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Differential Diagnosis of Septic and Aseptic Bone Lesions of the Foot in Patients With Diabetic Foot Syndrome: the Potential of Using a Standardized Uptake Value with Osteotropic Radiopharmaceuticals

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ABSTRACT

Aim. The study was conducted to calculate standardized uptake values (SUVs) for foot bones, determine the optimal SUV type in patients with degenerative changes, and assess the potential of quantitative single-photon emission computed tomography (SPECT/CT) in patients with diabetic foot syndrome (DFS) complicated by osteomyelitis.

Materials and methods. The study design was prospective. Patients with a documented clinical diagnosis of diabetic foot and confirmed or suspected osteomyelitis underwent SPECT/CT scanning after intravenous injection of the radiopharmaceutical (^{99m}Tc – Pyrophosphate). The calculation of standardized uptake values – mean SUV (SUVmean), maximum SUV (SUVmax), and peak SUV (SUVpeak) – was performed using the SyngoVia software. To calculate the threshold standardized uptake value, receiver operating characteristic analysis (ROC) was conducted, followed by the calculation of the area under the ROC curve (AUC).

Results. Forty-eight patients were examined: 28 people with septic foot lesions and 20 individuals with aseptic foot lesions. Calculations revealed no statistically significant differences between the SUV values (max, mean, and peak) for septic and aseptic lesions. However, the standardized uptake value normalized by lean body mass (SUVlbm (max)) demonstrated the largest ROC AUC. A threshold value for differentiating between pathological and healthy bone tissues was 1.64, with sensitivity of 93.5% and specificity of 95.6%.

The threshold value for distinguishing between septic and aseptic inflammations in patients with diabetic foot syndrome was 4.35, with sensitivity of 82.4% and specificity of 80.3%.

Conclusion. The study confirmed that the use of SUVlbm (max) threshold value of 4.35 (Se = 82.4%; Sp = 80.3%; AUC = 0.883) is possible for the differential diagnosis of osteomyelitis and Charcot foot in patients with diabetic foot syndrome. Additionally, to confirm inflammation, a SUVlbm (max) threshold value of 1.64 (Se = 93.5%; Sp = 95.6%; AUC = 0.983) is applicable.

Keywords: Charcot foot, inflammation, osteomyelitis, radionuclide diagnosis

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Дифференциальная диагностика септического и асептического поражений костных структур стоп у пациентов с синдромом диабетической стопы: возможности применения стандартизированного уровня захвата остеотропного радиофармпрепарата

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РЕЗЮМЕ

Цель. Провести исследование с целью расчета показателей стандартизированных уровней захвата (SUV) для костей стоп и определения оптимального типа SUV у пациентов с дегенеративными изменениями и определить возможности количественной оценки однофотонной эмиссионной компьютерной томографии (ОФЭКТ/КТ) у пациентов с синдромом диабетической стопы осложненным остеомиелитом.

Материалы и методы. Дизайн исследования – проспективное. Пациентам с документально подтвержденным клиническим диагнозом диабетической стопы и наличием остеомиелита или подозрением на его наличие было проведено ОФЭКТ/КТ сканирование после внутривенного ведения радиофармпрепарата (^{99m}Tc – пирфотех). Расчет показателей стандартизированных уровней захвата: среднего SUV (mean), максимального SUV (max) и пикового SUV (peak) производился при помощи программного обеспечения SyngoVia. Для вычисления порогового значения стандартизированного уровня захвата выполнялся ROC-анализ с последующим расчетом площади под ROC-кривой.

Результаты. Обследованы 48 пациентов (28 с септическим поражением и 20 с асептическим поражением стоп). Расчеты показали, что статистически значимых отличий между значениями SUV (max, mean, peak) септического и асептического поражения не выявлено, при этом наибольшей площадью под ROC-кривой обладает стандартизированный уровень захвата, нормированный по безжировой массе тела (SUVlbm (max)). Определено пороговое значение для разграничения патологического очага от здоровой костной ткани, равное 1,64, с чувствительностью 93,5% и специфичностью 95,6%. Пороговое значение для разграничения септических и асептических воспалительных процессов у пациентов с синдромом диабетической стопы равно 4,35 с чувствительностью 82,4% и специфичностью 80,3%.

Заключение. Для дифференциальной диагностики остеомиелита и стопы Шарко у пациентов с синдромом диабетической стопы возможно применение порогового значения SUVlbm (max), равного 4,35 (Se = 82,4%; Sp = 80,3%; AUC = 0,883), а для установления факта воспалительного процесса – порогового значения SUVlbm (max), равного 1,64 (Se = 93,5%; Sp = 95,6%; AUC = 0,983).

Ключевые слова: стопа Шарко, остеомиелит, радионуклидная диагностика, воспаление

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

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INTRODUCTION

Diabetes mellitus is an endocrine disease characterized by relative or absolute deficiency of insulin due to dysfunction or destruction of β-cells. It is one of the fastest growing global health concerns in the 21st century [1]. The primary pathogenetic mechanisms involve impaired insulin secretion and insulin resistance. Chronic hyperglycemia in diabetes mellitus leads to damage, dysfunction, and insufficiency of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels [2].

Diabetic foot syndrome (DFS) is one of the most dangerous and severe complications of diabetes mellitus. According to the World Health Organization, approximately 422 million people worldwide suffer from diabetes mellitus, with 15–25% developing diabetic foot ulcers [3]. Despite advancements in complex surgical interventions, a significant percentage of patients (25–30%) still require above-the-knee amputations. One-year mortality rate following major lower limb amputation reaches 50% [4]. This results in patient disability and a significant reduction in the patient's quality of life.

A gold standard for evaluating diabetic foot pathology is three-phase bone scintigraphy. However, the specific pattern of radiotracer accumulation in the region of interest often results in low specificity for this diagnostic technique. The specificity of radionuclide imaging for inflammation can be improved using labeled leukocyte scintigraphy, typically performed with single-photon emission computed tomography (SPECT) [5]. Additionally, there is a method using radiolabeled antimicrobial peptides. These peptides can selectively bind to pathogenic microorganisms and can be successfully used for differential diagnosis of osteomyelitis. Due to its high cost, this method is not widely available in routine clinical practice. Positron emission tomography / computed tomography (PET/CT) with 18F-fluorodeoxyglucose (18F-FDG) has gained importance in diagnosing infections and inflammations regardless of their etiology or location. PET/CT provides precise anatomical localization and allows to assess spread of infection to soft tissues or bone. However, 18F-FDG PET/CT imaging has limitations, particularly in the evaluation of Charcot foot due to the intense uptake of 18F-FDG in this condition.

In recent years, the role of quantitative analysis of PET results using standardized uptake values (SUVs) has been explored in patients with diabetic foot. Studies have shown higher SUV values in patients with osteomyelitis compared to those with Charcot foot, suggesting that SUV is a valuable parameter for differentiating these conditions [6]. Currently, this parameter is widely used in diagnosis of oncological diseases, for example, in the differential diagnosis of lung neoplasms. SUV is a crucial parameter and is broadly applicable in clinical practice [7].

There are new methods for quantitative assessment of hybrid SPECT/CT images using SUVs. One of these methods is xSPECT Quant, which has demonstrated accuracy and reproducibility with an error margin of up to 3% for standardized quantitative analysis of radionuclide images. The xSPECT Quant is applicable to Technetium-99m (99mTc) imaging and allows for clinical quantitative SPECT/CT assessment for more precise disease detection and improved therapeutic management [8].

Currently, there are no universally accepted interpreting criteria for differential diagnosis of inflammation, osteomyelitis, and Charcot foot. Quantitative analysis of hybrid SPECT/CT images holds promise for significantly improving the diagnosis of septic and aseptic lesions of the diabetic foot. This study is aimed at demonstrating that utilizing standardized uptake values can enhance diagnosis of complicated diabetic foot progression.

MATERIALS AND METHODS

The study design was prospective, non-randomized, and controlled. The study included 48 patients with DFS: 147 regions of radiopharmaceutical uptake were analyzed in 28 patients with septic lesions, 113 regions – in 20 patients with aseptic lesions, and 160 regions exhibiting normal bone metabolism. All patients were examined and treated at the clinics of Siberian State Medical University.

The study was conducted using a SPECT scanner (Siemens Symbia Intevo Bold), with an intravenous injection of the radiopharmaceutical (^{99m}Tc – pyrophosphate). The intensity of radiopharmaceutical uptake was evaluated using the SUV. The following types of SUV were distinguished: SUV Body Weight (SUVbw) – normalized by body weight; SUV Body Surface Area (SUVbsa) – normalized by body surface area; and SUV Lean Body Mass (SUVlbm) – normalized by lean body mass [9]. The analysis included maximum, mean, and peak SUV values.

Statistical data processing was performed using the Medcalc software (version 22.023). Descriptive statistics for quantitative variables not following normal

distribution were presented as the median and the interquartile range $Me\,[Q_1; Q_3]$. Intergroup comparisons were conducted using the non-parametric Mann – Whitney *U*-test, with a significance level set at $p < 0.05$. The ROC analysis was performed to evaluate the prognostic value of SPECT/CT parameters.

RESULTS

At the first stage of the study, SUVs across all three groups (areas of septic and aseptic inflammation, as well as regions with normal bone metabolism) were analyzed using the Kolmogorov – Smirnov test to assess the normality of distribution. The test results indicated that the distribution of the studied parameters was statistically significantly not normal. Consequently, the data were described using medians and interquartile ranges $Me\,[Q_1; Q_3]$ (Table 1–3), while non-parametric tests were employed for intergroup comparisons. Even though the data did not follow a normal distribution, the ROC analysis was used to evaluate diagnostic performance of the quantitative variables, since the ROC analysis itself does not require any specific data distribution.

Subsequently, SUVs of lesions (regardless of aseptic or septic origin) were compared with healthy bone tissues using the ROC analysis (Fig. 1–3).

Table 1

Standardized Uptake Values in Regions with Normal Bone Metabolism, $Me\,[Q_1; Q_3]$

Parameter	SUV bw	SUV lbm	SUV lbm janma	SUV bsa
Max	1.45 [1.01; 2.18]	1.00 [0.72; 1.43]	0.96 [0.67; 1.37]	0.34 [0.24; 0.50]
Mean	1.12 [0.69; 1.69]	0.81 [0.52; 1.14]	0.74 [0.48; 1.06]	0.27 [0.18; 0.39]
Peak	1.28 [0.91; 1.94]	0.92 [0.68; 1.29]	0.87 [0.64; 1.22]	0.32 [0.23; 0.47]

Table 2

Standardized Uptake Values in Areas with Septic Lesions, $Me\,[Q_1; Q_3]$

Parameter	SUV bw	SUV lbm	SUV lbm janma	SUV bsa
Max	11.17 [7.91; 17.32]	8.11 [5.48; 11.24]	7.27 [5.32; 10.89]	2.82 [1.99; 3.99]
Mean	9.18 [6.68; 13.69]	6.53 [4.51; 9.50]	6.21 [4.39; 9.32]	2.34 [1.63; 3.16]
Peak	9.73 [6.76; 14.91]	6.93 [4.50; 9.91]	6.41 [4.35; 9.74]	2.35 [1.61; 3.55]

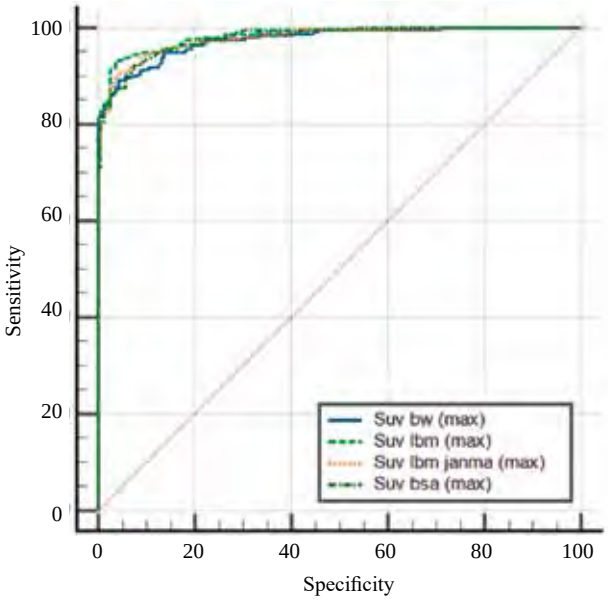


Fig. 1. Graph comparing ROC curves for maximum radiopharmaceutical SUVs: comparison of pathological areas with septic and aseptic inflammation with zones with normal bone metabolism – SUVmax. Here and in Fig. 2–5: X-axis – Specificity, Y-axis – Sensitivity

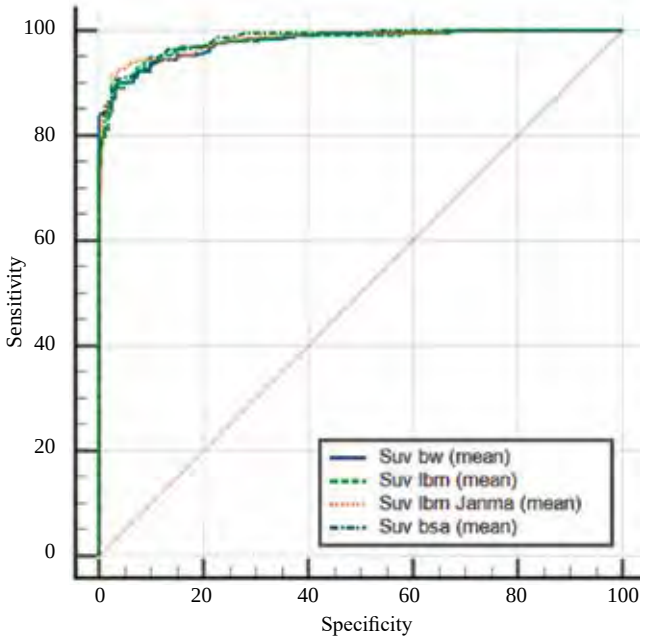


Fig. 2. Graph comparing ROC curves for mean radiopharmaceutical SUVs: comparison of pathological areas with septic and aseptic inflammation with zones with normal bone metabolism – SUVmean

Table 3

Standardized Uptake Values in Areas with Aseptic Lesions, *Me* [Q_1; Q_3]				
Parameter	SUV bw	SUV lbm	SUV lbm janma	SUV bsa
Max	5.84 [3.83; 7.84]	3.69 [2.78; 5.10]	3.59 [2.64; 4.90]	1.35 [0.88; 1.83]
Mean	4.80 [3.12; 6.24]	3.07 [2.09; 4.00]	2.94 [2.07; 3.89]	1.10 [0.71; 1.43]
Peak	4.91 [3.30; 6.80]	3.10 [2.22; 4.49]	3.13 [2.21; 4.31]	1.12 [0.77; 1.58]

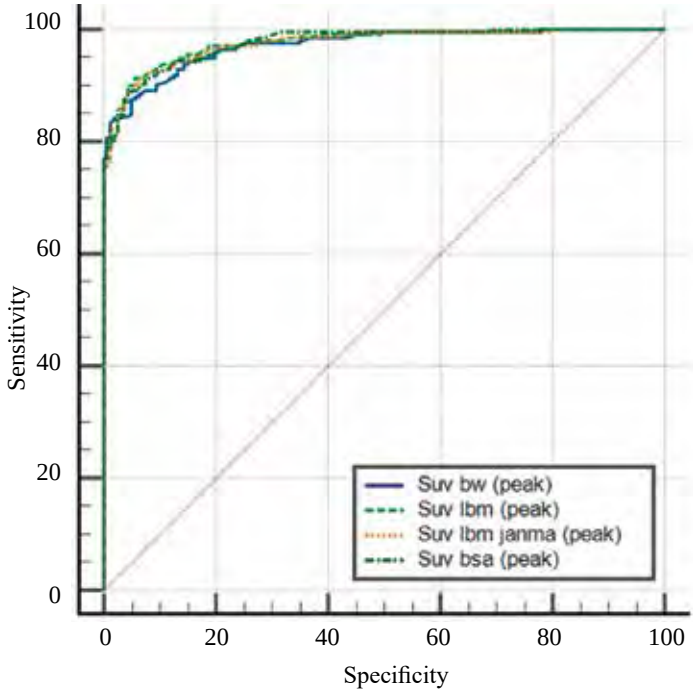


Fig. 3. Graph comparing ROC curves for peak radiopharmaceutical SUVs: comparison of pathological areas with septic and aseptic inflammation with zones with normal bone metabolism – SUVpeak.

Calculations revealed no statistically significant differences among the SUV parameters (Table 4). However, SUVlbm (max) demonstrated the largest area under the ROC curve (ROC AUC) and is suitable for establishing reference values.

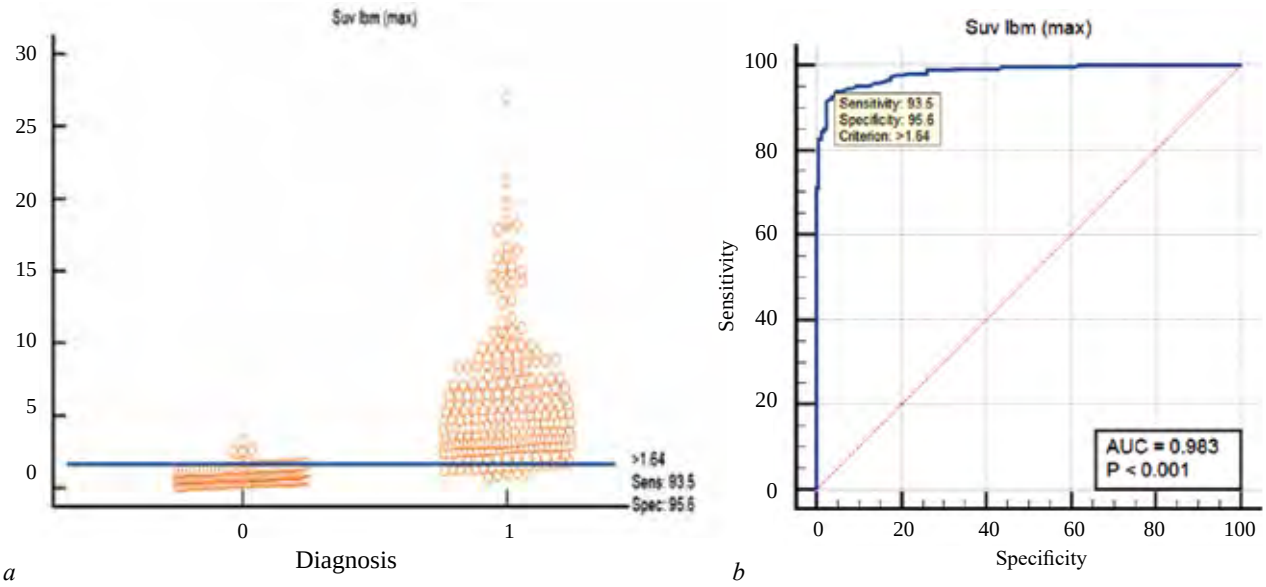
In the second stage of the study, the Mann – Whitney test revealed a statistically significant difference ($p < 0.05$) in SUVlbm (max) values in the pathological regions between patients with inflammation (septic/aseptic) and those with normal bone metabolism. Using the ROC analysis, a threshold value for SUVlbm (max) = 1.64 was established to differentiate inflammatory areas (septic or aseptic lesions) from areas with normal bone metabolism, with sensitivity (Sens) of 93.5% and specificity (Spec) of 95.6%, indicating that an uptake level at or above this threshold suggests inflammation (Fig. 4). The next stage of the study identified a threshold value for differential diagnosis of septic and aseptic bone inflammation in patients with diabetic foot syndrome (Fig. 5).

