissibility of the so-called tandem diagnostic conclusion, when two or more nosological units are indicated sequentially (often disorderly) in the rubric "Main disease". This is most often observed in a combination of coronary artery disease and arterial hypertension, when internists, usually referring to examples of non-categorized diagnostic conclusion findings presented in the Russian recommendations for the diagnosis and treatment of hypertension in 2010 (fourth revision), describe coronary disease between the degree of arterial hypertension and risk cardiovascular complications and death (we quote, *"essential arterial hypertension, stage III. Degree of arterial hypertension 2. Coronary heart disease. Angina of effort, II FC. Risk 4 (very high)"*). Recall that, in accordance with the rules of diagnosis [11], if any form of coronary heart disease is detected in a patient with arterial hypertension (the timing of the diagnosis does not matter), the last should be indicated in the diagnosis conclusion under the heading "Background diseases". It is impossible to correctly code the disease in any other way, since codes for diseases characterized by high blood pressure (I10–I15) should not be used in cases involving coronary vessels (I20–I25).

In order to make standardized diagnostic conclusions a reality, we call on colleagues of various specialties to be "law-abiding" and follow uniform rules of formulating clinical and pathoanatomical diagnoses, rather than creating their own.

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Allergic rhinitis and the phenomenon of entopy

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ABSTRACT

This article provides a review of the phenomenon of entopy or local atopy from the viewpoint of allergic phenotypes and endotypes. A clinical form of the entopy endotype is local allergic rhinitis, which is still a fertile area for research. The exact mechanisms in the breakdown of allergen tolerance in entopy remain unclear. The review focuses on the pathogenesis, diagnostic algorithm, and the choice of treatment strategies in local allergic rhinitis.

Key words: allergens, atopy, entopy, allergen tolerance, allergic rhinitis, local allergic rhinitis, phenotypes, endotypes, biomarkers, type 2 helper T-cells, T-regulatory cells.

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Аллергический ринит и феномен энтопии

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РЕЗЮМЕ

Представлен обзор современных исследований, посвященных недавно открытому явлению – феномену энтопии (локальной атопии), с точки зрения его фенотипов и эндотипов. Клиническим вариантом эндотипа энтопии является локальный аллергический ринит, новая патология, – объект исследований в современной иммунологии, аллергологии и оториноларингологии. Точные механизмы срыва толерантности к аллергенам при энтопии остаются неясными. Между тем феномен энтопии может стать ключом для расшифровки нерешенных вопросов срыва аллергической толерантности в разных анатомических сайтах. Обзор посвя-

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щен патогенезу, диагностическому алгоритму и проблеме выбора терапевтических подходов при локальном аллергическом рините.

Ключевые слова: аллергены, атопия, энтопия, толерантность к аллергенам, аллергический ринит, локальный аллергический ринит, фенотипы, эндотипы, биомаркеры, Т-хелперы 2-го типа, Т-регуляторные клетки.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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ATOPY AND ENTOPY (LOCAL ATOPY): HISTORY, MECHANISMS, BIOMARKERS, AND CLINICAL VALUE

Initially, the term “atopy” was denoted as “out of place” and “strange disease” by Coca and Cooke in 1923 in their seminal work [1, 2], when IgE was not discovered yet by Ishizaka et al. [3]. In 1963, Gell and Coombs [4] proposed an updated classification of known allergic phenomena where atopy was classified as type 1 hypersensitivity (immediate hypersensitivity). This classification is still used today. Taking into account the rising prevalence of many types of allergies in modern human populations, the hygiene and toxic hypotheses were proposed [5]. In particular, allergic reactions may be considered as maladaptive IgE immune responses towards environmental antigens [6]. Intriguingly, these mechanisms appeared to be very similar to those implicated in the acquisition of immunity against helminths and arthropods in human bodies. Based on the hypothesis that IgE-mediated immune responses evolved in humans and other mammals to provide extra protection against metazoan parasites rather than to cause allergy, the environmental allergens might share some properties with the metazoan parasite antigens, which are specifically targeted by IgE in infected human populations [6].

On the other hand, immediate hypersensitivity, or atopy, occurs in selected populations of *Homo sapiens*. It appears to be a polygeneously inherited disorder, as genome-wide association studies have convincingly detected a large number of loci associated with allergic diseases [7]. However, there are so-called primary atopic disorders based on monogenic inheritance [8]. In addition to that, epigenetic changes have been recently considered as a potential mechanism involved in the development of many disorders, including atopic diseases [9]. Atopy appears to show a strong hereditary component as a consequence of evolution (Fig. 1). From an evolutionary point of view, house dust mites, *Dermatophagoides pteronyssinus* (European species), and *Dermatophagoides farinae* (American species) are the “kings of allergens,” or panallergens [10, 11]. Likely, they used to be skin parasites in ancient humans in the Stone Age [12].

Nowadays, the term “atopy” is used by allergists and scientists for any hyper-IgE-mediated reaction induced by B-cell mediated Th2-dependent response to various allergens [12], such as household dust, house dust mites, animal hair and skin scales, pollens, flour, food proteins, insect venoms, molds, latex, penicillin, etc. There are also oligomeric components of allergen molecules and allergen-associated molecular patterns (AAMP), which may be responsible for effective cross-linking of allergen with the B cell receptor (BCR)/IgE [13]. Supposedly, a deficit of the AAMP leads to tolerance maintenance, whereas an AAMP excess results in tolerance breakdown. Exposure to allergens may occur during inhalation, ingestion, injection, or direct contact. In the course of B-cell-mediated immune response, plasma cells are stimulated by type 2 helper CD4+T cells to produce IgE antibodies specific to one allergen or allergen group. The difference between a normal B-cell-mediated response and a type 1 hypersensitivity response is that in type 1 hypersensitivity, the IgE antibodies predominate instead of IgM, IgG, or IgA immunoglobulins. The IgE antibodies bind to type 1 Fcε receptors (FcεRI) on the surface of mast cells and circulating basophils. After exposure to the same allergen, the allergen cross-links the bound IgE on target cells that result in degranulation and secretion of inflammatory mediators.

Type 1 hypersensitivity reactions may consequently be divided into two phases, the early phase reaction

and the late phase reaction. The early phase typically occurs within 10–20 minutes, or even seconds after the penetration of the allergen, which is associated with the release of preformed mediators, such as histamine, serotonin, chemotactic peptides for neutrophils and eosinophils, enzymes, etc. These mediators affect the nerve cells causing itching, smooth muscle contraction (e.g. asthmatic attack), mucus production by goblet cells, an increase in capillary permeability, and subsequent tissue edema with further recruitment of neutrophils and eosinophils in the focus. Mast cells located in the skin and lining epithelium of the respiratory, gastrointestinal, and genitourinary tracts are involved in recognizing signals coming from the external environment. Once activated, mast cells trigger the early phase of the atopic response, promote the recruitment of other inflammatory cells, such as eosinophils and neutrophils, and take part in the regulation of IgE adaptive immune response [14, 15]. Activated mast cells with their mediators, including tryptase and histamine, are the main biomarkers of the early phase.

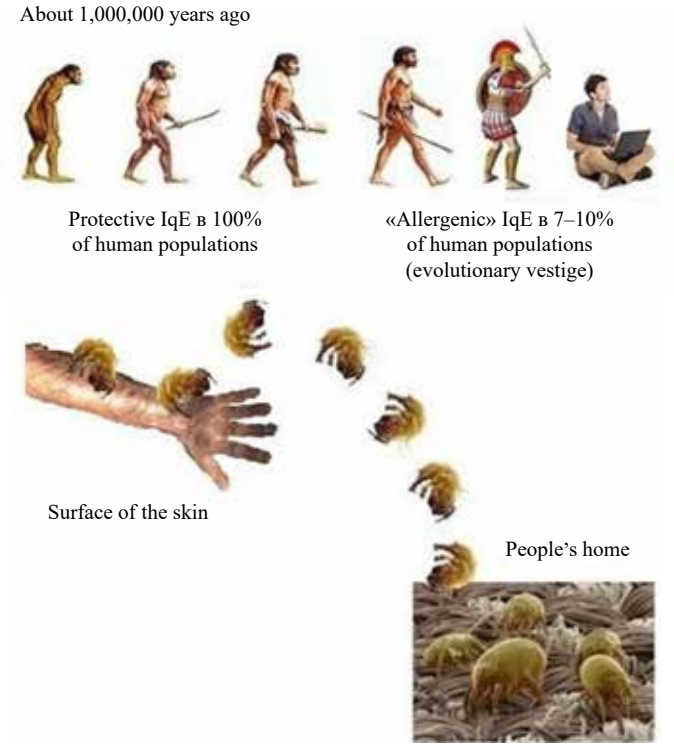

Fig. 1. Atopy as a vestige of evolution [12]

The **late phase** develops over 6–12 hours by the generation of newly formed mediators, such as thromboxane, leukotriene C4, leukotriene B4, prostaglandin D2, and platelet-, cytokine-, and chemokine-activating factors, which affect the surrounding tissues enhancing the inflammatory process. Endothelial cells express the adhesion molecules which facilitate the recruitment and activation of neutrophils, eosinophils, and type 2 innate lymphoid cells (ILC2) from the blood and other biological fluids into the site of the allergic inflammation. Commonly, the infiltrating cells contain a high proportion of eosinophils. The activated eosinophils release a variety of inflammatory molecules, including eosinophil cationic protein, major basic proteins, IL-5, etc. [14, 16]. The involved type 2 helper T cells secrete IL-4, IL-5, IL-6, IL-13, IL-33, etc. and affect plasma cells, which promote IgE isotype switching. The inflammatory process becomes chronic. Therefore, these cytokines, eosinophil mediators, and eosinophil surface molecules are the main biomarkers of the late phase.

Type 1 hypersensitivity is responsible for atopic dermatitis, perennial and seasonal allergic rhinitis, bronchial asthma, food allergies, insect allergies, anaphylactic shock, etc. In predisposed individuals, the term "atopic march" denotes a subsequent change of target organs in the following order: the skin, nose/conjunctiva, and bronchi, whereas some individuals may simultaneously develop all atopic disorders [12, 17]. Allergic skin tests and investigation of blood IgE are preferential methods for diagnosing atopic allergic conditions.

Recently, entopy, a new allergy-like phenomenon, has been identified [18], and the term came from the Spanish expression "*en topo*" that means "on-site." Interestingly, entopy sounds almost like ENT (Ear, Nose, and Throat). Initially, entopy was not related to atopy. Currently, the phenomenon is referred to an endotype of the atopic disorder which can develop local allergic rhinitis [19, 20], local allergic conjunctivitis [21], and local allergic asthma [22]. Among all entopic phenomena, the biggest number of investigations are performed in patients with local allergic rhinitis [19, 20; 23–26]. Patients with severe asthma having dramatic beneficial effects from Omalizumab treatment prove that the concept of local allergy (entopy) is worth discussing in severe asthma [27].

Detailed mechanisms of entopy remain unclear, and the phenomenon is a fertile area for further research. We suppose that, like any form of atopy, entopy is associated with allergen tolerance breakdown. Allergen tolerance is an active process which can be considered as a non-pathogenic immune response to the allergen. The development of allergen tolerance reflects immunoregulatory networks that recruit multiple secreted mediators, such as IL-10, IL-35, and

TGFβ, surface molecules, Treg, and other regulatory cell types. Allergen tolerance also occurs upon natural exposure to high levels of allergen in the environment, as typified by the modified type 2 helper CD4+ T cell-regulated response to this allergen [28]. Historically, there is classical differentiation of tolerance into central and peripheral mechanisms of tolerance induction [12]. The systemic (in the bloodstream) and local (in specific tissues) allergen tolerance is not the same differentiation. For understanding the mechanisms of tolerance induction and breakdown, it should be considered according to "systemic" and "local, or entopic" forms of atopy (Table) to clarify what factors of tissue microenvironment prevail. Specific anatomic sites, e.g., the mouth and respiratory tract, may provide favorable conditions for tolerance induction [28]. This point is open for debate, particularly due to the entopy phenomenon and sublingual allergen-specific immunotherapy.

Table

Forms of tolerance breakdown to allergens

Systemic forms	Local forms (entopy)
Dermal Oral (gastrointestinal) Genitourinary? Combined (may include conjunctival, nasal, and bronchial forms as a part of systemic forms)	Conjunctival Nasal Bronchial

ALLERGIC RHINITIS: PHENOTYPES, ENDOTYPES, AND BIOMARKERS

Allergic rhinitis is a global health problem [29, 30]. Allergic rhinitis (rhinoconjunctivitis) develops in predisposed individuals in two phenotypes: (1) perennial and/or (2) seasonal rhinitis (rhinoconjunctivitis) [25, 31]. Nowadays, both phenotypes of allergic rhinitis, in particular perennial rhinitis, cause considerable asthma prevalence in atopic individuals, impact on the quality of life, performance, sleep, exercise tolerance, and social functioning, and create a significant financial burden on healthcare systems throughout the world [29, 31].

In allergic rhinitis, patients complain of chronic symptoms of nasal obstruction, itching, rhinorhea, paroxysmal sneezing, and sometimes loss of smell, snoring and conjunctival redness and swelling [29, 32]. Clinically, phenotypes of "non-allergic rhinitis" almost do not differ from phenotypes of allergic rhinitis [33]. Perennial rhinitis commonly starts at the age of 3–5 years. It manifests itself through the mentioned symptoms all year round and sometimes throughout the life, being persistent and complicated by nasal polyps, sinusitis, and asthma. Sometimes the course of rhinitis can be mild and subtle. Allergens of *Dermatophagoides* house dust mites, cats, and other pets, feathers, cockroaches, and mold are the main responsible proteins for IgE dependent sensitization in persons with perennial allergic rhinitis [12, 34]. In seasonal rhinitis or "pollinosis", symptoms occur during certain periods of the year when trees, shrubs, and herbs pollinate, but the pathology may commence at any age, in any geographical location. Hypersensitivity is caused by allergens of birch, hazel tree, oak, fescue, ryegrass, timothy, ragweed, artemisia, and a wide variety of plants [12, 35, 36].

An initial diagnosis of allergic rhinitis is more likely when rhinitis is seasonal, or with a family history of atopy [37]. As a rule, examination and investigations include patient's history (family, past medical, social, occupational, etc.), allergic skin tests, serum/nasal secretion IgE assays, nasal secretion cytology, video rhinoscopy, acoustic rhinometry, tests for asthma, etc. [37, 38].

Cluster analysis for allergic rhinitis in adults identified 4 clusters [39]: (1) moderate childhood-onset rhinitis, (2) mild adolescence-onset female rhinitis, (3) severe early-onset rhinitis with asthma, and (4) moderate childhood-onset male rhinitis with asthma. The characteristics that distinguished patients with rhinitis and separated them into clusters were sex, the presence of asthma, and the severity and age of rhinitis onset. Seasonal allergic rhinitis predominated in all clusters. Several other clinical phenotypes and endotypes were subsequently described in various studies [40], whereas endotyping and confirmatory biomarkers showed a more significant impact on management and personalized therapy for patients with allergic rhinitis [41, 42].

The term "local allergic rhinitis" (LAR) was first proposed by Rondón et al. [19]. One group of researchers [43] just supposed that LAR did not fit into the systemic allergic (atopic) rhinitis vs. non-allergic rhinitis classification, and LAR was hence differentiated as a new rhinitis phenotype. However, the other group of researchers [20, 44, 45] substantiated LAR as an endotype of allergic rhinitis, since it displays all atopic biomarkers not at the systemic level but in the nasal mucosa, i.e. at the local level. Furthermore, recent evidence supports the existence of a bronchial counterpart of LAR named local allergic asthma [22], and its conjunctival counterpart defined as local allergic conjunctivitis [21].

Patients with LAR have the same classic symptoms typical of allergic rhinitis, such as nasal obstruction, sneezing, itching, and rhinorrhea. A study comparing patients with allergic rhinitis and LAR also showed that both groups of patients share a similar clinical phenotype. LAR is caused by sensitization to *Dermatophagoides* house dust mites, occurs mostly in nonsmokers, and displays a severe and persistent clinical picture, often with conjunctival and asthma symptoms, developed in both children and adults [44]. LAR can be verified by (1) detection of specific IgE house dust mite allergens and other aeroallergens in nasal secretion and (2) positive response to the nasal provocation test with the same allergens, whereas skin prick tests, intracutaneous tests, and serum IgE assay may be negative [25, 46, 47]. Incorvaia et al. [20] recommend a double nasal provocation test to diagnose LAR accurately. The algorithm for LAR diagnosis [45, 47] is shown in Fig.2.

Unfortunately, due to high cost and complexity, nasal provocation tests with house dust mite allergens and detection of specific IgE in nasal secretion are not yet recommended in everyday clinical practice. As a consequence, patients with LAR, including elderly persons [48], are still classified as individuals with non-allergic rhinitis in most hospitals [49].

As a result of a 7-year retrospective follow-up study, Sennekamp et al. [46] demonstrated conversion of LAR to conventional systemic respiratory allergic reactions in almost half of the observed patients. The conversion rate was higher in children and adolescents than in adults. On the other hand, following the results of a 10-year follow-up study of a cohort of 176 patients with LAR, Rondón et al. [50] insist on low conversion of entopy to systemic atopy and natural evolution of the disease towards allergic asthma.

Treatment for LAR is similar to that of conventional allergic rhinitis. It includes allergen avoidance/environmental controls, corticosteroid nasal sprays, antihistamines, leukotriene receptor antagonists, intranasal Cromolyn, medications containing immunosuppressive monoclonal antibodies like Omalizumab, and allergen-specific immunotherapy [45, 51, 52]. However, the problem of choice between subcutaneous or sublingual administration of allergens for allergen-specific immunotherapy or development of the other routes of their administration remains open for research and discussion [52].

In accordance with the concept of a single unified airway [52, 53], the upper and lower respiratory tracts are connected anatomically, functionally, and

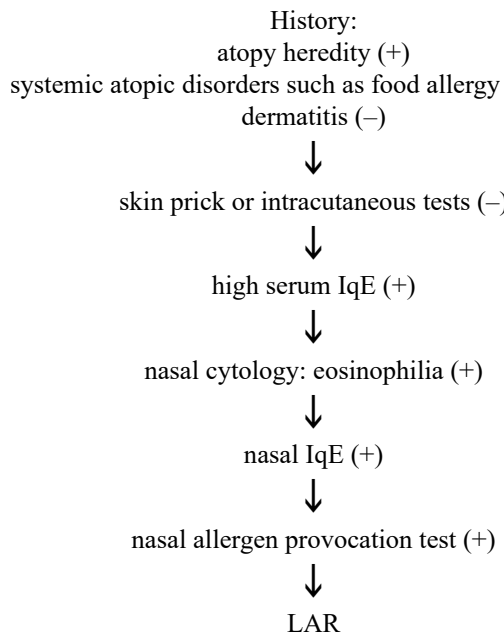

Fig.2. Diagnostic algorithm for local allergic rhinitis [45, 47]

immunologically, which is essential for the formation of a generalized inflammatory process. Disruption of systemic allergen tolerance often leads to the development of all forms of atopy and its complications (allergic rhinitis, rhinosinusitis, polyps, asthma) in the respiratory tract in combination with atopy in non-respiratory target organs. At present, it remains unclear how to relate the concept of a single unified airway and the phenomenon of entopy, its clinical manifestations, possible complications, dynamics of development, and the optimal choice of treatment strategies.

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Multiple subsets of regulatory T-cells

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ABSTRACT

Regulatory T-lymphocytes play a central role in the immunological tolerance system. To date, existence of many different subpopulations of regulatory T-cells have been described. However, a number of questions related to the function, differentiation, and homeostasis of these subpopulations in a body remain unclear. Interactions between the previously discovered pairs of helper and regulatory T-lymphocytes require further study. The main topic is identification and establishment of the functions of regulatory memory cells. Interstitial migration of activated regulatory T-lymphocytes is also a promising direction. In this review, we summarized the main findings in multiple subsets of regulatory T-lymphocytes, discussed unclear data that will require further studies, and showed an application for regulatory T-lymphocytes in medicine.

Key words: regulatory T-lymphocytes, immunological tolerance, memory Treg, effector Treg, central Treg, tissue-specific Treg.

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Разнообразие субпопуляций регуляторных Т-клеток

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РЕЗЮМЕ

Регуляторные Т-лимфоциты являются центральными клетками системы иммунологической толерантности. В настоящее время описано существование множества различных субпопуляций регуляторных Т-клеток (T-reg), однако большое количество вопросов, касающихся функционального назначения, путей дифференцировки и гомеостаза этих субпопуляций в организме, остаются неизученными. Продемонстрированные ранее пары хелперов и соответствующих им регуляторных Т-лимфоцитов требуют дальнейшего изучения их взаимодействий друг с другом. Актуальной темой является идентификация и установление функций клеток регуляторной памяти. Тканевая миграция активированных регуляторных Т-лимфоцитов также является перспективным направлением. В этом обзоре собраны и систематизированы данные о различных субпопуляциях регуляторных Т-лимфоцитов, выделены актуальные вопросы данной

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Ключевые слова: регуляторные Т-лимфоциты, иммунологическая толерантность, Treg памяти, эффекторные Treg, тканеспецифичные Treg.

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INTRODUCTION

The immune system is a complex and diverse structure with the task to maintain homeostasis. The main roles of the immune system are eliminating infectious agents, killing tumor cells, and immunoregulation. The system of immunological tolerance, which protects the body's own tissues from being attacked by immune cells, includes many different cells, such as tolerogenic antigen-presenting cells [1], regulatory T-cells, and B-cells [2].

Previously, it was believed that the population of regulatory T-cells (Tregs) was homogeneous, but over time the accumulated data contradicted this idea. Currently, the existence of various Treg subpopulations is accepted [3]. However, despite numerous studies, this area of immunology remains underdeveloped. There is little information on the differentiation of Tregs, the formation of their various subpopulations, and their interactions with other cells. The main mechanisms of tolerogenic action have been described (contact suppression due to suppressive molecules such as CTLA-4 [4] and PD-1 [5]; secretion of anti-inflammatory cytokines such as TGF-b [2], IL-10 and IL-35 [6]; sequestration of growth factors such as IL-2, necessary for activation of effector cells [7]; and metabolic activity, for example, the conversion of ATP to adenosine, which limits the pro-inflammatory effect of immune cells [8]). However, it is unclear how this happens *in vivo* and in what situations these mechanisms are implemented.

Unresolved issues in this area need to be studied, since their practical application can make a great contribution to solving many clinical problems. Currently, the treatment of autoimmune pathology is imperfect, and in some cases it cannot lead to compensation in patients. It is also associated with severe side effects, like infection and the risk of developing cancer [9]. Establishing the role of individual Treg subpopulations in the control of autoimmune processes can provide important information for new targets of therapeutic intervention and the creation of new effective and safe treatments for autoimmune diseases.

MULTIPLE SUBSETS OF REGULATORY T-CELLS

Treg cells can be classified as naive and activated, the latter of which have passed antigen recognition and proliferation processes in peripheral lymphoid organs. Naive Tregs can be designated as cells derived from the thymus (tTreg) [10] and these cells did not undergo TCR activation. They can be recognized by their expression of the CD45RA isoform, whereas previously activated cells have the CD45R0 isoform [11]. Treg cells that differentiate from Th0 (naive T-lymphocytes, but not Tregs) are referred to as peripheral (pTreg) in the literature, since they differentiate in peripheral lymphoid organs after TCR activation; these cells are also activated Treg cells [10].

The separation of tTreg and pTreg was based on the expression of the transcription factor Helios [12], which is expressed by Tregs of thymic origin. It has been shown that the level of Helios in tTreg was increased with activation; this makes possible to identify both naive (CD45RA+) and activated cells (CD45R0+) in the group of regulatory T-cells of thymic origin [12]. Naive thymic cells express CCR7 and CD62L, which allows them to migrate to the lymph nodes. Therefore, this population has been designated central Tregs (cTreg) in the literature [3]. These cells contain a large amount of CD25 (high affinity IL-2 receptor

alpha chains); this may allow them to deprive the surrounding T-cells of IL-2, limiting their proliferation [3]. Activated cells (CD45R0+) have other functional characteristics like the expression of suppressive molecules such as IL-10, CTLA-4, ICOS, TGIT, CD39 [13-15] and chemokine receptors which mediate their migration into various tissues [3]. Besides, in vitro induced Tregs (iTreg) [10], which are obtained by applying cultivation of T-lymphocytes outside the body, currently constitute a separate group; these cells may differ significantly from Treg cells in vivo. Among activated Tregs, differences were found between activated Treg cells derived from tTreg and pTreg cells. It was demonstrated that activated Helios+ Tregs (tTreg) and Helios$^-$ Tregs (pTreg) can differ in the cytokine profile [16] (described in more detail below in the section "Th-Treg pairs").

Based on tropism, Treg cells can be divided into tropic to lymphoid formations (peripheral lymph nodes) and tropic to non-lymphoid tissues [3]. Currently, there is data that allows determination of resident tissue-specific Tregs (section "Tissue-specific Tregs"). These cells are tropic to the microenvironment of certain tissues; however, they are not circulating (migrating) or recirculating cells. Therefore, Treg population may be further subdivided into circulating (recirculating) and resident cells.

It is difficult to identify which cells are recirculated (i.e. exit from one tissue and move through the bloodstream to the other tissue). It is also impossible to confidently determine which resident cells cannot leave tissues and recirculate under any circumstances. Treg cells studied in peripheral blood (i.e., circulating populations) also cannot be precisely defined as migrating in one direction or recirculating from one tissue to another.

Likely, these processes are quite dynamic and cells with the same origin and functional status can become both tissue-resident and recirculating populations depending on the context. Nevertheless, study in this area will provide much more understanding to the functioning of the tolerogenic system, since circulating and resident cells have different properties (described in more detail in the following sections). Cell tropism can be determined by the presence of appropriate tissue-specific chemokine receptors. The central (tropic to lymphoid formations) populations include naive thymic Tregs. However, there has recently appeared data to expand this group. According to the study by Wei X. et al. [6], there are two subsets of activated Treg in mice: IL-10+Bcl-6+ Tregs and IL-35+Ebi-35+ Tregs. IL-35+ Tregs demonstrate tropism for secondary lymphoid organs (these cells are localized in the peripheral lymph nodes / white pulp of the spleen and express CCR7 and CD62L), which identifies this subpopulation as central. According to the authors' assumptions, this subpopulation differentiates from thymic Tregs [6], which remain tropic to lymphoid organs after activation. There are some similarities between the functions of thymic naive Tregs and IL-35+ Tregs. These two subpopulations are located in the lymph nodes and are able to suppress initiation of the immune response. IL-35 promotes the differentiation of pTregs from naive T-lymphocytes [17]. Moreover, it was shown that differentiated cells are able to synthesize IL-35 themselves [17], which suggests that maintaining the constancy of IL-35+ Tregs can also occur due to the conversion of naive cells into IL-35 producing ones.

Activated Treg cells are referred to as effector Tregs in the literature [3]. These cells are a CD45RO+CD45RA-FOXP3^{high} subset and tropic to non-lymphoid tissues. IL-35+ Tregs are an exception to this condition. In addition, there is currently strong evidence for the existence of memory Tregs (Memory Tregs section), which also applies to activated cells. Activated non-regulatory T-lymphocytes are currently divided into various groups which include central memory T-cells, effector memory T-cells, and terminally differentiated T-cells (TEMRA) [18].

Based on these data, it is rational to distinguish between the group of effector Treg cells and Treg memory cells based on a number of features (for more details, see the Memory Tregs section). In addition, activated cells can be divided into tissue-specific cells and specific to subpopulations of helper T-lymphocytes. Thus, activated Tregs can be divided into tissue-specific, helper-specific Tregs and such separate groups as the effector Treg cells and Treg memory cells can also be identified.

TISSUE-SPECIFIC TREGS

The Treg tissue group is a subset of regulatory cells that suppress local tissue inflammation and provide homeostasis in peripheral tissues. The functions of these cells differ depending on the type of tissue. Thus, Treg cells of muscle tissue affect the repair of muscle fibers, accumulating in the tissue upon damage [19]. In adipose tissue, Tregs suppress

local inflammation, which is manifested by impaired glucose tolerance [20]. Intestinal Tregs regulate tolerance to antigens of food and commensal microflora of the gastrointestinal tract [21]. Therefore, different groups of tissue Treg cells perform various functions of maintaining homeostasis in the tissue, and not only monitor the activity of immune cells.

It should be noted that in some tissues, such as muscles or the central nervous system, the presence of resident Treg cells is limited; their accumulation is observed only when damage occurs [3]. However, for such tissues, Treg cells can play a significant role in regional lymph nodes, as they rapidly divide and migrate into the tissue if it is damaged. It can be assumed that resident Tregs exist for the lymph nodes of such tissues. It was shown that part of the memory T-cells is present in the peripheral lymph nodes [22].

TH-TREG PAIRS (HELPER-SPECIFIC TREGS)

There are subgroups of Tregs specific to certain groups of helper T-lymphocytes [16]. These cells specialize in suppressing a specific population of Th cells. The existence of Tregs specific for Th1, Th2, Th17 [16] and Tfh [23] was identified. Helper-specific Tregs are characterized by a specific set of chemokines and transcription factors. For example, Treg cells that suppress Th1: these cells express T-bet transcription factor (which is also expressed in Th1) and are dependent on cytokines associated with Th1: gamma interferon (IFNγ) and IL-27 [3, 16]. For the development of Th2-specific Treg cells, expression of the transcription factor of Th2 GATA3 cells is necessary [24]. Meanwhile, in order to suppress responses of the lymph node germinal center, which are provided by Tfh, Treg cells called T-follicular regulators (Tfr) are needed [23].

This cell population expresses Blimp-1, in contrast to Tfh cells, whose development is inhibited by Blimp-1 [25]. Further study of helper-specific subpopulations of Treg cells and their transcriptional program is necessary. It should also be noted that these cells have functional features similar to helper cells; they produce suppressive cytokines together with pro-inflammatory cytokines which are characteristic of their effector analogues (IL-17 for Th17, IL-4 for Th2, IFNγ for Th1) [16]. This circumstance can potentially disrupt the action of tolerogenic mechanisms of helper-specific regulatory T-cells due to the pro-inflammatory effects of these cytokines. Transition of Tregs to Th17 under the influence of various stimuli has been shown [26], which may indicate functional instability of these cells. An increase in such subpopulations has also been shown in autoimmune pathologies such as multiple sclerosis [27] and type 1 diabetes mellitus [28]. However, as it has been rightly noted in the work by T. Duhen et al. [16], the production of pro-inflammatory cytokines in Tregs may differ significantly from their effector analogues.

Thus, IL-22 was often co-produced with IL-17 in Th17 cells, which was not observed in the Th17-like Treg population [16]. All of these cytokines are produced together with IL-10 in Th17-like Tregs. It was also found that IFNγ and IL-17, under certain conditions, can have an immunoregulatory effect [29, 30]. It can be assumed that such Treg subpopulations are characterized by certain programs for establishing tolerance due to a combination of pro- and anti-inflammatory cytokines; these mechanisms need further study. In this regard, it is possible that an increase in the number of such Tregs in multiple sclerosis may be a compensatory body reaction to autoimmune inflammation [16].

It should be noted that, at the moment, it is impossible to accurately determine that the helper-specific Tregs existing in the norm and similar cells that increase in amount during autoimmune pathologies are identical. Helper-specific Tregs are heterogeneous in origin. Being activated (CD45R0+) cells, most of these Tregs expressed Helios [16], which may indicate their origin from the group of thymic Tregs. However, among the $CD25^{hi}$ $CD127^{lo}$ Th1- and Th17-like Tregs, a group of $Helios^{-}$ cells, probably pTregs, were also found [16]. These cells produced IL-10 in larger quantities than Helios+ cells [16]. Thus, helper-specific Treg cells of different origin could increase in amount during autoimmune diseases. It is possible that the expression of Helios factor is suppressed, and this is interconnected with a change in the cell functioning. A deeper analysis and comparison of different groups of helper-specific Tregs in normal and pathological conditions will help to better explain the role of these cells in the immune system.

MEMORY TREGS

Currently, cells of regulatory memory, memory Tregs, are being determined [22]. These cells can remain viable in the absence of stimulation by the

antigen (autoantigen) for a long time, and also effectively suppress the immune response when activated. Memory Tregs are characterized by an increased ability to suppress effector cells [22]. The existence of memory Treg cells was quite controversial because it is difficult to prove that memory Tregs are preserved in the absence of constant stimulation by the antigen, provided that normally autoantigens are constantly presented in the body [22]. The biological meaning of the existence of such cells can consist of the following points:

With age, there is a decrease in the thymus function and the production of naive T-lymphocytes [2], which suggests the existence of long-lived memory cells that support the lymphoid population in the absence of a constantly updated pool of naive T-lymphocytes.

In the absence of a pathological process, the presentation of autoantigens is performed by immature dendritic cells or specialized tolerogenic cells that contain a small number of costimulatory molecules and do not produce pro-inflammatory cytokines in sufficient quantities [1]. Such antigen presentation leads to anergy, apoptosis, or the formation of a regulatory phenotype in T-lymphocytes. When autoantigens with costimulatory molecules and pro-inflammatory cytokines are presented, activation of autoreactive T-cells occurs, which can lead to autoimmunity [2].

In this situation, the existence of Treg memory cells, which would be activated in parallel with autoreactive cells, would contribute to autoimmunity control. Thus, it is possible that the autoantigen presentation under normal conditions is not sufficient to activate memory Tregs, and these cells can only be activated under pro-inflammatory conditions when the autoantigen presentation can lead to the expansion of autoreactive cells.

Access of immune cells to tissues separated from the immune system by histological barriers (immunoprivileged tissues) is limited [2]. In case of damage to the barriers due to trauma or inflammation, autoantigens from these tissues become available for recognition by the immune system, and this situation can lead to the development of an autoimmune process [31]. To prevent this, Treg cells persisting in the absence of autoantigen presentation for a long time (for example, in regional lymph nodes) may exist; these cells, upon a repeated episode of damage, can migrate with effector cells into the tissue to prevent the autoimmune process.

Regulatory cells can be specific not only to autoantigens, but also to foreign antigens that are not expressed in the body. In a model of acute influenza infection, the number of virus-specific Treg cells was shown to increase 50-fold during the initial response [32]. Subsequently, like in the case of effector T-cell populations, the number of virus-specific Treg cells decreased after resolution of the primary infection.

However, a fraction of these Treg cells persisted for more than 50 days after infection. Upon re-infection, the pool of such Tregs underwent a 10-fold expansion, which is similar to an increase in the population of effector memory T-cells. In addition, Treg memory cells significantly inhibited the clonal expansion of the effector T-cell population and cytokine production. They also reduced tissue damage without impairing clearance of the virus [32]. These results were reproduced by another group using a similar infection model [33]. The mechanisms that allow Tregs to improve elimination of pathogens are currently unknown, but these experiments demonstrate the need for the interaction of various parts of the immune system for an adequate immune response, as well as the existence of not only suppressive, but also a regulatory function of Tregs.

Not all antigens can be constantly expressed in tissues; expression of some molecules may be activated during inflammation [34]. Tregs specific to such markers can also be memory cells.

A number of studies have been carried out proving the existence of Treg memory cells [32, 33, 35]. An experimental model has been created to suppress or activate the expression of a specific antigen in the skin [35]. Meanwhile, the expression of this antigen in the thymus was not suppressed. Upon presentation of this antigen, a group of regulatory T-cells that suppressed the immune response to the antigen developed in the skin. When its expression was turned off, the existence of regulatory T-lymphocytes specific to this antigen was detected, which remained for a long time in the skin in the absence of antigen presentation and, when its expression was re-activated, suppressed inflammatory reactions more efficiently than primary Treg cells [35].

The determination of memory Tregs in humans is somewhat more complicated. Human T-cells express the CD45RO isoform in the thymus and turn into CD45RA+ after migration to the peripheral lymph nodes [36]. After recognition of the antigen

at the periphery, these cells switch back to the form of CD45RO. Almost all *in vitro* CD45RA+CD4+ T-cells lose their expression of CD45RA and switch to the CD45RO+ phenotype after 4 days of TCR stimulation [37].

At this point, human memory Tregs are designated as T-cells expressing the CD45RO marker, indicating a previous activation. However, CD45RO expression alone does not define a T-cell as a true memory cell [22]. This marker does not distinguish between cells that persist in the absence of antigens and cells that constantly recognize antigens. However, CD45 isoforms are now widely used to distinguish between naive Tregs and cells activated by antigen recognition (among which memory cells are also represented). In the study by M. Miyara et al., based on the expression of CD25, CD45RA, and FOXP3, the peripheral blood T cells of healthy people were divided into two subsets of CD45RA+FOXP3^{low} and CD45RA-FOXP3^{hi}, which were called "resting" and "activated" Tregs [11]. It was demonstrated that, after antigen stimulation, resting Tregs proliferate and differentiate into activated Tregs [11]. It was shown that the number of CD45RA+ Treg cells in peripheral blood decreases with age, which is accompanied by an increase in the population of CD45RO+ Treg [38].

These results confirm the hypothesis that CD45RA+ Treg cells are a resting population that turns into activated CD45RO+ (among which there may be memory Treg cells) under the influence of antigen activation [22]. In turn, CD45RO+ Tregs can be divided into subpopulations in accordance with the expression of HLA-DR [39]. These groups differ in functional characteristics: suppressive ability and cytokine secretion. HLA-DR+ Tregs expressed higher levels of activation markers (CTLA-4, ICOS) and had a more pronounced suppressive effect *in vitro* but produced lower levels of cytokines. Perhaps, this group is Treg memory cells due to their more differentiated phenotype [22].

HLA-DR- cells can be considered recently activated, but not fully differentiated Treg cells. However, it has been shown that HLA-DR is expressed on recently activated conventional T-cells in humans [40]. In this regard, it is possible that CD45RA-HLA-DR+ Tregs are newly activated "effector" Treg cells, and not Treg memory cells [22]. It is also worth noting that most memory Tregs may be located in peripheral tissues [41], and in blood they can appear only when moving between tissues or between tissues and lymph nodes.

It was shown that almost all Treg cells in adult skin express CD45RO, while a significant part of the regulatory skin cells of the fetus were attributed to a subpopulation of CD45RA [42]. Tregs in adult skin also express high levels of other markers associated with memory T-cells, including CD27 and BCL2 [42]. It is important to note that, in comparison with effector T-cells, memory Tregs from human skin expressed unique tissue-specific TCR sequences, did not express CCR7, and could not migrate from skin [42]. All of this data shows that differentiated Treg memory cells may be located in tissues and do not appear in peripheral blood.

At present, there is no unified approach to the determination of regulatory memory T-cells, however, many distinctive features such as expression of activation molecules (CD45RO, HLA-DR), chemokine profile, and metabolic profile can help in solving this problem [22]. It is also important to note that memory Tregs may use other homeostatic factors. It was shown that memory Tregs are less dependent on IL-2 (which is necessary for the survival of naive and activated cells), but more sensitive to IL-7 (memory Tregs in the skin showed increased expression of IL-7R, i.e. CD127, which is usually low or not expressed at all on Treg cells in peripheral blood) [43]. This fact demonstrates different biology of ordinary Tregs and memory Tregs and can serve as one of the markers of regulatory memory T-cells.

RESULTS INTEGRATION

Based on the study of IL-35+ and IL-10+ Tregs, two main directions can be formulated: preventive, i.e. maintaining homeostasis by reducing the activation of non-regulatory T-lymphocytes or exposure to other cells (for example, antigen-presenting), and suppressive, aimed at limiting the already existing focus of inflammation. These two actions were divided between lymphoid IL-35+ Tregs (preventive) and non-lymphoid IL-10+ Tregs (suppressive) [6]. Such a separation is justified since an immune response is initiated in the lymph nodes and an inflammatory reaction occurs in tissues. However, in this review, using the preventive and suppressive effect of regulatory T-cells as a basis, a model is proposed, not of the anatomical distribution of these effects, but of a functional one, i.e. by the predominant type of cells that have these effects (Fig. 1).

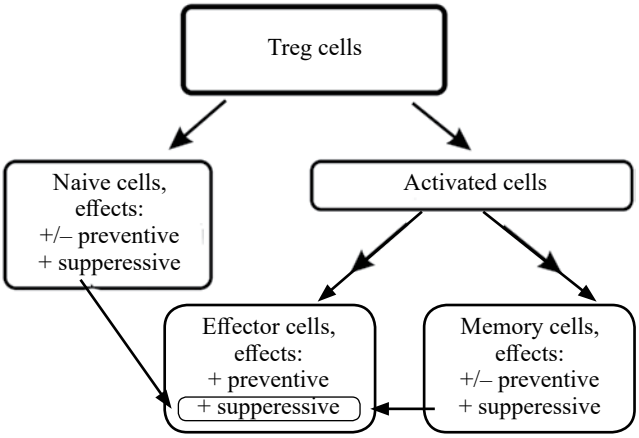

Fig. 1. Types of regulatory T-cells

By activation status, regulatory T-cells are divided into naive and activated. The activated cells include effector cells and memory cells. Each group has preventive and suppressive effects to various extents. Effector cells exhibit an active preventive effect or a suppressive effect depending on their number. Naive and memory cells have a passive preventive effect due to the competition for resources with other T-cells; upon activation, these populations divide and differentiate into effector cells and the suppressive effect is manifested due to their increased number.

Both lymphoid and non-lymphoid populations have both effects. Moreover, probably all populations (naive, effector, memory cells) have both effects, but they are realized to varying degrees. This is indirectly indicated by the results of the experiment [6], when one of the two populations (lymphoid or non-lymphoid) was removed, the development of autoimmune pathologies did not occur, which indicates a partial overlap in their function.

Naive Tregs have some preventive effect due to the competition with Th0 for IL-2 (a passive effect, since they do not synthesize anything) [7]. At the same time, these cells have a pronounced suppressive effect upon TCR activation due to proliferation and differentiation into Treg CD45R0 + effector cells [11]. Memory Treg cells may have similar properties like competing for IL-7 with other memory T-cells and providing proliferation and differentiation of effector Tregs upon re-activation. The effector Tregs, which constantly recognize antigens and are in an active functional state (produce suppressive cytokines and contact suppression molecules), have both preventive (active) and suppressive effects, which depends on the number of cells.

An increase in the number of effector Tregs during the proliferation of naive and memory cells translates the preventive effect into a suppressive one. It should be noted that due to the high expression of contact suppression molecules [22], memory cells may also have a preventive effect while maintaining a state of functional rest. However, this requires the presence of a sufficient number of these cells and this method can potentially lose the paracrine effects of suppressive cytokines to maintain a preventive effect. On the other hand, as a suppressive effect, this method may be more successful. As mentioned earlier, HLA-DR+ Tregs showed higher levels of contact suppression molecules CTLA-4 and had a more pronounced suppressive effect *in vitro*, but produced lower levels of cytokines [39].

The lymph nodes contain (at least in mice) naive Treg cells and activated IL-35+ Treg cells. IL-35+ cells exhibit effector features with functional activity (this is evidenced by gene expression profiles) [6]. Thus, lymphoid tissues contain groups of cells exhibiting both effects. Moreover, the existence of Treg memory cells in lymphoid tissue is not ruled out.

Various types of cells exhibiting both preventive and suppressive properties are also present in non-lymphoid tissues. Adipose tissue resident Treg cells are shown as functionally active cells that recognize local tissue antigens and, therefore, persist in adipose tissue and control homeostasis [20]. This description is suitable for effector regulatory T-cells that have a preventive effect. It was also demonstrated that with an increase in IL-33, which acts as an alarmin in tissue damage, these resident Tregs proliferate intensively, which demonstrates their suppressive effect [20]. It is not clear whether this population is homogeneous, and all of these effector cells have proliferative potential or there are resting memory cells among them.

Given the existence of several types of memory cells with different functional statuses in T-lymphocytes (Tcm, Tem) [18], such a division can also exist among Tregs. It may turn out that among adipose tissue resident Treg cells there are active effector cells with sufficient proliferative potential. However, an important feature of these resident cells is that adipose tissue Treg cells proliferate in response to exogenous administration of IL-33, while Treg cells in the lymph nodes did not show such proliferation [20]. This fact may mean that there are specific stim-

uli for each specific Treg group that induced their proliferation. Therefore, a suppressive effect can be observed under certain conditions. Treg IL-35 cells were practically not proliferated compared to IL-10 Tregs upon activation by monoclonal antibodies to CD3 [6]. This population may be effector with low proliferative potential or there may be a specific stimulus (stimuli) to which this population can respond by proliferation. This issue requires further study.

Despite the fact that in both lymphoid and non-lymphoid tissues there are cell populations responsible for preventive and suppressive effects, these tissues and Treg cells that they include should be considered as a single functional system for maintaining immunological tolerance. Some changes, for example, an increase in the level of pro-inflammatory IFNγ+ T-cells, were observed in the absence of IL-10+ Tregs alone [6], which indicates incomplete functional overlap between IL-10+ and IL-35+ Tregs. Autoimmune pathology did not occur in these mice, however, a background predisposing to this could be created (it has not been investigated). This can be considered as one of the links in the pathogenesis of autoimmune diseases.

The absence of such changes in mice with deletion of IL-35+ Tregs can be explained by the presence of additional functional reserves of lymphoid tissues in the form of naive populations of Tregs (also having both effects) or the effect of IL-10+ Tregs, which despite being directed to peripheral tissues (chemokine profile), were detected in the lymph nodes (perhaps, they were in the process of exiting the nodes) [6]. The chemokine profile of IL-35+ Tregs showed the orientation of these cells to lymphoid tissues, therefore, in the absence of IL-10+ Tregs, these cells could not migrate to peripheral tissues. This circumstance may explain the increase in the number of effector IFNγ+ T-cells. In the absence of lymphoid effector Tregs, non-lymphoid Tregs compensated for their function. The absence of non-lymphoid populations led to an increase in the level of pro-inflammatory cells (partial compensation), but not to the development of the disease. Spontaneous autoimmune colitis developed only in the absence of both populations [6], which indicates the existence of connections between tolerogenic systems of the lymph nodes and peripheral tissues.

To achieve immunological tolerance and control immunity, lymphoid (regional lymph nodes) and non-lymphoid tissues act in concert, partially compensating and complementing each other. The following scheme is proposed to describe the operation of this system (Fig. 2).

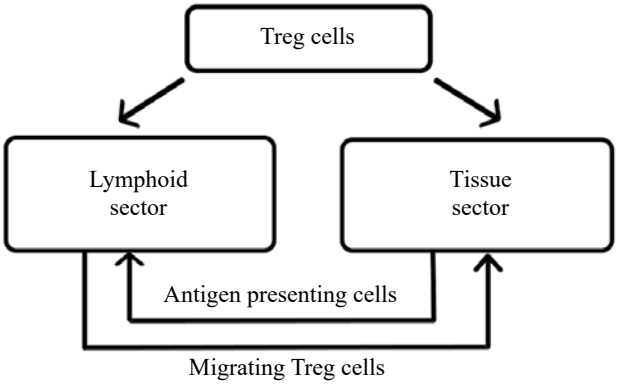

Figure 2. A unified system of immunological control

Lymphoid and tissue populations of regulatory T-cells act as a unified system of immunological control. Tissue sector affects lymphoid sector due to antigen-presenting cells. The lymphoid sector is a source of cells for suppressing tissue inflammation (the suppressive effect) and renewal of the tissue sector (transition of migrating populations to tissue resident ones after performing suppressive functions or outside of performing suppressive functions). Migrating cells compete with tissue resident cells; more adapted cells win the competition, which leads to dynamic immunoregulation under changing conditions.

An interaction takes place between the lymph nodes and peripheral tissues: antigen-presenting cells migrate from the tissues [2] and activated cells migrate from the lymph nodes to the tissues where they can become progenitors of tissue resident cells [22]. Adipose tissue resident Treg cells were capable of proliferation, i.e. self-renewal of the population [20].

This fact calls into question the need for migration of precursors from the lymph nodes. Nevertheless, in conditions of inflammation, due to the increase in the number of pro-inflammatory cells, the participation of Treg cells migrating into the tissue may be necessary to realize the suppressive effect. This may be especially important in relation to helper-specific populations that migrate to the places of accumulation of their helper analogues. Some of the migrating cells may remain after suppression of the immune response in tissues [22] to maintain homeostasis

(preventive effect). Due to the presence of competition for resources (cytokines and p-MHC complexes) in lymphocytes [44], these populations will compete with resident cells. As a result, dynamic regulation of the system of immunological tolerance can occur (more functional cells adapt to provide a preventive effect).