Table 4

Areas under the ROC Curves (ROC AUC) for Radiopharmaceutical SUVs				
Parameter	SUV bw	SUV lbm	SUV lbm janma	SUV bsa
Max	0.976	0.983	0.979	0.980
Mean	0.979	0.980	0.982	0.982
Peak	0.973	0.977	0.976	0.977

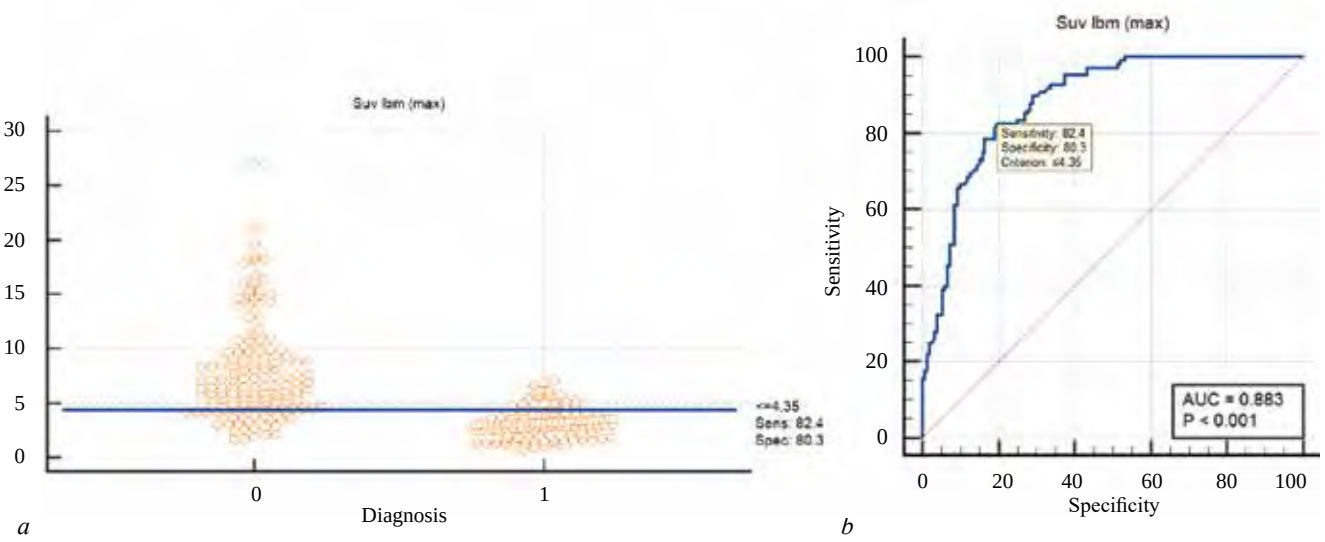
An uptake level equal to or exceeding 4.35 was associated with osteomyelitis, achieving sensitivity (Sens) of 82.4% and specificity (Spec) of 80.3%.

Below are clinical cases exemplifying septic and aseptic bone lesions in patients with type II diabetes mellitus. The cases presented similar clinical features, comparable DFS duration, and radiological evidence of destruction and disorganization of bone tissue in hindfoot (tarsal) and midfoot (metatarsal) regions. In patient N., as shown in Fig. 6, intense radiopharmaceutical hyperfixation was observed in cuneiform and metatarsal bones with SUVlbm (max) of 10.48, which exceeded the threshold of 4.35 and thereby confirmed osteomyelitis. Conversely, a clinical case of Charcot foot illustrating aseptic lesion in patients with DFS is presented in Fig. 7, where less intense radiopharmaceutical uptake (compared to septic lesions) with SUVlbm (max) = 3.87 was observed.



a *b*

Fig. 4. Results of comparing the difference in the radiopharmaceutical uptake in patients with diabetic foot syndrome (regardless of aseptic or septic lesions) and those with normal bone tissues: *a* – scatter chart showing coded areas (X-axis: 0 for normal radiopharmaceutical uptake, 1 for aseptic/septic bone lesions; Y-axis: standardized uptake value normalized by lean body mass); *b* – ROC curve illustrating differential diagnostic performance (X-axis – Specificity, Y-axis – Sensitivity)



a *b*

Fig. 5. Results of comparing the difference in radiopharmaceutical uptake in patients with diabetic foot syndrome with those with aseptic and septic lesions: *a* – scatter chart showing coded areas (0 for septic lesions, 1 for aseptic lesions; Y-axis: standardized uptake value normalized by lean body mass); *b* – ROC curve depicting differential diagnostic performance (X-axis – Specificity, Y-axis – Sensitivity)

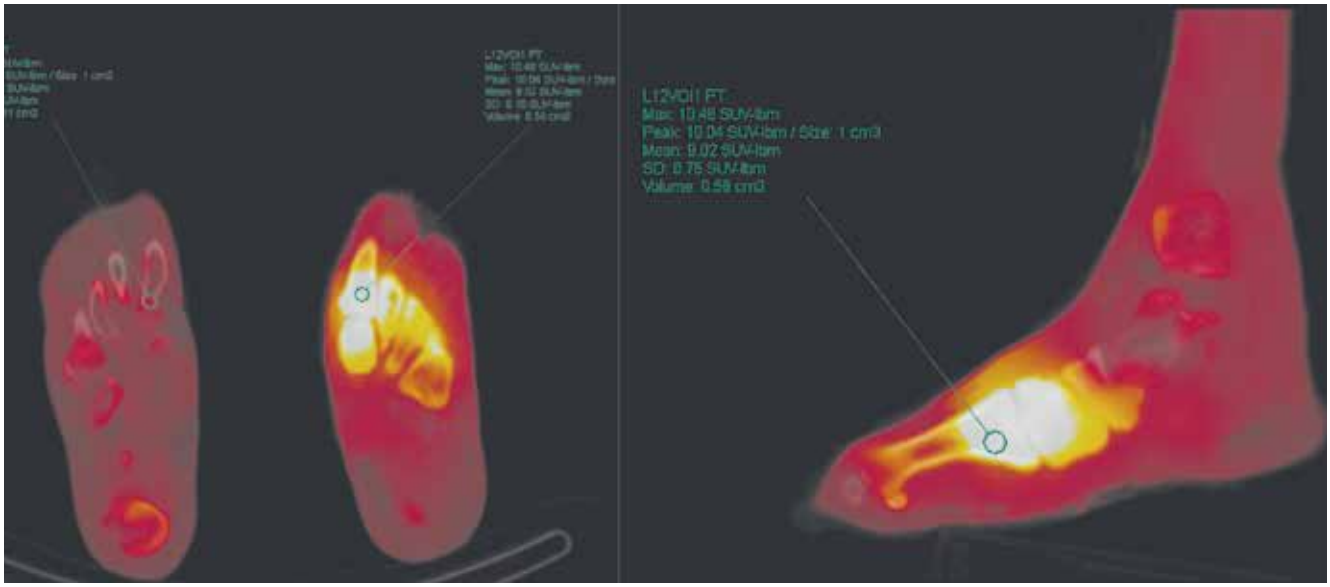


Fig. 6. Patient N., type II diabetes mellitus – osteomyelitis case: SPECT/CT images in axial and sagittal planes demonstrating intense radiopharmaceutical hyperfixation (SUVlbm (max) = 10.48) in medial cuneiform, base of first metatarsal, intermediate cuneiform, base of second metatarsal, stumps of third and fourth metatarsals, and base of fifth metatarsal – with signs consistent with marginal lytic lesion (septic lesion)

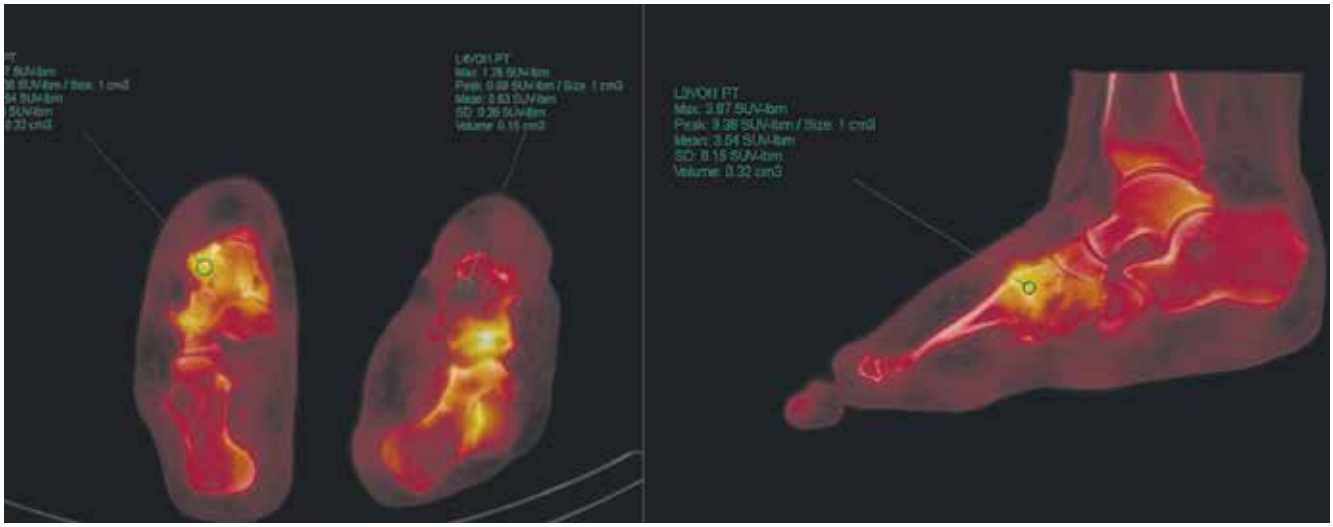


Fig. 7. Patient N. – Charcot foot case: SPECT/CT images in axial and sagittal planes showing intense radiopharmaceutical hyperfixation (SUVlbm (max) = 3.87) in ankle joint, Lisfranc and Chopart joints on the left foot. Similar, but less intense radiopharmaceutical hyperfixation is seen in Lisfranc and Chopart joints on the right foot indicating aseptic inflammation

DISCUSSION

The study evaluated the potential application of SUV in SPECT/CT imaging. It was established that SUV could assist in the differential diagnosis of septic and aseptic lesions of foot bones in patients with DFS.

The results of the study by M. Yoshiyuki [10] demonstrated high efficacy of SPECT/CT imaging using SUVs for the differential assessment of chronic osteomyelitis, osteoradionecrosis, and medication-related osteonecrosis of the jaw. K Kazuhiro et al. [11] investigated the use of osteoscintigraphy for dynamic evaluation of a treatment response in a patient with mandibular osteomyelitis, showing that quantitative SPECT/CT-derived parameters, such as SUV, can be useful for assessing inflammatory activity during therapy.

The clinical significance of these findings lies in the fact that utilizing the SUV in SPECT/CT imaging can serve as a diagnostic tool for osteomyelitis in patients with diabetic foot. This approach has the potential to reduce diagnostic and treatment times in this patient group.

CONCLUSION

This study analyzed the potential application of the standardized uptake value in SPECT/CT imaging for the differential diagnosis of septic and aseptic lesions of foot bones in patients with diabetic foot syndrome. The analysis demonstrated that standardized uptake values allow for the differentiation between inflammatory and non-inflammatory bone tissue lesions. SUVlbm (max) values exceeding 5 are associated with septic inflammation, supporting the clinical utility of this parameter. The data obtained indicate that the standardized uptake value in SPECT/CT imaging possesses high information value for diagnosing osteomyelitis in diabetic foot, which may facilitate more accurate choice of a treatment strategy and reduce the number of invasive procedures.

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Anatomical Substantiation of the Thoracodorsal Nerve as a Donor Nerve and the Musculocutaneous Nerve as a Recipient Nerve

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ABSTRACT

Aim. To identify the correspondence in the diameter of the thoracodorsal and musculocutaneous nerves, depending on the level of branching.

Materials and Methods. Using 121 preparations of the brachial plexus from 105 corpses of men and women aged 40–97 years, the diameter of the thoracodorsal nerve was measured at five levels, and the diameter of the musculocutaneous nerve was determined at two levels. For each parameter, the median and the interquartile range *Me* [Q_1; Q_3] were determined. The significance of differences between the groups was found by the Mann – Whitney test. The differences were considered significant at $p < 0.05$. The correlation was evaluated by the Spearman's rank correlation coefficient. At $0.7 \leq r_s < 0.9$, the correlation was regarded as strong, at $0.5 \leq r_s < 0.7$ – as moderate.

Results. The diameter of the thoracodorsal nerve varied throughout its length: in the initial section, it was 1.66 [1.66; 1.99] mm, before branching – 3.00 [2.65; 3.50] mm, at the first- and second-order extramuscular branches – 4.2 [3.2; 5.0] mm and 5.25 [4.50; 6.50] mm, at the first-order intramuscular branches – 4.00 [3.50; 4.66] mm. The diameter of the musculocutaneous nerve in the initial section was 3.0 [2.6; 3.3] mm, and before the coracobrachialis muscle – 2.7 [2.4; 3.0] mm.

The total diameter of the extra- and intramuscular branches of the thoracodorsal nerve was equal to or greater than the thickness of the musculocutaneous nerve in 90.1–95.0% of cases. Excess total diameter of the branches of the thoracodorsal nerve (0.05–8.0 mm) and fascicular dissection make it possible to preserve 1–2 first- and second-order extramuscular branches and 1–4 first-order intramuscular branches.

Conclusion. The diameter of the thoracodorsal nerve in the initial section is smaller than that of the musculoskeletal nerve, but the total thickness of its extra- and intramuscular branches is equal to or greater by 0.05–8.0 mm in 90.1–95.0% of cases. Different levels of branching of the thoracodorsal nerve contribute to extended transfer, and an excess diameter with fascicular dissection will preserve the function of the latissimus dorsi muscle.

Keywords: thoracodorsal nerve, musculocutaneous nerve, levels of branching, latissimus dorsi muscle, fascicular dissection

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Анатомическое обоснование грудоспинного нерва как нерва-донора и мышечно-кожного нерва как нерва-реципиента

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РЕЗЮМЕ

Цель – выявить соответствие диаметров у грудоспинного и мышечно-кожного нервов в зависимости от уровня ветвления.

Материалы и методы. На 121 препарате плечевого сплетения от 105 трупов мужчин и женщин в возрасте 40–97 лет измерен диаметр грудоспинного нерва на пяти, а мышечно-кожного – двух уровнях. У каждого показателя определена медиана межквартильного интервала *Me* [Q_1; Q_3]. Значимость различий в группах находили по *U*-тесту Манна – Уитни. Различия считались значимыми при $p < 0,05$. Сопряженность оценивали по коэффициенту Спирмена. При значении $0,7 \leq rs < 0,9$ связь расценивали как сильную, $0,5 \leq rs < 0,7$ – средней силы.

Результаты. Диаметр грудоспинного нерва изменяется на всем протяжении: в начальном отделе – 1,66 [1,66; 1,99] мм, перед разделением на ветви – 3,00 [2,65; 3,50] мм, на уровне внемышечных ветвей первого и второго порядков – 4,2 [3,2; 5,0] мм и 5,25 [4,50; 6,50] мм, внутримышечных ветвей первого порядка – 4,00 [3,50; 4,66] мм. Диаметр мышечно-кожного нерва в начальном отделе равен 3,0 [2,6; 3,3] мм, а перед клювовидно-плечевой мышцей – 2,7 [2,4; 3,0] мм. Общий диаметр вне- и внутримышечных ветвей грудоспинного нерва равен или больше толщины мышечно-кожного в 90,1–95,0%. Избыток общего диаметра ветвей грудоспинного нерва (0,05–8,0 мм) и фасцикулярная диссекция позволят сохранить по 1–2 внемышечные ветви первого и второго порядков, 1–4 внутримышечные ветви первого порядка.

Заключение. Диаметр грудоспинного нерва в начальном отделе меньше, чем у мышечно-кожного, но общая толщина его вне- и внутримышечных ветвей равна или больше на 0,05–8,0 мм в 90,1–95,0%. Разные уровни ветвления грудоспинного нерва способствуют протяженному переносу, а избыток диаметра с фасцикулярной диссекцией позволит сохранить функцию широчайшей мышцы спины.

Ключевые слова: грудоспинной нерв, мышечно-кожный нерв, уровни ветвления, широчайшая мышца спины, фасцикулярная диссекция

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. Протокол исследования одобрен этическим комитетом КрасГМУ им. проф. В.Ф. Войно-Ясенецкого (№ 127/24 от 25.09.2024).

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как нерва-реципиента. *Бюллетень сибирской медицины.* 2025;24(4):14–20. https://doi.org/10.20538/1682-0363-2025-4-14-20.

INTRODUCTION

Despite the encouraging and predictable results of surgical treatment for injured nerves, questions regarding the choice of a donor remain relevant. [1, 2]. Countless studies specify the main requirements that potential nerve donors must meet [3–5]. First of all, they must be sufficient in length, match the diameter of the recipient, and minimally disrupt the function of the muscles innervated by the donor nerve. [6].

Thoracodorsal nerve (TDN) is a mixed nerve that contains a sufficient amount of sensory (85%) and motor (15%) fibers and has a convenient location and optimal size, which allows it to be used as a donor, including for transfer to the position of the damaged musculocutaneous nerve (MCN) [7–9]. The anatomy of the TDN has been studied in great detail. It has been established that this nerve is formed from the spinal nerves C7, C8 and less frequently from C6–C8. Its length ranges from 12.3 to 14.1 cm, with a diameter of 2.1 to 3.0 mm. The number of extramuscular branches is 1–4, and the number of myelinated fibers ranges from 1,530 to 9,974. [8, 10, 11]. However, despite the conducted research, there is no information about the diameter of the TDN at different levels of branching, which complicates its selection as a donor nerve.

Considering the above, the aim of this study was to identify the correspondence of the diameters of TDN and MCN depending on the level of branching.

MATERIALS AND METHODS

Anatomical dissection was conducted on 105 human cadavers (66 men and 39 women) aged 40–97 years, with 121 specimens of the brachial plexus (105 from the right side and 16 from the left) at the Department of Forensic Examination of the Krasnoyarsk Regional Bureau of Forensic Medical Expertise and at the Department of Operative Surgery and Topographic Anatomy of the Krasnoyarsk State Medical University named after Professor V.F. Voyno-Yasenetsky. The time from the death of individuals to the examination was up to 20 hours, and the bodies were stored in a refrigeration chamber at a temperature of 3–5 °C. The cause of death for all individuals was systemic disease without head, neck, upper limbs, and thorax injuries. The research protocol was approved by the Ethics Committee at Krasnoyarsk State Medical University named after Professor V.F. Voyno-Yasenetsky (Minutes No. 127/24 dated September 25, 2024).

Anatomical layer-by-layer dissection of all elements of the brachial plexus was performed on the human corpses with the isolation of TDN and MCN (Figure). Special attention was paid to the extra- and intramuscular branches of TDN. Using an NTB-4B microscope (China), the epineurium was removed from TDN and MCN, leaving the perineurium intact. The length of various segments of TDN along its entire length was measured with an electronic caliper.

The diameter of TDN was measured at five levels using the eyepiece scale of the microscope: 1 – immediately after branching from the posterior bundle; 2 – before splitting into extramuscular branches; 3 – after splitting into first-order extramuscular branches; 4 – after splitting into second-order extramuscular branches; 5 – after splitting into first-order intramuscular branches. At the last three levels, the total diameter of all branches was determined.

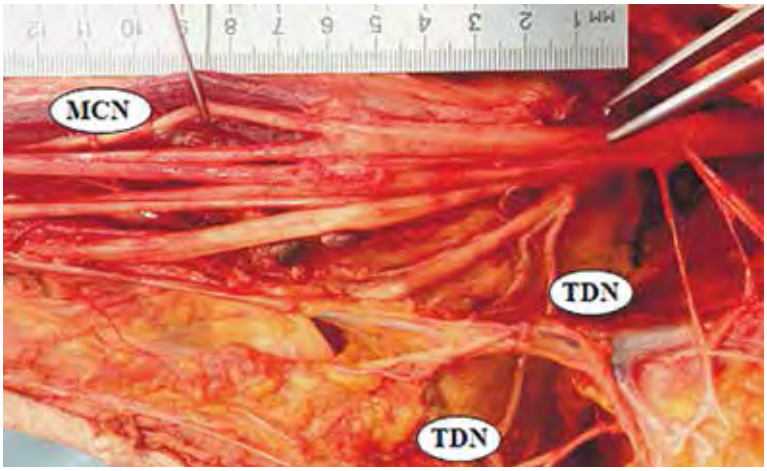

Figure. Musculocutaneous nerve (MCN) and thoracodorsal nerve (TDN) nerves in the right brachial plexus of the male corpse aged 62 years

The diameter of MCN was measured at two levels: 1 – after branching from the lateral bundle; 2 – before entering the coracobrachialis muscle.

After measuring the diameters of the nerves in each brachial plexus specimen (n = 121), a paired comparison was made between the thickness measurements of the donor nerve – TDN at five levels and the corresponding measurements of the recipient nerve – MCN at two levels. The absolute and relative (%) number of specimens where the diameter of the donor nerve was equal to, greater than or less than that of the recipient nerve was determined.

The conclusions of the study were obtained on the basis of statistical processing of data obtained from the entire sample population, since no significant gender, age, or bilateral features of the TDN and MCN diameters (from p = 0.08 to p = 1.0) were revealed. All obtained data were entered into MS Excel 12.0 software (Microsoft Corporation, USA). Using Statistica for Windows 12.0 (StatSoft, USA), normality of distribution was tested using the Shapiro – Wilk test, and thereafter non-parametric methods were employed. Minimum and maximum values, as well as the median and the interquartile range Me [Q_1; Q_3] were determined for each parameter. The significance of differences between nerve diameters was assessed using the Mann – Whitney U-test. The differences were considered significant at $p < 0.05$. The correlation between the diameter of TDN and the length of its segments was evaluated using the Spearman's rank correlation coefficient (rs). A coefficient value of $0.7 \leq rs < 0.9$ indicated a strong correlation, while $0.5 \leq rs < 0.7$ indicated a moderate correlation.