Thus, peripheral tissues affect the lymphatic population of Treg cells due to antigen-presenting cells stimulating both the preventive (presentation of antigen in small quantities to maintain the persistence of effector populations) and suppressive effects (presentation of antigen in significant quantities together with inflammatory signals to activate naive cells and memory cells). The lymph nodes provide a suppressive effect for tissues through the production of effector Tregs and regulate the preventive effect due to tissue resident progenitor cells that compete with local Treg cells for resources. As a result, cells more adapted to tissue stimuli will persist in the tissues, providing local homeostasis and immunological tolerance.

It is also important to emphasize the specificity of the preventive action. Its focus of reducing activation and preventing the development of the immune response is non-specific. However, the methods by which this effect is achieved may be different for each Treg group. This is indicated by the fact that activated Helois+ and Helios⁻ Tregs, being effector cells, differed in the amount of IL-10 produced [16]. Additionally, in the study by X. Wei et al. [6], the authors note that activated cells producing IL-10 act on this type of cytokine to affect many types of cells. This is due to the broad expression of IL-10R, while IL-35 acts mainly on T-cells. Thus, it can be assumed that due to differences in functional modes (cytokine spectrum), the preventive effect of a certain Treg group will be selective for different cell populations; it makes the regulation of immunological tolerance more adaptive to changing conditions.

APPLICATION PROSPECTS

The separation of different populations of regulatory T-lymphocytes entail many significant consequences. Different populations are able to act as diagnostic and prognostic markers. For example, insufficiency of specific populations may be a prognostic marker for the development of pathologies associated with this group of cells (insufficiency of Treg subpopulations that suppress Th2 may be a risk factor for the development of allergic diseases).

Therapy of autoimmune diseases is currently an urgent problem, but the currently existing methods based solely on suppressing the immune response are imperfect because they do not always allow disease control and at the same time have a large number of serious side effects [9]. Thus, it is necessary to create new methods for the treatment of autoimmune diseases based not on full immunosuppression, but on correction of the immune response. One of the varieties of new techniques is tolerogenic cell vaccines [45].

These methods are based on the introduction of autologous tolerogenic cells specific to a causally significant autoantigen (i.e., autoantigen to which the development of an autoimmune reaction is expected) [45]. In autoimmune diseases, there are impaired regulations of the immune response including impaired control of immunological tolerance [2]. The regulatory system, as shown above, includes the joint work of regulatory cells of lymphoid and non-lymphoid tissues. Accordingly, the impact on both levels of the immunological tolerance system should be more effective than on any one level. This approach should be true for the use of tolerogenic cell vaccines. The impact on both levels of immunological regulation, either using two types of tolerogenic populations or using agents that affect both types of populations, can significantly improve the methodology of tolerogenic cell vaccines.

To implement this strategy for the treatment of autoimmunity, further study of various populations and levels of immunological tolerance, as well as their disorders and shifts in autoimmune pathologies is necessary. The result of such studies may be the emergence of new immunotherapy techniques that can restore the system of immunological tolerance and are devoid of the disadvantages of immunosuppressive therapy.

CONCLUSION

Currently, new groups of regulatory T-cells, their influence on the processes of immunological tolerance, the immune response, and the role of these cells in pathological conditions are being investigated. However, at the moment there is no clear structuring of various subpopulations and their roles in the implementation of immunological control. In this review, attempts were made to theoretically system-

atize data on Treg subpopulations. Further study and systematization of various Treg groups may open up many new practical directions in the diagnosis and treatment of various diseases, especially autoimmune ones.

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Rehabilitation possibilities for children with cerebral palsy through the use of robotic devices and biofeedback

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ABSTRACT

This article overviews and systemizes published data on the ways of implementing different methods of biofeedback, robotic devices, and brain-computer interfaces (BCI) for rehabilitation of children with cerebral palsy (CP).

Aim. To survey implementation practices and clinical outcomes of rehabilitation technologies and possible neurophysiological mechanisms underlying their efficacy in patients with CP. We searched PubMed, Web of Science and eLIBRARY.ru databases for relevant publications using specified keywords.

Results. The analysis of relevant literature has shown that robotic technologies and BCIs with biofeedback based on electroencephalography and electromyography parameters are rapidly developing and implemented for the rehabilitation of children with CP. The first evidence of effectiveness for such methods and approaches has been found. However, there is a lack of fully developed conventional standards for the use of such rehabilitation methods and protocols in children. Control groups comprising of children with CP are often absent in such studies. In many cases, the variations of neurophysiological and neurochemical parameters before and after a course of rehabilitation are not evaluated. Having such data would help clarify physiological mechanisms underlying effective rehabilitation of motor functions and then design more adequate rehabilitation procedures and medication protocols.

Key words: children, cerebral palsy, biofeedback, robotic exoskeleton, brain-computer interfaces.

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Возможности реабилитации детей с синдромом ДЦП с применением роботизированных устройств и биологической обратной связи

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РЕЗЮМЕ

Обзор литературы посвящен систематизации имеющихся данных о применении методики биологической обратной связи, роботизированных устройств и интерфейсов «мозг – компьютер» в реабилитации детей с синдромом детского церебрального паралича (ДЦП).

Цель – изучить опыт применения, клиническую эффективность реабилитационных технологий у пациентов с ДЦП и возможные нейрофизиологические механизмы, лежащие в их основе. Поиск по ключевым словам (дети, ДЦП, биологическая обратная связь, роботизированные устройства, интерфейс «мозг – компьютер», экзоскелеты) был проведен с использованием баз научной литературы Pubmed, Web of Science, eLIBRARY.ru.

Результаты. Проведенный анализ данных литературы показывает, что в настоящее время в реабилитации детей с синдромом ДЦП активно развивается применение роботизированных устройств и интерфейсов «мозг – компьютер» с биологической обратной связью по параметрам электроэнцефалограммы и электромиограммы. Получены первые доказательства эффективности указанных методов и подходов. В то же время не полностью разработаны стандарты использования таких методов в реабилитационной практике и протоколы работы с детьми. Не всегда создавались контрольные группы из детей с ДЦП. Во многих исследованиях не оценивалась динамика нейрофизиологических и нейрохимических показателей до и после курса реабилитации. Такие данные позволили бы уточнить физиологические механизмы восстановления моторных функций и более корректно подходить к назначению реабилитационных процедур и медикаментозного лечения.

Ключевые слова: дети, ДЦП, биологическая обратная связь, роботизированные устройства, интерфейс «мозг – компьютер», экзоскелеты.

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

Источник финансирования. Обзор подготовлен в рамках выполнения темы «Разработка комплекса экзоскелета кисти с внешним программным управлением и биологической обратной связью для процедуры реабилитации детей с синдромом ДЦП» при финансовой поддержке Министерства науки и высшего образования Российской Федерации (RFMEFI60519X0186).

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INTRODUCTION

Cerebral palsy, among all diseases of the nervous system, is the main cause of disabilities in children [1, 2]. In the Russian Federation, the prevalence of reported cases of cerebral palsy is 2.2–3.3 cases per 1000 newborns [3].

Cerebral palsy is described in modern special literature as a group of non-progressive syndromes caused by impaired development or damage of brain motor centers in antenatal, intranatal or neonatal periods of individual development that vary widely in etiology, clinical manifestations, severity and prognosis [4–6]. Multiple reasons underpinning the emergence of this disorder have been discovered. There are several risk factors for pregnancy, childbirth and the perinatal period, which include premature birth, multiple pregnancy, prenatal development disorders, intrauterine infection, placental pathology, congenital malformations, asphyxia, perinatal infection, perinatal stroke, cervical spine injuries and many others [7].

Cerebral palsy is characterized by impairments in motor functions associated with abnormal development of statokinetic reflexes, muscle tone pathology, and paresis. Secondary changes in nerve and muscle fibers, joints, ligaments, and cartilage develop in the course of a patient's lifetime. Various cognitive and mental disorders are often present in the disease as well [8]. The severity of such disorders can vary from

mild emotional abnormalities to severe cognitive impairments. Movement disorders in cerebral palsy are often combined with mental retardation, epileptic seizures, and learning difficulties. Sometimes children have concomitant pathological changes in vision, hearing, sensitivity and various pathologies in internal organs, which exacerbate delays in psychomotor development [9].

Due to the variety of clinical manifestations of cerebral palsy in children, the widely used medical and physiotherapeutic methods of care can sometimes be seen as lacking efficiency. Many authors emphasize the importance of searching for novel recovery techniques and evaluating their efficiency [10]. Of particular relevance are various methods of rehabilitation based on the use of biofeedback (BFB), robotic devices, and brain-computer interfaces, because such methods allow enabling natural physiological resources in a child's brain.

BIOFEEDBACK BASED ON ELECTROMYOGRAM

The technique of electromyographic biofeedback (EMG-BFB), or functional biocontrol, has been used for rehabilitation of patients with cerebral palsy since the 1980s [11]. The method is built up on the principle of training active motor control based on visual and sound information about the produced movements in real time. Using this information, patients are able to deliberately adjust their movement patterns [12, 13]. In early studies, automated devices informing patients of an optimal level of muscle activation or relaxation with sound or color signals were implemented. The EMG in antagonist muscles (flexors and extensors in hand and fingers, shoulder biceps and triceps muscles, tibia and calf muscles, etc.) during movement in children with cerebral palsy was used as signals to control devices. In one of such works [11], an automated device was used in the treatment of 53 patients with spastic diplegia aged 8–14 years. Control group consisted of 15 patients with the same condition. A series of EMG-BFB training sessions (on average, 20 trials, 10–30 minutes long) significantly improved functional properties of affected muscles, their control and coordination. The same improvement was not seen in the control group.

A recent study conducted in the Republic of Korea [14] demonstrated the advantages of using the EMG-BFB technique when the feedback signal is given in a form of a visual image in a virtual reality environment. The study involved 18 children aged 7–15 with a spastic cerebral palsy and 8 healthy children of the same age, whose movement characteristics and EMG activity were used as control values. All children with cerebral palsy first underwent an EMG-BFB session (30 minutes long), with the EMG in biceps and triceps during elbow flexion and extension being represented in the form of a simple graph on a computer screen. In a week, the next EMG-BFB session with the same duration was conducted, with the feedback signal in the form of a video game depicting a character inflating a balloon. The balloon size depended on the EMG power in antagonist muscles when the arms moved. This type of biofeedback led to a significantly greater improvement in movement parameters and ability to achieve neuromuscular balance in the elbow joint, compared to the use of a simple EMG graph as feedback. The authors believe that implementing a video game as a feedback signal improved motivation and provided positive emotions for children, giving more efficiency to the processes of multisensory integration for planning and executing movement.

IMPLEMENTING ROBOTIC DEVICES IN TREATMENT OF GAIT DEVIATIONS

Recently, robotic devices have been widely used for motor function correction and to help patients with social adaptation. Rehabilitation with the use of these devices is based on motor learning [15]. Exoskeletons directly controlling limb joint movements are considered the most physiologically fit mechanism for motor disorders rehabilitation [16]. Such devices facilitate long workouts, improve movement patterns, increase motor activity and endurance. Although movement patterns are produced externally, without biological signals from the patient's body, limb movements provide a flow of reverse afferentation, which positively affects neocortex status.

Wearable leg exoskeletons with built-in electric motors controlled by signals from the child's body have been designed to correct gait deviations in cerebral palsy. They help in correcting crouch-gait in patients with cerebral palsy. Engines are started by means of mechanical sensors due to the child's limb movements. This type of exoskeleton was first tested on a six-year-old subject with spastic diplegia. The results of the study showed that the child's gait parameters improved. The use of exoskeleton entailed no adverse side effects, as the activity of muscles in knee extensors did not decrease [17]. The follow-up study involved six subjects aged 6–19 who had the same condition [18]. After six sessions of 2–4 hours each, half of the participants had improved knee extension parameters. Analysis of brain activity showed that the

pattern of electroencephalogram (EEG) changes in the child's neocortex during exoskeleton movements corresponded to that associated with self-initiated movement launch and execution processes. Therefore, it proved that the involvement of the cerebral cortex in organizing movement acts was not reduced. It should be noted that the results of these studies are still of pilot, preliminary nature, and are based on a small sample of children with cerebral palsy.

Recently, Locomat (Switzerland) stationary robotic devices have widely been used. They provide a system for bodyweight support, automated leg orthoses and a treadmill. A group of Italian researchers (University of Verona) used Lokomat in combination with traditional methods of therapy (20 sessions of robotic walking and 20 of physiotherapy, 60 minutes each) in treatment of a small group of children composed of 16 boys and girls 4–18 years old [19]. The treatment resulted in an increase in endurance in a six-minute walking test, but the Gross Motor Function Measure (GMFM) and the modified Ashworth scale did not show a significant improvement in children's condition.

The most representative retrospective study, with the largest sample of patients, was carried out by a group of Italian authors from the IRCCS "E. Medea" Institute (Lecco) and Bambino Gesù Children's Hospital (Rome) [20]. They analyzed the effects of the Locomat-based robotic rehabilitation carried out in 2012–2017, in 72 children with cerebral palsy and 110 with brain injuries acquired postnatally which caused motor disorders (patients aged 4–18). For a month-long period, the children underwent 20 sessions of robotic walking and 20 sessions of physiotherapy lasting 45 minutes each. Assessment of motor functions with the six-minute walk test showed a significant improvement in both groups of children. However, the GMFM test revealed a statistically significant, compared to their initial state, improvement only in a group of children with acquired brain damage.

The most recent version of Lokomat, which displayed motion parameters in a virtual reality environment, was used in a study conducted by researchers from the University of Munich [21]. This allowed increasing the degree of children's involvement in controlling their walk. For 24 months, 20 children with cerebral palsy (mean age being 5.9 years) underwent three stages of treatment including 12 sessions (30–60 minutes duration) of robotic walking each. All types of traditional therapy assigned to them were still preserved. The Gross Motor Function Classification System (GMFCS) test showed a significant improvement in motor functions both after each block and after the entire course of treatment.

It is noteworthy that in each of the studies mentioned above, the use of robotic devices was carried out in combination with other traditional methods of treatment and physiotherapy, in particular. Control groups of children with cerebral palsy were not used. A randomized crossover study is suggested as an evaluation method to measure robotic walking techniques' actual efficiency [22]. The effects of conventional therapy and the use of robotic devices are to be compared, after being studied separately. However, to our knowledge, such studies are yet to be conducted.

IMPLEMENTING ROBOTIC DEVICES IN TREATMENT OF HAND MOVEMENT DEVIATIONS

To help correct deviations in hand motor functions in children with cerebral palsy, a number of robotic devices have been developed. The most popular are the following: InMotion 2 (a commercial version is named MIT MANUS), NJIT-RAVR, and CosmoBot [23].

Implementation of the InMotion 2 system (Fig. 1) helps children with cerebral palsy increase the accuracy of movements in achieving goals with an orthotic robotic arm. Children learn the training technique of the "arm reaches the object" movement in certain directions with a given level of support from the device [24–26]. Several studies [27–29] showed a positive change after a 6-8 week course of using the InMotion 2 system in children with cerebral palsy. A decrease in muscle tone and an improvement of the following kinematic parameters were found: an increase in speed and an improvement in the smoothness of arm movements.

The NJIT-RAVR system combines a robotic arm with virtual reality games to train movements in children with hemiplegia. Similar to InMotion2, the NJIT-RAVR system can both assist and resist movements produced by children. For example, among other virtual reality games, the "Get the mug" game was used in one of the studies [30]. In this game, a three-dimensional room is displayed on the screen with specially designed shelves and a table (Fig. 2). The goal of the game is to perform movements to place mugs on shelves. The study involved four children with cerebral palsy and four healthy children. After a three-week rehabilitation course, one-hour-long sessions

three times a week, positive dynamics were found for a duration of time needed to achieve the goal, movement accuracy rate and movement trajectory [30].

Fig. 1. InMotion 2 system (MIT-MANUS commercial version) [26]

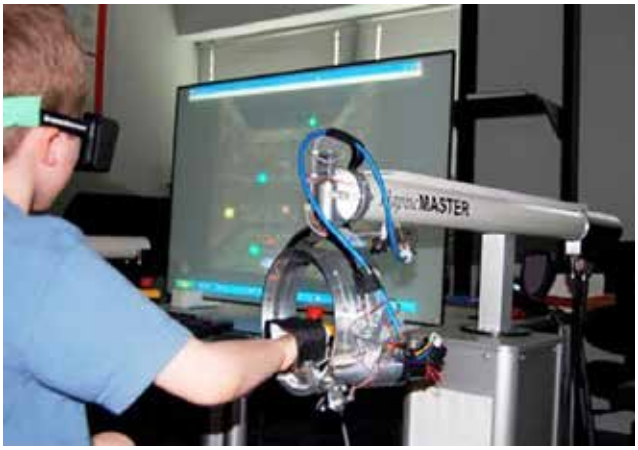

Fig. 2. NJIT-RAVR system [30]

One more version of a robotic rehabilitation system is the CosmoBot system (Fig. 3), developed by AnthroTronix (USA). This remote-control system serves to provide automatic visual and auditory feedback to patients when they try to solve a set of motor tasks. The system evaluates changes in the angle of movement (supination and pronation) in relation to the neutral primary position, which is adjusted individually for each patient.

A study with the CosmoBot system involved six children aged 5–18 with varying degrees of spastic quadriplegia and hemiplegia. Children underwent physiotherapy and robotic rehabilitation (crossover design). The CosmoBot-based rehabilitation was carried out for 20 minutes twice a week for five weeks. After the robotic therapy in children, their movement performance indicators improved to a greater extent in comparison to the traditional treatment [31].

a

b

Fig. 3. CosmoBot feedback system: *a* – graphical user interface allows therapists to set child's motion thresholds required to enable robot's motion; *b* – a child equipped with reflective markers for simultaneous measurement of movements for right shoulder, elbow, forearm and wrist during the "pull up" task [31]

Although positive effects have been identified for each of the mentioned robotic devices, it is not possible to compare them directly in terms of their efficacy. Experimental protocols in each study were different both in session and the entire course duration.

Besides, while some studies were conducted by implementing the InMotion 2 [27–29, 32] or NJIT-RAVR systems with virtual reality games [30, 33, 34], the others combined the InMotion 2 robotic therapy with injections of botulinum toxin type A (BTX-A) [35]. In most studies, children had varied diagnoses, and the sample sizes were extremely small (up to 10 people). These facts impose significant limitations on the possibility of comparing the results of the published studies.

BRAIN-COMPUTER INTERFACES AND THEIR IMPLEMENTATION IN REHABILITATION OF MOTOR FUNCTIONS

The efficiency of rehabilitation procedures, as noted by A.A. Frolov et al. [16], depends on the degree to which they are able to trigger mechanisms of brain plasticity for rebuilding its sensorimotor system. Exoskeleton movements must occur exactly at times when the brain is most susceptible to receiving peripheral signals, or, more precisely, when the patient is trying to make a movement. This approach still cannot be used in completely paralyzed patients or when a normal muscle co-activation process is impaired, which is present in many patients with cerebral palsy. In some studies dedicated to rehabilitation of stroke patients [36–38], brain-computer interfaces (BCIs) based on kinesthetic movement imagery were used to identify patients' intentions.

BCI is a combined hardware and software technology that allows for the control of external technical systems using signals registered in one's brain. A general BCI set includes a system for recording biopotentials and sending them to a computer, tools for filtering signals and selecting activity parameters most indicative of identifying human intentions, and an activity classifier and a tool for its pairing with an external technical device, which may be a prosthesis, exoskeleton or monitor screen [38]. When controlling the BCI, subjects receive feedback from the technical device, allowing them to compare their action with their intention. This approach ensures that the subject is focused on controlling the BCI and reinforces the successful completion of the task. Visual information is typically used as a feedback signal. In case the BCI is designed to control an exoskeleton, proprioceptive afferentation is used as well.

BCIs are classified according to the necessity of surgery procedures to record brain signals (invasive vs non-invasive IMC). Electrocorticogram or neural activity is used as signals of electrophysiological activity for invasive BCIs. For non-invasive BCIs, EEG and magnetoencephalogram are used. According to pattern types, non-invasive BCIs are divided into synchronous and asynchronous [38]. Synchronous BCIs are based on the analysis of EEG activity patterns in response to external stimuli; asynchronous BCIs are based on the analysis of EEG patterns that occur voluntarily following the emergence of subjects' intentions. Most BCIs that control movements of an external technical device are based on fulfilling the task of mental imagery in response to an external command. The practice of controlling such a BCI is an effective procedure promoting the rehabilitation of motor functions in post-stroke and post-traumatic patients [15].

Many present-day BCIs that are parts of systems designed for rehabilitation of motor functions are based on the analysis of the EEG sensorimotor rhythm patterns. This rhythm includes alpha and beta components [39]. The alpha component (10–12 Hz in adults), or the mu-alpha rhythm, is thought to reflect the level of activation of the postcentral somatosensory cortex, while the mu-beta component (peak frequency of about 20 Hz) is indicative of the precentral motor cortex activity. The response of the EEG mu rhythm in the form of desynchronization is considered to be an indicator of activation of corresponding zones of the cerebral cortex. Such a reaction manifests itself when the subject performs movements, imagines them, observes movements performed by others, and hears sounds characteristic of certain movements. The mu rhythm desynchronization starts about 1.5–2 seconds before the start of the movement. The individual frequency of mu rhythm depends on the age of subjects. During the first year of a child's life, the peak frequency of this rhythm increases from 3 to 8 Hz. In subsequent years, the increase in frequency gradually slows down and stabilizes in around 10 Hz by adulthood [40].

The mu-alpha rhythm is considered to have at least two components. The low-frequency component (8–10 Hz) is associated with "non-specific" desynchronization occurring in various motor tasks. The 10–12 Hz desynchronization of the mu-alpha rhythm is focused and specifically localized; it can be clearly identified in the movements of one's fingers and feet [41]. Since the representations of various organs (for example, arms and legs, right and left body parts) are distributed in the cortex over relatively large spans, it appears possible, by means of localizing this component of the mu rhythm, to quite accurately find the organ, the movement of which is being imagined by the subject [38]. The classifier of brain activity, in this case, activates an external robotic device or starts the exoskeleton movement. It has been demonstrated that intentions to make a movement in a stroke patient can be effectively associated with real movements made by an exoskeleton [42].

There are two possible types of BCIs clinical use: neurorehabilitation and social rehabilitation. Neurorehabilitation implies an improvement of motor functions as a result of BCI training, while social reha-

bilitation helps patients effectively adapt to real life, improving their self-care in everyday life and communication with other people [16, 43].

Despite some authors [44, 45] foreseeing excellent prospects for BCIs in the rehabilitation of children with cerebral palsy, there are only a few experimental works in this area. One of the first research works was a study showing the BCI potential use for patients with cerebral palsy based on the analysis of sensorimotor rhythm patterns and visual EEG-evoked potentials triggered by external stimuli [46]. The mu and beta rhythm modulations, associated with the task of imagining the process of executing certain movements with the subject's upper and lower limbs, were properly estimated by the classifier program.

Researchers from South Korea [47] used the BCI integrated with an electric stimulator of wrist extensor muscles. Electrical stimulation started based on an online analysis of EEG parameters: an increase in beta to theta rhythm powers ratio in frontal leads (an increase of the so-called attention index) when the patient imagined his hand being extended. A series of sessions for children with cerebral palsy resulted in improved parameters of children's hand movements, as well as an enhanced capacity to focus attention. This study proves that the technique of BCI-controlled electrostimulation can be effectively used in the neurorehabilitation of patients with cerebral palsy.

In the context of social rehabilitation, there is evidence of successful use of the BCI, controlled by the P300 potential parameters, when performing cognitive tasks on a computer by children with severe forms of cerebral palsy with both motor and speech disorders [48]. Similar results were collected when using a hybrid BCI system controlled simultaneously by the P300 event-related potential and EMG parameters in subjects with severe motor disorders. This system was developed to control a program designed to assist in writing words [49]. The authors of the work demonstrated that the use of the two-signal processing algorithm improves the accuracy of writing words and reduces the number of errors.