RESULTS

The conducted study revealed that the diameter of the TDN significantly changes along its entire length up to the latissimus dorsi muscle. After branching from the posterior bundle of the brachial plexus, the diameter of the TDN ranges from 0.83 to 3.33 mm, with a median of 1.66 [1.66; 1.99] mm. In the distal section, the diameter of the TDN increases and reaches 3.00 [2.65; 3.50] mm ($p < 0.001$) before splitting into extramuscular branches, at a distance of 9.5 [8.3; 11.0] cm from the point of origin. After the splitting, the total diameter of the first-order extramuscular branches at a distance of 12.5 [11.5; 14.3] cm is 4.2 [3.2; 5.0] mm ($p < 0.001$), while for the second-order branches at 14.1 [11.5; 15.5] cm, it is 5.25 [4.50; 6.50] mm ($p < 0.001$), and for the first-order intramuscular branches at 18.7 [16.3; 21.0] cm, it is 4.00 [3.50; 4.66] mm ($p < 0.001$). Correlation analysis revealed a strong but insignificant correlation between the length and diameter of the TDN (rs = 0.828; p = 0.083).

The diameter of the MCN after branching from the lateral bundle varies from 1.5 to 5.0 mm, with a median of 3.0 [2.6; 3.3] mm, and at a distance of 6.0 [4.5; 7.8] cm, before reaching the coracobrachialis muscle, it makes 2.7 [2.4; 3.0] mm ($p < 0.001$). Statistical analysis showed that these values are greater than the diameter of the TDN in the initial segment ($p < 0.001$), equal to and smaller than the TDN diameter before splitting into branches (p = 0.167 and $p < 0.001$), and smaller than the TDN diameter at all subsequent levels ($p < 0.001$).

In pairwise comparisons of the two nerves in each specimen of the brachial plexus, it was established that TDN is qualified as a donor nerve at the level of first- and second-order extramuscular branches and first-order intramuscular branches, with total diameters that are equal to or greater by 0.05–8.0 mm than the diameter of the MCN in the initial segment in 90.1–92% of cases and before reaching the coracobrachialis muscle in 93.4–95% of cases (Table).

Choosing TDN with branches at different levels expands the surgeon's options and allows for selecting a longer and appropriately sized donor nerve, transferring it as close as possible to the denervated muscle, which will shorten the path and time for regeneration. A positive aspect of using TDN as a donor nerve is that its excess diameter of 0.05–8.0 mm allows for fascicular dissection and transfer of individual branches, thereby preserving the function of the latissimus dorsi muscle.

Table

Compliance of Diameters of TDN and MCN at Different Levels, *n (%)*

Diameter of TDN at the level:	Diameter of MCN at the level:	
	initial segment	in front of the coracobrachialis muscle
– Initial section (n = 121): matches or is greater by 0.06–1.82 mm,	7 (5.8)	10 (8.3)
smaller by 0.01–3.51 mm.	114 (94.2)	111 (91.7)
– Before splitting (n = 113): matches or is greater by 0.06–2.8 mm,	69 (61.1)	81 (71.7)
smaller by 0.1–2.0 mm.	44 (38.9)	32 (28.3)
– First-order extramuscular branches (n = 113): matches or is greater by 0.1–4.3 mm,	102 (90.3)	106 (93.8)
smaller by 0.05–1.2 mm.	11 (9.7)	7 (6.2)
– Second-order extramuscular branches (n = 64): matches or is greater by 0.3–8.0 mm,	59 (92)	61 (95)

End of table

Diameter of TDN at the level:	Diameter of MCN at the level:	
	initial segment	in front of the coracobrachialis muscle
smaller by 0.1–2.5 mm.	5 (8)	3 (5)
– First-order extramuscular branches (n = 121): matches or is greater by 0.05–4.66 mm,	109 (90.1)	113 (93.4)
smaller by 0.07–1.87 mm.	12 (9.9)	8 (6.6)

DISCUSSION

Restoration of flexion function in the elbow joint for patients with brachial plexus injury is of primary importance [12, 13]. To restore the function of the elbow flexor muscles, the clinical practice of transferring bundles from the ulnar and median nerves has shown excellent functional results [14, 15]. If the motor function of these nerves is not preserved, alternative donor nerves include intercostal nerves, the phrenic nerve, the accessory nerve, the medial pectoral nerve, the contralateral spinal C7, and the TDN [10, 16–18].

We have chosen TDN as the donor nerve for transfer to the position of the MCN for two reasons. First of all, existing studies have demonstrated that the lengths of TDN with extramuscular branches are sufficient for transfer to the position of MCN in 95% of cases [8]. Secondly, there are conflicting data regarding the diameter and sufficiency of the fiber ratio between these two nerves [7, 10].

Although TDN has fewer motor fibers than the MCN, there is evidence that normal muscle activity can be achieved with approximately 30% innervation of motor neurons [19]. In TDN, the number of motor fibers is 58% from that in MCN, and, therefore, it is sufficient to maintain the function of the shoulder flexors. In another study, when comparing the number of axons with clinical outcomes for elbow flexion strength recovery, a threshold ratio of motor fibers in the donor nerve to the recipient nerve was recommended at 0.7:1.0 [20]. For TDN, this ratio is 0.6:1.0, which is below the required norm. A double transfer of bundles from the ulnar and median nerves has been developed for restoring elbow flexion [21]. Considering these results, it can be suggested to use TDN as an additional donor nerve.

On the other hand, the number of axons is proportional to the diameter of the nerve, and, therefore, the thickness of the donor and recipient must match [6]. M.S. Sporer et al. noted without specifying to which nerve TDN is transferred that its length and cross-sectional area are not suitable for fascicular transfer [22].

Considering the contradictory literature data, we studied the diameter of TDN on 121 specimens of the brachial plexus from 105 human cadavers at five levels, while for MCN, the diameter was studied at two levels. It was established that the diameter of TDN varied from 0.83 to 3.33 mm, with a median of 1.66 [1.66; 1.99] mm. Comparing our data with known studies revealed inconsistencies in results. For example, M. Samardzic et al. found that in 15 cadavers, the diameter of TDN ranged from 2.1 to 3.0 mm [23]. After removing the epineurium and in some cases perineurium on 20 specimens from 17 cadavers, K.S. Lee found that the diameter of TDN ranged from 1.16 to 1.92 mm, with a median of 1.45 [1.33; 1.65] mm, which was significantly ($p < 0.001$) smaller than our findings [24]. M. Dancker et al. identified on 28 specimens from 14 cadavers that the diameter of TDN and the lower subscapular nerve was 2.5 ± 0.4 mm (range of 1.6–3.5 mm) [25].

The diameter of MCN in the initial segment varied from 1.5 to 5.0 mm, with a median of 3.0 [2.6; 3.3] mm, which was significantly greater ($p < 0.001$) than that of TDN at a ratio of 0.6:1.0. Previous studies also report conflicting results. For instance, V. Macchi et al. determined that in 6 cadavers, the average diameter of MCN before branching was 1.96 ± 0.2 mm, while in a trunk variant (6 cadavers), it was 2.86 ± 0.3 mm [26]. H. Namazi et al. found that on 10 specimens of the brachial plexus, the diameter of MCN was 1.8 ± 0.7 mm [27]. E. Clarke et al. reported diameters of MCN of 2.49 mm on one cadaver and of 4.87 mm on another [28]. L. Foroni et al. determined on 26 cadavers that the diameter of the nerve ranged from 2 to 4 mm [29]. According to A. Hansasuta et al., after dissecting 35 specimens from 18 human cadavers, it was found that the diameter varied from 3.0 to 5.5 mm, with a median of 4.3 mm [30]. J.P. Lee et al. established sex differences showing that in men (n = 6), the diameter of MCN was 4.3 ± 1.1 mm (range of 2.5–6.0), while in women (n = 6), it was 3.1 ± 1.5 mm (range of 1.6–4.0) [31].

The variability in the measurements is clearly related to the different number of specimens, levels of measurement, and the dissection techniques used, where some researchers remove the epineurium while others preserve the nerve sheath.

The conducted study revealed that the closer to the latissimus dorsi muscle, the significantly greater the total diameter of the extra- and intramuscular branches of TDN ($p < 0.001$), which is sufficient for transfer to the position of MCN. The significant predominance

of the diameters of TDN branches (0.05–8.0 mm) in 90.1–95.0% of cases allows the surgeon to approach fascicular transfer individually while maximizing the preservation of latissimus dorsi function. Thus, the excess total diameter of TDN will allow, through fascicular dissection, to preserve 1–2 first-order extramuscular branches (diameter of 1.00 [0.75; 1.25] mm) from 2–4 ones, 1–2 second-order branches (diameter of 0.75 [0.5; 1.0] mm) from 2–4 ones, and 1–4 first-order intramuscular branches (diameter of 0.57 [0.5; 0.66] mm) from 2–7 ones.

Therefore, the conducted study demonstrates that TDN as a donor nerve exceeds MCN as a recipient nerve in diameter at the levels of extra- and intramuscular branches, and its fascicular dissection with account for the branching level will allow for the preservation of latissimus dorsi function.

CONCLUSION

The diameter of the thoracodorsal nerve at the initial section is smaller than that of the musculocutaneous nerve, but the overall thickness of its extra- and intramuscular branches is equal to or greater by 0.05–8.0 mm in 90.1–95.0% of cases. Different branching levels of the thoracodorsal nerve contribute to extensive transfer, and the excess diameter with fascicular dissection will allow for the preservation of latissimus dorsi muscle function.

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Echocardiographic Predictors of Undiagnosed Heart Failure with Preserved Ejection Fraction in Hospitalized Atrial Fibrillation Patients with Dyspnea

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ABSTRACT

The aim was to evaluate the prevalence and echocardiographic predictors of previously undiagnosed heart failure with preserved ejection fraction (HFpEF) in patients with atrial fibrillation (AF) and chronic dyspnea.

Material and methods. This prospective observational study included 85 patients hospitalized for cardioversion with paroxysmal or persistent AF and chronic dyspnea. All participants underwent transthoracic speckle-tracking echocardiography of left atrial longitudinal strain (LAS). HFpEF probability was assessed using the HFA-PEFF algorithm. Dynamic follow-up of diastolic function was performed at three predefined time points: during AF paroxysm, 24 hours post-cardioversion, and one-month post-cardioversion.

Results. High probability of HFpEF was identified in 78.7% of patients (67 out of 85). These patients exhibited significantly higher NT-proBNP levels, greater CHA_2DS_2-VASc score, as well as more impaired LAS parameters and elevated left atrial stiffness index compared to low-intermediate HFpEF probability groups. At one-month follow-up after cardioversion (n = 55), while NT-proBNP levels significantly declined, overall HFpEF probability remained unchanged. Left atrial stiffness index demonstrated the strongest independent predictive value in verifying high probability HFpEF, with remarkable discriminative capacity both during AF (OR = 34.5; 95% CI 2.5–478.7) and after sinus rhythm restoration (OR = 193.1; 95% CI 7.3–1,207).

Conclusion. This study reveals high prevalence of undiagnosed HFpEF among AF patients undergoing cardioversion, with disease probability persisting despite rhythm control during one-month follow-up. The left atrial stiffness index is as a valuable diagnostic marker for HFpEF detection in this population, potentially enhancing standard HFA-PEFF algorithm.

Keywords: atrial fibrillation, heart failure with preserved ejection fraction, cardioversion, left atrial strain, HFA-PEFF score

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee of RUDN University Medical Institute (Minutes No. 16 dated March 16, 2023).

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Эхокардиографические предикторы недиагностированной сердечной недостаточности с сохраненной фракцией выброса у госпитализированных пациентов с фибрилляцией предсердий и жалобами на одышку

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РЕЗЮМЕ

Цель: оценить частоту встречаемости и эхокардиографические предикторы ранее недиагностированной сердечной недостаточности с сохраненной фракцией выброса (СНсФВ) у пациентов с фибрилляцией предсердий (ФП) и хронической одышкой.

Материалы и методы. В проспективное наблюдательное исследование включены 85 пациентов с пароксизмальной или персистирующей формой ФП и хронической одышкой, госпитализированных для проведения кардиоверсии. Всем участникам выполнялась трансторакальная эхокардиография в сочетании с методом спекл-трекинга для анализа продольной деформации левого предсердия (ПД ЛП). Вероятность СНсФВ оценивалась с помощью алгоритма HFA-PEFF. Для динамического наблюдения за пациентами были выделены три временные точки: во время фибрилляции предсердий, через 24 ч после кардиоверсии и через 1 мес наблюдения.

Результаты. Высокая вероятность СНсФВ была выявлена у 78,7% пациентов (67 из 85). В этой группе зарегистрированы статистически значимо более высокие уровни NT-proBNP, более высокие баллы по шкале CHA_2DS_2-VASc, а также более низкие показатели ПД ЛП, более высокий индекс жесткости ЛП по сравнению с пациентами с низкой и промежуточной вероятностью СНсФВ. При динамическом наблюдении через 1 мес после кардиоверсии (n = 55) количество баллов по алгоритму HFA-PEFF оставалось неизменным, несмотря на статистически значимое снижение уровня NT-proBNP. Наибольшую прогностическую значимость для выявления пациентов с высокой вероятностью сердечной недостаточности продемонстрировал индекс жесткости ЛП, с отношением шансов 34,5 (95%-й доверительный интервал (ДИ) 2,5–478,7) во время ФП и 193,1 (95% ДИ 7,3–1207) при синусовом ритме.

Заключение. Полученные данные свидетельствуют о высокой распространенности ранее недиагностированной СНсФВ среди пациентов с ФП, госпитализированных для кардиоверсии. Вероятность СНсФВ существенно не изменялась в течение месяца после восстановления синусового ритма. Индекс жесткости ЛП обладает высокой диагностической ценностью для верификации СНсФВ у данной категории пациентов и может рассматриваться как дополнительный критерий при использовании стандартного алгоритма HFA-PEFF.

Ключевые слова: фибрилляция предсердий, сердечная недостаточность с сохраненной фракцией выброса, кардиоверсия, деформация левого предсердия, шкала HFA-PEFF

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

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

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом Медицинского института РУДН (протокол № 16 от 16.03.2023).

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INTRODUCTION

Verification of the diagnosis of heart failure with preserved ejection fraction (HFpEF) in patients with atrial fibrillation (AF) and complaints of dyspnea can be significantly challenging, as both conditions are highly prevalent in the elderly, have similar symptoms, and may directly cause each other [1–5]. At present, questions regarding the reversibility of heart failure after cardioversion as left atrial (LA) function normalizes and, consequently, the appropriateness of diagnosing HFpEF during hospitalization for cardioversion remain insufficiently studied.

Moreover, this patient population often have comorbid conditions (such as chronic obstructive pulmonary disease, anemia, and obesity), which complicate the accurate interpretation of the underlying cause of dyspnea. Given the abovementioned, optimizing the detection of HFpEF in this patient group is critically important to enable timely adjustments in pharmacological therapy [1, 2, 6].

Patients with HFpEF are often characterized by cardiac fibrosis, remodeling, overload, and impaired LA function. These changes can be assessed using parameters already widely employed in clinical practice and metrics whose role requires further investigation. Among these, the evaluation of LA longitudinal strain (LAS) via speckle-tracking echocardiography is of particular interest [7–10].

LAS is a promising echocardiographic marker that reflects not only LA function but also left ventricular (LV) filling pressure. It may serve as a complementary parameter to validated clinical tools, such as the H2FPEF and HFA-PEFF scores, which are used for diagnosing HFpEF [11, 12]. Moreover, LAS is likely a more sensitive marker of diastolic dysfunction compared to conventional parameters assessing LA and LV morphology [3, 13]. For instance, some studies have demonstrated high accuracy of LAS in differentiating between HFpEF and other causes of dyspnea in patients with sinus rhythm [14–16]. However, data on the utility of this method in patients with AF remain limited and require further investigation.

The aim of this study was to assess the prevalence and echocardiographic predictors of previously undiagnosed HFpEF in patients with atrial fibrillation and chronic dyspnea.

MATERIALS AND METHODS

After screening 171 patients, this prospective observational study included 85 patients aged ≥ 55 years with paroxysmal or persistent AF and complaints of chronic dyspnea, who were consecutively admitted to the Cardiac Intensive Care Unit at Vinogradov City Clinical Hospital (a branch of RUDN University) for cardioversion between March 2023 and May 2024. The final analysis included 55 patients who underwent a one-month follow-up. The method for sinus rhythm restoration (either pharmacological or electrical cardioversion) was selected according to current clinical practice guidelines of the Russian Ministry of Healthcare.

The main exclusion criteria were previously diagnosed heart failure, inability to restore and maintain sinus rhythm during follow-up (AF episodes requiring repeat cardioversion), left ventricular systolic dysfunction, presence of potentially reversible causes of AF (such as electrolyte disturbances or thyrotoxicosis), and severe comorbidities (including severe anemia, chronic obstructive pulmonary disease, or active malignancy). A follow-up visit was scheduled one month after discharge to assess changes in laboratory and echocardiographic parameters.

The study database recorded the main clinical and demographic characteristics of the study sample. Additional parameters included duration of medical history and AF type, along with presence of comorbidities. Dyspnea severity was classified according to NYHA criteria. Standard laboratory parameters were documented at hospital admission, and changes in NT-proBNP were reported at admission, next day, and one month post-cardioversion. All patients underwent thromboembolic and bleeding risk assessment using CHA_2DS_2-VASc and HAS-BLED scales, respectively, based on clinical and laboratory parameters.

Electrocardiography (ECG) was used to assess QRS complex morphology and basic interval durations (PQ, QRS, and QT). Transthoracic echocardiography was performed using expert-class equipment (GE Vivid E90, GE Healthcare, Horten, Norway) with standard imaging planes ensuring optimal visualization and recording of required parameters. The method for assessing global LV contractility (the Teichholz method or Simpson's method) was selected based on visualization quality and presence of regional LV wall motion abnormalities, following the clinical practice adopted at the institution.

Diastolic function was evaluated using tissue Doppler imaging in apical four-chamber view by measuring peak mitral inflow E-wave velocity (E) and averaging lateral and septal mitral annular early

diastolic velocities (e'), with subsequent calculation of E/e' ratio. Automated strain analysis was conducted using specialized software. For LV global longitudinal strain (LVGLS) assessment, automatic tracking between endocardial and epicardial borders was performed followed by bull's eye diagram generation and GLS calculation. Left atrial strain was evaluated using ECG-gated images from apical 4- and 2-chamber views. The zero-reference point for atrial strain curves was set at the ECG R-wave followed by analysis of reservoir (LASr), conduit (LAScd), and contractile (LASct) phases, with the latter parameter assessed only during sinus rhythm [17]. Left atrial stiffness index was calculated as E/e' divided by LASr.

The likelihood of HFpEF was assessed using the HFA-PEFF score. In cases of intermediate HFpEF probability, a diastolic stress test (DST) was performed according to protocols outlined in current Russian and international guidelines [12, 18, 19]. DST was positive if: 1) there was an increase in E/e' ratio ≥ 15 (2 points), or 2) there was an increase in E/e' ratio ≥ 15 with a peak tricuspid regurgitation velocity ≥ 3.4 m/s (3 points).

Statistical analysis was performed using IBM SPSS Statistics software (v. 27.0). Quantitative data are presented as median and interquartile range *Me* [*IQR*], while qualitative variables are expressed as absolute numbers and percentages *n* [%]. To assess statistical significance of intergroup differences, we used the Mann–Whitney *U* test for independent samples, Wilcoxon signed-rank test for two related samples, and Friedman test with subsequent post-hoc pairwise comparisons (with Bonferroni correction) for three related samples. Differences were considered statistically significant at $p < 0.05$ (with Bonferroni correction at $p < 0.017$).

To identify independent predictors of persistent high HFpEF probability at one-month follow-up, we performed logistic regression analysis separately for parameters recorded during AF episodes and after cardioversion. Variables showing statistical significance at $p < 0.1$ were included in the multivariate analysis. For each independent predictor, ROC analysis was performed. The predictive value of variables was assessed based on the area under the curve (AUC), with optimal cutoff values determined using the Youden index.

All patients provided a written informed consent to participate in the study. The study protocol was approved by the Ethics Committee of the Medical Institute at RUDN University (Minutes No. 16 dated March 16, 2023).

RESULTS

High probability of HFpEF according to the HFA-PEFF score was identified in 67 patients (78.7%), including 47 patients with initially high HFpEF probability and 20 patients with intermediate probability and positive diastolic stress test (DST). Intermediate and low HFpEF probabilities were recorded in 16 and 2 cases, respectively. At one-month follow-up, AF episodes were documented in 30 patients (35.3%), including 25 from the group with high HFpEF probability and 5 from the group with low/intermediate probability. Thus, the final analysis included 55 patients (42 with high HFpEF probability, including those with positive DST – Group 1; and 13 with low/intermediate HFpEF probability – Group 2). Group 1 patients demonstrated higher NT-proBNP levels (both at admission and during follow-up) and higher CHA_2DS_2-VASc scores (Table 1).

Data on the dynamics of HFA-PEFF score components and LA strain parameters in patients with HFpEF are presented in Table 2. Over the one-month follow-up period, the median HFA-PEFF score remained unchanged (5→5 points, $p > 0.05$). Transition from high to intermediate HFpEF probability occurred in only three patients, driven exclusively by reductions in NT-proBNP levels. During follow-up, we noted statistically significant positive changes in some algorithm parameters (NT-proBNP, lateral e') and in all LA strain parameters.

Statistically significant differences: * – for FU-1 vs. FU-2 and FU-3; FU-2 vs. FU-3; # – for FU-2 vs. FU-3; ** – for FU-1 vs. FU-2; ## – for FU-1 and FU-2 vs. FU-3.

Logistic regression analysis demonstrated that the left atrial stiffness index (E/e'/LASr) was the only independent predictor of HFpEF persistence at one-month follow-up in AF patients undergoing cardioversion, showing significant associations both during the AF episode (odds ratio (OR) = 34.5 [95% CI 2.5–478.7; $p = 0.008$]) and after sinus rhythm restoration (OR = 193.1 [95% CI 7.3–1207; $p = 0.008$])

ROC analysis identified the following cutoff values for the left atrial stiffness index (see Figure): > 1.10 (area under the curve [AUC] 0.83; sensitivity 49.1%; specificity 100%) during AF and > 0.48 (AUC 0.86; sensitivity 91.2%; specificity 66.7%) during sinus rhythm.

Table 1

Baseline Clinical and Demographic Parameters of Study Participants at the Time of Inclusion

Parameter	Group 1 (*n* = 42)	Group 2 (*n* = 13)	*p*
Age, *Me* [*IQR*], years	75.5 [68;82]	73 [67;75]	0.18
Female sex, *n* (%)	29 (69.0)	6 (46.1)	0.24
Smoking, *n* (%)	3 (7.1)	0 (0)	0.77
Heart Failure Characteristics			
HFA-PEFF, *Me* [*IQR*], points	6.0 [5.0;6.0]	4.0 [3.0;5.0]	<0.0001
NYHA FC, *n* (%) – II – III	 29 (69) 13 (31)	 11 (84.6) 2 (15.4)	 0.46
NT-proBNP, *Me* [*IQR*], pg/ml	1,225 [568; 2,225]	226 [171;694]	<0.0001
Atrial Fibrillation Characteristics			
AF subtype, *n* (%) – newly diagnosed (paroxysmal or persistent); – paroxysmal; – persistent;	 9 (21.4) 34 (81.0) 8 (19.0)	 4 (30.8) 11 (84.6) 2 (15.4)	 0.75 0.91
Duration of AF history (including patients with newly diagnosed AF), *n* (%) – less than 1 year; – 1–3 years; – more than 3 years	 11 (26.2) 13 (31.0) 18 (42.8)	 5 (38.5) 5 (38.5) 3 (23.0)	 0.95
AF paroxysm duration ≥48 hours, *n* (%)	17 (40.5)	2 (15.4)	0.18
CHA_2DS_2-VASc, *Me* [*IQR*], points	4.0 [3.0;5.0]	3.0 [2.0;3.0]	0.014
HAS-BLED, *Me* [*IQR*], points	2.0 [1.0;2.0]	2.0 [1.0;2.0]	0.66
Comorbidities			
Hypertension, *n* (%)	42 (100.0)	13 (100)	0.87
Coronary artery disease, *n* (%)	7 (16.6)	1 (7.7)	0.73
Obesity, *n* (%)	17 (40.5)	3 (23.1)	0.42
Diabetes mellitus, *n* (%)	6 (14.3)	0 (0)	0.3
Stroke, *n* (%)	4 (9.5)	1 (7.7)	0.73
CKD (GFR-EPI <60 ml/min/1,73 m^2), *n* (%)	24 (57.1)	4 (30.8)	0.18
Pharmacological Therapy			
ACEI/ARBs, *n* (%)	28 (66.7)	9 (69.2)	0.87
Beta-blockers, *n* (%)	13 (31.0)	7 (53.8)	0.24
MRAs, *n* (%)	2 (4.8)	1 (7.7)	0.77
Thiazide diuretics, *n* (%)	5 (12.0)	3 (23.1)	0.58
Loop diuretics, n (%)	6 (14.3)	0 (0)	0.35
CCBs, *n* (%)	9 (21.4)	4 (30.8)	0.75
AAD, *n* (%)	18 (42.9)	4 (30.8)	0.65
Anticoagulants, *n* (%)	25 (59.5)	7 (53.8)	0.97

Note. AAD – antiarrhythmic drugs; ACEI – angiotensin-converting enzyme inhibitors; AF – atrial fibrillation; ARB – angiotensin II receptor blockers; CCB – calcium channel blockers; CKD- chronic kidney disease; FC – functional class; GFR – glomerular filtration rate; HFA-PEFF – Heart Failure Association score for HFpEF diagnosis; MRA – mineralocorticoid receptor antagonists; NT-proBNP – N-terminal pro-brain natriuretic peptide; NYHA – New York Heart Association functional classification. The results were considered statistically significant at $p < 0.05$.

Table 2

Longitudinal Changes in Laboratory and Echocardiographic Parameters

Parameter	FU-1	FU-2	FU-3	*p*
NT-proBNP, Me [*IQR*], pg/ml	1,225 [560; 2,297]	899.0 [330.5; 412.5]	374 [133; 1,099]	<0.001*
LAVI, *Me* [*IQR*], ml/m^2	40.0 [31.9;45.2]	40.8 [33.7;44.2]	37.0 [34.0; 43.0]	0.03#
IVSd, *Me* [*IQR*], cm	1.3 [1.1;1.4]	–	1.2 [1.1;1.3]	0.01
LVPWd, *Me* [*IQR*], cm	1.1 [1.0;1.2]	–	1.1 [1.0;1.2]	NS
LVMI, Me [*IQR*], g/m^2	96.9 [82.3;108.5]	–	96.0 [77.5;108.5]	0.02
RWT, *Me* [*IQR*]	0.48 [0.43;0.55]	–	0.48 [0.42;0.55]	NS
PASP, *Me* [*IQR*], mm Hg	35.0 [28.7;41.2]	–	35.5 [28.7;41.2]	H3
TRV, *Me* [*IQR*], m/s	2.8 [2.1;3.6]	–	3.0 [2.4;3.5]	NS
Lateral e', *Me* [*IQR*], cm/s	0.06 [0.05; 0.09]	0.07 [0.06; 0.09]	0.07 [0.05; 0.08]	<0.001**

End of table 2

Parameter	FU-1	FU-2	FU-3	*p*
Septal e', *Me* [*IQR*], cm/s	0.06 [0.05; 0.08]	0.07 [0.05; 0.08]	0.05 [0.04; 0.06]	<0.001##
E/e' ratio, *Me* [*IQR*]	11.4 [9.0;14.0]	12.0 [9.3;14.5]	11.6 [9.0; 14.5]	NS
GLS, Me [*IQR*], %	16 [14;18]	–	–	–
LASr, *Me* [*IQR*], %	11.5 [8.0;14.0]	15.5 [11.7;21.2]	21.0 [17.0;24.0]	<0.001*
LAS cd, *Me* [*IQR*], %	–7.0 [–10.0; –4.0]	–10.5 [–13.0; –8.0]	–13.0 [–14.5; –10.5]	<0.001*
LAS ct, *Me* [*IQR*], %	–	–5.0 [–8.0; –3.0]	–7.0 [–12.0; –4.5]	0.006
E/e' / LASr, *Me* [*IQR*]	1.0 [0.68;1.7]	0.70 [0.52;1.0]	0.53 [0.41;0.78]	<0.001*

Note. FU – follow-up; FU-1, FU-2, and FU-3 represent time points corresponding to parameter measurements at admission (during AF episode – FU-1), 24 hours post-cardioversion – FU-2, and 30 days post-cardioversion – FU-3, respectively. LVMMI – left ventricular mass index; LA – left atrium; NS – not significant; PASP – pulmonary artery systolic pressure; TRV – peak tricuspid regurgitation velocity; LVPWd – left ventricular posterior wall thickness; IVSd – interventricular septum thickness; E/e' – ratio of early diastolic mitral inflow velocity to average mitral annular tissue Doppler velocity; E/e'/LASr – left atrial stiffness index; LV GLS – left ventricular global longitudinal strain; LAScd – left atrial conduit strain; LASct – left atrial contraction strain (assessed in sinus rhythm only); LASr – left atrial reservoir strain.

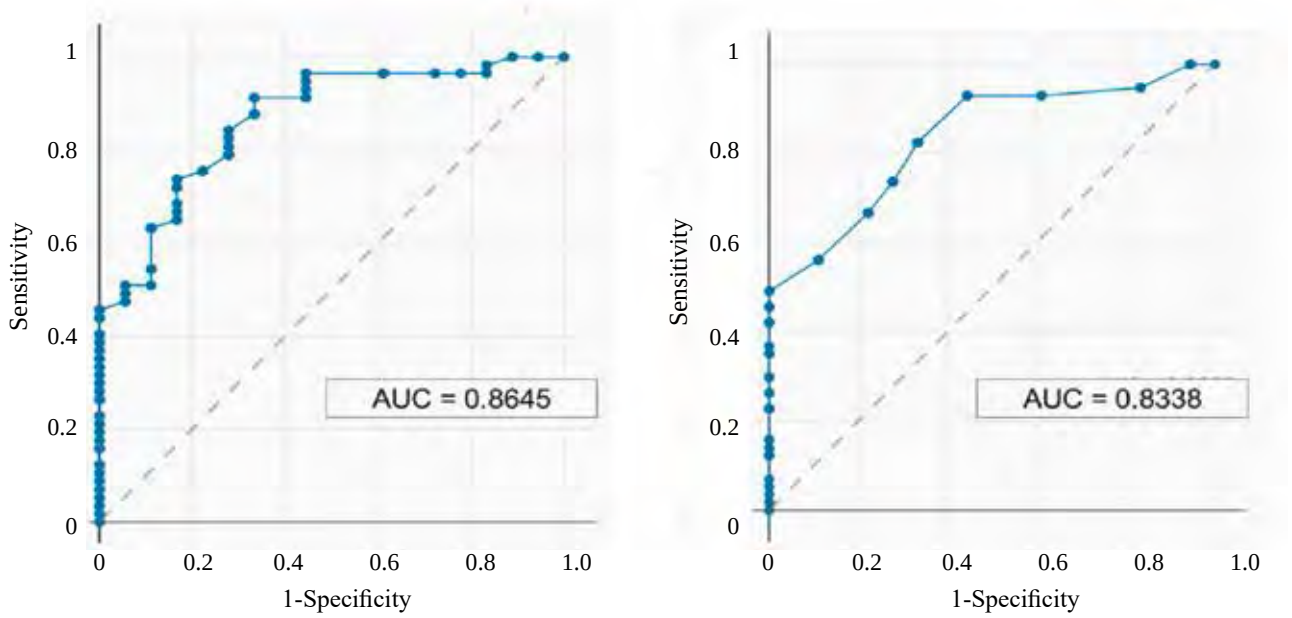

Figure. ROC curve for the left atrial stiffness index measured during: *a* – sinus rhythm; *b* – atrial fibrillation episodes

DISCUSSION

HFpEF often remains underdiagnosed in AF patients, since in clinical practice dyspnea, which is the cardinal symptom of heart failure, may be attributed solely to AF [20, 21]. Specifically, J.A.Naser et al. et al. (2023) reported high HFpEF probability in 62% of patients with this arrhythmia, yet only 5% had corresponding ICD-10 codes in medical records at enrollment [20].

Y.N.V. Reddy et al. (2018) demonstrated hemodynamic evidence of HFpEF via right heart catheterization in 64% of AF patients with unexplained dyspnea [21]. Our study identified high HFpEF probability using HFA-PEFF score in most elderly AF patients without prior heart failure history but with chronic dyspnea, aligning with invasive diagnostic data. Indeed, when using the mainstay – exercise stress right heart catheterization – HFpEF can be detected in 65–94% of AF patients [21–23]. Furthermore, AF is associated with a 2.5-fold increased risk of developing clinically significant LV diastolic dysfunction (with 3.4% annual incidence) during follow-up compared to sinus rhythm [20]. These findings together underscore the imperative for active HFpEF screening in AF patients with chronic dyspnea [20–24].

HFpEF in AF patients may be the sole thromboembolic risk factor requiring anticoagulant therapy and itself constitutes an indication for prognosis-modifying medications [11, 12, 25]. Moreover, AF episodes can induce changes that promote the

development and progression of heart failure [24, 26]. Consequently, post-cardioversion changes in both individual HFpEF markers and overall HFpEF probability are of particular interest.

Indeed, sinus rhythm restoration may be accompanied by reverse remodeling of cardiac chambers [27–29], where functional improvements in LA and LV (E/e' and LASr) along with reductions in HF biomarkers typically precede morphological changes (LA volume index), which may require months of maintained sinus rhythm. Some studies have demonstrated restored atrial mechanical synchrony [30] and increased LASr shortly after cardioversion [29, 31]. The latter rarely normalizes completely, likely due to atrial myocardial stunning whose duration varies depending on AF episode characteristics, atrial size, and underlying structural heart disease [32, 33].

In our study, despite successful sinus rhythm restoration in all cases (main inclusion criterion), only three patients (4.5%) showed reduced HFpEF probability (from high to intermediate) based on NT-proBNP reduction. The overall study population exhibited bidirectional changes in medial/lateral mitral annular velocities alongside improvements in LA reservoir, conduit, and contractile functions. These findings supported by existing evidence [24–33] suggest limited short-term reversibility of HFpEF parameters post-cardioversion and highlight the need for early initiation of prognosis-modifying therapies (anticoagulants, SGLT2 inhibitors, and non-steroidal MRAs) [11, 12]. Improved LA function following rhythm control supports the early rhythm control strategy demonstrated in EAST-AFNET 4 [34], where HF hospitalizations were part of the primary endpoint.

Among echocardiographic markers of HFpEF, the stiffness index (E/e'/LASr) deserves special attention as it represents an integrated measure of left heart diastolic function [6, 35–42]. Several studies have demonstrated in HFpEF patients associations of this parameter both with AF recurrence after cardioversion or catheter ablation [35, 36] and with long-term prognosis [37–39]. Furthermore, the stiffness index has been investigated for HFpEF screening as a potential adjunct to currently used diagnostic probability models [6, 40, 41]. While standardized reference values are lacking, this index typically does not exceed 0.3 in healthy individuals.

HFpEF leads to increased stiffness index values, with additional contribution from impaired LA reservoir function during AF episodes, explaining the varying cutoff values identified by ROC analysis in our study [31, 42]. Although this parameter showed slightly greater accuracy immediately after cardioversion, its potential utility during AF is particularly clinically relevant, as Russian practice typically involves only a single pre-cardioversion echocardiogram. In our study, the stiffness index was the sole predictor of persistent HFpEF during follow-up. Consequently, this indicator may be valuable both as an adjunct to other HFpEF probability assessment methods and as a potential alternative to diastolic stress testing in cases of intermediate HFpEF probability. However, the precise role of the stiffness index in contemporary HFpEF diagnostic algorithms requires further investigation.

Study limitations include the relatively small sample size, short follow-up period (one month), and lack of a control group with permanent AF. A promising direction for future research involves investigating the long-term effects of cardioversion combined with HFpEF-targeted therapy on left atrial remodeling and clinical outcomes.

CONCLUSION

In this study, the majority of elderly patients with atrial fibrillation and chronic dyspnea demonstrated high probability of HFpEF according to the HFA-PEFF score, which persisted during follow-up after cardioversion. The limited reverse remodeling of cardiac chambers observed despite maintained sinus rhythm supports the importance of active HFpEF screening in this population to enable early initiation of prognosis-modifying therapies. The stiffness index appears particularly valuable in this context as a potential adjunct to current guideline-recommended diagnostic algorithms for early HFpEF detection.

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Author Contribution

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Genetic and Functional Features of Peripheral Blood Leukocyte Mitochondria in Patients with Coronary Heart Disease and High Risk of Sudden Cardiac Death

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ABSTRACT

Aim. To assess the relationship between the respiration of mitochondria of peripheral blood leukocytes and mitochondrial DNA (mtDNA) polymorphism in patients with coronary heart disease (CHD) depending on the risk of developing sudden cardiac death (SCD).

Materials and methods. We formed two groups of patients: the main group – patients with CHD and the high risk of SCD (n = 107); the comparison group – patients with stable course of CHD without the risk of SCD (n = 50). Using methods of high-throughput sequencing, we determined patients' haplogroup and carriage of mtDNA polymorphisms A2706G, G3010A, and G9055A. The respiratory activity of isolated mitochondria from peripheral blood leukocytes was assessed by amperometric method using NAD- and FAD-dependent oxidation substrates.

Results. In both studied groups, H, U, and J haplogroups were predominant (74.5% and 92.5%, respectively, for the main group and the comparison group). There were more minor haplogroups in the main group than in the comparison group. The frequencies of occurrence of polymorphisms A2706G, G3010A, and G9055A did not significantly differ in intergroup comparison. In the main group, carriage of the A2706G polymorphism was associated with a decrease in the respiratory control ratio (RC) in FAD-dependent respiration (p = 0.05), and in the comparison group, it was associated with a decrease in oxygen consumption rate (OCR) in the V4 metabolic state in both NAD- and FAD-dependent respiration (p = 0.002 and p = 0.008, respectively) without changes in RC. In the main group, carriage of the G9055A polymorphism was associated with a decrease in OCR in the V3 metabolic state (p = 0.037) in FAD-dependent respiration. For the G3010A polymorphism, no association with mitochondrial respiration was found in the studied groups.

Conclusion. In patients with CHD, regardless of the risk of SCD, the frequencies of haplogroups H, U, and J and mtDNA polymorphisms A2706G, G3010A, and G9055A do not differ significantly. In patients with high risk of SCD, carriage of the A2706G polymorphism is associated with a decrease in RC in FAD-dependent respiration, and the G9055A polymorphism is associated with a decrease in OCR in V3 during FAD-dependent respiration.

Keywords: mitochondria, peripheral blood mononuclear cells, coronary heart disease, sudden cardiac death, mtDNA polymorphism

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Biomedical Ethics Committee of Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences (Minutes No. 20 dated February 14, 2024).

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Генетические и функциональные особенности митохондрий лейкоцитов периферической крови пациентов при ишемической болезни сердца с высоким риском внезапной сердечной смерти

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РЕЗЮМЕ

Цель. Оценить взаимосвязь дыхательной активности митохондрий лейкоцитов периферической крови с полиморфизмом митохондриальной ДНК (мтДНК) у пациентов с ишемической болезнью сердца (ИБС) в зависимости от наличия риска развития внезапной сердечной смерти (ВСС).

Материалы и методы. Были сформированы две группы пациентов: основная группа – пациенты с ИБС и высоким риском ВСС (n = 107), группа сравнения – пациенты со стабильным течением ИБС без риска ВСС (n = 50). Пациентам определяли гаплогруппу, носительство полиморфизмов A2706G, G3010A и G9055A мтДНК методами высокопроизводительного секвенирования. Оценивали дыхательную активность изолированных митохондрий из лейкоцитов периферической крови амперометрическим методом при использовании NAD- и FAD-зависимых субстратов окисления.

Результаты. В обеих исследованных группах гаплогруппы H, U, J являлись превалирующими (74,5 и 92,5% для основной группы и группы сравнения соответственно). В основной группе минорных гаплогрупп было больше, чем в группе сравнения. Частоты встречаемости полиморфизмов A2706G, G3010A, G9055A не имели значимых межгрупповых различий. В основной группе носительство замены A2706G ассоциируется со снижением коэффициента дыхательного контроля (ДК) при FAD-зависимом дыхании (p = 0,05), а в группе сравнения – со снижением скорости потребления кислорода (СПК) в метаболическом состоянии V4 при NAD- и FAD-зависимом типах дыхания (p = 0,002 и p = 0,008 соответственно) без изменения ДК. Носительство замены G9055A в основной группе ассоциировано со снижением СПК в метаболическом состоянии V3 (p = 0,037) при FAD-зависимом дыхании. Для полиморфизма G3010A мтДНК не выявлено связи с респираторной активностью митохондрий в исследованных группах.

Заключение. У пациентов с ИБС вне зависимости от риска развития ВСС частоты гаплогрупп H, U, J и полиморфизмов A2706G, G3010A, G9055A мтДНК не имеют значимых различий. У пациентов высокого риска ВСС носительство полиморфизма A2706G связано с падением ДК при FAD-зависимом дыхании, а полиморфизма G9055A – со снижением СПК в V3 при FAD-зависимом дыхании.

Ключевые слова: митохондрии, лейкоциты периферической крови, ишемическая болезнь сердца, внезапная сердечная смерть, полиморфизм мтДНК

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

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено комитетом по биомедицинской этике НИИ медицинской генетики Томского НИМЦ (протокол № 20 от 14.02.2024).