It has already been noted that the use of exoskeletons controlled by BCIs is considered to be the most optimal method for neurorehabilitation [38]. In this way, a central motor command is seconded by afferent kinesthetic signals related to its execution by an exoskeleton, thus, it is complemented by biofeedback. However, to our knowledge, an analogous system was used to help correct motor functions of upper limbs in children with cerebral palsy in only one clinical study so far, which was performed by researchers of the V.I. Vernadsky Crimean Federal University [50]. In this work, the Exokist 2 set was used together with a non-invasive BCI (Fig. 4) collecting data from the EEG in frontal, central, and parietal cortex areas. This set was manufactured by a consortium uniting the Android Technics SPA, N.I. Pirogov Russian National Research Medical University and the Institute of Higher Nervous Activity and Neurophysiology of RAS.

Fig. 4. Rehabilitation session with the use of Exohand-2 set.

The study involved 50 boys and girls with cerebral palsy (30 subjects in the main group and 20 in the control group) who had a level of motor activity not higher than III according to the Gross Motor Function Classification System (GMFCS). All patients underwent a standard course of health resort rehabilitation for 21 days. The patients of the main group were additionally rehabilitated with the help of the Exokist-2 complex paired with a non-invasive BCI. The results of the complex use showed that 70% of the main group patients had a significant decrease in spasticity according to the Ashworth (MAS) and Tardieu (MTS) Scales. In half of the patients, the paretic arm muscle strength significantly increased according to the Medical Research Council Scale for Muscle Strength (MRC-SS). The Modified Franchay Scale (MFS) showed an improvement in the manipulative capabilities of the hand. According to the ABILHAND-Kids Scale, a positive change in patients' ability to perform everyday activities appeared to be the most prominent. Changes in motor functions in the control group of patients with standard therapy were not statistically significant. It should be noted that modulations of the EEG and EMG parameters in children who used the Exokist-2 complex have not been analyzed in this publication.

CONCLUSION

The analysis of available publications shows that there is currently an increased interest in the use of robotic devices in the rehabilitation of children with cerebral palsy. The possibility to control robotic devices based on the analysis of patients' brain activity has been demonstrated. There is evidence of the efficacy of various methods and approaches based on the biofeedback method. On the other hand, standards for the use of such methods in rehabilitation practice and protocols for working with children are yet to be developed. Many studies do not provide data on modulations of neurophysiological indicators (EEG, EMG) and neurochemical parameters before and after a rehabilitation course with the implementation of robotic devices. Such data would help analyze physiological mechanisms underlying rehabilitation of motor functions and approach the assignment of rehabilitation procedures and medical treatment more accurately.

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Authors contribution

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Chronic endometritis and reproductive disorders: versions and contraversions (review)

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ABSTRACT

Among married couples of childbearing age, the frequency of infertility in different regions of Russia and the world ranges from 10 to 21%. The effectiveness of the results of in vitro fertilization (IVF) and embryo transfer is determined by two factors: the functional completeness of the embryo at the blastocyst stage and the absence of intrauterine pathology. One of the main causes of imperfect or unsuccessful implantation is an impaired function and damaged endometrial structure, which is often caused by a chronic inflammatory process in the endometrium.

Chronic endometritis (CE) is a condition associated with a violation of the coexistence between microorganisms and the immune system of a macroorganism in the endometrium. A majority of CE cases produce no noticeable clinical signs or mild symptoms and the CE prevalence rate is approximately 10% based on the histological findings of an endometrial biopsy.

The interconnection between CE and reproductive dysfunctions, such as implant damage and repeated miscarriage, has been studied by many researchers at the present stage. Chronic endometritis is common among patients with unexplained infertility. Diagnosis and treatment of chronic endometritis increase the frequency of spontaneous pregnancies and live births in such patients. The diagnosis of chronic endometritis is not simple, often contradictory, and, thus, requires close cooperation between the fertility specialist and the pathologist. In this study, we reviewed the literature on the pathophysiology of chronic endometritis and how it may be associated with infertility, as well as the literature regarding the diagnosis and treatment of CE, published at PubMed as on May 2019 in a version and contra-version format.

Key words: ART, chronic endometritis, endometrium, female infertility, hysteroscopy, IVF, repeated implantation failure, recurrent pregnancy loss.

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Хронический эндометрит и репродуктивные нарушения: версии и контраверсии

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РЕЗЮМЕ

Частота бесплодия в разных регионах России и в мире среди супружеских пар репродуктивного возраста колеблется от 10 до 21%. Существует два основных фактора, определяющих эффективность результатов экстракорпорального оплодотворения (ЭКО) и переноса эмбрионов: функциональная полноценность эмбриона на стадии бластоцисты и отсутствие внутриматочной патологии. Одной из главных причин неполноценной или неудачной имплантации являются нарушенная функция и поврежденная структура эндометрия, часто обусловленная хроническим воспалительным процессом в эндометрии. Хронический эндометрит (ХЭ) – это состояние, связанное с нарушением сосуществования между микроорганизмами и иммунной системой макроорганизма в эндометрии. В большинстве случаев ХЭ не имеет заметных клинических признаков, а его распространенность на основании гистологического заключения биопсии эндометрия составляет около 10%. Связь между ХЭ и репродуктивными нарушениями, такими как имплантационная недостаточность и повторный выкидыш, стала предметом пристального внимания многих современных исследований. Обращает на себя внимание распространенность хронического эндометрита у пациенток с необъяснимым бесплодием, а диагностика и лечение хронического эндометрита повышают частоту спонтанных беременностей и живорождений у таких пациенток. Диагноз хронического эндометрита является не простым, а зачастую противоречивым и требует тесного сотрудничества специалистов – репродуктологов и патоморфологов.

В этом обзоре мы рассмотрели литературу по вопросам патофизиологии ХЭ, возможных причин, ассоциированных с бесплодием, а также привели результаты научных исследований, касающихся диагностики и лечения ХЭ, которые были опубликованы в коллекции PubMed по состоянию на май 2019 г. в формате изложения версий и контраверсий.

Ключевые слова: хронический эндометрит, эндометрий, оплодотворение, ВРТ, ЭКО, гистероскопия, повторная имплантационная недостаточность, периодическая потеря беременности.

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

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INTRODUCTION

Among married couples of childbearing age, the frequency of infertility in different regions of Russia and the world ranges from 10 to 21% [1–3]. The effectiveness of in vitro fertilization (IVF) and embryo transfer (ET) is determined by two factors: the functional completeness of the embryo at the blastocyst stage and the absence of intrauterine pathology. One of the decisive and main reasons for imperfect or unsuccessful implantation is an impaired function and damaged endometrial structure, often caused by a chronic inflammatory process in the endometrium. Chronic endometritis is a clinico-morphological syndrome, in which multiple secondary morphofunctional changes, disrupting cyclic biotransformation and uterine mucosa receptivity, occur as a result of persisting endometrial damage by an infectious factor [1].

NOSOLOGICAL FORM OF CHRONIC ENDOMETRITIS AND ITS PREVALENCE

The question of the necessity of distinguishing CE in a separate nosological definition has been discussed at length in the scientific society; the reason for this discussion is the existence of two divergent views on the importance of the infectious factor in CE genesis. Some researchers adhered to the point of view that the uterine cavity is sterile and in cases of penetration of conditionally pathogenic microflora (CPM) into the endometrium, a pathological process occurs in about 60% of cases [4]. Other researchers [5] argued that the uterine mucosa cannot be sterile due to permanent microbial colonization from the lower genital tract.

Currently, CE is identified in the International Classification of Diseases and Causes of Death (CDC) of the 10th revision: Class XIV (N071.1.) as an independent nosological unit [1].

The prevalence of CE varies from 10 to 85%, due to the difficulties of clinical and morphological verification of the disease [6, 7]. According to various sources, among women with infertility, the number of CE cases is on average 10% (from 7.8 to 15.4%). 80–90% of CE is detected in women of reproductive age. It eventually causes menstrual and reproductive function disorders, leading to the development of infertility, failures in IVF programs and embryo transfer, miscarriage and complications in the course of the gestational process and childbirth [6, 7]. Infertility is diagnosed in 60% of women with CE (in primary 22.1%, in secondary 36.5%), unsuccessful attempts of IVF and embryo transfer were noted in 40% of women with CE. Some unsuccessful IVF attempts reach 80% in women with CE in the medical history, and the average number of failures in assisted reproductive technologies (ART) programs is approximately 3 per woman. According to other studies [8], CE is the only diagnosed cause of miscarriage in 47–52% of cases.

Foreign studies declare a CE prevalence rate of 10–11% based on the histological conclusion of an endometrial biopsy of patients who underwent hysterectomy due to benign gynecological conditions [9]. CE was diagnosed in 15% of infertile women who underwent IVF cycles, and the prevalence of CE was 42% in women with recurrent implantation failure (RIF) [10]. The prevalence of CE was 14% and 27% in the group of recurrent pregnancy loss (RPL) and women with RIF in a modern prospective study [11]. Thus, CE should be considered as a gynecological disease that cannot be ignored in the context of infertility treatment and assisted reproductive technologies.

Another point of view of J.C. Kasius et al. [12] is that the clinical signs of CE are minimal as they diagnosed this condition in only 2.8% of asymptomatic infertile women without abnormalities during transvaginal ultrasound examination (TUE). The opinion of these authors is that chronic endometritis does not adversely affect the reproductive outcome during normal cycles of in vitro fertilization or intracytoplasmic sperm injection (ICSI). At the same time, they emphasize that low detection and unknown clinical significance of chronic endometritis require further research [12].

Nevertheless, many researchers believe that CE usually occurs without clinical manifestations or has non-specific clinical signs, such as abnormal uterine bleeding, chronic pelvic pain and leukorrhea [13]. Chronic endometritis is associated with RPL, defined as three or more pregnancy losses before 20 weeks of pregnancy, which occurs in 3% of all couples [14].

It was observed that women with repeated abortions have chronic endometritis (68.3%) and women who received adequate antibiotic treatment had a significantly higher frequency of successful pregnancies compared to women who were not treated. The authors report that in this population the most common infectious agents were disruptive pathogenic bacteria and mycoplasmas [14]. Other symptoms that are associated with chronic endometritis were noted [13]. One of them is abnormal uterine bleeding (such symptoms may appear as intermenstrual discharge or metrorrhagia), however, to date, the relationship between abnormal uterine bleeding and chronic endometritis is not completely clear. Another symptom is dysmenorrhea. The current hypothesis defines prostaglandins, released through the endometrial cell membranes damaged by the inflammatory process, as the main cause of dysmenorrhea. Symptoms also include dyspareunia (pain during intercourse), genitourinary symptoms and leukorrhea. Sometimes there is unpleasant, purulent vaginal discharge with an increased frequency of urination and/or symptoms similar to the symptoms of cystitis, as well as concomitant irritation of the bladder. Fever usually occurs in the acute phase and

in some cases mild fever can be noted in the chronic form of CE.

PATHOGENETIC ASPECTS OF CHRONIC ENDOMETRITIS

The traditional idea that the uterine cavity is sterile underwent reanalysis when microorganisms were detected in the uterine cavity of non-pregnant women [15]. Ascending from the lower parts of the genital tract, microorganisms can colonize the uterine cavity, in which case the host's protective mechanisms must limit both the invasion and reproduction of bacteria [15, 16]. Protective factors include the cervical mucosa [17, 18], endometrial epithelium and its immune cellular components (neutrophils, macrophages and natural killer cells), as well as elements of the innate immune system, including the natural antimicrobial peptides present in the endometrium [18]. In 95% of cases, CE is primary, developing in the endometrium as a result of the introduction of sexually transmitted microorganisms, either exogenous strains or the multiplication of CPM in the endometrium after intrauterine treatment and diagnostic procedures. Only 5% of endometritis is secondary, developing when infection enters the endometrium from extragenital foci by hematogenic, lymphogenic or descending pathways [19, 20].

The study of the microbial landscape of the endometrium has a relatively short history. For the first time, the persistence of mycoplasma in the endometrium is mentioned in the work of Z. Koren in 1978 [21]. The persistence of more than 20 types of microorganisms of the opportunistic group was found in the endometrium. A total of 129 strains was identified, including obligate anaerobes – 64% (bacteroids, eubacteria, peptostreptococci, clostridia), microaerophiles – 31.8% (genital mycoplasmas and diphteroids), and facultative anaerobes – 12%. Monocultures were identified in only 14% of women; in the rest, associations of 2–6 types of microorganisms were found.

The vaginal microbiome is characterized by a deficiency of lactobacilli (52.5%), a low concentration of lactobacilli (44.3%), a high frequency of excretion of enterococci (59%), coagulase-negative staphylococci (57.4%) and multicomponent associations (67.2%) in women with chronic endometritis. The microbiota of the intestinal biotope was characterized by a high frequency of detection of enterococci (62.2 %) and dysbiosis of the 1st and 2nd degree (89.2%); the microbiota of the nasopharyngeal biotope was characterized by a deficit of indigenous flora and a high frequency of S. pneumoniae (25.5%) [22, 23].

It has been hypothesized that in women with chronic endometritis, normobiota representatives have a symbiotic relationship with CPM in all open biotopes, which confirms the decrease in normobiotic colonization resistance and its inability to suppress pathogenic biota [22, 23].

At the present stage, it is known that microorganisms form biofilms (dense shells of polymer compounds) to counteract the immunological mechanisms of the host; the effect of biofilms counteracting natural and synthetic antibiotics is known [24]. It was found and proved that chronic infections, such as valvular endocarditis, otitis media, chronic bacterial prostatitis and periodontitis, are associated with the presence of bacterial biofilms that contribute to subclinical colonization of the uterine cavity [25, 26].

One of the main and complex problems in the treatment of infertility, miscarriage and premature birth is the persistent effect of the microbial factor on the quality of the endometrium [6, 27]. Microorganisms are only a part of the problem; septic conditions are also associated with problems of inflammation control and/or anti-inflammatory response of the host [28]. There are studies of the mechanisms of preterm delivery, which showed that microorganisms on the surface of the endometrium do not cause a pro-inflammatory reaction and support peaceful coexistence with the host during normal pregnancy [24, 29, 30]. However, if the host organism (in this context, the mother, embryo/fetus or both) "learned" about microbial carriage through recognition receptors and initiated a pro-inflammatory response, peaceful coexistence will no longer be possible. Changes in virulence patterns, such as planktonic bacteria released from biofilms, have also caused a change in the balance between microorganisms and the host, leading to preterm delivery caused by inflammation.

The result of successful implantation and prolongation of pregnancy is a delicate balancing mechanism between the embryo and endometrium, which is expressed in the predominance of the TH2 profile compared to the profile of TH1 cytokines in the endometrium. Thus, all the reasons that upset this balance can affect endometrial susceptibility.

It was found that immunocompetent cells in the endometrium secrete chemokines, attracting natural killers and macrophages from the circulating peripheral blood into the endometrium [31, 32]. In turn, trophoblasts organize the production of pro-inflammatory cytokines from monocytes and macrophages, which are also of great importance in implantation and formation of the placenta [32].

An increase in the number of NK cells in the peripheral blood, leading to an increase in TH1 cytokines, has a negative effect on the invasion and implantation of trophoblast, which increases the likelihood of premature pregnancy loss [33].

A study of 438 cases of hysteroscopically diagnosed CE confirms the theory that the infectious factor is crucial in the pathogenesis of CE [34]. The researchers reported that 73.1% of women with CE showed ≥1 positive detection of pathogens. The structure of endometrial infections was as follows: 58% of common bacterial infections, including gram-negative bacteria, 10% of Ureaplasma urealyticum, and 2.7% of Chlamydia trachomatis. It has been established that gram-negative bacterial colonization of the endometrium can reduce the rate of implantation of embryos, leading to an increase in the frequency of miscarriages. Gram-negative bacteria endotoxins elicit a predominant TH1 response to decidual tissue to stimulate the production of pro-inflammatory cytokines. Thus, a paracrine medium is formed, which can cause damage to the embryo, impaired implantation, or spontaneous miscarriage [35].

Thus, chronic inflammation of the inner lining of the uterus disrupts the production of endometrial cytokines and, accordingly, endometrial function [36], leading to the formation of pathological lymphocytes and a change in the secretion of paracrine factors. As a result of this, the susceptibility of embryos to the endometrium decreases [33] and, in fact, in cases of CE, delayed differentiation of the endometrium in the middle secretory phase was observed [37]. Endometrial proliferation was detected in the secretory phase due to an increase in estrogen receptors and nuclear expression of the Ki-67 marker in patients with CE [38].

E. Cicinelli et al. [39] demonstrated that CE was a condition often associated with RIF (66.0%), which was 2 times greater than 30.3% in a study by E.B. Johnston-MacAnanny et al. [40]. The most common infectious agents were common bacteria and mycoplasmas in this population-based study. In addition, antibiotic treatment was associated with the normalization of the endometrial pattern on hysteroscopy and a significant improvement in the reproductive results of IVF.

DIAGNOSIS OF CHRONIC ENDOMETRITIS

The generally accepted histological diagnostic criterion for CE is the presence of plasma cells in the stroma of the endometrium during endometrial biopsy [9, 12]. However, the frequency of an erroneous diagnosis may be higher than ideal [12, 13]. The accuracy of morphological conclusions can be called into question due to the following conditions: proliferation of stromal cells, infiltration of mononuclear inflammatory cells, plasmacytoid cells of the stroma, or a pronounced pre-relapse reaction in the late secretory endometrium. In addition, histological examination is a very time-consuming and invasive method.

The method of staining with hematoxylin and eosin (H & E) had a low level of CE verification (<10%) in women with infertility and repeated spontaneous miscarriages in the medical history [41]. Immunohistochemical (IHC) studies can detect specific antigens for CD38 and CD138 plasma cells inside the endometrium [42]. IHC showed a significantly higher sensitivity for the diagnosis of CE (56% versus 13% for H & E strain) [43]. Plasma endometritis showed no correlation with bacterial colonization of the endometrium or the clinical picture of pelvic inflammatory diseases [44]. Plasma endometritis was histologically diagnosed in 39% of women who underwent endometrial biopsy, but 82% of women had positive results in microbial cultures of CE biopsy samples. Haggerty et al. [45] reported that histological endometritis revealed no association between reproductive diseases and antibiotic treatment in randomized controlled trials (RCTs). These experts believe that the histological diagnosis of CE cannot determine which patients can benefit from further antibiotic treatment and which cannot do this in terms of fertility outcomes.

An effective diagnostic method for the verification of CE is liquid hysteroscopy [46, 47]. Signs of CE in liquid hysteroscopy are endometrial polyps, stromal edema, focal or diffuse hyperemia. The diagnostic value of liquid hysteroscopy concerning CE is manifested in the great sensitivity of the method for diagnosing CE, in comparison with the microbio-

logical culture method for studying the endometrium [48]. Studies comparing the accuracy of the histological diagnosis of CE and fluid hysteroscopy showed a very high diagnostic accuracy of the latter (93.4%) [46, 47, 49]. Apparently, the discrepancies between hysteroscopic observations and histological studies can be associated with many limitations characteristic of the CE histological diagnosis [50].

Modern research studies the changes in the qualitative and quantitative composition of the vaginal and other biotopes of the body's open cavities and the presence of pathogenicity genes in microorganisms, as well as clarification of their relationship with inflammatory markers and factors contributing to the chronic process in the uterus. Researchers note an increased level of pathogenicity in the dominant E. faecalis and E. faecium species in different biotopes (vaginal, intestinal and nasopharyngeal) in women with CE, which is manifested in an increase in the number of enterococcal autostrains with nucleotide sequences of the pathogenicity gene synthesizing serine proteinase (sprE) (penetration, colonization, tissue damage). The presence of morphological signs of CE (inflammatory infiltrates, endometrial stromal fibrosis, sclerotic changes in the walls of arteries and plasma cells) is associated with the presence of genaserin proteinase (sprE) in the prevailing enterococci in the vaginal, intestinal, and nasopharyngeal biotopes [22, 23, 51].

TREATMENT OF A CHRONIC ENDOMETRITIS

All the studies of foreign colleagues that we have discovered come down to the fact that chronic endometritis therapy is based on the use of broad-spectrum antibiotics [52, 53]. Typically, the drug of choice is doxycycline in doses of 100 mg every 12 hours for 14 days or, alternatively, the introduction of cephalosporins, macrolides or quinolones is possible. It is also preferable for the partner to undergo the same antibiotic treatment. In case of failure of antibiotic therapy and/or preservation of endometritis at the same, an endometrial culture with a relative antibiogram should be considered and appropriate antibiotic treatment should be prescribed.

The Centers for Disease Control (CDC) recommend the following treatment options [39]: with a positive result for gram-negative bacteria: ciprofloxacin 500 mg 2 times a day 10 days as first-line therapy; for gram-positive bacteria: amoxicillin + clavulanate 1g two times a day for 8 days; infections of Mycoplasma and U. Urealyticum: Josamycin 1 g two times a day for 12 days; in case of persistence: minocycline 100 mg two times a day for 12 days; gram-negative cultures: ceftriaxone 250 mg IM in a single dose plus doxycycline 100 mg orally two times a day for 14 days with metronidazole 500 mg orally two times a day for 14 days.

If the signs of chronic endometritis remain in the following hysteroscopy, the protocol can be repeated up to three times. If there is confirmed tuberculosis endometritis, the patient should be given special antibacterial therapy for tuberculosis (isoniazide, ethambutol, rifampicin and pyrazinamide for 2 months, then isoniazid and rifampicin for another 4 months) [54, 55].

Cicinelli et al. [14] reported in their study of patients with unexplained recurrent implantation failure and chronic endometritis diagnosed by hysteroscopy that the incidence of clinical pregnancy in the group where hysteroscopic values were normalized 1 year after antibiotic treatment was significantly higher than in the comparison group: 74.8% (88 out of 118) versus 24.4% (22 out of 90). A study by McQueen et al. [41] showed that in patients with RPL and chronic endometritis, the frequency of live births per pregnancy increased significantly to 56% after antibiotic treatment compared with 7% before treatment.

According to a retrospective study by R. Yang et al. [56], in patients with RIN and CE undergoing in vitro fertilization cycles – embryo transfer (IVF-Cryo transfer) the implantation success rate increased up to 18.6% (18 out of 97) versus 4.9% (3 out of 61]) and the pregnancy rate 29.3% (12 out of 41) versus 7.4% (2/27). IVF cycles increased significantly after antibiotics treatment compared to the results before treatment. Cicinelli et al. [56] conducted a retrospective study of patients with RIN who underwent new IVF cycles with Cryo-transfer. They found that the frequency of clinical pregnancies and the rate of live births in the group with normal hysteroscopic indices after antibiotic treatment was significantly higher than in the group with sequential results of CE 65 % versus 33% and 60.8% versus 13.3%, respectively. The above values indicate that CE has an important role in infertility.

Diagnostic hysteroscopy is actively discussed in the treatment of chronic endometritis. Many studies have suggested that endometrial damage associated

with diagnostic hysteroscopy can increase the frequency of implantation and the frequency of clinical pregnancies in women with previous failed IVF-Cryo transfer attempts [57–59]. The hypothetical biological basis for this assumption is as follows [60]: first, local trauma in the endometrium can increase the implantation speed, which will lead to decay; secondly, cytokines and growth hormone released during the restoration after artificial damage to the endometrium, may have a beneficial effect on the implantation of embryos. Third, artificial endometrial damage may delay earlier endometrial maturation associated with hyperstimulated ovaries in the next IVF-Cryo transfer cycle.

Meta-analyzes [61, 62] regarding artificial endometrial injuries during ART or hysteroscopy reported a significant improvement in the clinical pregnancy rate. However, this possibility has not yet been confirmed by well-developed RCTs, so this assumption should remain hypothetical.