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INTRODUCTION

Chronic coronary heart disease (CHD) is recognized as a primary pathogenetic factor in the development of not only heart failure (HF) [1], but also an increased risk of life-threatening ventricular arrhythmias, such as ventricular tachycardia [2]. The latter could be the cause of sudden cardiac death among above-mentioned patient population [3]. The search for the markers that can improve the stratification of patients with a high risk of sudden cardiac death (SCD) is actively ongoing, since the markers available to cardiologists are not always sufficient [4].

According to some data, mitochondrial DNA (mtDNA) polymorphism may contribute to the development of pacemaker cell dysfunction resulting in cardiac arrhythmias including life-threatening ones [5]. It is not possible to directly assess the function and genome of cardiac myocytes in routine clinical practice. However, if somatic mutations of mtDNA are not taken into account, DNA isolated from peripheral blood leukocytes can be used for genotyping.

The aim was to assess the relationship between the respiration of peripheral blood leukocyte mitochondria and mitochondrial DNA polymorphism in patients with CHD depending on the risk of SCD.

MATERIALS AND METHODS

As part of the study, we divided patients into two groups. The main group encompassed 107 patients diagnosed with CHD, who underwent implantation of cardiac resynchronization therapy devices with a defibrillator function as primary and secondary prevention of life-threatening ventricular tachyarrhythmias according to clinical guidelines [6]. The comparison group encompassed 50 patients with stable CHD (without a history of cardiovascular events, such as myocardial infarction, stroke, thromboembolism, and sudden circulatory arrest) and without indications for implantation of resynchronization therapy devices. Clinical and laboratory characteristics of the patients are summarized in Table 1. The study protocol was approved by the local Ethics Committee of the Research Institute of Medical Genetics, Tomsk National Research Medical Center (Minutes No. 20 dated February 14, 2024). A signed informed consent was obtained from each patient included in the study.

Table 1

Clinical and Laboratory Characteristics of the Patients at the Time of Enrollment

Parameters	Main group (n = 107)	Comparison group (n = 50)	p-value
Age, *Me* (Q_1; Q_3), years	64.0 (59.0; 71.0)	67.0 (63.0; 72.0)	0.147
Men, *n* (%)	83 (77.6%)	22 (44.0%)	<0.001
Angina pectoris, *n* (%)	83 (77.6%)	36 (72.0%)	0.320
Myocardial infarction, *n* (%)	72 (67.3%)	0 (0%)	
Coronary atherosclerosis, *n* (%)	47 (43.9%)	35 (70.0%)	0.003
LVEF, *Me* (Q_1; Q_3), %	42 (33; 58)	65 (64; 67)	<0.001
NYHA HF FC I, *n* (%)	11 (10.3%)	20 (40.0%)	<0.001
NYHA HF FC II, *n* (%)	58 (54.2%)	21 (42.0%)	0.121
NYHA HF FC III, *n* (%)	38 (35.5%)	9 (18.0%)	0.036
Hypertension, *n* (%)	99 (92.5%)	49 (98.0%)	0.552
Body mass index, *Me* (Q_1; Q_3), kg/m^2	29.1 (26.2; 33.1)	31.2 (26.5; 34.5)	0.326
Obesity, *n* (%)	47 (43.9%)	25 (50.0%)	0.699
Dyslipidemia, *n* (%)	78 (72.9%)	28 (56.0%)	0.020
Diabetes mellitus, *n* (%)	23 (21.5%)	7 (14.0 %)	0.244
Carotid artery atherosclerosis, *n* (%)	53 (49.5%)	40 (80.0%)	<0.001
Femoral artery atherosclerosis, *n* (%)	35 (32.7%)	25 (50.0%)	0.076
Thyroid gland diseases, *n* (%)	10 (9.3%)	11 (22.0%)	0.068
ACEi/ARA, *n* (%)	85 (79.4%)	38 (76.0%)	0.310
BAA, *n* (%)	88 (82.2%)	29 (58.0%)	<0.001
Anticoagulants, *n* (%)	44 (41.1%)	12 (24.0%)	0.029
SGLT2i, *n* (%)	24 (22.4%)	5 (10.0%)	0.055
Statins, *n* (%)	93 (86.9%)	43 (86.0%)	0.403
Diuretics, *n* (%)	49 (43.9%)	18 (36.0%)	0.208
Antiarrhythmics, *n* (%)	37 (34.6%)	10 (20.0%)	0.053
CCBA, *n* (%)	15 (14.0%)	21 (42.0%)	<0.001
Antiplatelets, *n* (%)	68 (63.6%)	33 (66.0%)	0.693
Glucose, *Me* (Q_1; Q_3), mmol/l	5.69 (5.22; 6.60)	5.57 (5.05; 6.19)	0.204
Total cholesterol, *Me* (Q_1; Q_3), mmol/l	4.14 (3.62; 5.00)	4.35 (3.60; 5.50)	0.466

End of Table 1

Parameters	Main group (n = 107)	Comparison group (n = 50)	p-value
Triglycerides, *Me* (Q_1; Q_3), mmol/l	1.27 (0.92; 1.86)	1.47 (1.13; 1.87)	0.307
HDL, *Me* (Q_1; Q_3), mmol/l	1.21 (0.93; 1.46)	1.15 (1.03; 1.45)	0.879
LDP, *Me* (Q_1; Q_3), mmol/l	2.25 (1.55; 3.21)	2.40 (1.70; 3.10)	0.855

Note. LVEF – left ventricular ejection fraction; FC – functional class; CHF – chronic heart failure; NYHA – New York Heart Association; ACEi – angiotensin-converting enzyme inhibitor; ARA – angiotensin II receptor antagonist; BAA – beta-adrenoreceptor antagonist; SGLT2i – sodium-glucose transporter type 2 inhibitors; CCBA – calcium channel-blocking agent; HDP – high-density lipoproteins; LDP – low-density lipoproteins.

All patients had blood collected in vacutainers with EDTA. Isolation of peripheral blood leukocytes was carried out using Histopaque-1077 density gradient (Sigma, USA). The resulting "ring" containing leukocytes was washed in phosphate buffered saline (pH = 7.40) (Sigma). Isolated mitochondria were obtained using the commercial Mitochondria Isolation Kit for Cultured Cells (ThermoScientific, USA) according to the manufacturer's instructions. The resulting pellet was resuspended in a minimum volume of 0.25 M sucrose for further work. The methodology of studying respiration during NAD-dependent (pyruvate + malate) and FAD-dependent (succinate) substrate oxidation was described previously [7]. The assessed parameters of mitochondrial respiration were V3 – phosphorylating metabolic state (in the presence of oxidation substrates, inorganic phosphate and ADP), V4 – non-phosphorylating metabolic state (after ADP depletion), and respiratory control coefficient (RC) – V3/V4.

To study mtDNA, total DNA was isolated from peripheral blood leukocytes. The complete mtDNA sequence was determined using high-throughput sequencing as described previously [8]. The mtDNA haplogroup of each patient was determined using the mtDNA-Server 2 program [9]. The search for associations with cellular respiration was carried out for three mtDNA polymorphisms: A2706G (marker of haplogroup H), G3010A (marker of haplogroup H1), and G9055A (marker of haplogroup K).

The choice of the above-noted polymorphisms for analysis was determined by the results of our own research and literature data. For haplogroup H, associations with myocardial infarction, ischemic cardiomyopathy, and postoperative atrial fibrillation were previously found [10–12]; for haplogroup H1, associations with increased risk of cardiovascular accidents, including sudden cardiac death, were found [13; 14]; haplogroup K, according to the results of several studies, showed a protective effect, but only in neurodegenerative disease [15; 16], whereas we previously revealed a higher frequency of the G9055A polymorphism in individuals who survived cardiac arrest [17].

Statistical data processing was carried out using STATISTICA 10.0 software package. The hypothesis of normal distribution of quantitative data was tested using the Shapiro – Wilk test. The differences between quantitative variables were assessed using the non-parametric Mann – Whitney test. The results were presented as median, upper and lower quartiles (*Me* (Q_1; Q_3)). Differences in frequencies were studied using the Pearson's chi-square test. The results were presented as absolute frequencies (n) and percentages (%). Differences were considered statistically significant at $p < 0.05$.

RESULTS

According to the data presented in Table 1, men predominated in the main group (80.6% vs. 44.0% compared to the comparison group, $p < 0.001$). Patients of this group more often had NYHA functional class II HF ($p = 0.036$), dyslipidemia ($p = 0.020$), and their LVEF was on average classified as mildly reduced, whereas in the comparison group, all patients had preserved LVEF. Also, patients in the main group more often took β-blockers ($p < 0.001$) and anticoagulants ($p = 0.029$), and less often took calcium antagonists ($p < 0.001$).

Genotyping and determination of mtDNA haplogroups were carried out in samples of 102 patients of the main group and 40 patients of the comparison group (Table 2). The frequencies of predominant haplogroups among patients in both studied groups corresponded to the population distribution [8]. The highest frequencies of occurrence were registered for haplogroups H, U, and J, and the total contribution of the listed mtDNA variants in the comparison and main groups was 92.5% and 74.5%, respectively. Also, haplogroup T was one of the most common mtDNA haplogroups in the main group of patients (10.8%). In the comparison group, the minor haplogroups were the following: T, D, and M-G. Carriage of these haplogroups was established in only 3 patients (1 case for each haplogroup). In the main group, there were more minor mtDNA haplogroups. In this group, these

included the following haplogroups: M-G, V, W, A, F, N, I, X, HV, C, and R. The obtained results are consistent with previous data obtained on a sample of patients with ischemic heart failure [8].

Table 2

Frequencies of Occurrence of mtDNA Haplogroups Among the Studied Patients, *n* (%)

mtDNA haplogroup	Comparison group ($n = 40$)	Main group ($n = 102$)	*p*-value
H, *n* (%)	19 (47.5%)	42 (41.2%)	0.86
U, *n* (%)	13 (32.5%)	25 (24.5%)	0.87
J, *n* (%)	5 (12.5%)	9 (8.8%)	0.45
T, *n* (%)	1 (2.5%)	11 (10.8%)	0.22
D, *n* (%)	1 (2.5%)	0 (0%)	0.14
A/M-G, *n* (%)	1 (2.5%)	1 (1.0%)	0.58
V, *n* (%)	0 (0%)	2 (2.0%)	0.32
W, *n* (%)	0 (0%)	1 (1.0%)	0.49
A, *n* (%)	0 (0%)	2 (2.0%)	0.32
F, *n* (%)	0 (0%)	1 (1.0%)	0.49
N, *n* (%)	0 (0%)	2 (2.0%)	0.32
I, *n* (%)	0 (0%)	1 (1.0%)	0.49
X, *n* (%)	0 (0%)	2 (2.0%)	0.32
HV, *n* (%)	0 (0%)	1 (1.0%)	0.49
C, *n* (%)	0 (0%)	1 (1.0%)	0.49
R, *n* (%)	0 (0%)	1 (1.0%)	0.49

Note. Here and in Table 3: mtDNA – mitochondrial DNA.

Analysis of the carriage of mtDNA A2706G, G3010A, and G9055A polymorphisms was carried out in 97 samples from the main group and 40 samples from the comparison group. Results are presented in Table 3. There were no significant differences in the occurrence frequencies of these mtDNA variants between the studied groups.

Table 3

Frequencies of mtDNA A2706G, G3010A, and G9055A Polymorphisms in the Studied Groups, *n* (%)

mtDNA polymorphism	Comparison group ($n = 40$)	Main group ($n = 94$)	*p*-value
A2706G, *n* (%)	23 (60.0%)	62 (66.0%)	0.91
G3010A, *n* (%)	12 (30.0%)	20 (21.3%)	0.36
G9055A, *n* (%)	4 (10.0%)	10 (10.6%)	0.92

Table 4 demonstrates the results of the assessment of the respiration of peripheral blood leukocyte mitochondria in carriers of the A2706G polymorphism in both studied groups. The presented data show that in carriers of this polymorphism in the comparison group, oxygen consumption rate in V4 metabolic state significantly decreased in both NAD- and FAD-dependent substrate oxidation ($p = 0.002$ and $p = 0.008$, respectively). In both cases, there were no significant changes in RC. In the main group, carriage of this polymorphism was associated with a decrease in RC in FAD-dependent substrate oxidation ($p = 0.05$).

The presence of guanine at position 2706 of mtDNA was associated with a decrease in the efficiency of phosphorylation only in succinate oxidation in patients of the main group, whereas in the comparison group, it exerted a protective effect reducing oxygen consumption rate in non-phosphorylating state. This is consistent with the data indicating increased production of reactive oxygen species as a result of electron leakage from the respiratory chain in carriers of haplogroup H (haplotype 2706A [18]), despite high levels of oxygen consumption (VO2max) during physical activity [19].

It is likely that carriage of this haplogroup is very common among patients with cardiovascular diseases, since long-term exposure to reactive oxygen species is damaging, including in relation to cardiomyocytes and their mitochondria. Together with risk factors and comorbidity, this results in a progressive decrease in myocardial contractile activity and the development of arrhythmias, including life-threatening ones.

In carriers of the G9055A polymorphism, a simultaneous decrease in mitochondrial respiration in V3 ($p = 0.037$) and V4 ($p = 0.037$) metabolic states with a fall in RC ($p = 0.13$), which did not reach statistical significance, was shown among patients with high risk of sudden cardiac death in FAD-dependent substrate oxidation (Table 5).

Carriage of the G3010A mtDNA polymorphism was not associated with a significant change in mitochondrial respiration in any of the studied groups in either NAD- or FAD-dependent substrate oxidation (Table 6).

Table 4

Peripheral Blood Leukocyte Mitochondria Respiration in the Presence and the Absence of mtDNA A2706G Polymorphism, (*Me* (Q_1; Q_3))

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	A2706	G2706	*p*-value	A2706	G2706	*p*-value
V3, nmol O_2/min/mg mitochondrial protein	196.44 (125.42; 245.15)	125.19 (93.69; 137.09)	0.07	123.18 (82.57; 194.71)	104.93 (61.81; 161.98)	0.52

End of table 4

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	A2706	G2706	*p*-value	A2706	G2706	*p*-value
V4, nmol O_2/min/mg mitochondrial protein	67.19 (52.08; 123.06)	41.47 (34.90; 47.78)	0.002	42.64 (30.38; 54.69)	41.87 (27.50; 55.15)	0.87
RC, RU	2.25 (1.89; 3.07)	2.47 (1.92; 3.57)	0.54	2.57 (2.47; 2.70)	2.35 (2.23; 2.88)	0.22
	FAD-dependent substrate					
V3, nmol O_2/min/mg mitochondrial protein	235.75 (156.25; 312.50)	175.93 (108.42; 193.73)	0.19	82.57 (71.88; 150.24)	140.31 (74.22; 169.96)	0.74
V4, nmol O_2/min/mg mitochondrial protein	87.50 (52.82; 106.21)	36.46 (28.91; 61.34)	0.008	29.20 (20.90; 54.69)	49.46 (32.50; 66.96)	0.19
RC, RU	2.96 (2.58; 3.38)	2.91 (2.45; 3.80)	1.00	3.23 (2.30; 4.18)	2.57 (2.16; 2.76)	0.05

Note. Here and in Tables 5 and 6. V3 – phosphorylating metabolic state; V4 – non-phosphorylating metabolic state; RC – respiratory control ratio (V3/V4)

Table 5

Peripheral Blood Leukocyte Mitochondria Respiration in the Presence and the Absence of mtDNA G9055A Polymorphism, (*Me* (Q_1; Q_3))

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	G9055	A9055	*p*-value	G9055	A9055	*p*-value
V3, nmol O_2/min/mg mitochondrial protein	125.32 (85.33; 199.42)	131.32 (127.81; 140.54)	0.79	107.95 (76.39; 161.98)	53.35 (50.65; 57.40)	0.09
V4, nmol O_2/min/mg mitochondrial protein	44.95 (34.09; 56.25)	51.53 (47.67; 55.13)	0.62	45.96 (30.27; 54.69)	23.95 (23.95; 25.95)	0.09
RC, RU	2.32 (1.88; 3.10)	2.55 (2.34; 2.87)	0.62	2.53 (2.30; 2.88)	2.23 (2.15; 2.61)	0.13
	FAD-dependent substrate					
V3, nmol O_2/min/mg mitochondrial protein	165.18 (72.62; 225.00)	254.32 (154.18; 260.33)	0.15	128.68 (75.76; 162.55)	32.67 (28.51; 45.29)	0.037
V4, nmol O_2/min/mg mitochondrial protein	56.25 (29.71; 88.14)	84.77 (51.46; 92.52)	0.52	43.66 (26.69; 64.41)	15.24 (14.40; 29.87)	0.037
RC, RU	2.67 (2.33; 3.53)	3.00 (2.41; 3.37)	0.46	2.64 (2.28; 3.23)	2.14 (2.01; 2.56)	0.13

Table 6

Peripheral Blood leukocyte Mitochondria Respiration in the Presence and the Absence of mtDNA G3010A Polymorphism, (*Me* (Q_1; Q_3))

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	G3010	A3010	*p*-value	G3010	A3010	*p*-value
V3, nmol O_2/min/mg mitochondrial protein	131.32 (120.31; 199.42)	164.30 (93.16; 235.86)	0.96	120.86 (64.95; 203.61)	106.06 (79.78; 114.48)	0.56
V4, nmol O_2/min/mg mitochondrial protein	51.86 (44.94; 78.13)	41.59 (36.32; 71.35)	0.26	48.65 (27.50; 91.02)	33.44 (30.13; 50.89)	0.10
RC, RU	2.29 (1.88; 3.57)	2.50 (2.29; 3.07)	0.35	2.52 (2.16; 2.70)	2.49 (2.30; 3.32)	0.52
	FAD-dependent substrate					
V3, nmol O_2/min/mg mitochondrial protein	170.55 (69.90; 254.32)	132.33 (106.25; 172.28)	0.57	128.68 (65.00; 178.31)	133.93 (81.21; 163.10)	0.69
V4, nmol O_2/min/mg mitochondrial protein	58.80 (29.71; 95.24)	43.03 (28.91; 87.50)	0.66	43.66 (29.20; 66.96)	38.07 (23.36; 65.10)	0.65
RC, RU	2.94 (2.57; 3.81)	2.95 (2.34; 3.16)	0.48	2.61 (2.17; 2.87)	3.17 (2.43; 4.07)	0.10

DISCUSSION

The search for associations between the carriage of mtDNA polymorphisms, the belonging of mtDNA to a certain haplogroup, and cardiovascular disease progression type is an actively developing field today. The aim of the study was to reveal the relationship between the carriage of single polymorphisms, A2706G, G3010A, and G9055A, as well as the belonging of mtDNA of individuals of the studied groups to certain haplogroups and the risk of developing sudden cardiac death in patients diagnosed

with ischemic heart disease. When comparing the group of patients without risk of sudden cardiac death and the group of patients with high risk of sudden cardiac death, no differences in the frequencies of mtDNA haplogroups were found. The predominant mtDNA haplogroup in both groups was haplogroup H (47.5% in the comparison group and 41.2% in the main group), which is consistent with literature data.

In point of fact, this haplogroup is the main one for our population; its frequency of occurrence in our country reaches an average of 40% [20]. Less frequently, patients were identified as carriers of haplogroups U (32.5% and 24.5% for the comparison group and the main group, respectively) and J (12.5% and 8.8%, respectively), which also correlates with previous literature data. The main group was characterized by a greater variety of minor mtDNA haplogroups, such as A/M-G, C, D, F, HV, I, N, R, V, W, and X.

The analysis of available literature data did not reveal any studies aimed to identify the role of the relationship between the carriage of the A2706G polymorphism (encodes the 12S rRNA subunit; marker polymorphism of haplogroup H) and mitochondrial respiration. In our study, this polymorphism was associated with a decrease in oxygen consumption rate in V4 metabolic state in oxidation of various substrates ($p = 0.002$ and $p = 0.008$ in NAD- and FAD-dependent respiration, respectively) in patients diagnosed with CHD without the risk of sudden cardiac death. It could be assumed that in this group of patients, the carriage of this polymorphism partially exerts a protective effect aimed to reduce respiratory activity in V4 metabolic state to reduce oxygen consumption for processes not associated with the synthesis of ATP by mitochondria.

In patients with CHD and high risk of sudden cardiac death, the carriage of the A2706G polymorphism was associated with lower RC ratio in FAD-dependent substrate oxidation. With respect to other respiration parameters, carriage of this polymorphism did not make a significant contribution to changes in mitochondrial respiratory parameters in patients of this group. It may be concluded that the carriage of this polymorphism has a probable protective effect in patients with CHD without the risk of developing life-threatening tachyarrhythmias. This effect is the opposite in patients with high risk of developing life-threatening arrhythmias.