The analysis of Russian literature and studies on the principles and methods of treatment of chronic endometritis is characterized by the presence of the second stage of treatment (after antibiotic therapy) and the variety of methods used. A large number of authors point to the high effectiveness of physiotherapy: they use electro-pulse therapy, interference currents, infrared laser irradiation, magnetotherapy, and hirudotherapy. Physiotherapeutic methods help stimulate receptor function, improve the pelvic hemodynamics, accelerate endometrial regeneration processes, and increase the immune status [63]. An advisability of hormone therapy is under discussion. Some authors consider hormone therapy ineffective in this pathology, except in cases of patients having ovarian hypofiction or anovulation [64]. Others claim that in case of chronic inflammation, in the presence of pathological tissue regeneration, hormone replacement therapy has a positive anti-inflammatory effect [6, 65]. Correction of immune status occupies a special place in the treatment of chronic endometritis in the domestic literature. In the case of infection persistence in the body, the use of inducers of interferongenesis is important. Based on the data of studying the immune and interferon status of patients with chronic endometritis, the correction of immune disorders is carried out using a number of drugs: glavit, immunomax, and polyoxidonium [65, 66]. Sessions of intrauterine ozone laser therapy and intrauterine endometrial laser therapy using the He-Ne laser were effective in women with infertility and miscarriage [66, 67]. There are observational studies of bacteriophage therapy, which had a good therapeutic effect in 78.3–93.6% of cases; there is evidence of intrauterine irrigation with a liquid bacteriophage at a dose of 20 ml daily for 5 days with a pronounced clinical effect [68]. The therapeutic effect of bacteriophages is associated with lytic activity, immunomodulating the antigenic property of components of destroyed microbial cells located in the phage lysates, especially with repeated administration of the drug [69]. Several observational studies indicate the effectiveness of metabolic therapy, including the use of riboxin, wonbenzyme, vitamin therapy, glutamic acid, systemic enzyme therapy and actovegin [65]. Some researchers consider taking probiotics the second stage after antibiotic treatment, explaining that the risk of developing gastrointestinal disorders caused by antibiotic therapy is reduced and the restoration of intestinal microbiocenosis and other disturbed physiological processes in the body is initiated [69]. However, most authors agree on the opinion that the use of bacteriophage therapy does not mean a complete rejection of antibiotics, however, it will assist their strict prescription according to indications [65, 66]. Monitoring the effectiveness of complex therapeutic measures is recommended not earlier than 2 months after the end of the entire course of treatment, taking into account physiotherapy. At the same time, the dynamics of clinical symptoms, echographic and dopplerometric indicators, the elimination of microbial agents, and the restoration of the morphological structure of the tissue according to the control aspiration biopsy are evaluated.

Criteria of the clinical effectiveness of chronic endometritis are reduction of clinical manifestations combined with elimination of pathogenic microflora from uterine cavity against the background of normalization of immunocompetent cell and pro-inflammatory cytokine levels in the endometrium, restoration of endometrial microcirculation, improvement of blood rheological properties, and reduction of the intensity of fibrosation and sclerosis processes. The final criterion of successful treatment is the restoration of the reproductive function followed by pregnancy and consecutive live births [70].

CONCLUSION

Currently, limited evidence confirms that hysteroscopy can be a powerful tool for the physical

removal of entrometrial bacterial biofilms, which contribute to the pathogenetic development of CE. After hysteroscopy, reproductive results in subsequent IVF cycles may improve in patients with RIFs and latent CE. Recent meta-analyses regarding hysteroscopy or artificial endometrial injuries in ART (hatching) have reported a significant improvement in the clinical pregnancy rate. However, this possibility has not yet been confirmed by well-developed RCTs, so this proposal should remain hypothetical.

To date, CE remains a rather difficult problem from the point of view of nosology, pathogenetic mechanisms, diagnosis and treatment in terms of reproductive disorders. This was associated among other things with poor reproductive results in the context of ART. In cases of CE, the peaceful coexistence between host immunity and microorganisms is impaired, the distribution of lymphocytes involved in embryo implantation, and, ultimately, endometrial susceptibility is reduced due to inadequate secretion of various cytokines. Recent clinical trials of patients with RPL have shown that antibiotic treatment for CE can lead to significant changes in future pregnancy outcomes. The use and effectiveness of various treatment methods as the second stage of treatment of CE requires well-developed RCTs. Due to the lack of qualitative data in the published literature, such treatment methods are still hypothetical and empirical. Well-planned prospective studies or RCTs should be conducted to clarify the possible correlations between CE and poor reproductive outcomes, as well as the effectiveness of endometrial interventions. CE is a clinically significant nosological unit from the perspective of reproductive medicine, further study of the features of its etiology and pathogenesis is required in order to improve understanding of the course of the inflammatory process and improve treatment and prevention methods.

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Molecular characteristics of anaplastic astrocytomas and isolation of molecular subgroups of their IDH1 mutant forms using *in silico* analysis

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ABSTRACT

Aim. The problem of anaplastic astrocytomas is quite relevant today. The WHO classification distinguishes *IDH1*/*IDH2* mutant anaplastic astrocytomas, anaplastic astrocytomas without *IDH1*/*IDH2* mutations, and anaplastic astrocytomas not otherwise specified. The aim of this work was to cluster *IDH1*-mutant anaplastic astrocytomas based on their cytogenetic profile to select prognostically significant molecular subgroups, which can have both clinical and fundamental scientific value.

Materials and methods. In this work, we performed a cluster analysis of anaplastic astrocytomas according to their cytogenetic profiles based on available genetic databases of tumors and large cohort studies, as well as a comparison of Kaplan – Meyer survival curves for various molecular subgroups of patients.

Results. We studied the main genetic features of the inter-tumor heterogeneity of anaplastic astrocytomas and distinguished seven molecular subgroups based on the cytogenetic profile: embryo-like, inflammatory-like, deletion, matrix, cyclin, *GATA3*-dependent and tyrosine kinase. Moreover, each of these subgroups has not only distinctive molecular characteristics, but also important clinical features.

Conclusion. A detailed study of the molecular properties of anaplastic astrocytomas will not only optimize the process for predicting treatment outcomes, but also create innovative formats for targeted therapy within the framework of the concept of personalized medicine.

Key words: anaplastic astrocytoma, *IDH1* mutation, inter-tumor heterogeneity.

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Молекулярно-биологические особенности анапластических астроцитом и выделение молекулярных подгрупп их IDH1-мутантных форм с помощью анализа *in silico*

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РЕЗЮМЕ

Актуальность. Проблема анапластических астроцитом достаточно актуальна в наши дни. В классификации Всемирной организации здравоохранения выделяются анапластическая астроцитома с мутацией в генах *IDH1* и *IDH2*, анапластическая астроцитома без мутаций в генах *IDH1* и *IDH2*, анапластическая астроцитома без дополнительного генетического уточнения.

Целью данной работы стало кластерирование анапластических астроцитом с мутацией в гене *IDH1* на основе их цитогенетического профиля для выделения прогностически значимых молекулярных подгрупп, которые могут иметь как клинико-практическое, так и фундаментально-научное значение.

Проведен кластерный анализ анапластических астроцитом по их цитогенетическим профилям на основе доступных генетических баз данных опухолей и крупных когортных исследований, а также сравнение кривых выживаемости Каплана – Мейера для различных молекулярных подгрупп пациентов.

Результаты. Нам удалось изучить основные генетические особенности межопухолевой гетерогенности анапластических астроцитом и выделить на основе цитогенетического профиля семь молекулярных подгрупп – *эмбриональноподобную*, *инфламмоподобную*, *делеционную*, *матриксную*, *циклиновую*, GATA3-зависимую и *тирозинкиназную*. При этом каждая из этих подгрупп имеет не только отличительные молекулярные характеристики, но и важные клинические особенности.

Заключение. Детальное изучение молекулярных свойств анапластических астроцитом позволит не только оптимизировать процесс прогнозирования исходов лечения, но и создать инновационные форматы для таргетной терапии в рамках концепции персонализированной медицины.

Ключевые слова: анапластическая астроцитома, мутация гена *IDH1*, межопухолевая гетерогенность.

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

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INTRODUCTION

The problem of anaplastic gliomas is quite relevant today. Anaplastic gliomas in the WHO classification of tumors of the central nervous system (CNS), 4th revision 2016, are represented by anaplastic astrocytoma and anaplastic oligodendroglioma [1]. According to statistics, the average incidence of anaplastic astrocytoma is 1.7% of the total number of central nervous system tumors, the prevalence of anaplastic astrocytoma is 1,307 cases per 100,000 people [2, 3].

The WHO classification distinguishes anaplastic astrocytoma with a mutation in the *IDH1* and *IDH2* genes, anaplastic astrocytoma without mutations in the *IDH1* and *IDH2* genes, and anaplastic astrocytoma not otherwise specified. Thus, mutations in the *IDH1* and *IDH2* genes are key genetic classification indicators [4]. The *IDH1* and *IDH2* genes serve as a template for the production of two isoforms of the isocitrate dehydrogenase enzyme. The *IDH1* gene encodes the cytoplasmic form, and the *IDH2* gene encodes the mitochondrial form. Both enzymes are involved in the oxidative decarboxylation of isocitrate with its conversion to 2-oxoglutarate [5]. It was shown that *IDH1* and *IDH2* mutations are the most accurate prognostic factors for astrocytic tumors; the presence of a mutation in these genes is associated with better patient survival [6]. It was also found that in cells carrying a mutation of the *IDH1* or *IDH2* gene, hyperproduction of oncometabolite 2-hydroxyglutarate (2HG) occurs, which leads to significant rearrangements in the epigenetic landscape of the tumor genome [7]. Moreover, the absence of the mutation results in a significant increase in the proliferative potential of astrocytic glioma cells [8]. A number of studies have revealed both the activating and inactivating effects of *IDH1* and *IDH2* mutations on different protooncogenes, such as *PIK3CA*, *KRAS*, *AKT*, *N-MYC* and others [9]. Some of the effects of these mutations are realized through metabolic molecular pathways, primarily through modification of lipid metabolism [10].

In modern oncology, the issues of tumors' molecular heterogeneity play a significant role. Genetic, epigenetic, and proteomic features of the pathological process can have significant individual characteristics in each specific tumor. Only with these features taken into account is it possible to form a truly personalized approach to the diagnosis and treatment of tumors, within the framework of which modern high-tech methods of diagnosis and treatment will allow the most precise assessment of the main properties and characteristics of the tumor process and, based on this, implement effective targeted therapy programs and individualized treatment approach. All of the above is extremely relevant for anaplastic gliomas, since the existing diagnostic and therapeutic approaches are obviously not effective enough [11].

In this work, we decided to analyze and review data from several large studies of the genetic characteristics of anaplastic astrocytomas, including the aim of identifying various patterns that can have both clinical, practical, and fundamental scientific value.

MATERIALS AND METHODS

An analytical study was conducted in accordance with the international principles of observational studies in epidemiology (MOOSE) [12]. To assess the genetic heterogeneity and related features of the clinical aspects of anaplastic astrocytomas, data from large multicenter studies were analyzed, including data from The Cancer Genome Atlas.

Search and selection of literature

We conducted a thorough literature search using the PubMed, Medline, Scopus, Embase, and Cochrane Library databases. The search keywords used were "astrocytoma OR anaplastic astrocytoma OR diffuse anaplastic astrocytoma OR astrocytoma Grade III" (all fields) AND "genomic data OR genome-wide analysis OR mutations OR multi-omics OR genome sequencing" (all fields). In order not to miss work on this topic, the list of links of full-text articles has also been fully checked.

The inclusion criteria were the presence of the data of full genome sequencing with the determination of mutational events and cytogenetic rearrangements of at least part or the entire studied group of patients; the presence of clinical data in the same patients, including indicators of overall survival (OS) and relapse-free survival (RFS); the presence in patients of an identified mutation in the *IDH1* gene; sufficient data to assess the risk ratio (RR) and 95% confidence intervals. Articles were excluded from the analysis if they were presented by reviews, abstracts, letters to the editor or experimental work on animals; if more than one study was conducted in the same group of patients, only the most recent or complete study was included in the analysis. The full text of the articles accepted for analysis has been carefully studied for a comprehensive assessment.

Data extraction and quality assessment

The two authors retrieved the data independently. The information retrieved included the name of the first author, year of publication, country of origin of the article, histological type of tumor, time of observation, methodological features of whole-genome sequencing, and OS and RFS indices. The study cohort included only patients with an established histological diagnosis of anaplastic astrocytoma, WHO Grade III, carrying the *IDH1* or *IDH2* gene mutation. The quality of each study was assessed independently by two researchers using the Newcastle-Ottawa Quality Assessment Scale [13]. Data from patient cohorts The Cancer Genome Atlas [14, 15], Glioma (MSK) [16], Low-Grade Gliomas (UCSF) [17], and Merged Cohort of LGG and GBM (TCGA) [18] were extracted using CBioPortal instrument (Memorial Sloan Kettering Cancer Center, USA). The analysis also included data from another 9 large, genome-wide studies of gliomas of various degrees of malignancy [19–27].

Statistical Data Analysis

A cluster analysis of all cases of anaplastic astrocytomas included in the study was performed. For cluster analysis, the k-means method was used. Cluster analysis was carried out on the basis of data on cytogenetic rearrangements in tumor samples. To determine the number of clusters, a hierarchical analysis was initially performed, indicating the possibility of separation with the greatest reliability of 6, 7 or 8 clusters. Cluster analysis was carried out with the isolation of 5, 6, 7, 8 and 9 clusters, the highest accuracy was observed in the identification of 7 clusters (molecular subgroups). In each cluster (molecular subgroup), the frequency of cytogenetic modifications and mutations was evaluated separately. Separate evaluation of OS and RFS for each molecular subgroup using Kaplan-Meyer curves, as well as a comparative analysis of survival rates was performed using the logarithmic rank criterion (LRC), the Cox-Mantel criterion (CMC), and the Gehan-Wilcoxon (GW) criterion. Adjusted risk ratios with 95 percent confidence intervals were used. A level of p less than 0.05 was considered statistically significant.

Statistical analysis was performed using SPSS Statistics 23 software (IBM, USA).

RESULTS

General characteristics of the cohort

In the cohort of patients included in the study and meeting all inclusion criteria, including the criterion for the presence of in the *IDH1* gene mutation, there were a total of 886 patients, accounting for 69.66% of all considered cases of anaplastic astrocytomas. The average age of the patients was 36.72 ± 4.58 years. Men accounted for 58.24% of the total cohort, while women accounted for 41.76%. The average OS rate was 9.18 ± 0.24 years, and the average RFS level was 2.34 ± 0.18 years.

Brief molecular characterization

In anaplastic astrocytomas with *IDH1* mutation, based on our analysis, the most frequent concomitant mutation was the point modification of the *TP53* gene, which was detected in 96.15% of cases. The group of tumors that simultaneously carry mutations in the *IDH1* and *TP53* genes was characterized by a high frequency of the *ATRX* gene mutation, which occurs in 64% of cases. In addition, mutations of the *SMARCA4*, *APOB*, and *FLG* genes were relatively often detected, each of which was observed in 10% of cases. Anaplastic astrocytomas with a mutation in the *ATRX* gene showed a higher mutation rate in the *CDKN2A* gene (8.57%) compared with tumors that did not carry this mutation (3.85%). The epidermal growth factor receptor (EGFR) mutated in 4.26% of cases, however, in combination with the *ATRX* mutation, it was not found. Among the cytogenetic events that accompanied the *ATRX* gene mutation, the most frequent was the amplification of the *EXT1* gene, which occurred in 21.43% of cases.

Molecular subgroups of anaplastic astrocytomas, IDH1-mutatant, and their characteristics

The first subgroup that can be distinguished among anaplastic astrocytomas with *IDH1* gene mutation based on the cytogenetic profile was anaplastic astrocytomas carrying amplifications of the *EXT1* and *MYC* genes. The *EXT1* gene encodes exostosin glycosyltransferase 1 protein, which is required for exosomal release of SDCBP, CD63, and syndecan factors, and plays a role in early tissue development and tumor progression [28]. The participation of the *MYC* in the implementation of genetic proliferative programs is widely known, as well as its participation in the pathogenesis of tumor diseases of different localization [29]. At the same time, activation of *MYC* gene in relation to CNS tumors is most often found in tumors and in individual populations of tumor cells having embryonic properties, in particular, medulloblastomas [30, 31]. Moreover, as in the case of anaplastic astrocytomas, the activation of this gene occurs most often precisely due to its amplification. Thus, this molecular subgroup can be arbitrarily called an embryonic-like subgroup. It occurs in 21.67% of analpastic astrocytomas. It is interesting to note that in this subgroup, *PTK2* gene amplification is revealed in 86.5% of cases. This gene product is tyrosine kinase type 2 protein, which is also associated with cells exhibiting embryonic properties to varying degrees and it is actively involved in the proliferation and stabilization of neuronal and glial elements in early stages of central nervous system development [32].

Moreover, clinically, this group, as in the case of medulloblastomas, is characterized by a worse prognosis for OS and RFS. Thus, OS (LRC: $p = 0.0086$; CMC: $p = 0.00051$; GW: $p = 0.00038$) along with RFS (LRC: $p = 0.00776$; CMC: $p = 0.00138$; GW: $p = 0.00368$) is significantly lower in the embryonic-like subgroup compared with similar indicators in the rest of the analyzed cohort (Fig. 1).

The second subgroup includes tumors carrying amplification of the *ERC1* gene. In the aspect of carcinogenesis, the participation of ERC1 protein in the activation of the transcription factor NF-κB is extremely important; increased activity of this mechanism has been identified as one of the key molecular events in breast tumors [33, 34]. Under physiological conditions, NF-κB is a key factor in the implementation of inflammatory programs and cell stress response [35]. Subsequently, the role of this transcription factor in the carcinogenesis of different tumors was shown [36]. Therefore, this molecular subgroup can be called *inflammatory-like*, and is detected in 18.51% of cases. This subgroup is characterized by a high frequency of amplification of the cyclin D2 gene (*CCND2*) in 94.36% of cases. The cyclin D2 gene is a regulatory component of the cyclin D2-CDK4 complex, inhibiting the members of retinoblastoma protein family (RB), including RB1 protein, and causing the cell transition to the S phase of the cell cycle. This increases the proliferative activity of cells [37]. In addition, there was a high frequency of amplification of genes belonging to the fibroblast growth factor family, fibroblast growth factor 6 (*FGF6*) and 23 (*FGF23*) types. These factors lead to the activation of the tyrosine

kinase cascade with a significant increase in mitotic activity and cell survival [38]. The clinical features of this subgroup consist in a similar level of OS and RFS in comparison with the embryonic like subgroup (Fig. 1). The observational data are also confirmed by statistical criteria, which do not reveal significant differences both in relation to RFS (LRC: $p = 0.0878$; CMC: $p = 0.0615$; GW: $p = 0.05895$) and OS (LRC: $p = 0.0781$; CMC: $p = 0.05845$; GW: $p = 0.0568$).

The third subgroup includes tumors carrying deletions of the *BRSK1*, *ZNF331*, *TFPT* and *U2AF2* genes. The *BRSK1* gene encodes a brain-specific serine/threonine protein kinase 1 that phosphorylates and activates a number of secondary messengers and acts as a key regulator of the polarization of cortical neurons, as well as the formation of the gliocyte cytoskeleton. In addition, it is involved in the implementation of a number of other functions, in particular, it can play the role of a negative regulator of the cell cycle, inhibiting its development in case of DNA chain damage and simultaneously facilitating its rapid repair [39]. The *TFPT* gene ensures the development of apoptosis regardless of the mutational status of the *TP53* gene,

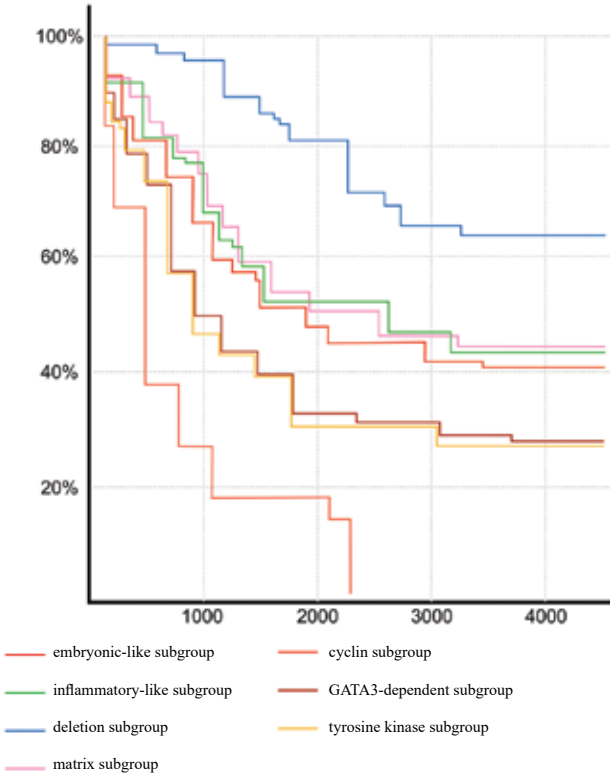

Fig. 1. Kaplan – Meyer curves for the overall survival of patients separately in each molecular subgroup. The abscissa shows the survival time in days. In the upper right corner is the color designation for the various subgroups

which is extremely important for anaplastic astrocytomas carrying the *TP53* mutation in 96.15% of cases [40]. The *U2AF2* gene encodes an auxiliary factor of small nuclear RNA type 2, which plays an important role in the splicing processes of a number of genes, including those associated with the cell's proliferative potential [41]. Since the whole set of combined deletions is the most characteristic from the genetic point of view for this type, the considered subgroup can be conditionally called deletion. It is detected in 17.49% of cases.

Such cytogenetic changes lead to better clinical outcomes (Fig. 1). The OS in this subgroup is not only higher than the average level for all molecular subgroups, but also significantly higher than in embryonic-like (LRC: $p = 0.00864$; CMC: p = 0.00546; GW: $p = 0.000324$) and the inflammatory-like subgroup (LRC: $p = 0.00953$; CMC: $p = 0.00486$; GW: $p = 0.000472$). RFS is also significantly higher in comparison with embryonic-like (LRC: $p = 0.00623$; CMC: $p = 0.00399$; GW: $p = 0.000285$) and inflammatory-like (LRC: $p = 0.00694$; CMC: $p = 0.00512$; GW: $p = 0.000421$) subgroups.

The following group contains cases with the amplification of the *MSN* gene and combined amplification of the *AMER1* gene in 83.33% of cases. The *MSN* gene encodes a moesin protein involved in providing a connection between the components of the cytoskeleton of the cell and the cytoplasmic membrane. Moreover, it can participate in the regulation of contact inhibition of certain cells' proliferation and their motility [42]. The protein encoded by the *AMER1* gene is one of the key regulators of the Wnt/beta-catenin cascade, capable of both increasing and decreasing its activity. Elements of this cascade, as well as the product of the *MSN* gene, are involved in cell contacts with the intercellular matrix and other cells; this cascade likewise takes part in the processes of contact matrix and intercellular regulation of cell proliferation [43]. In connection with the described features, this subgroup can be designated as matrix. It occurs in 14.79% of cases. The clinical features of this subgroup are close to embryonic like and inflammatory-like; OS was similar to both embryonic-like (LRC: $p = 0.84651$; CMC: $p = 0.8245$; GW: $p = 0.6278$) and inflammatory-like subgroups (LRC: $p = 0.8231$; CMC: $p = 0.6731$; GW: $p = 0.7392$), RFS was also similar to embryonic-like (LRC: $p = 0.09284$; CMC: $p = 0.07645$; GW: $p = 0.06245$) and inflammatory-like subgroups (LRC: $p = 0.06127$; CMC: $p = 0.06045$; GW: $p = 0.06012$).

Furthermore, a subgroup of anaplastic astrocyto-

mas was detected in which deletions of the *MTAP*, *CDKN2A* and *CDKN2B* genes were combined. The *CDKN2A* encodes several transcript variants that differ in the composition of their first exons and act as regulators of the cell cycle, inhibiting the transition of the cell to mitosis. In addition, under the influence of the proteins encoded by the *CDKN2A* gene, the p53 protein is stabilized and activated [44]. The product of the *CDKN2B* gene is a cyclin-dependent kinase inhibitor, which is complexed with *CDK4* or *CDK6* and prevents their activation; therefore, the encoded protein also functions as a cell growth regulator that slows down the progression of the G1 phase of the cell cycle [45]. The *MTAP* gene encodes a methylthioadenosine phosphorylase protein, which plays an important role in the metabolism of polyamines. A decrease in the activity of this gene is observed in many tumors due to frequent co-deletion with the *CDKN2A* and *CDKN2B* genes. Due to the fact that cyclin system regulating proteins play a significant role in the pathogenesis of this subgroup of tumors, the *cyclin* subgroup seems to be the most suitable variant. This subgroup presented in 14.33% of cases. Interestingly, the *ATRX* gene mutation occurs in 100% of tumors of this subgroup.