There is no valid evidence in the available sources on the influence of the G3010A polymorphism carriage (replacement in the 12S rRNA; marker polymorphism of haplogroup H1) on mitochondrial function in cardiovascular diseases. A number of studies have only revealed associations between an unfavorable course of cardiovascular diseases, including the development of sudden cardiac death [13, 14]. In this study, carriage of mentioned mtDNA polymorphism was not associated with any changes in mitochondrial respiration or oxidation-phosphorylation coupling, neither in NAD-dependent nor in FAD-dependent substrates oxidation, both in the group of patients without the risk of sudden cardiac death and in the group of patients with high risk of sudden cardiac death.

The G9055A mtDNA polymorphism determines an amino acid substitution in the ATPase-6 subunit of ATP synthase. Only a small number of studies aimed to evaluate the effect of this polymorphism on energy metabolism in various diseases not related to cardiovascular pathology (Parkinson's disease, autism, and breast cancer) have been published [21, 22]. The results of the study showed that the contribution of this mtDNA polymorphism to the mitochondrial respiration resulted in a significant decrease in oxygen consumption rate in succinate oxidation in patients of the main group who carry this polymorphism, i.e., it had a negative effect on the functioning of the electron transport chain. Otherwise, in patients with complicated CHD, this polymorphism was not associated with any significant changes in mitochondrial respiration. In patients of the comparison group, carriage of this polymorphism was not accompanied by any significant changes in mitochondrial respiration. Most likely, for these patients, the G9055A polymorphism is neutral and does not make a significant contribution to the course of the underlying disease.

CONCLUSION

In this study, a comprehensive study of peripheral blood leukocyte mitochondria was carried out in cardiac patients diagnosed with CHD and high risk of developing life-threatening arrhythmias, namely their functional activity, haplogroups, and certain mtDNA polymorphism (A2706G, G3010A, and G9055A). The frequency distribution of haplogroups in CHD patients with and without high risk of developing life-threatening tachyarrhythmias corresponds to the population distribution. As in the population, haplogroups H, U, and J were predominant for CHD patients regardless of the risk of developing sudden cardiac death. The presence of guanine at position 2706 of mtDNA was associated with a decrease in the

activity of non-phosphorylating state of mitochondrial respiration only in case of CHD without the risk of developing sudden cardiac death. In case of CHD with high risk of sudden cardiac death, carriage of the G9055A polymorphism was associated with a decrease in the intensity of mitochondrial respiration in both phosphorylating and non-phosphorylating states in FAD-dependent substrate oxidation. For the G3010A mtDNA polymorphism, no association with changes in respiration of peripheral blood leukocyte mitochondria was revealed.

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Neurocognitive Deficits in Schizophrenia and Polymorphic Variants of Protein Kinase Signaling Pathway Genes: Search for Associations

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ABSTRACT

Aim. To study the associations of polymorphic variants in the *BDNF, GSK3B, AKT1, MAPK,* and *CREB1* genes with neurocognitive deficits (NCD) in patients with schizophrenia.

Materials and methods. The study included 148 patients with schizophrenia, who underwent psychometric examination and genotyping. The Brief Assessment of Cognition in Schizophrenia (BACS) was used to assess neurocognitive functioning indicators. Ten polymorphic variants in the genes *BDNF*, *GSK3B*, *AKT1*, *MAPK*, and *CREB1* were genotyped. Statistical processing was carried out using the $\chi 2$ goodness-of-fit test, Fisher's exact test, cluster analysis, the Kruskal – Wallis test, and multivariate analysis of variance.

Results. The CT genotype of the *BDNF* rs6265 polymorphic variant was more common in the group of patients with severe NCD, while the CC genotype was more typical of patients with moderate and mild NCD. In patients with severe and moderate NCD, the AG *MAPK* rs8136867 genotype was predominant, while in patients with mild NCD, the GG genotype was predominant. A statistically significant effect of polymorphic variants of the *BDNF* gene on performance in the Token motor task (rs6265: $p = 0.025$ and rs11030104: $p = 0.027$) and the Tower of London subtests (rs6265: $p = 0.016$ and rs11030104: $p = 0.037$) was found. There was also a significant effect of *MAPK* gene polymorphisms on the performance in the Token motor task subtest (rs8136867: $p = 0.003$) and *CREB1* on the Tower of London test (rs6740584: $p = 0.022$).

Conclusion. For the first time, associations of *BDNF* rs6265 and *MAPK* rs8136867 polymorphisms with neurocognitive deficit in patients with schizophrenia, as well as *BDNF* rs6265, *BDNF* rs11030104, *MAPK* rs8136867, and *CREB1* rs6740584 polymorphisms with performance in the BACS battery subtests were found.

Keywords: schizophrenia, neurocognitive deficits, molecular genetics, protein kinases, BDNF, single nucleotide polymorphism

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

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

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Нейрокогнитивный дефицит при шизофрении и полиморфные варианты генов протеинкиназных сигнальных путей: поиск ассоциаций

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РЕЗЮМЕ

Цель. Изучить ассоциации полиморфных вариантов в генах *BDNF*, *GSK3B*, *AKT1*, *MAPK* и *CREB1* с нейрокогнитивным дефицитом (НКД) у больных шизофренией.

Материалы и методы. В исследование включены 148 пациентов с шизофренией, у которых было проведено психометрическое обследование и генотипирование. Для оценки показателей нейрокогнитивного функционирования использовалась краткая шкала оценки когниции при шизофрении (BACS). Подвергнуты генотипированию 10 полиморфных вариантов в генах *BDNF*, *GSK3B*, *AKT1*, *MAPK* и *CREB1*. Статистическая обработка осуществлена с помощью критерия согласия $\chi 2$, точного критерия Фишера, кластерного анализа, критерия Краскела – Уоллиса и многофакторного дисперсионного анализа.

Результаты. Генотип CT полиморфного варианта *BDNF* rs6265 чаще встречался в группе пациентов с выраженным НКД, в то время как для пациентов с умеренным и легким НКД был более характерен генотип CC. У пациентов с выраженным и умеренным НКД преобладал генотип AG *MAPK* rs8136867, тогда как у пациентов с легким НКД – генотип GG. Обнаружено статистически значимое влияние полиморфных вариантов гена *BDNF* на результативность в субтестах «Двигательный тест с фишками» (rs6265: $p = 0{,}025$ и rs11030104: $p = 0{,}027$) и «Башня Лондона» (rs6265: $p = 0{,}016$ и rs11030104: $p = 0{,}037$). Также наблюдался значимый эффект полиморфных вариантов гена *MAPK* на показатели в субтесте «Двигательный тест с фишками» (rs8136867: $p = 0{,}003$) и *CREB1* – в субтесте «Башня Лондона» (rs6740584: $p = 0{,}022$).

Заключение. Впервые обнаружены ассоциации полиморфных вариантов *BDNF* rs6265 и *MAPK* rs8136867 с нейрокогнитивным дефицитом у больных шизофренией, а также полиморфных вариантов *BDNF* rs6265, *BDNF* rs11030104, *MAPK* rs8136867 и *CREB1* rs6740584 с результативностью в субтестах батареи BACS.

Ключевые слова: шизофрения, нейрокогнитивный дефицит, молекулярная генетика, протеинкиназы, BDNF, однонуклеотидный полиморфизм

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

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

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INTRODUCTION

Despite the fact that antipsychotic therapy effectively relieves the main clinical symptoms of psychosis (hallucinations, psychomotor agitation, delusions, and mental health conditions), the problem of developing neurocognitive deficit (NCD) in patients with schizophrenia is still relevant in psychiatry. NCDs are often some of the causes of reduced social and work adaptation of patients, as well as disability [1]. Despite the fact that NCD does not belong to the fundamental criteria of schizophrenia in

modern diagnostic guidelines [2, 3], it is a persistent symptom complex that forms at the very beginning of the disease and makes a significant contribution to its clinical polymorphism [4].

It is believed that NCDs in schizophrenia are based on dopamine neurotransmission dysfunctions, as well as the psychotomimetic and cognitive-disruptive effects of N-methyl-d-aspartate (NMDAR) receptor antagonists [5]. In this regard, it is currently becoming relevant to search for opportunities not only for targeted drug therapy, but also for mechanisms to increase the neuroplasticity of people with schizophrenia.

The *BDNF* gene encodes a brain neurotrophic factor that supports brain development, survival of nerve cells [6], branching of their dendrites, and neuroplasticity in general [7]. BDNF is expressed in all areas of the brain, with its highest concentration in the hippocampus and the cerebral cortex [8]. When BDNF binds to its high-affinity TrkB receptor (tropomyosin-related kinase B), this leads to phosphorylation of the latter and activation of intracellular cascades, such as PI3K/AKT and MAPK/ERK signaling pathways [9].

The product of the *AKT1* gene, protein kinase type 1, regulates cell growth, proliferation, and metabolism, apoptosis, and angiogenesis [10, 11], and also plays an important role in the negative regulation of GSK-3ß (glycogen synthase 3ß kinase). By activating AKT, BDNF is able to block GSK-3ß, thereby increasing neuronal polarization, growth, and branching of neuronal axons.

The MAPK pathway is the main point of convergence in all protein kinase signaling pathways. It is responsible for regulating cell growth and differentiation and neuroplasticity [12]. The downstream target of the MAPK pathway is the transcription factor CREB, which regulates many cellular functions: neurotransmission, transcription, neuroplasticity, and metabolism. There is evidence confirming the contribution of CREB to the development of addictive disorders, subclinical and clinical manifestations of anxiety and depression [13].

The contribution of the genetic component to the development of schizophrenia has been repeatedly confirmed in research [14, 15]. A number of publications have demonstrated that BDNF, AKT, and GSK-3 can be considered as potential biomarkers of schizophrenia [16–18]. In this regard, we hypothesized that polymorphic variants in the *BDNF*, *GSK3B*, *AKT1*, *MAPK*, and *CREB1* genes may contribute to the formation of NCD as a component of the clinical pattern of schizophrenia.

The aim of the research was to study the associations of polymorphic variants in the *BDNF*, *GSK3B*, *AKT1*, *MAPK*, and *CREB1* genes with neurocognitive deficits in patients with schizophrenia.

MATERIALS AND METHODS

The study was conducted at the clinic of the Mental Health Research Institute of Tomsk National Research Medical Center of the Russian Academy of Sciences. The study included 148 patients born and living in the Siberian Federal District of the Russian Federation with an established diagnosis of schizophrenia (F20 in accordance with the criteria of the International Classification of Diseases, 10th Revision (ICD-10)). Inclusion criteria were as follows: age of patients from 18 to 55 years, belonging to the Slavic ethnic group, established diagnosis of schizophrenia according to the ICD-10 criteria, and consent to participate in the study. Exclusion criteria were the following: mental retardation, dementia, severe organic pathology or somatic-symptom somatic disorders in the stage of decompensation, and refusal to participate in the study.

All patients included in the sample received basic antipsychotic therapy with conventional or atypical antipsychotics. To unify the assessment of pharmacotherapy, the doses of the drugs were converted to the chlorpromazine equivalent (CRZeq, mg/day).

The Basic Map of Socio-demographic and Clinical-dynamic Characteristics for Patients with Schizophrenia [19], which had been previously tested in clinical trials, was filled out for all subjects. The severity of the patients' psychopathological symptoms was verified by the Positive and Negative Syndrome Scale (PANSS) [20] in the adapted Russian version (SCI-PANSS) [21].

The assessment of neurocognitive functioning indicators was carried out using the Brief Assessment of Cognition in Schizophrenia (BACS) in an adapted Russian-language version [22] using normative indicators for the Tomsk population [23]. The scale consists of six subtests: 1) List Learning (verbal memory); 2) Digit Sequencing Task (working memory); 3) Token Motor Task (motor functions); 4) Controlled Oral Word Association Test (semantic fluency); 5) Symbol Coding (attention); 6) Tower of London (executive functions).

Blood sampling for genotyping was performed on an empty stomach (after 12 hours of fasting, between 7.00 am and 9.00 am) through ulnar venipuncture into

BD Vacutainer tubes. Ten polymorphic variants of five genes were genotyped using polymerase chain reaction methods on a QuantStudio 3D Digital PCR System (Applied Biosystems, USA): *BDNF* (rs6265, r s11030104), *GSK3B* (rs13321783, rs6805251, rs334558), *AKT1* (rs1130233, rs3730358), *MAPK* (rs8136867, rs3810608), and *CREB1* (rs6740584).

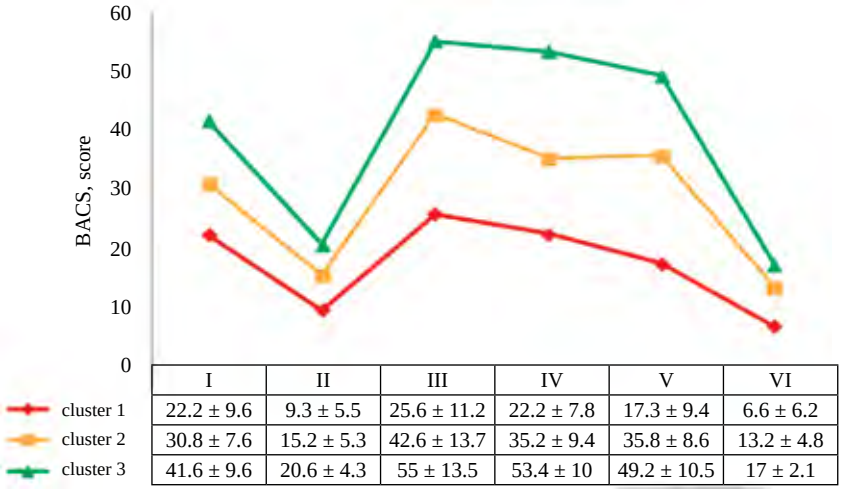
The statistical analysis was performed using the software Statistica 12.0 and R 4.4.3. The nature of the distribution of variables (agreement with the law of normal distribution) was assessed using the Shapiro–Wilk test. Data with a normal distribution were presented as the mean and standard deviation – $M \pm SD$, in the absence of a normal distribution – as the median of the interquartile range $Me\ [_{Q1;\ Q3}]$. Qualitative variables were represented as absolute (n) and relative (%) units.

The frequency analysis was carried out using the agreement $\chi 2$ test and the Fisher's exact test (in the case of frequencies less than 5). K-means clustering was used to identify the severity of NCD. The Kruskal–Wallis test was used to compare several independent samples. The genetic balance was calculated in the R program using the "genetics" package. Multifactorial analysis of variance (factorial ANOVA) was used to study the relationship between polymorphic variants and indicators of cognitive functioning. The value of $p < 0.05$ was considered statistically significant.

RESULTS

Based on the BACS, we identified three clusters of neurocognitive disorders registered across all subtests, which differed in clinical severity: cluster 1 (38 (25.7%) patients) with severe NCD, cluster 2 (67 (45.3%) patients) with moderate NCD, and cluster 3 (43 (29%) patients) with mild NCD ($p < 0.001$) (Fig. 1).

The socio-demographic and clinical indicators of the selected clusters of patients with schizophrenia are presented in Table 1.



	I	II	III	IV	V	VI
cluster 1	22.2 ± 9.6	9.3 ± 5.5	25.6 ± 11.2	22.2 ± 7.8	17.3 ± 9.4	6.6 ± 6.2
cluster 2	30.8 ± 7.6	15.2 ± 5.3	42.6 ± 13.7	35.2 ± 9.4	35.8 ± 8.6	13.2 ± 4.8
cluster 3	41.6 ± 9.6	20.6 ± 4.3	55 ± 13.5	53.4 ± 10	49.2 ± 10.5	17 ± 2.1

Fig. 1. The cognitive profile of the identified variants of the severity of neurocognitive deficits according to BACS in the group of patients with schizophrenia: I – List Learning, II – Digit Sequencing Task, III – Token Motor Task, IV – Controlled Oral Word Association Test, V – Symbol Coding, VI – Tower of London

Table 1

Socio-demographic and Clinical Parameters of Patients in the Identified Clusters according to BACS

Parameter		Cluster 1, $n = 38$	Cluster 2, $n = 67$	Cluster 3, $n = 43$	p
Sex (male/female), n		20/18	39/28	22/21	0.735
Age, $Me\ [Q_1; Q_3]$, years		38 [33; 47]	34 [30; 38]	35 [29; 40]	0.107
Age of symptom manifestation, $Me\ [Q_1; Q_3]$, years		23 [18; 28]	24 [20; 30]	23 [20; 29]	0.621
Duration of the disease, $Me\ [Q_1, Q_3]$, years		12 [4; 21]	7 [3; 16]	10 [5; 14]	0.124
PANSS, $Me\ [Q_1; Q_3]$	Positive symptoms	25 [20; 29]	26 [19; 30]	23 [18; 28]	0.104
	Negative symptoms	26 [24; 29]	26 [24; 29]	25 [21; 28]	0.161
	General psychopathological symptoms	57 [51; 62]	55 [50; 60]	53 [48; 60]	0.146
	Total score	111 [101; 119]	108 [97; 114]	100 [92; 107]	0.073
Duration of baseline therapy, $Me\ [Q_1, Q_3]$, years		4 [0.5; 17]	3 [0.5; 9]	3.5 [0.6; 8]	0.308
Type of antipsychotic, n (%)	Atypical	16 (42.1)	32 (47.8)	31 (72.1)	0.012
	Conventional	22 (57.9)	35 (52.2)	12 (27.9)	
CPZeq, $Me\ [Q_1; Q_3]$, mg / day		379 [225; 800]	400 [250; 599]	300 [125; 599]	0.105

The selected clusters were comparable in terms of sex, age, age of manifestation of the schizophrenic process, duration of the disease, and severity of psychopathological symptoms. The duration of the baseline therapy received by the patients, as well as the overall antipsychotic load, did not differ between the clusters, however, in patients with mild NCD, atypical antipsychotics were used as baseline therapy compared with the rest of the study participants (p = 0.012).

The frequencies of the studied polymorphic variants in the general sample of patients with schizophrenia followed the Hardy–Weinberg equilibrium (all p > 0.05). Analysis of the genotype frequency distribution revealed statistically significant differences between patient clusters for the polymorphic variant *BDNF* rs6265 and *MAPK* rs8136867 (Table 2).

The CT *BDNF* rs6265 genotype was more common in the group of patients with severe NCD, while the CC genotype was more typical of patients with moderate and mild NCD. However, in patients with severe and moderate NCD, the AG *MAPK* rs8136867 genotype prevailed, whereas in patients with mild NCD, the GG genotype prevailed.

Table 2

Genotype Frequencies in Patients with Schizophrenia Depending on the Severity of NCD, units

Polymorphism	Genotype	Cluster 1	Cluster 2	Cluster 3	$\chi 2$/F	p
BDNF rs6265	CT	13 (34.2)	11 (16.4)	9 (20.9)	8.546	0.045
	CC	23 (60.5)	56 (83.6)	33 (76.8)		
	TT	2 (5.3)	0 (0)	1 (2.3)		
MAPK rs8136867	AG	27 (71.1)	36 (53.7)	15 (34.9)	12.829	0.012
	GG	5 (13.2)	15 (22.4)	18 (41.9)		
	AA	6 (15.7)	16 (23.9)	10 (23.2)		

Next, the influence of the studied polymorphic variants on the indicators of neurocognitive functioning in patients with schizophrenia was analyzed using multifactorial analysis of variance (factorial ANOVA). A statistically significant effect of polymorphic variants of the *BDNF* gene on performance was found in the subtests: Token Motor Task (rs6265: p = 0.025 and rs11030104: p = 0.027) and Tower of London (rs6265: p = 0.016 and rs11030104: p = 0.037). There was also a significant effect of the polymorphic variant *MAPK* rs8136867 on the indicators in the Token Motor Task subtest (p = 0.003) and *CREB1* rs6740584 on the Tower of London subtest (p = 0.022) (Fig. 2).