The OS in patients of this subgroup is extremely low, while its OS and RFS differ for the worse from all the subgroups indicated above: embryonic-like (LRC: $p = 0.00852$; CMC: $p = 0.00712$; GW: $p = 0.00556$ and LRC: $p = 0.00973$; CMC: $p = 0.00848$; GW: $p = 0.00468$, respectively), inflammatory-like (LRC: $p = 0.00751$; CMC: $p = 0.00706$; GW: $p = 0.00513$ and LRC: $p = 0.00957$; CMC: $p = 0.00759$; GW: $p = 0.00365$, respectively), matrix (LRC: $p = 0.00725$; CMC: $p = 0.00606$; GW: $p = 0.00472$ and LRC: $p = 0.00908$; CMC: $p = 0.00704$; GW: $p = 0.003145355$, respectively) and deletion (LRC: $p = 0.00453$; CMC: $p = 0.00317$; GW: $p = 0.00159$ and LRC: $p = 0.00509$; CMC: $p = 0.00251$; GW: $p = 0.00084$, respectively). Thus, belonging to the cyclin subgroup is an extremely unfavorable factor of prognosis of patients (Fig. 1). These findings are consistent with a recent study by Shirahata et al., which showed that deletion of *CDKN2A* and *CDKN2B* is an unfavorable prognostic molecular event for anaplastic astrocytomas [46].

A small subgroup of patients had amplification of the *GATA3* gene. The product of this gene is a transcriptional activator that binds to an enhancer of T-cell receptor genes. The pro-carcinogenic effects of *GATA3* can be associated with deregulation of three genes, *BCL2*, *DACH1*, *THSD4*, which are involved in cell differentiation processes [47]. The *GATA3-dependent* subgroup is not numerous, it was revealed in 11.63% of cases, but, like the cyclin one, it is characterized by an extremely unfavorable prognosis. Nevertheless, the prognosis of OS is somewhat better than in the cyclin subgroup (LRC: $p = 0.03499$; CMC: $p = 0.03207$; GW: $p = 0.03168$), but RFS does not significantly differ in these subgroups (LRC: $p = 0.07207$; CMC: $p = 0.06628$; GW: $p = 0.06266$). At the same time, OS and RFS were significantly lower in comparison with embryonic-like (LRC: $p = 0.00824$; CMC: $p = 0.00723$; GW: $p = 0.00649$ and LRC: $p = 0.00948$; CMC: $p = 0.00813$; GW: $p = 0.00689$, respectively), inflammatory-like (LRC: $p = 0.00809$; CMC: $p = 0.00749$; GW: $p = 0.00582$ and LRC: $p = 0.00942$; CMC: $p = 0.00765$; GW: $p = 0.00667$, respectively), matrix subgroups (LRC: $p = 0.00784$; CMC: $p = 0.00689$; GW: $p = 0.00381$ and LRC: $p = 0.00935$; CMC: $p = 0.00749$; GW: $p = 0.00648$, respectively).

The last subgroup was characterized by combined amplification of the *FIP1L1*, *CHIC2*, *PDGFRA*, *KIT* and *KDR* genes. The *FIP1L1* gene encodes a protein performing polyadenylation of the 3′end of pre-mRNA [48]. *PDGFRA* encodes a type A thrombocyte growth factor receptor, a tyrosine kinase cell membrane receptor that has pronounced mitogenic effects through the activation of the RAS/RAF/MAPK cascade [49]. The product of the *KIT* gene, c-kit protein, is a tyrosine protein kinase that acts as a cell surface receptor for KITLG/SCF cytokines and plays a significant role in the regulation of cell survival and proliferation, hematopoiesis, and maintenance of stem cells, as well as their migration. Moreover, like PDGFRA, the c-kit realizes a significant part of its effects through the activation of the RAS/RAF/MAPK tyrosine kinase cascade [50]. In this regard, conditionally this subgroup can be called tyrosine kinase. It was detected in 1.58% of cases.

The tyrosine kinase subgroup is characterized by similarities in both OS and RFS with the GATA3-dependent subgroup, and the statistical criteria do not reveal significant differences between the subgroups in these indicators (LRC: $p = 0.37488$; CMC: $p = 0.28652$; GW: $p = 0.25913$ and LRC: $p = 0.34975$; CMC: $p = 0.27728$; GW: $p = 0.25105$, respectively).

DISCUSSION

Thus, according to the results of our analysis, 7 molecular subgroups of anaplastic astrocytomas were identified that differ in their cytogenetic profile, as well as different prognosis and features of mutational changes. Embryonic and inflammatory-like subgroups

are the most frequent and occur in 21.67% and 18.51% of cases, respectively. The deletion subgroup makes up a total of 17.49% of cases, the matrix subgroup consists of 14.79% of cases, the cyclin subgroup with 14.33% of cases and the *GATA3*-dependent subgroup, which occurs in 11.63%, are located nearby. The rarest is the tyrosine kinase subgroup, as it is detected in only 1.58% of cases. The worst prognosis is observed in the cyclin subgroup, a relatively poor prognosis is revealed in the *GATA3*-dependent and tyrosine kinase subgroups, the middle prognosis in the embryonic, inflammatory-like and matrix subgroups, the best prognosis is found in the deletion subgroup (Fig. 2).

What can be responsible for such inter-tumor heterogeneity within the framework of one nosological unit? We can try to trace the potential pathways for the occurrence of genetic diversity of anaplastic astrocytomas by examining the currently available literature data on the carcinogenesis of gliomas and by sketching a possible pathway for the progression of these tumors.

The earliest and most important mutational event

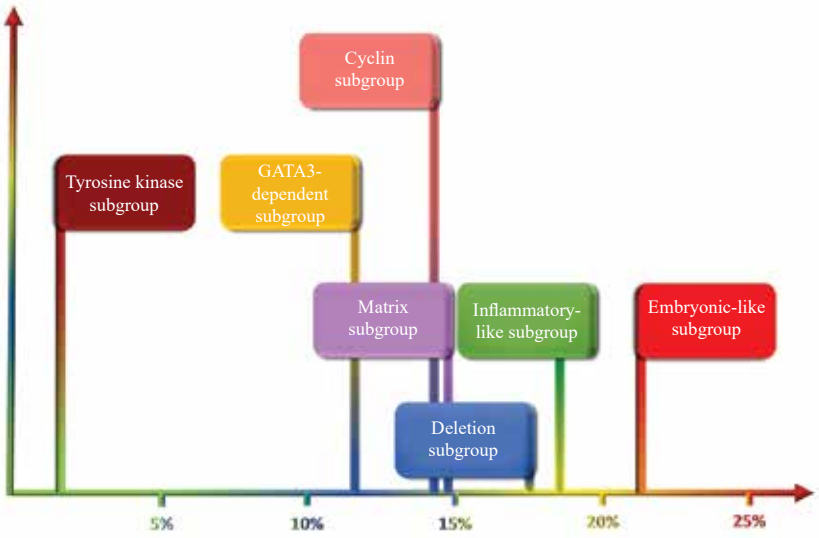

Fig. 2. The correlation of different subgroups according to the degree of tumors aggressiveness and the frequency of occurrence. The increase in the degree of tumor aggressiveness in each subgroup is the higher, the higher this subgroup is located relative to the ordinate axis. The prevalence of molecular subgroups is shown on the abscissa as a percentage of the total number of cases analyzed

in anaplastic astrocytomas is the mutation of the *IDH1* gene [51]. This event primarily affects neuronal stem cells, which may be the primary tissue source of the tumor [52]. However, tumor cells can follow different paths under the influence of many factors, including genetic and epigenetic constitutional features and stochastic effects in gene expression, leading to the emergence of not only inter-tumor, but also intratumoral heterogeneity. Various types of cells arise; in particular, three principal cell populations appear in the composition of anaplastic astrocytoma: astrocyte-like cells, oligodendro-like cells and progenitor cells with stem properties [19]. It is curious that amplification of the *PDGFRA* gene acts as a marker genetic event for oligodendro-like cells, the content of which is extremely low in anaplastic astrocytomas. In the cohort analyzed, within the framework of this study, the tyrosine kinase subgroup, for which this also serves as one of the marker events, is extremely rare. In our study, the worst prognosis was observed in patients whose tumors belong to the cyclin subgroup, which is characterized primarily by deregulation of cell cycle proteins. It is curious that similar changes in the application of single-cell technologies are found in tumor glioma cells, called mesenchymal-like cells. This type of cell practically does not occur in classical anaplastic astrocytomas and is more characteristic of glioblastomas, for which a higher degree of malignancy is typical.

Thus, the tumor cell subclones within the same tumor create the mosaic picture that is assembled into a single molecular tumor pattern, which served as the basis for the selection of subgroups in our study. Differences in the details of this mosaic can produce different molecular subtypes of anaplastic astrocytomas. Each subtype will have different biological properties

and different aggressiveness, which is reflected in the prognostic aspects.

CONCLUSION

Diffuse glial tumors are a difficult problem both in fundamental and in clinical terms. The significant heterogeneity of the molecular properties of anaplastic astrocytomas affects not only the rate of progression of the pathological process, but also the effectiveness of different types of treatment. Moreover, such heterogeneity is a reflection of the individual characteristics of the tumors in each individual patient. Consideration of such features is extremely important for the development of truly effective personalized approaches to the diagnosis and treatment of such patients. Molecular clustering of tumors will not only optimize the prognosis of treatment outcomes, but also create innovative formats for targeted, precise therapy within the framework of the concept of personalized medicine.

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Adiponectin and insulin: molecular mechanisms of metabolic disorders

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ABSTRACT

Adiponectin, the most common plasma adipocytokine, plays a crucial metabolic and anti-inflammatory role. With insulin resistance associated with obesity, an increase of adiponectin concentration, which leads to the activation of signaling pathways involved in the regulation of metabolism, occurs. Currently, adiponectin is being investigated as a potential therapeutic target for metabolic syndrome, although more research is required to understand the underlying mechanisms controlling its levels. In this review, we will examine the main mechanisms that control adiponectin levels in blood serum and its role in insulin-sensitizing effect, as well as evaluate the potential use of adiponectin and its receptors as a potential therapeutic target.

Keywords: adiponectin, insulin, adipocytes, obesity.

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Адипонектин и инсулин: молекулярные механизмы реализации метаболических нарушений

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РЕЗЮМЕ

Адипонектин – самый распространенный адипоцитокин в плазме крови, который играет критическую метаболическую и противовоспалительную роль. При инсулинорезистентности, связанной с ожирением, происходит увеличение концентрации адипонектина, что приводит к активации сигнальных путей, участвующих в регуляции метаболизма. В настоящее время адипонектин исследуется в качестве потенциальной терапевтической мишени для метаболического синдрома, хотя необходимы дополнительные исследования, чтобы понять основные механизмы, контролирующие уровень адипонектина в крови. В этом обзоре мы представим основные механизмы, контролирующие уровень адипонектина в сыворотке крови, и его роль в

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инсулин-сенсибилизирующем действии, а также оценим потенциальное использование адипонектина и его рецепторов в качестве потенциальной терапевтической мишени.

Ключевые слова: адипонектин, инсулин, адипоциты, ожирение.

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INTRODUCTION

Adiponectin is one of the most studied adipocytokines. Since the discovery of adiponectin in adipose tissue, it has been shown that it can be secreted in skeletal muscle, osteoblasts, and lymphocytes [1, 2]. Nevertheless, adipose tissue remains the main source of adiponectin in the serum, and its concentration in the serum varies from 2 to 26 μg/ml, making up > 0.01% of serum protein [3, 4, 5].

In the last few years, the relationship between adiponectin and insulin has been widely studied since the sensitizing effect of adiponectin on insulin by binding to its receptors leads to the activation of adenosine monophosphate-activated protein kinases (AMPK), receptors activated by peroxisome proliferators (PPAR-α) and, possibly, other molecular ways which have not been studied; therefore, it is necessary to research it further. With insulin resistance (IR) associated with obesity, the content of adiponectin decreases, which leads to the activation of signaling pathways that regulate metabolism [3, 4]. At the same time, there is insufficient data on the effect of insulin on the synthesis and secretion of adiponectin. This review summarizes the latest findings on the effects of insulin on serum adiponectin levels and discusses the relationship between the adiponectin system and IR. In addition, the possible use of adiponectin or its receptors as a therapeutic target in cardiovascular diseases (CVD) was considered.

THE PRIMARY STRUCTURE OF ADIPONECTIN

Adiponectin is a glycoprotein [6] encoded by a single gene transcript and consisting of 247 amino acids. It has an N-terminal signal peptide (~28–32 amino acids) followed by a hypervariable region (12 amino acids) containing conservative amino acids necessary for oligomerization and a collagen region containing 22 Gly-X-Y repeats where X and Y are most often proline, isoleucine, or hydroxylisine (66 amino acids) and the C-terminal globular domain, which makes up 55% (136–137 amino acids) of the total number of amino acids [7]. Interestingly, the globular domain of adiponectin is a structural homolog to TNFα. However, despite the structural homology, there are not so many homologous sequences, with the exception of the four conservative residues responsible for maintaining structural folds. The collagen domain of adiponectin has a common homology with the complement protein C1q [7]. Thus, adiponectin belongs to the paralogous protein family known as C1Q/TNF-linked proteins or CTRPs [7].

MULTIMERIC FORMS OF ADIPONECTIN (OLIGOMERIZATION)

Serum adiponectin exists in several oligomeric complexes: trimer (LMW or low molecular weight form), hexamer (MMW or medium molecular weight form), and a 12- or 18-measure form (HMW or high molecular weight form) [7, 8]. In addition to these forms of adiponectin, there is a small form called gAd (globular adiponectin). The gAd mainly consists of 3 C-terminal globular domains held together by the strong hydrophobicity of the inner trimmer core [8]. It has been suggested that the HMW-adiponectin can also serve as a form of gAd storage that can be obtained from HMW [9].

The existence of multiple oligomeric structures contributes to the multifaceted activity of adiponectin, so that different oligomers act on different target tissues with diverse biological effects. The HMW adiponectin acts on liver and reduces the level of glucose in the blood serum, while the LMW or MMW adiponectin does not have similar effects [10]. The HMW

oligomer is necessary for the sensitizing effect of adiponectin on insulin by inhibiting gluconeogenesis in the liver [7]. On the other hand, gAd enhances fatty acid oxidation and insulin sensitization in skeletal muscles [11, 12]. In cultured myotubes, C2C12 HMW and MMW adiponectin activate NF-κB; while the trimer form activates AMPKα [13, 14]. On the contrary, Hada et al. showed that HMW adiponectin binds well to the membrane fraction of C2C12 muscle tubes [15]. In the central nervous system, HMW oligomers (large sizes) do not cross the blood-brain barrier. Thus, the main actions of adiponectin are mediated only by trimer and hexamer oligomers.

It is noteworthy that exogenous oligomers of adiponectin do not undergo conversion in blood serum to other oligomeric forms [16]. It has been proven that the intracellular production of oligomers is crucial to maintaining their serum ratio. Moreover, the affinity of binding of various adiponectin oligomers to its receptors, as well as the tissue-specific distribution of receptors, apparently contribute to the differentiated action of adiponectin [17].

RECEPTORS OF ADIPONECTIN

AdipoR1 (Adiponectin Receptor 1) and AdipoR2 (Adiponectin Receptor 2) belong to the PAQR family (family of progestin and adipoQ (adiponectin, C1Q and collagen domain containing) receptors)), consisting of 7 transmembrane domains similar to G-protein coupled receptors (GPCR). However, unlike GPCR, adipoR is not associated with the G-protein and has an extracellular C-terminus and an intracellular N-terminus [18]. Skeletal muscles are the main tissue expressing AdipoR1, and liver cells express primarily AdipoR2. In addition, AdipoR1 and AdipoR2 bind to oligomeric forms of adiponectin with different affinities, for example, gAd mainly binds to AdipoR1, while full-sized adiponectin mainly binds to AdipoR2 [18]. Then, activation of intracellular signaling pathways occurs after binding to the receptor, which leads to the appearance of various physiological effects.

PROTEINS INTERACTING WITH ADIPONECTIN RECEPTORS

It is known that AdipoR1 and AdipoR2 do not have their own kinase/phosphorylating activity. Mutagenesis of intracellular tyrosine residues, which usually act as initiator signaling residues in other receptors, does not block adiponectin signaling [19]. So far, APPL1 (an adapter protein containing the homologous domain of plextrin), ERp46 (endoplasmic reticulum protein 46), Rack1 (receptor for activation of C-kinase 1) and CK2β (casein kinase 2β) have been identified for direct interaction with AdipoRs (Fig. 1).

APPL1 protein was identified as an adapter protein that binds to AdipoR, acting on both AdipoR1 and AdipoR2 to facilitate intracellular signaling. Binding of the ligand to AdipoR1 enhances the relationship of AdipoR1-APPL1 [20]. Overexpression of APPL1 in C2C12 muscle tubes increases the basal and adiponectin-induced phosphorylation of AMPK, p38 and ACC (acetyl-CoA carboxylase), which mediates the meta-

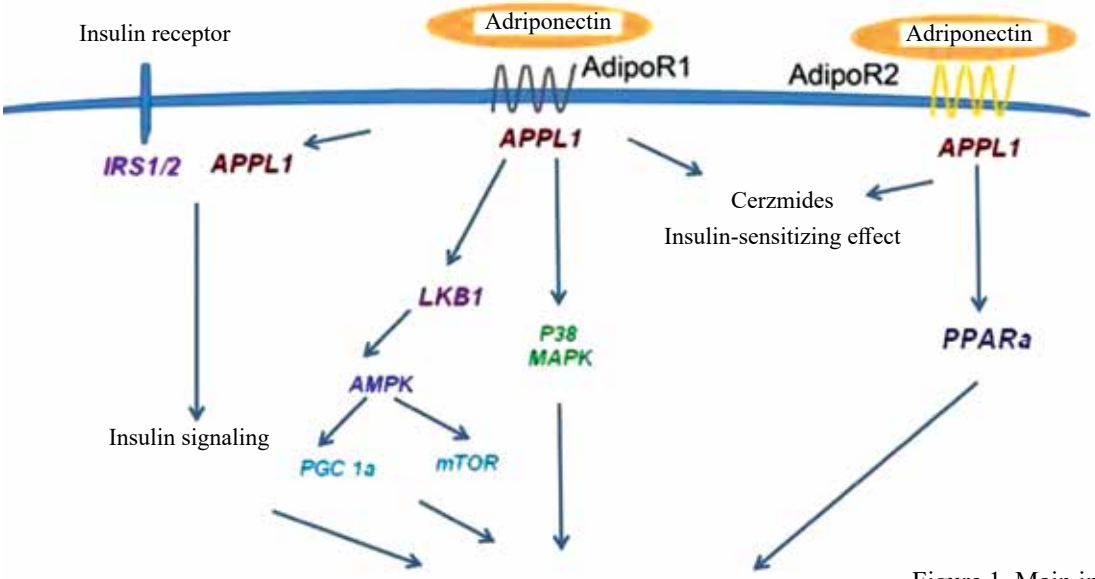

Figure 1. Main intracellular signaling pathways induced by AdipoR1 and AdipoR2

bolic functions of adiponectin. Accordingly, APPL1 knockout has been shown to inhibit AdipoR1-mediated signaling [21].

APPL2 is an isoform of APPL1 with 54% sequence similarity and similar domain organization. It acts as an inhibitor of APPL1 activity [22]. In addition, it is able to communicate with AdipoR through its BAR (Bin/Amphiphysin/Rvs) domain. Transgenic expression of APPL2 prevents the binding of APPL1-AdipoR1, which occurs either by competition of APPL1 for binding to AdipoR1, or by the formation of a heterodimer with APPL1, thereby inhibiting the interaction of APPL1-AdipoR1. Suppression of APPL2 expression improves adiponectin-induced fatty acid oxidation and glucose uptake [19]. APPL1 also enhances eNOS activation and NO production in endothelial cells by blocking by direct competition the compound Akt (a signal intermediate in the signaling pathway of insulin with its endogenous inhibitor 3 (TRB3)). In adipocytes and muscle cells, APPL1 forms a complex with Akt2, which dissociates when stimulated by insulin to regulate insulin-stimulated translocation of the GLUT4 membrane. APPL1 also facilitates the binding of IRS1/2 to the insulin receptor.

INTERMEDIATE SIGNAL MOLECULES

LKB1 (liver b1-kinase): after activation by ligand-bound AdipoR1 in the cytoplasm, APPL1 interacts and activates PP2A (phosphatase 2A protein). Activated PP2A deactivates PKC (protein kinase C) through dephosphorylation (threonine 410). PKC is a serine/threonine kinase that phosphorylates LKB1 to serine (307) and promotes its nuclear translocation. Deactivation of PKC leads to the accumulation of LKB1 in the cytoplasm, and also increases its interaction with APPL1. In the cytoplasm, APPL1 binds LKB1 and phosphorylates AMPK [22].

CaMKK (calcium/calmodulin dependent kinase kinase) has significant sequence and structural homology with LKB1 [23]. There are two CaMKK isoforms: CaMKKα and CaMKKβ. They are encoded by two different genes and have 70% amino acid sequence homology. Adiponectin has been shown to enhance the production of cytoplasmic Ca^{2+} either by releasing Ca^{2+} from the sarcoplasmic reticulum, or by an extracellular influx of Ca^{2+} [24]. Unlike LKB1, CaMKK-mediated phosphorylation of AMPK depends only on Ca^{2+} [25]. In addition, in an experimental study performed on mice knocked out by AdipoR1, Iwabu et al. demonstrated that binding of AdipoR1 to the ligand induces Ca^{2+} influx and activates CaMKKβ, which leads to AMPK phosphorylation [26].

AMPK is a serine/threonine protein kinase, also called a metabolic cell sensor. Functional AMPK protein is a heterotrimer consisting of α, β and γ subunits. The catalytic α subunit also has a threonine phosphorylation site (172), while β- and γ- subunits act as regulators. The α subunit has two options: α1 (is exclusively cytoplasmic) and α2 (localized in the nucleus). In addition to the kinases listed above (LKB1 and CaMKKβ), AMPK is also activated by allosteric binding of AMP, the cellular level of which increases in a state of energy depletion [27].

Adiponectin induces AMPK activation in major peripheral target tissues. AdipoR1-mediated phosphorylation of AMPK was shown to inhibit skeletal muscle glycogen synthesis [28]. It is likely that the inhibition is due to phosphorylation of glycogen synthase to serine (7) only with AMPK α2 [29]. AMPK can also phosphorylate and activate PGC1α, receptors activated by peroxisome proliferators γ-coactivated 1α [30]. There are also data on an alternative pathway for PGC1α activation by adiponectin, including deacetylation of PGC1α through activation of sirtuin 1 (SIRT1). Subsequently, sirtuin 1-mediated activation of PGC1α activates gluconeogenic genes and hepatic glucose level, as well as PGC1α-mediated inhibition of glycolysis [31]. Activated CaMKKβ can also activate PGC1α independently of AMPK [31]. In addition, activated PGC1α enhances mitochondrial biogenesis and mitochondrial respiration, which consequently enhances muscle fatty acid oxidation [31].

Activated AMPK also induces the translocation of the insulin-dependent glucose transporter GLUT4 (type 4 glucose transporter) to the cell surface of various cell types, including skeletal muscles, adipocytes and cardiomyocytes, thereby increasing glucose uptake, which is one of the insulin-sensitizing effects of adiponectin. S.L. Torn et al. proposed a GLUT4 translocation model in which AMPK was activated directly or via mTOR the AS160 inactivation pathway (substrate for Akt protein kinase 160 kDa), similar to the one transmitting insulin signals [32], thereby initiating GLUT4 translocation to the cell surface [33]. Moreover, through AMPK-mediated Rheb phosphorylation (Ras homolog), adiponectin inhibits the p70 S6 kinase, which is unable to phosphorylate serine (302) and activate IRS1 (insulin receptor substrate 1). Finally, this contributes to the insulin-sensitizing effect of adiponectin [34].

The addition of various oligomeric forms of adiponectin (HMW or LMW) to the culture medium containing hepatocytes leads to a decrease in glucose production in the medium due to transcriptional suppression of G6P (glucose 6 phosphate) and PEPCK (phosphoenolpyruvate carboxylase), which is responsible for gluconeogenesis and glycogenolysis, respectively, via the AMPK dependent mechanism [35]. In addition, data appeared on the independent inhibition of LKB1-AMPK gluconeogenesis [35].

AMPK signaling also activates autophagy by phosphorylation of Ulk1 (Unc-51-like kinase 1 or a kinase that initiates mammalian autophagy) under conditions of nutrient deficiency, i.e. serine (317) and serine (777). Moreover, AMPK phosphorylates and thereby deactivates mTOR (mammalian rapamycin target), which is a known inhibitor of autophagy. In nutrient-rich environments, mTOR inhibits the induction of autophagy by phosphorylating serine (757) Ulk1, thereby preventing AMPK binding and subsequent activation of Ulk1 [36]. Adiponectin was shown to induce autophagy in an AMPK-dependent manner in various cell types, including cardiomyocytes and skeletal muscle [37]. It also induces the differentiation of vascular smooth muscle cells through AMPK-mediated inhibition of the mTOR complex [38]. It is noteworthy that autophagy is an important mechanism for the differentiation of cells of various types [38].

p38 MAPK: APPL1 acts as an anchor for adiponectin-mediated activation of the p38 MAPK pathway. In experimental studies, it was shown that p38-MAPK and signal components of the cascade were combined using APPL1. Under basal conditions, TAK1 (transforming growth factor β-activated kinase) is found with APPL1, while MKK3 (MAP kinase-3) and p38 MAPK remain weakly bound to APPL1 [38]. Adiponectin-activated APPL1 further activates TAK1, and subsequently a complex consisting of MAPK AdipoR1, APPL1, TAK1, MKK3 and p38 is formed. Activated TAK1 then phosphorylates MKK3, which in turn phosphorylates p38 MAPK. After phosphorylation, MKK3 and TAK1 dissociate from APPL1, and TAK1 activity rapidly decreases. TAK1 can also directly phosphorylate AMPK. It was also shown that activated p38 MAPK has an antilipogenic effect on muscles [40].

PPAR activates ACO (acetyl CoA oxidase) and UCPs (uncoupling proteins), which ultimately promotes the fatty acid oxidation and increased energy expenditure in skeletal muscles [41]. PPARα signaling increases the sensitivity of hepatic insulin and therefore improves glucose uptake in the liver [41]. PPAR-mediated signaling also activates catalase and SOD1 (type 1 superoxide dismutase) in hepatocytes, which additionally contributes to the insulin-sensitizing effect of adiponectin in the liver by reducing oxidative stress [41]. On the other hand, PPARγ, which induces adiponectin expression in adipose tissue, is also activated by adiponectin [41].

INSULIN-SENSITIZING EFFECT OF ADIPONECTIN

Adiponectin has a sensitizing effect on insulin and other beneficial metabolic effects, inhibiting hepatic gluconeogenesis and enhancing fatty acid oxidation by activating AMP-activated protein kinase (AMPK) and proliferator-activated peroxisome α receptor (PPARα) [42, 43], as well as inhibition of acetyl-coenzyme A-carboxylase (ACC) in the liver and muscles [44]. Moreover, its anti-inflammatory effect is due to a decrease in the migration of macrophages and foam cells through the vascular wall and the polarization of macrophages [44]. A study by Fu et al. showed that overexpression of adiponectin in adipocytes increases insulin sensitivity by modulating proliferation, differentiation, and lipid accumulation [45].

With obesity, there is an active growth in adipose tissue: hyperplasia and hypertrophy. In response to energy balance, adipocytes produce and secrete various peptides. Studies have shown that adiponectin is a potential key mediator of glucose homeostasis in obesity and IR [46]. However, the autocrine actions and functions of adiponectin for adipocyte insulin signaling and glucose transport are not fully understood. In an experimental study, Chang et.al added insulin to a culture medium containing adipocytes, which induced a decrease in adiponectin mRNA expression [47]. In turn, adiponectin deficiency in the culture medium led to a decrease in insulin-stimulated glucose uptake and a decrease in the activation of AMPK in insulin-sensitive adipocytes.

At present, it is known that IRS proteins are of the greatest importance for insulin signal transmission; IRS-1 and IRS-2 are expressed in almost all types of cells and tissues [43]. IRS-1 mediates the regulatory effects of insulin on peripheral metabolic and growth processes, while IRS-2 is more responsible for the central effects of insulin, including control of differentiation and growth of neuronal cells, central regulation of eating behavior, glucose homeostasis and endocrine functions. It has been proven that a decrease in the content of IRS-1 is associated with IR and T2DM

[48]. In addition, Yamauchi et al. found that administration of globular adiponectin to lipotropic mice improved insulin sensitivity by enhancing the insulin-stimulated tyrosine phosphorylation of IRS-1 [43]. In C2C12 myotubes, adiponectin treatment reduces the IRS-1 phosphorylation to serine (636/639), which inhibits the subsequent insulin-stimulated IRS-1 tyrosine phosphorylation using the insulin receptor. It was proved that the presence of IR leads to a change in the expression of GLUT4 in the plasma membrane and intracellular compartments of adipocytes with a deletion of the adiponectin gene [49].

AKT (protein kinase B), the next target for insulin signaling in the cell, also causes various metabolic effects mediated by insulin, and AKT activity has been shown to decrease markedly in T2DM [50]. In their study, Chang et al. showed that the addition of insulin to a culture medium containing adipocytes is accompanied by activation of AKT. However, the adiponectin deletion did not lead to further AKT activation compared to control cells transfected with siRNAs (a double-stranded RNA class, 20–25 nucleotides long) [47].

These results suggest that AKT signaling is not involved in adiponectin-induced reduction in insulin-stimulated glucose transport. GLUT 4 plays a key role in this process, which is involved in the clearance of glucose, and GLUT1 plays a secondary role, mainly for glucose uptake during non-insulin stimulation [50]. In IR conditions, including obesity and T2DM (type 2 diabetes mellitus), the expression of GLUT4 in adipocytes decreases [50]. Overexpression of GLUT4 in adipose tissue leads to an increase in glucose tolerance [50].

It should be noted that the expression of AdipoR1/R2 in insulin target tissues appears to be inversely correlated with plasma insulin levels, since insulin negatively regulates adiponectin receptor expression levels via the PI3 kinase/Foxo1 pathway. Thus, it is both AdipoR1/R2 agonism and strategies to increase AdipoR1/R2 that can be logical approaches to provide a new method for the treatment of IR and T2DM, as we have shown that in patients with visceral obesity in the late post-infarction period manifestation of T2DM [52].

It is also known that adiponectin indirectly regulates insulin sensitivity by modulating immune responses. It is noteworthy that adiponectin has an antiapoptotic effect on cardiomyocytes and β-cells of the pancreas and reduces oxidative stress in endothelial cells [43]. Despite these well-known endocrine effects of adiponectin, its autocrine/paracrine effects are still to be further researched. For example, adiponectin reduces ceramide in the liver by enhancing their catabolism and the production of the antiapoptotic metabolite sphingosine-1-phosphate (S1P), thereby improving insulin sensitivity, inhibiting inflammation. However, the role of adiponectin in controlling fatty ceramides is unclear. Overexpression of adiponectin in the adipose tissue of ob/ob mice reduces the thickness of AT and systemic inflammation and promotes the accumulation of fat in subcutaneous fatty deposits, including smaller adipocytes, which leads to improved sensitivity to systemic insulin and pancreatic β-cell survival [43]. However, the physiological effect of endogenous adiponectin derived from adipocytes on AT is not known.

When studying the molecular mechanisms underlying the insulin-sensitizing effect of adiponectin, there is growing evidence that adiponectin activates intracellular signaling pathways by activating AMPK and p38MAPK in skeletal muscle cells [53]. Stimulation of glucose utilization and fatty acid oxidation by adiponectin is mediated by AMPK and p38MAPK [44]. An increased expression of AMPK in C2C12 myotubes reduces the insulin-sensitizing effect of adiponectin [44]. In addition, blocking of the AMPK pathway inhibits adiponectin-induced insulin-sensitizing effects [54]. Thus, at present, the insulin-sensitizing effects of adiponectin have been studied only on peripheral tissues such as muscles and liver. It has been proven that a deletion of the adiponectin gene impairs insulin signaling simultaneously with a decrease in AMPK activation in insulin-sensitive, but not insulin-resistant, adipocytes. However, the autocrine effects of adiponectin on glucose uptake by adipocytes and insulin signaling have not been fully elucidated.

ADIPONECTIN AND ITS RECEPTORS AS A THERAPEUTIC TARGET IN CVD AND T2DM

Several strategies are considered to enhance the beneficial effects of adiponectin, including increasing both its plasma level and its activity. The levels of circulating adiponectin can be increased either by directly using exogenous adiponectin, for example, by injection, or by increasing endogenous adiponectin through treatment. Due to a high level of circulating blood and multimeric conformations of adiponectin, the direct use of exogenous adiponectin is difficult. Thus, increasing the level of endogenous adiponectin through the use of pharmacological agents, nutraceutical compounds and lifestyle modifications remains

the best option. Pharmaceutical products effective to increase circulating adiponectin include PPAR-α thiazolinedione (TZD) agonists, renin-angiotensin inhibitors such as angiotensin converting enzyme inhibitors (ACE inhibitors) and angiotensin II receptor blockers (ARBs) [55, 56].

In addition, the effect of statins is being actively discussed, and so recently we have shown that early use of statins in patients with MI leads to a significant increase in adiponectin levels and the adiponectin/leptin ratio, which is considered as a favorable effect of atorvastatin, which helps to reduce adipokine imbalance, normalize lipid exchange and reduce IR [57].

Among nutraceutical compounds, fish oil, linoleic acid, green tea extract, polyphenol resveratrol and osmotin, a representative of plant defense proteins, have recently been identified as potential adiponectin receptor agonists that can increase the concentration of adiponectin [58]. In addition, weight loss or physical activity can increase adiponectin levels, especially among people with obesity or diabetes [58].

An alternative approach to enhancing the beneficial effects of adiponectin is to enhance its transmission through compounds that may affect AdipoR. Treatment with PPARγ agonists, such as pioglitazone and rosiglitazone, increases plasma adiponectin levels and also increases insulin sensitivity in patients with IR and diabetes [58]. In addition, treatment with pioglitazone increases plasma adiponectin levels. Lin et al. showed that administration of rosiglitazone increases adiponectin mRNA levels in differentiated 3T3-L1 adipocytes for 1 day [59].

It has also been shown that insulin negatively regulates the HMW adiponectin complex. Accordingly, thiazolidinedione (TZD) mediated improvement in insulin sensitivity correlated with HMW concentration of adiponectin [59]. Although TZD is a widely used class of antidiabetic drugs, most patients do not show an improvement in insulin sensitivity [49]. The mechanism by which TZD stimulates an increase in adiponectin levels is unknown, but the secretory pathway of adipocytes appears to be the main site of action.

Pharmacological activation of AMPK by metformin has therapeutic potential for eliminating metabolic disorders, such as T2DM and non-alcoholic fatty liver disease. AMPK directly phosphorylates enzymes and transcription factors involved in the absorption of glucose and fatty acids and their mitochondrial metabolism by switching catabolic pathways [60]. It also disables the synthesis of glucose, glycogen and lipids in the liver through anabolic pathways and promotes the absorption of glucose in the muscles. It is also reported that metformin improves insulin sensitivity by activating AMPK, thereby inhibiting the synthesis of fatty acids and triglycerides and promoting fat oxidation [61].

CONCLUSION

Over the last few years, adiponectin has been of greatest clinical interest due to its positive regulatory effect in certain conditions, including IR. Some of the problems associated with the molecular and cellular mechanisms underlying the functioning of the insulin-adiponectin system can be taken into account as potentially useful in the development of new pharmacological approaches. Further study of the effect of insulin on adipokines is needed to fully elucidate the molecular mechanisms of biosynthesis, secretion and signal transduction and their potential therapeutic value.

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Emergency roentgen-endovascular clot aspiration in cardioembolic stroke

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ABSTRACT

A case report of successful intravascular aspiration in a patient with acute cardioembolic stroke is presented. Non-occlusive critical thrombosis of the extracranial segment of the left internal carotid artery with distal total embolization of the medial cerebral artery was verified. During the intervention, the dislocation of thrombotic masses into the supraclinoid segment occurred.

Key words: atrial fibrillation, cardiac embolism, ischemic stroke, cerebral angiography, thromboembolism, thrombectomy, internal carotid artery.

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Неотложная рентгенэндоваскулярная тромбаспирация при ишемическом кардиоэмболическом инсульте

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РЕЗЮМЕ

Представлено клиническое наблюдение успешной внутрисосудистой аспирации у пациента с острым ишемическим инсультом кардиоэмболического генеза. Верифицирован неокклюзивный критический тромбоз экстракраниального сегмента левой внутренней сонной артерии с дистальной тотальной эмболизацией средней мозговой артерии. В ходе проведения вмешательства произошла дислокация тромботических масс в супраклиноидный сегмент.

Ключевые слова: фибрилляция предсердий, кардиоэмболия, ишемический инсульт, внутренняя сонная артерия, тромбоаспирация, тромбоэкстракция.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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INTRODUCTION

Stroke remains one of the leading causes of death worldwide, being only slightly behind coronary heart disease. Disability, rehabilitation costs, loss of social activity and work capacity are factors that strike both the patient's family and the economy of the state as a whole.

Annually in the United States, about 800,000 strokes are recorded, in the European Union there are about 1,000,000 [1], and in Russia, more than 450,000. Ischemic stroke accounts for 80% of them. Elimination of acute thrombogenic occlusion of the intracranial artery as a cause of cerebral infarction is the main goal of endovascular intervention [2], and introduction of intravascular methods for treating ischemic stroke (IS) in the last decade has led to a decrease in mortality from acute ischemic cerebrovascular accident (CVA) of cardioembolic or atherothrombotic genesis [1].

Restoration of blood flow through a stroke-dependent artery at the early stages provides reduction of the infarction area due to preservation of the penumbra, the zone of damage to the brain matter. The immediate outcome of patient's rehabilitation depends on this restoration [3].

Intravenous thrombolytic therapy (ITT) is recommended as a standard method of treatment in the absence of contraindications in the acute period of IS [4, 5]. Thrombolysis within 4.5 hours from the onset of clinical manifestations significantly improves the outcome of the disease [6].

Endovascular contact (aspiration) thromboextraction and mechanical thrombectomy (MTE) are modern methods of treating IS contributing to rapid recovery of patients and reducing the risk of an unfavorable outcome.

CLINICAL CASE

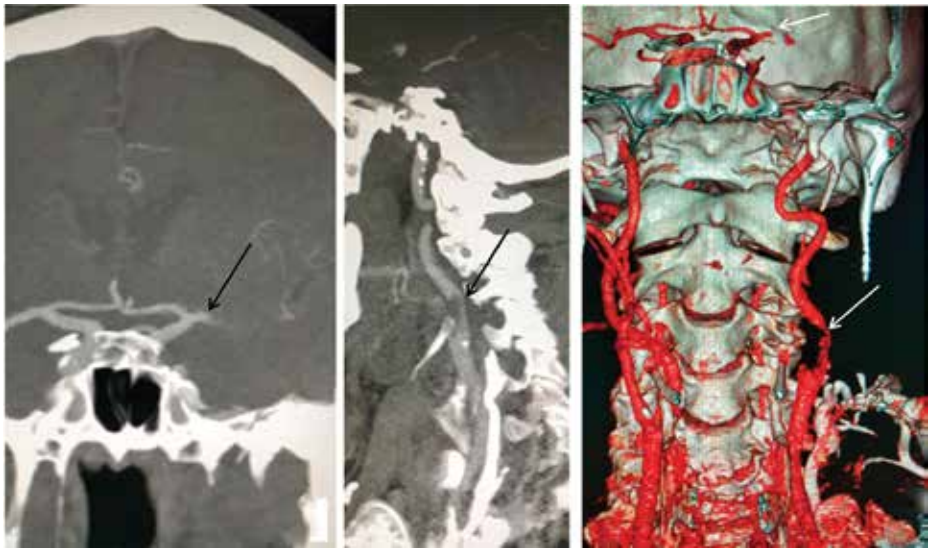
A 64-year-old patient was delivered by an ambulance team to the Irkutsk Regional Clinical Hospital with the acutest stage of ischemic stroke, 1 hour 45 minutes after the onset of clinical manifestations. Examination by a neurologist revealed right-sided hemiparesis, aphasia, and stupefaction. Medical history included persistent atrial fibrillation and irregular intake of anticoagulants.

Computed tomography (CT) of the brain was performed, including CT angiography. According to clinical guidelines, blood was taken to perform a set of emergency laboratory tests.

CT angiography showed thrombosis of the M1 segment of the middle cerebral artery (MCA) on the left, critical stenosis with signs of an ulcerated plaque in the initial segment of the internal carotid artery (ICA) on the left with a loss of lumen up to 90%, and occlusion of the external carotid artery on the left (Fig. 1).

The assessment of early signs of cerebral ischemia according to the Alberta Stroke Program early computed tomography scale (ASPECTS) [7] reached 8 points. The total score according to the National Institutes of Health Stroke Scale (NIHSS) was 19, according to the Glasgow Coma Scale, it was 10–14 (moderate to severe stupefaction). The index according to the modified Rankine scale was 5 (dramatically impaired vital activity, the patient is bedridden and constantly needs assistance of medical personnel).

The main vital signs were assessed: the level of blood pressure, heart rate, and an ECG was performed.



a *b* *c*

Fig. 1. CT angiogram upon admission: occlusion of the M1 segment of the middle cerebral artery (arrow, *a*); clot in the internal carotid artery (arrow, *b*); 3D reconstruction, occlusion of the M1 segment of the middle cerebral artery and clot in the internal carotid artery (arrows, *c*)

The principal diagnosis: ischemic (probably cardioembolic) stroke in the MCA basin on the left; atherosclerosis of the cerebral vessels; hypertensive disease, stage 3-1, risk 4; severe right-sided hemiparesis; aphasia; stupefaction. Secondary diagnosis: ischemic heart disease; persistent atrial fibrillation; hypertension, stage 3, risk 4.

Taking into account the clinical data and the results of X-ray and laboratory studies (the international normalized ratio of 1.5; VTT is contraindicated), it was decided to carry out endovascular reperfusion. The patient was delivered to the interventional radiology suite.

Puncture of the femoral artery was performed 3 hours after the onset of clinical manifestations of IS. After insertion of the 8F-introducer, under intravenous sedation, polypositional carotid angiography was performed on the left. Common carotid artery (CCA) was without pathology. External carotid artery showed occlusive thrombosis. In the projection of the initial extracranial segment of the ICA from the orifice, non-occlusive 18 × 5 mm thrombosis with a loss of arterial lumen up to 90% was visualized (blood flow according to the arterial occlusive lesion (AOL) score was 1). Pronounced tortuosity of the extra- and intracranial segments of the ICA was detected without stenotic-occlusive lesions. The blood flow through the anterior cerebral artery (ACA) was without changes. Occlusive thrombosis of the M1 segment of the MCA was visualized (AOL score – 0). There were no visible collaterals in the ischemic zone (collateral flow grading system – 0).

It was decided to perform contact thromboaspiration (CTA) under endotracheal anesthesia. Blood pressure was 140–150 mm Hg. A Neuron MAX guiding catheter (Penumbra, USA) was placed in the left CCA. An ACE68 reperfusion catheter (Penumbra) and a 3MAX microcatheter (Penumbra) were passed to the ICA thrombosis using a Fielder FC coronary guide wire (Asahi). After removing the microcatheter and the guide wire, the aspiration catheter was fixed at the blood clot and connected to the Penumbra MAX pump. Aspiration took place for 5 min. The catheter was removed, no thrombotic masses were produced. Control angiograms showed displacement of the blood clot into the supraclinoid segment of the ICA. ACA and MCA on the left are not contrasted (Fig. 2).

A triaxial system was installed in the ICA, an aspiration catheter was passed to the clot, and double thromboaspiration (up to 4 min) was performed from the supraclinoid section of the ICA. During the second attempt, a clot up to 4 cm long was aspirated (Fig. 3).

Control carotid angiography revealed restoration of blood flow along the ICA and ACA. MCA was occluded in M1. Intravascular thromboaspiration was performed from the MCA basin (M1) for 4.5 minutes. A blood clot up to 7 cm long was aspirated. Control angiograms showed complete restoration of blood flow in the ICA, ACA, and MCA on the left (AOL

score – 3, mTICI scale – 3). There was no extravasation of the contrast, and the venous phase of contrasting occurred in a timely manner (Fig. 4).

Blood pressure at the time of recanalization was 110–115 mm Hg. The time from the onset of IS to complete restoration of blood flow in the basin of the left internal carotid artery was 4 hours 47 minutes.

The patient was transferred to the intensive care unit and extubated 15 hours later. The patient's status was adequate and he was available for contact. Speech disorders included dysarthria and elements of aphasia with pronounced regression. Right-sided hemiparesis decreased to moderate.

CT was performed: hemorrhagic transformation in the area of the basal nuclei on the left (size 14 × 13 mm), manifestation of cerebral atherosclerosis and dyscirculatory encephalopathy, external and internal hydrocephalus (Fig. 5).

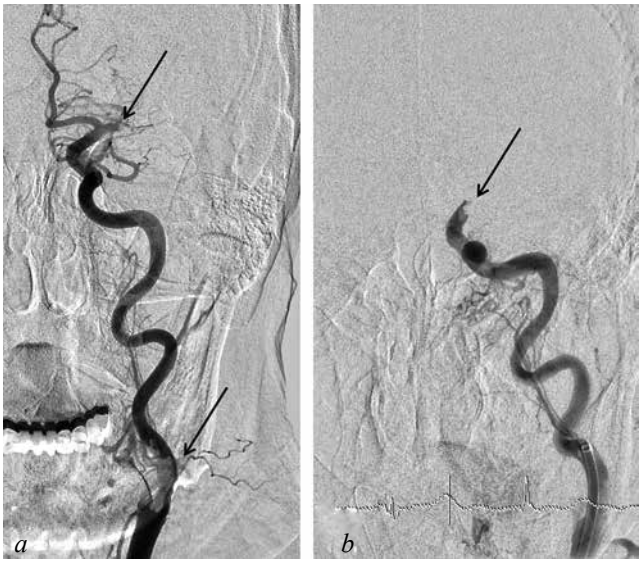


Fig. 2. Initial carotid angiography on the left, clot in the internal carotid artery and occlusion of the M1 segment of the middle cerebral artery (arrows, *a*); displacement of a clot in the internal carotid artery into the supraclinoid section of the internal carotid artery (arrow, *b*)

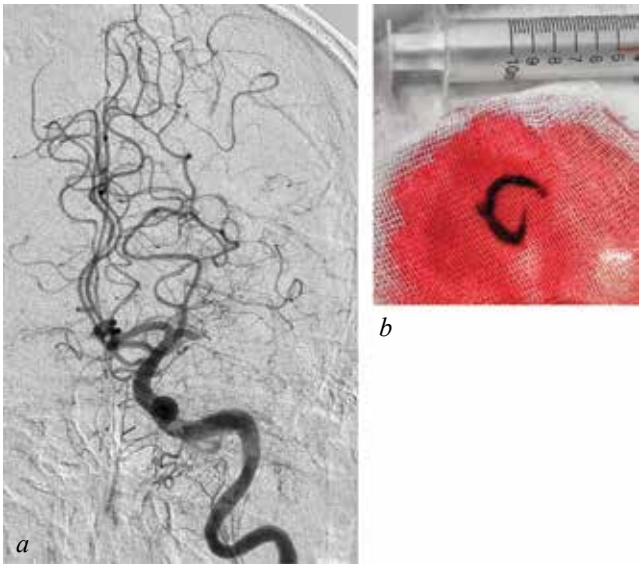


Fig. 3. Carotid angiography on the left after thromboaspiration from the supraclinoid section of the internal carotid artery (*a*); aspirated clot (*b*)

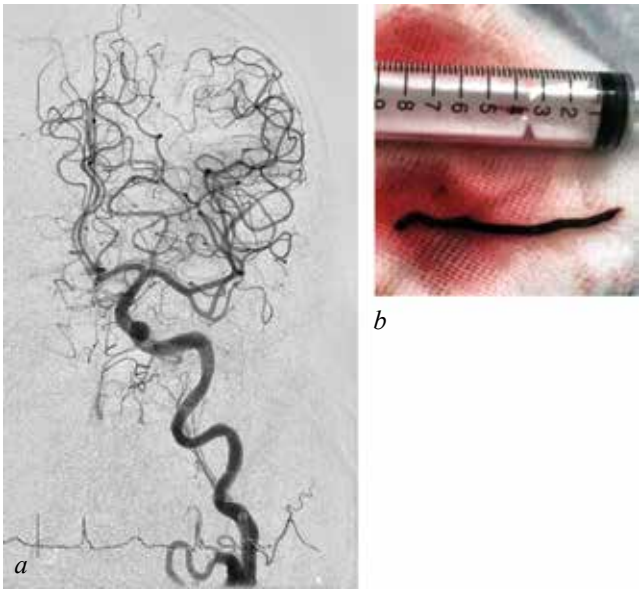


Fig. 4. Carotid angiography on the left after thromboaspiration from the M1 segment of the middle cerebral artery on the left (*a*); aspirated clot (*b*)

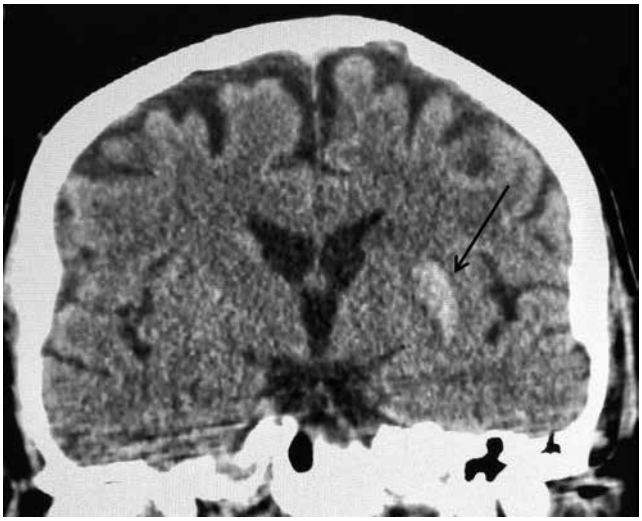


Fig. 5. CT scan 1 day after endovascular thromboaspiration. The focus of hemorrhagic transformation (arrow)

After 3 days the patient was transferred from the intensive care unit to the hospital. At the time of discharge, no speech disorders were detected. Moderate right-sided hemiparesis was observed. The total score according to the NIHSS Stroke Severity Scale was 7. The index according to the modified Rankine scale was 3.