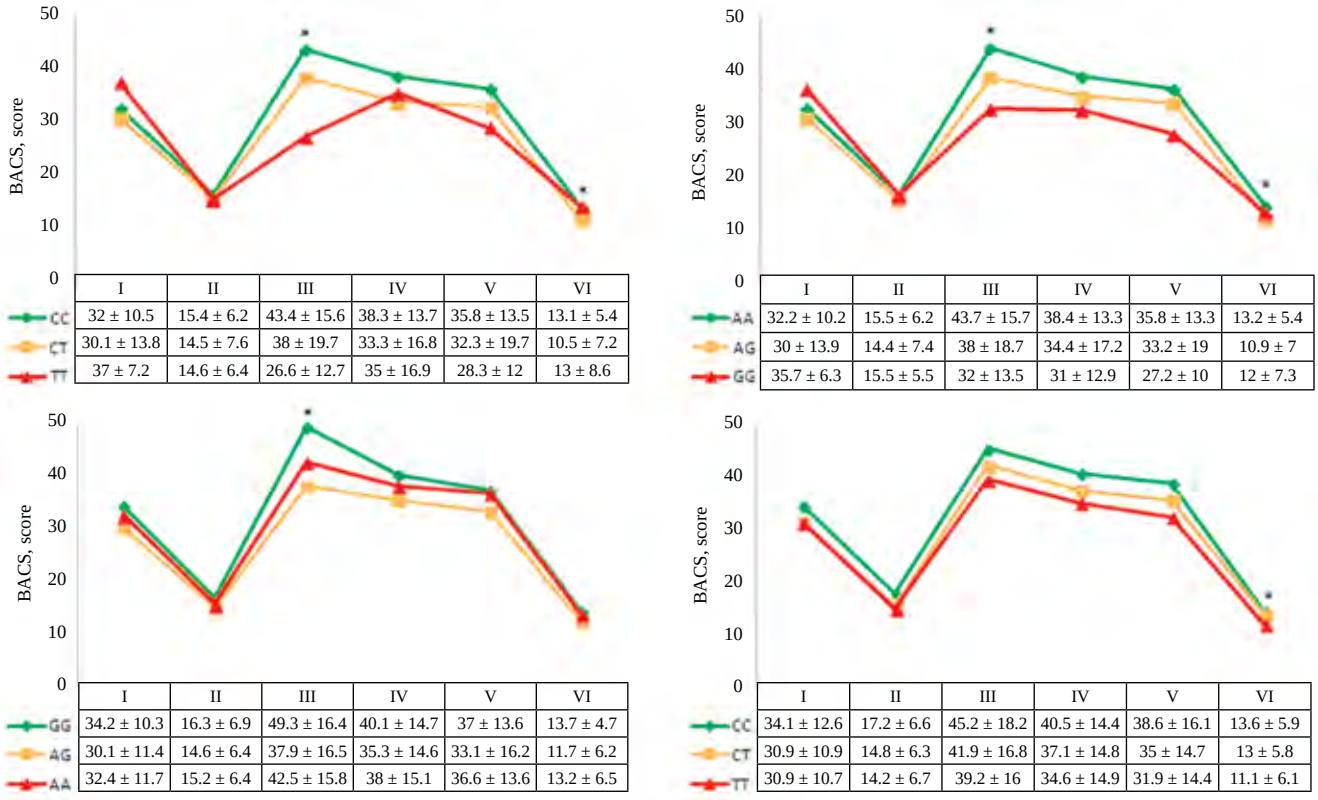


Fig. 2. Associations of polymorphic variants of the *BDNF*, *MAPK*, and *CREB1* genes with cognitive functioning in patients with schizophrenia: *a* – *BDNF* rs6265; *b* – *BDNF* rs11030104; *c* – *MAPK* rs8136867; *d* – *CREB1* rs6740584. * – the level of statistical significance

DISCUSSION

The problem of a genetic predisposition to NCD in schizophrenia is no less acute than a predisposition to the disease itself or the undesirable effects of its pharmacotherapy. Polymorphic variants of the studied genes have been repeatedly tested for associations with the clinical pattern of mental and addictive disorders. In addition to the above, our study raises the issue of neuroplasticity as a protective factor against neurocognitive disorders.

We found that the CT genotype of the polymorphic variant of *BDNF* rs6265 was more common in the group of patients with severe NCD. The C allele of the polymorphic variant of *BDNF* rs6265 encodes the amino acid valine, the T–methionine allele. The expression of the latter leads to a decrease in BDNF production [24]. RS6265 also contributes to the formation of the hippocampus and prefrontal cortex, two brain regions that have the highest expression of BDNF and are centers of learning and memory processes [25]. In 2005, E. Dempster et al. [26] showed the association of the T allele with lower values on the Wexler Memory Scale compared to the C allele. A study was also conducted where apparently healthy subjects with different *BDNF* rs6265 genotypes were presented with verbal recognition tasks similar to the Controlled Oral Word Association test [27]. Then it turned out that the best results are shown by carriers of the CC genotype. These data are partially consistent with ours.

NCD is the third domain of schizophrenia after positive and negative. Schizophrenia itself was described in the late 19th century. century as "early dementia." There is an interpretation that Emil Kraepelin introduced this phrase as the equivalent of dementia in Alzheimer's disease for young patients [4]. There is biological evidence for this interpretation, in particular, an association with Alzheimer's disease has been shown for the polymorphic variant of *BDNF* rs6265 [28]. In another study, no such association was found, but the authors were able to show an increased incidence of the heterozygous rs6265 genotype and the diplotype of three polymorphic *BDNF* variants (rs6265, rs11030104, rs2049045; H1-GTC/H2-ACG) in the subgroup without the carriage of apolipoprotein E4 (APOE 4), which increases the risk of developing Alzheimer's disease [29].

Attention deficit hyperactivity disorder (ADHD) is another clinical problem associated with cognitive impairment. There is an association of the polymorphic variant of *BDNF* rs6265 with both ADHD and general intellectual disabilities [30]. In both groups of patients, the G allele and GA genotype of this polymorphic variant were more common than in the control group, which is consistent with the results of our study. The association of rs6265 with ADHD is also confirmed by the fact that medications used to treat this disorder can increase BDNF levels in the central nervous system [31]. Based on this, it can be assumed that the decrease in indicators for individual subtests of the BACS is associated with attention instability, which is an important link in the pathogenesis of ADHD, especially the subtype predominantly with attention disorders [32].

Concentration of attention plays an important role in the performance in the BACS subtests, especially Towers of London and Symbol Coding, so we consider the hypothesis to be well-founded. It is worth clarifying that there are also opposite data [33, 34] indicating the absence of an association between ADHD and the polymorphic variant of *BDNF* rs6265, as well as the variant rs11030104 in the same gene [35]. In 2010, a meta-analysis was conducted [36], which showed that the contribution of the *BDNF* gene to the development of ADHD was not statistically significant when sex or comorbidity with mood disorders were taken into account.

The presented facts suggest that the involvement of this genetic mechanism is most likely not specific to schizophrenia, but is responsible for cognitive functioning in general. The transdiagnostic comparison of the results obtained in other mental disorders with the data obtained by us is an argument for supporters of the dimensional model of psychiatry over the categorical one.

In the course of this study, it was found that the AG *MAPK* rs8136867 genotype prevailed in patients with severe and moderate NCD. Previously, polymorphic variants of the *MAPK* gene were studied mainly in European populations in the context of affective disorders: major depressive and bipolar disorder. In one study, the MAPK1 variant rs8136867 showed an association with bipolar disorder [37]. In another [38], polymorphic variants *MAPK1* rs8136867 and *CREB1* rs6740584 demonstrated a contribution to resistance to antidepressant treatment in patients with major depressive disorder, and the heterozygous MAPK1 rs8136867 genotype also contributed to the duration and quality of remissions in both groups of patients.

Previously, this was explained by the relatively general thesis that changes in neuroplasticity are

necessary for a therapeutic response, and treatment of affective disorders is most often prolonged and depletes the plasticity potential in neurons [39, 40]. Later, information appeared that the MAPK pathway is the target of several antidepressants [41, 42]. So, G. Mercier et al. showed that fluoxetine itself rapidly activates MAPK cascades in rat astrocytes [40]. However, it was also shown that the MAPK signal prevented the inhibition of glutamate release by bupropion [43].

In modern psychiatry, the concept of overlapping symptoms of schizophrenia and bipolar disorder has been repeatedly confirmed [44, 45], which allowed us to hypothesize common pathogenetic pathways of these disorders. Like schizophrenia, depressive states are also accompanied by cognitive impairments associated with the death of neurons in the prefrontal cortex and hippocampus [46]. In light of this, it is reasonable to conclude that genes whose products are involved in protein kinase signaling pathways contribute to the pathogenesis of not only affective disorders, but also schizophrenia. This is demonstrated, among other things, by the results of our study showing the effect of polymorphic variants of the *MAPK* and *CREB1* genes on the performance in individual BACS subtests. This is the second transdiagnostic dimensional perspective, which is the result of comparing the data we have obtained with studies performed in other mental disorders.

Among the limitations of this study, a small sample size is worth mentioning, but it was, but sufficient for correct statistical processing reflecting objective clinical and neuropsychological data.

Our hypothesis was confirmed with respect to polymorphic variants in the *BDNF*, *MAPK*, and *CREB1* genes, which may contribute to the formation of NCD as a component of the clinical picture of schizophrenia. The results obtained suggest the specificity of the effect of the considered polymorphic variants on impaired neuropsychological development, which plays an important role in the etiology of schizophrenia and confirms the validity of identifying its cognitive phenotypes.

CONCLUSION

For the first time, we found associations of polymorphic variants *BDNF* rs6265 and *MAPK* rs8136867 with neurocognitive deficits in patients with schizophrenia, as well as polymorphic variants *BDNF* rs6265, *BDNF* rs11030104, *MAPK* rs8136867, and *CREB1* rs6740584 with performance in the BACS subtests. The data obtained once again prove the involvement of genetic factors in NCD in schizophrenia. Further research will reveal reliable genetic markers of schizophrenia and related disorders, which will prevent an unfavorable outcome of schizophrenia and complicate the social readaptation of patients.

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Kornetov A.N. – conception and design, drafting of the manuscript. Tiguntsev V.V. – laboratory research, drafting of the manuscript. Galkin S.A. – statistical data analysis. Mikhalitskaya E.V. – sample preparation, laboratory tests. Agarkov A.A. – clinical, psychopathological and psychometric examination of the sample. Boyko A.S. – review of publications on the topic of the article, maintaining a database. Kornetova E.G. – conception and design, critical revision for important intellectual content. Ivanova S.A. – conception and design, editing of the manuscript, final approval of the manuscript for publication.

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Correlation of Vascular Endothelial Growth Factor Receptor-2 Expression and Morphological Changes in the Myocardium of Rats on a High-Carbohydrate High-fat Diet

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ABSTRACT

Aim. To evaluate the relationship between the expression of vascular endothelial growth factor receptor 2 (VEGFR2) in the myocardium and its association with morphological changes in cardiac muscle cells in rats on a high-carbohydrate high-fat diet with regard to the age using the immunohistochemical method.

Materials and methods. The study was conducted on male Wistar rats aged 5 and 18 months, some of which were fed with a standard diet, while the other previously received a high-carbohydrate and high-fat diet (HCHFD) for 90 days. VEGFR2 was detected by immunohistochemical staining of myocardial sections, signs of myocardial damage were assessed by the presence of perinuclear depletion (edema) of the sarcoplasm and contracture changes in cardiac muscle cells, karyopyknosis, and changes in the specific volumes of the stroma.

Results. An increase in the specific volume of VEGFR2 positive cardiomyocytes occurs in young (5 months old) rats on HCHFD, in old (18 months old) rats on a standard diet, and, to the greatest extent, in aged animals receiving HCHFD. The change in the proportion of cardiomyocytes expressing VEGFR2 correlates with the content of cardiomyocytes with morphological signs of damage in the form of karyopyknosis, contracture, and depletion of the perinuclear zone of sarcoplasm. According to multiple regression analysis, karyopyknotic disorders made the greatest contribution to the change in VEGFR2 expression in cardiomyocytes in older animals.

Conclusion. HCHFD induces predictable changes in VEGFR2 expression in cardiac muscle cells, depending on age and the severity of myocardial damage. The study results suggest that the protective effect of VEGFR2 expression may be disrupted in HCHFD and with age.

Keywords: age-related myocardial changes, high-carbohydrate high-fat diet, vascular endothelial growth factor receptor-2 (VEGFR2), contracture changes in cardiac muscle cells

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

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Корреляция экспрессии рецептора-2 сосудистого эндотелиального фактора роста и морфологических изменений миокарда крыс на высокоуглеводной высокожировой диете

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РЕЗЮМЕ

Цель: с помощью иммуногистохимического метода оценить взаимосвязь экспрессии рецептора-2 сосудистого эндотелиального фактора роста (VEGFR2) в миокарде и с морфологическими изменениями кардиомиоцитов у крыс на высокоуглеводной высокожировой диете в возрастном аспекте.

Материалы и методы. Исследование проведено на самцах крыс линии Вистар в возрасте 5 и 18 мес, одна часть которых содержалась на стандартном пищевом рационе, другая – предварительно находилась на высокоуглеводной и высокожировой диете (ВУВЖД) в течение 90 дней. VEGFR2 выявляли при иммуногистохимическом окрашивании срезов миокарда, признаки повреждения миокарда оценивали по наличию перинуклеарного опустошения (отека) саркоплазмы и контрактурных изменений кардиомиоцитов, кариопикноза, изменений удельных объемов стромы.

Результаты. Увеличение удельного объема VEGFR2 иммуногистохимически позитивных кардиомиоцитов возникает у молодых (5 мес) крыс на ВУВЖД, у старых крыс (18 мес) на стандартной диете и, в наибольшей степени, у возрастных животных, содержавшихся на ВУВЖД. Изменение доли кардиомиоцитов, экспрессирующих VEGFR2, коррелирует с содержанием кардиомиоцитов с морфологическими признаками повреждения в виде кариопикноза, контрактуры и опустошения перинуклеарной зоны саркоплазмы. По данным множественного регрессионного анализа, у старых животных наибольший вклад во влияние на изменение экспрессии VEGFR2 в кардиомиоцитах оказали кариопикнотические нарушения.

Заключение. ВУВЖД вызывает закономерные изменения экспрессии VEGFR2 в кардиомиоцитах, зависящие от возраста и степени поражения миокарда. Результаты исследования позволяют предполагать, что протекторная направленность экспрессии VEGFR2 может быть нарушена при ВУВЖД и с возрастом.

Ключевые слова: возрастные изменения миокарда, высокоуглеводная высокожировая диета, рецептор-2 сосудистого эндотелиального фактора роста (VEGFR2), контрактурные изменения кардиомиоцитов

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

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INTRODUCTION

Cardiovascular diseases are largely the result of an unhealthy lifestyle, including an unbalanced diet with high levels of fats and carbohydrates, which affects both general metabolism and endothelial metabolism in particular [1, 2]. A key link in this process is impaired angiogenesis and endothelial dysfunction, which has been shown in experiments with metabolic disorders in animals: a decrease in microvessel density and VEGFR2 expression underlies the process of disruption of signaling along the VEGF/VEGFR pathway in cardiac endothelial cells [2, 3]. Moreover, an imbalance in the VEGF system, which acts as a key regulator of angiogenesis and cardiomyocyte survival, can aggravate the course of cardiovascular diseases [4]. The importance of the VEGF/VEGFR pathway is highlighted by the fact that bidirectional signaling between endothelial cells and cardiac muscle cells via VEGFR2 coordinates physiological cardiac growth, and its disruption contributes to the transition to pathological hypertrophy and heart failure [2].

In the myocardium, cardiac muscle cells are a source of VEGF, along with the endothelium [4]. Saturated fatty acid overload increases cardiac muscle cells apoptosis *in vitro* [5], while lipotoxic effects on the myocardium are mitigated by VEGF overexpression, significantly restoring cardiac muscle cells viability. The protective effect of VEGF in combating cardiac muscle cell apoptosis may identify therapeutic targets in diseases caused by fatty acid stress [6].

However, the effect of a high-carbohydrate high-fat diet (HCHFD) on VEGFR2 expression in the myocardium remains unstudied. Identification of this link is critical for complete understanding of the pathogenesis and the development of new strategies for the treatment and prevention of cardiovascular complications in metabolic disorders associated with excessive consumption of saturated fatty acids and carbohydrates, which determines the high relevance of this study.

The aim of this work is to evaluate, using the immunohistochemical method, the relationship between VEGFR2 expression in the myocardium and morphological changes in cardiac muscle cells in rats on HCHFD with regard to age.

MATERIALS AND METHODS

The study was conducted on male Wistar rats aged 5 and 18 months. All procedures complied with Directive 2010/63/EU of the European Parliament and the FASEB statement on the use of animals in research and education. The study was approved by the Ethics Committee of Cardiology Research Institute of Tomsk NRMC (Protocol No. 201 dated July 30, 2020).

The experimental groups were formed as follows: group 1 (n = 14) – intact 150-day-old (5-month-old) rats receiving a standard diet for 90 days (starting at 60 days of age); group 2 (n = 14) – 150-day-old rats receiving a HCHFD for 90 days (starting at 60 days of age); group 3 (n = 14) – intact 540-day-old (18-month-old) rats fed with a standard diet for 90 days (starting at 450 days of age). Group 4 (n = 14) included 540-day-old rats maintained on a high-frequency intrauterine fluid (HIF) for 90 days (starting at 450 days of age). The HCHFD contained 16% protein, 21% fat, 46% carbohydrates, including 17% fructose, and 0.125% cholesterol. Water was replaced with a 20% fructose solution. Rats from groups 1 and 3 (intact animals) were given standard rodent chow (24% protein, 6% fat, 44% carbohydrates) and clean water *ad libitum*.

Animals were sacrificed from the experiment by decapitation after preliminary anesthesia with chloralose (100 mg/kg intraperitoneally). For histologic examination, the heart wall was cut into 2-3-mm-thick slices, fixed in 10% buffered formalin for 24 h, washed in running water, and dehydrated in Izoprep (BioVitrum, St. Petersburg), a solution for histological processing based on absolute isopropyl alcohol. After dehydration, the myocardial samples were embedded in BioPlast homogenized paraffin embedding medium (BioOptica, Italy).

Histological sections (5–7 μm thick) obtained using HM 325 rotary mechanical microtome (Thermo Scientific, USA) were stained with hematoxylin and eosin according to Van Gieson's stain (stains from BioVitrum, St. Petersburg). Expression of VEGFR2 in cardiac muscle cells was assessed by immunohistochemistry using rabbit polyclonal antibodies at a 1:50 dilution (E3712, Spring Bioscience, USA) according to the manufacturer's instructions. 3,3'-Diaminobenzidine was used as a chromogen, and counterstaining was performed with hematoxylin. Stained preparations were embedded in BioMount synthetic mounting medium (BioOptica, Italy) and examined under Axio Lab.A1 light microscope (Carl Zeiss, Germany). Micrographs of histological preparations were obtained using Axiocam 105 color camera (Carl Zeiss, Germany).

In the left ventricular myocardium, the average number of cardiac muscle cells with karyopyknosis, perinuclear depletion (edema) of the sarcoplasm, and contracture changes, as well as the number of

unchanged cardiac muscle cells, were counted per field of view. Cardiac muscle cells were counted in 20 independent fields of view of each left ventricular myocardium section at 400x magnification; the field of view area was 0.196 mm^2. Using the ocular grid proposed by Avtandilov, we determined the specific volumes (%) of myocardial stromal connective tissue stained according to Van Gieson's stain technique and VEGFR2-positive cardiac muscle cells, the expression of which was detected during the immunohistochemical reaction.

Statistical data processing was performed using STATISTICA 13.0 (StatSoft Inc., USA). The obtained data were tested for compliance with the normal distribution using the Shapiro–Wilk test. Data that did not correspond to the normal distribution were presented as the median and interquartile range (Me (Q_1–Q_3)). Homogeneity of variances was tested using Levene's test. The statistical significance of differences in indicators between groups was assessed using the the Mann–Whitney test with Bonferroni correction for multiple comparisons. For six pairwise comparisons, differences were considered statistically significant at $p = 0.05$, if the hypothesis of equality of the mean values of the indicators was rejected at $p = 0.05/6 \approx 0.0127$.

To assess the relationship between the indicators, the Spearman's rank correlation coefficient was calculated. Multiple regression analysis was performed to identify the dependence of VEGFR2 expression in cardiac muscle cells on such variables as karyopyknosis, contracture, perinuclear depletion of the cytoplasm of cardiac muscle cells, and the specific volume of stroma. A standardized regression model was built using indicators pre-transformed so that the mean value of each indicator is 0, and the standard deviation is 1:

$$VEGF = B_1 \times X_1 + B_2 \times X_2 + B_3 \times X_3 + B_4 \times X_4,$$

where X_1 is karyopyknosis, X_2 is perinuclear cytoplasmic depletion (edema), X_3 is contracture, and X_4 is stroma.

In this case, the coefficients B_i allow us to estimate the relative contribution K_i of each independent variable to the prediction of the dependent variable VEGF as a percentage using the formula:

RESULTS

In Group 1 (5 months old, standard diet), immunohistochemical analysis of VEGFR2 expression revealed weak staining of a small proportion of cardiac muscle cells and more intense staining of endothelial cells in some myocardial capillaries (Fig. 1, *a*). The proportion of VEGFR2-positive cardiac muscle cells was 15.3% (13.2–16.3%). In Group 2 rats (5 months old, on HCHFD), immunohistochemical staining was mosaic. Light-brown stained cardiac muscle cells alternated with unstained myocardial cells, with a generally distinct border between them (Fig. 1, *b*).

The proportion of VEGFR2-positive cardiac muscle cells increased compared to Group 1 and was 32.2% (27.2–34.8%) ($p_{1\text{-}2} = 0.048$). In group 2, an increase in the number of cardiac muscle cells with karyopyknosis to 1.3 (1.2–1.5) in the field of view versus 0.4 (0.3–0.4) in group 1 ($p_{1\text{-}2} = 0.0003$) was revealed using histologic examination. It also revealed a rise in the number of cardiac muscle cells with lytic changes in the form of depletion (edema) of the cytoplasm in the perinuclear zone to 1.2 (0.9–1.3) in the field of view versus 0.5 (0.3–0.6) in group 1 ($p = 0.0003$).