DISCUSSION

The cardioembolic type of acute cerebrovascular events makes up to 20% of all transient ischemic attacks and 12–31% of all IS. The main risk factors for cardioembolic strokes are atrial fibrillation and myocardial infarction [8, 9].

In the presented clinical case of a patient with atrial fibrillation against the background of irregular intake of anticoagulants, an acute cerebrovascular event developed, which made it possible to suspect the cardioembolic nature of IS. The hypothesis was confirmed by the results of CT angiography, where both occlusion of the external carotid artery and tandem lesion of the ICA (non-occlusive thrombosis in the initial section with distal total MCA embolization) were found. Taking into account the therapeutic window, it was decided to perform reperfusion of the target artery. In the presence of contraindications to ITT, on the one hand, and low efficiency of ITT with cardioembolic lesions, on the other, endovascular reperfusion is the most substantiated method.

Endovascular thrombectomy should be performed by experienced interventional radiologists in an operating room equipped with the necessary angiographic equipment and supplies. Endotracheal anesthesia is often required. During the intervention, a hard-to-stop spasm of the carotid artery may occur along with a need for its dissection and fragmentation of a clot with distal embolization and aggravation of neurologic deficit.

To date, two most well-studied methods of endovascular reperfusion in IS are contact thromboaspiration and mechanical thrombectomy (MTE). MTE showed the most promising results when using the Solitaire FR (EV3, USA) and Trevo Pro (Stryker, USA) stent retrievers. No significant differences were established between the Penumbra thromboaspiration system and stent retrievers [10].

In the "Trevo versus Merci retrievers for thrombectomy revascularization of large vessel occlusion (TREVO-2)" study, the Trevo stent retriever achieved TICI 2–3 recanalization rate in 86% of cases, and a good clinical outcome after 90 days was noted in 40% of patients with 90-day mortality of 34.1% [4, 11]. The use of the Penumbra aspiration system in a clinical trial allowed for recanalization of the obstructed artery to TIMI 2–3 rate in 87% of cases with a good clinical outcome after 90 days in 41% of patients and a mortality rate of 20% [2, 12, 13].

At the initial stage, the tactics of contact thromboaspiration with the use of a Penumbra large-bore aspiration catheter was chosen (the unit has necessary tools for performing mechanical thrombectomy, including a balloon guide catheter). After the catheter was passed through to the clot and aspiration was performed, a complication arose: dislocation of the clot with total occlusion of the supraclinoid segment. It is possible that MTE with a stent retriever against the background of reverse blood flow using a balloon catheter made it possible to perform the intervention more effectively at the initial stage. However, another strategy was chosen. At the same time, after three additionally performed sessions of thromboaspiration, complete restoration of cerebral blood flow in the left carotid basin was achieved with regression of neurologic deficit and restoration of the main vital functions.

CONCLUSION

The presented clinical case demonstrates the likelihood of dislocation of thrombotic masses during contact thromboaspiration in patients with complicated tandem lesions of ICA. Only multiple, sequential thromboextractions can help restore the main blood flow.

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Optimization of immunosuppressive therapy during the third kidney transplant in the early postoperative period. Clinical observation

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ABSTRACT

The choice of immunosuppressive therapy is determined by the degree of sensitization to the histocompatibility gene complex on chromosome 6 (HLA). The risk of rejection in the early periods after surgery increases for the patients with repeated kidney transplantation. Optimizing immunosuppressive therapy is the only way to prolong the life of a patient with a terminal stage of chronic renal failure. имости на 6-й хромосоме (HLA). The analysis of a clinical case of a 47-year-old patient who was undergoing treatment at the N.V. Sklifosovsky Scientific Research Institute of Emergency Medicine after the third allotransplantation of a cadaveric kidney in 2016 was performed. The patient was diagnosed with chronic glomerulonephritis (IgA-nephropathy) chronic end-stage renal failure; in the early postoperative period, in addition to basic immunosuppression, anti-lymphocytic polyclonal antibodies were prescribed in combination with plasmapheresis sessions for the treatment and prevention of acute rejection crisis in the early postoperative period. For the first time, in order to prevent the development of an acute rejection crisis and minimize infectious complications of immunosuppressive therapy in the recipient after the third kidney transplant, plasmapheresis sessions were used using a plasmapheresis filter with a polymethylacrylate membrane in combination with a short course of polyclonal antibodies.

Key words: kidney transplantation, immunosuppressive therapy, sensitization of the patient.

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Оптимизация иммуносупрессивной терапии при выполнении третьей пересадки почки в раннем послеоперационном периоде. Клиническое наблюдение

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РЕЗЮМЕ

Выбор иммуносупрессивной терапии определяется степенью сенсибилизации к комплексу генов гистосовместимости на 6-й хромосоме (HLA). У пациентов при повторной пересадке почки риск отторжения в ранние сроки после операции увеличивается. Оптимизация иммуносупрессивной терапии – единственный путь продления жизни пациента с хронической почечной недостаточностью в терминальной стадии. Проведен анализ клинического случая пациента 47 лет после выполнения третьей аллотрансплантации трупной почки в 2016 г., находившегося на лечении в НИИ СП им. Н.В. Склифосовского с диагнозом «хронический гломерулонефрит (IgA-нефропатия), хроническая почечная недостаточность, терминальная стадия». В раннем послеоперационном периоде помимо базовой иммуносупрессии были назначены антилимфоцитарные поликлональные антитела в сочетании с сеансами плазмафереза для лечения и профилактики острого криза отторжения в ранние сроки после операции.

Впервые с целью профилактики развития острого криза отторжения, минимизации инфекционных осложнений иммуносупрессивной терапии у реципиента после третьей пересадки почки были применены сеансы плазмафереза с использованием плазмофильтра с полиметокрилатной мембраной в сочетании с коротким курсом поликлональных антител.

Ключевые слова: трансплантация почки, иммуносупрессивная терапия, сенсибилизация пациента.

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INTRODUCTION

Every year the number of patients on the waiting list for a second kidney transplant increases. Despite the emergence of new generations of immunosuppressive drugs (mycophenolic acid, daclizimab, basilixiimab, and tacrolimus) and plasmapheresis using a plasma filter with polymethylacrylate membrane (PMMA), the question of the second kidney transplantation remains open. Patients in this category can be considered as belonging to a group with a high risk of developing an acute rejection crisis (ARC) in the early postoperative period. Rejection is the main problem in the early postoperative period and is one of the causes of early graft loss. Circulating immune complexes and antibodies being directed at endothelial, HLA or other renal antigens are involved in the rejection mechanism. As a result, acute angitis with damage to small and medium arteries of the kidney transplant, often with associated glomerulitis [1].

In clinical transplantology, various methods have been proposed for the prevention of ARC in this category of patients. Extracorporeal therapies have been successfully used in combination with basic immunosuppressive therapy or anti-lymphocytic drugs have been used [2, 3]. However, these treatments were not always effective, and given their high cost, they could not be used in all cases. It should be borne in mind that it is impossible to prescribe mono- or polyclonal anti-lymphocytic drugs in order to prevent ARC in sensitized recipients during repeated kidney transplantations due to the formation of an antibody titer [1, 3, 4].

CLINICAL OBSERVATION

Patient D., 47 years old (born in 1969), was admitted in May 2016 to the Department of Kidney and Pancreas Transplantation to undergo third kidney transplantation. The clinical diagnosis was “chronic glomerulonephritis (IgA nephropathy). Chronic renal failure (CRF), end stage. Condition after two kidney allotransplantations (ATP) (in 1997, 2008). Subcompensated steroid diabetes mellitus. Secondary anemia. Secondary arterial hypertension. Condition after tumour excision in the parietal region. Chronic viral hepatitis B and C. Superficial gastritis. Chronic reflux esophagitis. Axial cardiac hernia of the esophageal diaphragm”.

From the anamnesis of the disease, it is known that the patient has been ill since 1996, when he was first diagnosed with chronic glomerulonephritis with signs of CRF. In 1997, the first allotransplantation of a cadaveric kidney was carried out. In the early postoperative period, the patient was diagnosed with ARC, and pulse therapy with methylprednisolone at a dose of 3 g was carried out, antilympholine was used with the total dose of 1.6 g. After therapy, there was a recovery in daily diuresis and a decrease in creatinine to 0.15 mmol/L.

The patient was discharge from the hospital with satisfactory kidney transplant function. Since 2000, signs of kidney transplant dysfunction have been noted in the form of the appearance of proteinuria and an increase in creatinine to 0.250 mmol/L. In 2001, a biopsy of a kidney transplant was performed for diagnostic purposes, which showed morphological signs of recurrent glomerulonephritis (IgA nephropathy) of the kidney transplant. Symptomatic and immunosuppressive therapy was carried out. In 2006, pain in large and small joints increased, and gross hematuria was intermittently observed. When examined, the patient was diagnosed with gouty arthritis, allopurinol was prescribed.

Over the next few months, creatinine level increased in blood to 0.68 mmol/L and glomerular filtrate rate (GFR) dropped to 8 ml/min. Recurrent CRF of a kidney transplant was diagnosed, and renal replacement therapy was prescribed. Hemodialysis therapy continued.

In July 2009, the second allotransplantation of a cadaveric kidney was performed. The immediate function of the graft with a gradual decrease of azotemic wastes and prolonged healing of the postoperative wound was noted. The patient was prescribed a 3-component immunosuppression scheme: cyclosporine (CyA), mycophenolic acid (MF), and prednisolone (PM). When discharged from the hospital on day 42, the creatinine level was 0.08 mmol/L. The concentration of cyclosporine in the blood (CyA) was 144 ng/ml. Two months later, the patient noted pain in the area of the postoperative wound. The ultrasound data revealed an increase in the size of the kidney transplant, with creatinine increase to 0.15 mmol/L, the appearance of proteinuria up to 0.3 g per day and erythrocyturia. In this regard, the patient underwent a kidney transplant biopsy. Based on the biopsy results, an acute rejection crisis was verified. The patient was prescribed a pulse therapy with methylprednisolone (MP), the total dose of which was 1.5 g. Since 2013, recurrent chronic renal failure was diagnosed and treatment with program hemodialysis (PHD) was started. Then, the indications for the third allotransplantation of a cadaveric kidney were determined and the patient was referred to N.V. Sklifosovsky Scientific Research Institute of Emergency Medicine.

In May 2016, the third allotransplantation of a cadaveric kidney was performed on the left. The term of cold ischemia of the kidney transplant was 20 hours, crossmatch test was negative, mismatch A, A, B, Drb1. Given the high risk of developing ARC in the early postoperative periods, to prevent ARC, the patient was prescribed intravenous thymoglobulin at a dose of 50 mg per day for 5 days as well as MP pulse therapy (at the total dose of 1 g). Due to the increase level of azotemic residues, early after the transplantation the patient underwent hemodialysis using dialyzers based on polymethylacrylate membranes (HD-PMMA) No. 4. During the first 18-24 hours after the surgery, tacrolimus was prescribed at a dose of 0.1 mg/kg per day. From the first day after surgery, the recipient received a 3-component scheme of immunosuppression: prednisolone at a dose of 0.6 g/kg per day, CellCept at a dose of 2 g per day, and tacrolimus. During therapy, the initial function of the graft with a slow decrease in azotemic wastes was noted. Recovery of diuresis was traced from 2 days after surgery. Daily diuresis was sufficient, up to 2000–2500 ml per day with stimulation with loop diuretics from 120 to 60 mg on the first day after surgery. Blood pressure (BP) in the postoperative period changed with the use of combined antihypertensive therapy; it was not higher than 115/75 mm Hg. Within one month after surgery, the blood pressure remained stable, not lower than 110/65 mm Hg, not exceeding 125/75 mm Hg, the weight was from 65 to 63 kg. The healing of postoperative wound was carried out by secondary intention, on the 21st day, a divergence of skin sutures in the upper and middle third of the wound was revealed.

In ultrasound examination of the kidney transplant, the dimensions remained the same throughout the entire observation period in hospital: 134 × 60 × 17 mm; the contours of the kidney transplant were clear and even; the calices-pelvis system was not expanded; the pelvis size was not more than 1.4 cm, and the resistance index was 0.52–0.68. The main arteries in the opening was not located, and the venous outflow was not disturbed. On the 14th day after surgery, dynamic nephroscintigraphy of the kidney transplant was

performed, which showed satisfactory perfusion and moderate impairment of the excretory function of the graft, GFR was 42 ml/min. X-ray examination of the chest organs revealed an expansion of the shadow of the heart in diameter due to the left sections and signs of calcification of the aorta. The patient was discharge in a satisfactory condition on day 31. Observations of the patient in the next 12 months revealed intact graft function with satisfactory condition and well-being of the patient.

DISCUSSION

The patient was assessed in the "waiting list" as a recipient with a very high immune risk of developing ARC in the early periods after allotransplantation. Given acute and chronic rejection crises in the anamnesis, confirmed by biopsy, and also a high titer of preexisting antibodies before performing a third kidney transplantation. Intravenous administration of polyclonal antibodies and plasmapheresis sessions are used as prevention and therapy of acute rejection reactions in repeated kidney transplantations [1, 4, 5].

We used methylprednisolone pulse therapy without the use of polyclonal antibodies as a treatment and prevention of ARC during the first two allotransplantations. Therefore, for the third allotransplantation of a cadaveric kidney, thymoglobulin and hemodialysis (HD-PMMA) were used as the fourth component of immunosuppressive therapy. The mechanism of thymoglobulin effect causes a decrease in the number of lymphocytes involved in the cascade of T-cell activation in the graft rejection reaction, such as CB2, CB3, CB4, CB8, CB11a, CB, B 25, HLA DR- and HLA Dr1-class.

In addition, thymoglobulin causes the activation of the functions of lymphocytes associated with their immunosuppressive activity. Therefore, *in vitro* thymoglobulin at a concentration of about 0.1 mg/ml activated T-lymphocytes and stimulated their proliferation (the same is for CD4+ и CD8+ subpopulations) with the synthesis of interleukin-2 and expression of CD-25. This mitogenic activity is mainly realized through CD-2 [2]. In our case, thymoglobulin was prescribed to prevent ARC of the kidney transplant at a dose of 1 mg/kg per day for 5 days after the kidney transplantation with preliminary intravenous administration of glucocorticoids and antihistamines. In addition to the use of thymoglobulin, dialyzers with PMMA membranes were used for the first time during four hemodialysis sessions. Hemodialysis was carried out on the Artificial Kidney apparatus for 4 hours with dialyzer type BK-2,1 F TORAY [5]. During the first two weeks of treatment, the patient showed a pronounced decrease in the absolute number of all lymphocyte populations by more than 80%. 21 days after surgery, the level of leukocytes in the blood did not exceed 2.78×10^9/L, with the number of lymphocytes not exceeding 4.3%. At the same time, the amount of antibodies to HLA was monitored weekly in the course of treatment. Before the procedure, the class II antibody titer was more than 8585 [3]. With the complex therapy and after four sessions of hemodialysis the antibody titer decreased to 1468.

Considering such significant predictors as the initial kidney transplant function, the absence of an acute rejection crisis in the early postoperative periods, normalization of azotemic wastes and the absence of proteinuria, it is possible to assume an optimistic prognosis for assessing the outcome of the third kidney transplantation. The use of hemodialysis sessions using dialyzers based on PMMA membranes and prescription of short courses of polyclonal antibodies made it possible to avoid the development of irreversible acute rejection, the development of infectious complications and loss of kidney transplant function.

CONCLUSION

Prevention of acute rejection crises made it possible to perform a third kidney transplantation to the patient with a high immune risk of developing ARC. In order to optimize immunosuppressive therapy in the early postoperative period, the patient underwent hemodialysis sessions with dialyzers based on PMMA membranes. The complex therapy made it possible to prevent the development of acute rejection crisis in the early period after allotransplantation of a kidney and to minimize infectious complications of the 4-component scheme of immunosuppressive therapy.

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АВТОРОМ ПЕРВОГО В РОССИИ УЧЕБНИКА ПО БИОИНФОРМАТИКЕ ДЛЯ МЕДИЦИНСКИХ СПЕЦИАЛЬНОСТЕЙ СТАЛ УЧЕНЫЙ СИБГМУ

Издание и. о. заведующего кафедрой медицинской и биологической кибернетики Сибирского государственного медицинского университета, доктора медицинских наук Наталии Часовских впервые в России опубликовано в качестве учебника по биоинформатике для медицинских специальностей.

Наталия Часовских

«Основательная работа с изданиями и учебными материалами по биоинформатике ведется мной на протяжении пяти лет. Несмотря на перспективность данной области знаний, существует нехватка информационных ресурсов по теме, и, как следствие, возникает необходимость обращения к зарубежным источникам. Поэтому актуальным стал вопрос о создании такого труда, который позволил бы на доступном уровне познакомиться с основами биоинформатики. При подготовке учебника использовался материал ранее изданных собственных пособий, а также тематические публикации на английском языке и информация от разработчиков биоинформационных инструментов», – отметила Наталия Часовских.

Это первый учебник, рекомендованный Координационным советом по области образования «Здравоохранение и медицинские науки» к использованию в образовательных учреждениях для таких направлений подготовки, как «Медицинская биохимия», «Медицинская биофизика», «Медицинская кибернетика». Учебник также может быть рекомендован студентам технических специальностей, интересующимся медико-биологическими дисциплинами.

«Для медицинских вузов отечественных учебников по биоинформатике не существовало, в образовательной сфере применялись книги зарубежных авторов, переведенные на русский язык достаточно давно (5–10 лет), что для стремительно развивающейся дисциплины является крайне неэффективным. С появлением учебника основная задача – поддерживать актуальным его содержание, так как биоинформатика – одна из динамично развивающихся областей», – подчеркнула Наталия Часовских.

Современное издание составлено не только с учетом систематизации имеющегося опыта, но и адаптировано для медицинской науки. Такая практика позволит студентам медицинских вузов освоить междисциплинарную область в контексте фундаментальной медицины и биологии, что способствует оптимизации образовательного процесса.

«Специальность «Медицинская кибернетика», обучение по которой ведется на кафедре медицинской и биологической кибернетики СибГМУ, является современной и перспективной, так как учитывает основные тренды развития нашего общества: цифровизацию, нарастание объемов данных, персонализированную медицину. Помимо учебного процесса, на кафедре ведутся биоинформационные исследования механизмов социально-значимых заболеваний, к которым привлекаются и обучающиеся. Наши выпускники востребованы в разных сферах деятельности: как системные аналитики в здравоохранении, специалисты по работе с медицинскими данными (статистики), разработчики и аналитики программного обеспечения в биомедицинской сфере, специалисты по биоинформационной аналитике, в том числе для фармацевтической промышленности. Очевидно, что с каждым годом потребность в таких специалистах будет только нарастать», – добавила автор.

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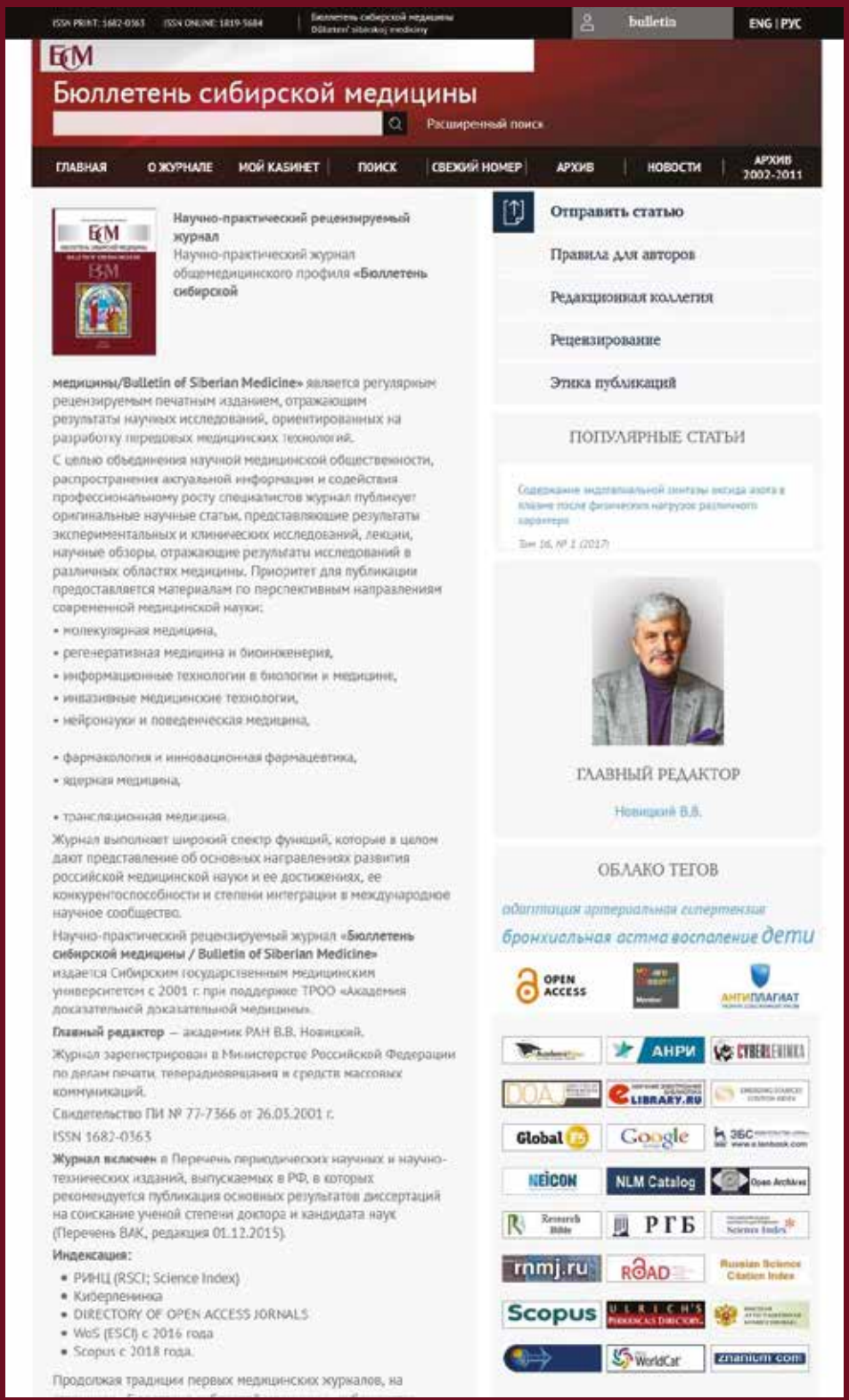

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