These morphological changes were illustrated in one of our previous reports [11]. The number of cardiac muscle cells with contracture changes was 0.1 (0.1–1.0) in the field of view; there were no significant differences in this indicator in groups 1 and 2. In general, the content of cardiac muscle cells with damage to the nucleus and cytoplasm in group 2 increased to a level of 8% of all cardiac muscle cells versus 3% in group 1.

In group 3 (18 months, standard diet), the content, staining pattern, and distribution of VEGFR2-positive cardiac muscle cells were comparable with those observed in group 2, and their specific volume was 36.4% (33.6–38.2%; $p_{1\text{-}3} = 0.018$). In group 3, compared with groups 1 and 2, the content of karyopyknotic cardiac muscle cells significantly increased to 2.5% (2.2–2.7%; $p_{1\text{-}3} = 0.0003$), the proportion of cardiacmuscle cells with lytic changes rose to 1.5% (1.4–1.5%; $p_{1\text{-}3} = 0.0003$), and the content of cells with contracture per field of view increased to 1.1% (1.1–1.5%; $p_{1\text{-}3} = 0.0003$). In total, altered cardiac muscle cells in group 3 accounted for approximately 16%.

In group 4, the specific volume of VEGFR2-positive cardiac muscle cells (Fig. 1, c) increased to 79.4% (62.0–82.2%; $p_{1\text{-}4} < 0.0001$; $p_{2\text{-}4} = 0.00264$; $p_{3\text{-}4} > 0.05$). In this group, the content of karyopyknotic cardiac muscle cells was 3.1 (1.9–6.0; $p_{3\text{-}4} = 0.025$) per field of view, with lytic changes in the cytoplasm – 1.4 (1.2–2.1; $p_{3\text{-}4} = 0.6$), and with contracture changes – 1.3 (0.9–2.5; $p_{3\text{-}4} = 0.3$). Along with cardiac muscle cells, immunopositive staining for VEGFR2 characterizes the cytoplasm of a significant proportion of endothelial

cells in myocardial arterioles, capillaries, and venules (Fig. 1, *d*). Overall, damaged cardiac muscle cells in group 4 accounted for 19% of the total population.

Correlation analysis revealed significant positive and negative associations between various parameters in group 1 (Fig. 2).

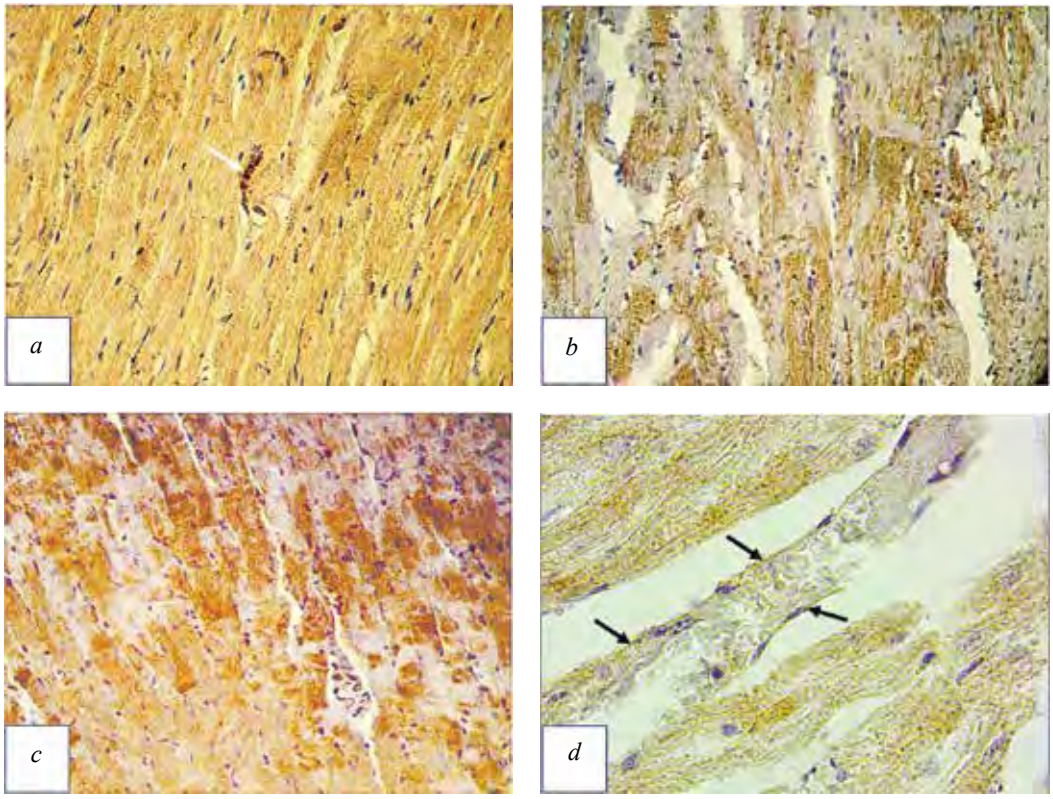

Fig. 1. Immunohistochemical detection of VEGFR2 in rat myocardium: *a* – myocardium of 5-month-old rats receiving a standard diet (group 1), weak VEGFR2 expression in cardiac muscle cells and more intense staining of the hemocapillary endothelium (arrow); *b* – mosaic VEGFR2-positive staining of cardiac muscle cells in 5-month-old rats receiving a HCHFD; *c* – intense VEGFR2 staining of the majority of cardiac muscle cells in 18-month-old rats receiving a HCHFD (group 4); *d* – VEGFR2 expression in cardiac muscle cells and endothelium of blood vessels of the myocardium of 18-month-old rats receiving a HCHFD (group 4). Magnification: x400

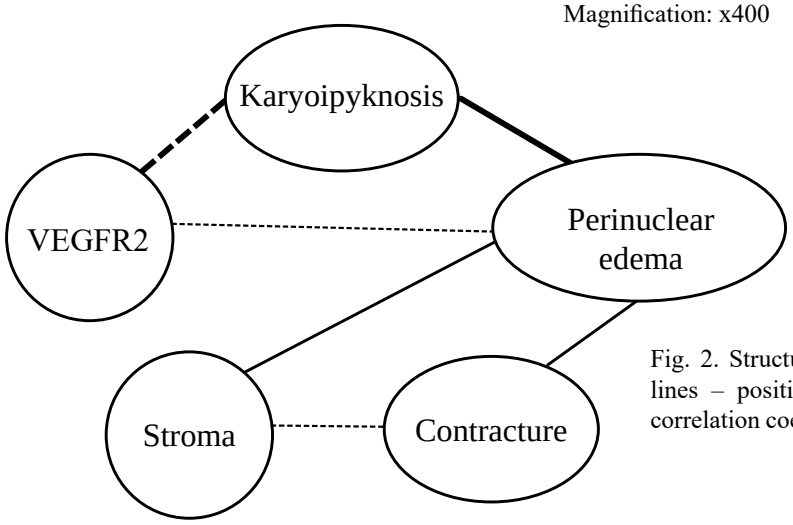

Fig. 2. Structural diagram of correlation links for group 1: solid lines – positive correlation coefficient, dotted lines – negative correlation coefficient; thick lines – strong link $|r| > 0.7$, thin lines – average link $0.3 < |r| < 0.7$.

Thus, a strong positive correlation was noted between the content of cardiac muscle cells with karyopyknosis and their lytic changes in the form of depletion (edema) of the perinuclear zone of the cytoplasm (Spearman's $r = 0.89$; $p = 0.0001$), an average positive correlation was observed between karyopyknotic disorders and contracture of the cytoplasm ($r = 0.69$; $p = 0.0136$), but a strong negative

correlation was observed between the content of cardiac muscle cells with karyopyknosis and the specific volume of cardiac muscle cells with VEGF expression ($r = –0.80$; $p = 0.017$). An average negative correlation was observed between lytic changes in the form of depletion of the perinuclear zone of the cytoplasm and VEGFR2 expression ($r = –0.66$; $p = 0.0193$), as well as between contracture of cardiac muscle cells and the specific volume of myocardial stroma ($r = –0.61$; $p = 0.0349$).

In groups 2–4, the correlations between the parameters were moderate or weak, and they were not statistically significant, often at the trend level. Thus, in group 4, positive correlations of moderate strength were recorded between karyopyknosis and VEGF expression ($r = 0.36$; $p = 0.2769$), and between contracture and VEGFR2 ($r = 0.38$; $p = 0.2466$).

Figure 3 shows the relative contribution of K_i for each independent variable to predicting the dependent variable VEGFR2 in the study groups. If the K_i coefficient is positive, then the higher the parameter, the higher the VEGFR2 value, and vice versa.

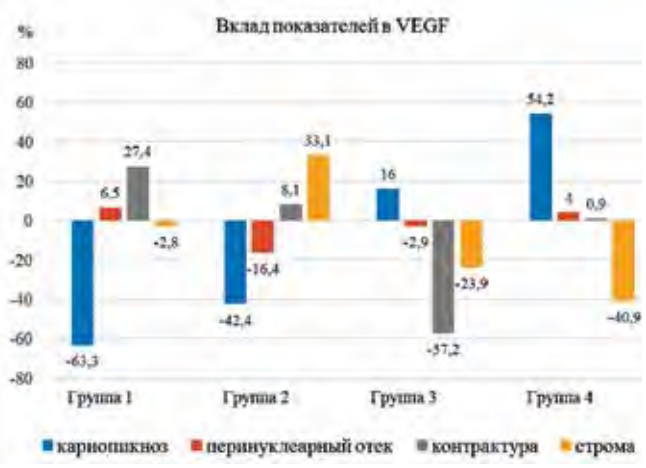

Fig. 3. Multiple regression analysis to identify the dependence of VEGF on the indicators: X_1 – karyopyknosis, X_2 – perinuclear depletion (edema) of the cytoplasm, X_3 – contracture, X_4 – stroma (specific volumes).

Thus, in group 1, contracture of cardiac muscle cells had the greatest positive effect at 27.4%, while a relatively low level of karyopyknosis had a negative effect on VEGFR2 expression. In group 2, the increase in stromal specific volume increased to 33%, while karyopyknosis demonstrated a negative correlation (–42.4%). In groups 3 and 4, with aged (18 months) animals, the positive correlation between karyopyknosis and VEGFR2 expression increased. Thus, in groups 3 and 4, the contribution of karyopyknosis was 16.6 ans 54.2%, respectively. While fibrotic changes in the stroma had a negative effect on VEGFR2 expression, in groups 3 and 4 the stromal contribution was 23.9 and 40.9%, respectively.

DISCUSSION

A large body of evidence has accumulated indicating that VEGF receptor expression is not limited to endothelial cells and that their ligands perform a variety of fundamental functions in other cell types. Non-angiogenic functions of VEGF include the ability to prevent neuronal death due to ischemia and stimulate neurogenesis, stimulate hepatocyte regeneration after liver injury, and stimulate osteoblast migration and differentiation [7–10].

Cardiac muscle cells are a source and target of VEGF-A and express its receptors VEGFR1 and VEGFR2 on their cell surface. It should be noted that although VEGFR1 strongly binds VEGF-A, it has a much weaker kinase activity compared to VEGFR2. Therefore, the biological effect of VEGF-A through VEGFR1 activation is much weaker than that exerted through VEGFR2 in metabolic syndrome [1, 3]. We would like to emphasize that one of our previous reports provided data showing that maintenance on HIF a similar experiment modeled the development of metabolic syndrome in old rats with an increase in the weight of animals, the mass of adipose tissue, an increase in serum glucose, insulin, endothelin 1, insulin resistance, and other indicators of carbohydrate metabolism disorders, as well as destructive changes in the aortic wall and persistent hypertension [11, 12].

The function of VEGF-A in the myocardium is multivector. On the one hand, VEGF-A activates cardiac muscle cells, inducing morphogenesis, contractility, and regeneration, causes vasculogenesis [13], recruitment of stem cells [14], reduction of apoptosis [15], and enhancement of vasodilation [16]. On the other hand, VEGF-A is produced by cardiac muscle cells during inflammation, mechanical stress, and cytokine stimulation. High concentrations of VEGF-A have been found in patients with various cardiovascular diseases and often correlate with the prognosis and severity of the disease [4].

In this study, we identified a relationship between VEGFR2 expression, age, and diet. The specific volume of VEGFR2-positive cardiac muscle cells significantly increased in 18-month-old rats compared to 6-month-old animals. The use of HCHFD resulted in an increase in this parameter in both 6-month-old and, to a significantly greater extent, 18-month-old animals.

Using correlation and multiple regression analysis, we established the relationship between VEGFR2 expression and the type of structural damage to cardiac muscle cells and its severity in HCHFD in relation to age. Distinct and quantifiable changes, such as karyopyknosis, contracture, and lytic disorders of cardiac muscle cells, and stromal fibrosis, were used as histological criteria for myocardial alteration. The total content of variously altered cardiac muscle cells across the experimental series was approximately 3% in group 1, 8% in group 2, 16% in group 3, and 19% in group 4. Accordingly, the specific volume of VEGFR2-positive cardiac muscle cells was 15.3 (13.2–16.3)% in group 1, 32.2 (27.2–34.8)% in group 2, 36.4 (33.6–38.2)% in group 3, and 79.4 (62.0–82.2)% in group 4. Consequently, a direct positive relationship is evident between the degree of cardiac muscle cells damage and VEGFR2 expression. At the same time, it is noteworthy that the proportion of VEGFR2-positive cardiac muscle cells in all experimental groups was significantly higher than that of cardiac muscle cells with signs of damage. This indicates that VEGFR2 expression encompasses cardiac muscle cells adjacent to the affected ones. Thus, the proportion of cardiac muscle cells with karyopyknosis, which is an indicator of cell death by both apoptosis and necrosis, in group 4 with the most severe myocardial damage was 3.1 (1.9–6.0) per field of view, or approximately 10.5%, while the specific volume of VEGF-positive cardiac muscle cells was 79.4%.

In 6-month-old animals receiving a standard diet, which can be considered controls, the content of VEGFR2-positive cardiac muscle cells was low – 15.3%. This is consistent with literature data on the low level of VEGF expression in normal cardiac muscle cells and an increase in the content of VEGF-positive cardiac muscle cells in various pathologies, in particular, with VEGFR2 expression in cardiopathy caused by antitumor drugs [18] and in acute myocardial ischemia induced by coronary artery ligation [19]. VEGFR2-immunopositivity of cardiac muscle cells, as a rule, has a spotty mosaic character, and immunopositive cardiac muscle cells alternate with unstained cells.

Thus, in acute myocardial infarction, immunohistochemical staining of cardiac muscle cells was intense in the ischemic zone without necrosis, weak in the area of coagulative necrosis without inflammatory infiltration, and absent in the foci of developed necrosis with inflammatory infiltration. Forensic studies show "patchy" myocardial immunopositivity for VEGF in cases of rapid death due to a stab wound to the heart, limited to the area surrounding the wound, but no VEGF staining in cases of asphyxia, drowning, or in cases of sudden cardiac arrest without prior ischemic-related cardiac pathology. The authors conclude that ischemic myocardial changes precede the development of VEGF immunopositivity in cardiac muscle cells, which is often accompanied by eosinophilia of cardiac muscle cells [20].

In this study, the relationship between VEGFR2 expression in cardiac muscle cells and contracture changes was assessed. Of particular note is the increased sarcoplasmic eosinophilia observed in cardiac muscle cells, typically in the form of wide transverse bands. This is a characteristic type of morphological change in cardiac muscle cells, described in literature as contracture band necrosis, which occurs during reperfusion following hypercontraction and leads to sarcolemmal rupture [21]. The mechanism by which reperfusion of ischemic heart muscle induces contracture necrosis involves the influx of calcium after a period of relative deprivation. A sudden influx of calcium, associated with excessive amounts of locally released norepinephrine, can cause irreversible contractures leading to necrosis.

In the present study, a moderate positive correlation between contracture and VEGFR2 expression in cardiac muscle cells was found in 18-month-old animals fed with HCHFD (group 4). In young rats (6 months) receiving a standard diet, VEGFR2 expression showed the greatest correlation with contracture changes, according to multiple regression analysis. Meanwhile, in aged rats receiving the HCHFD, karyopyknotic changes had the greatest impact on VEGFR2 expression.

The identified clear positive correlation between the specific volume of cardiac muscle cells expressing VEGFR2 and the content of alternatively altered cardiac muscle cells likely indicates an adaptive-compensatory role of VEGFR2 in myocardial injury. Thus, it is known that intramyocardial injection of VEGF165 cDNA can significantly improve cardiac function, stimulate angiogenesis, reduce infarct size and apoptosis of cardiac muscle cells, inhibit the expression of myocardial p53, Fas and Bax, and increase the expression of VEGF and Bcl-2 in the myocardium in an acute infarction model [22–24]. However, the exact mechanism by which VEGF DNA causes these effects is still unclear.

At the same time, a number of studies have shown that VEGF reduces damage to cardiac muscle cells caused by unsaturated fatty acids, in particular

palmitate, via the JNK signaling pathway. High-fat diet induced JNK activation [25], which was abolished by TLR4 knockout [26–28]. Cellular ceramide accumulation activated JNK signaling and apoptosis, which was prevented by ceramide synthase 5 knockout [29, 30]. JNK activation was observed in palmitate-treated cardiac muscle cells and was attenuated by protein kinase R inhibition [31–33]. Enhanced cardiac muscle cells apoptosis upon saturated fatty acid overload may lead to myocardial infarction and cardiac dysfunction. However, viability of cardiac muscle cells was restored by VEGF overexpression during 0.5 mM palmitate treatment. This process was accompanied by a decrease in the apoptosis rate and the expression of caspase 3, Bax, and NF-κB p65. These results indicate protective effects of VEGF in combating lipotoxicity-induced cardiac muscle cells apoptosis and may identify therapeutic targets for cardiovascular protection in combating fatty acid stress [6].

CONCLUSION

HCHFD induces predictable changes in VEGFR2 expression in cardiac muscle cells, depending on age and the degree of myocardial injury. An increase in the proportion of VEGFR2-immunohistochemically positive cardiac muscle cells occurs in young (6 months) rats fed with HCHFD, in old rats (18 months) receiving a standard diet, and, to the greatest extent, in older animals maintained on HCHFD. Changes in the proportion of cardiac muscle cells expressing VEGFR2 correlate with the proportion of cardiac muscle cells with morphological signs of injury, such as karyopyknosis, contracture, and depletion of the perinuclear zone of sarcoplasm. According to multiple regression analysis, karyopyknotic abnormalities had the greatest impact on changes in VEGFR2 expression in cardiac muscle cells in old animals.

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Logvinov S.V. – conception and design, drafting of the morphological part of the article, justification of the manuscript, critical revision for important intellectual content, and final approval of the manuscript for publication. Mustafina L.R. – conducting morphological and immunohistochemical studies, data interpretation, translating the article into English. Fokin V.A. – preparation of the statistical part of the article text, statistical analysis and interpretation of data, critical revision for important intellectual content, and final approval of the manuscript for publication. Akbasheva O.E. – conception and design, drafting of the article, justification of the manuscript, critical revision for important intellectual content, and final approval of the manuscript for publication. Gerasimov A.V. – drafting of the morphological part of the article, justification of the manuscript, critical revision for important intellectual content. Potapov A.V., Gereng E.A. – conception and design, morphological research, statistical analysis and interpretation of data. Lasukova T.V., Tikhonovskaya O.A. – conception and design, drafting of the article, justification of the manuscript. Naryzhnaya N.V. – conception and design, conducting research, drafting of

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Effect of Idelalisib on Cytokine Production by Blood Mononuclear Cells in Patients With Allergic Rhinitis

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ABSTRACT

Aim. To assess the ability of phosphatidylinositol 3-kinase δ inhibitor (idelalisib) to suppress cytokine production by peripheral blood mononuclear cells (PBMCs) of patients with allergic rhinitis.

Materials and methods. PBMCs of AR patients (n = 17) were incubated with idelalisib (0.5 μM) and recombinant proteins for induction of type 2 immune response (IR). Secretion of cytokines by PBMCs was determined by enzyme-linked immunosorbent assay. Intracellular production of cytokines in blood T-helper cells (CD4+) and cytotoxic (CD8+) T lymphocytes was analyzed by flow cytometry.

Results. Idelalisib significantly inhibited the secretion of interleukins (IL) 4, 8, 9, 13, 17A, interferon γ, and tumor necrosis factor α by PBMCs from patients with allergic rhinitis exposed to recombinant proteins (IL-2, IL-25, IL-33, and thymic stromal lymphopoietin) inducing type 2 IR. This drug also significantly suppressed the intracellular production of IL-4, IL-5, IL-13, and IL-17A by blood CD4+ and CD8+ T lymphocytes activated by type 2 IR.

Conclusion. The obtained data justify the need to conduct further clinical trials using idelalisib for the treatment of allergic rhinitis.

Keywords: allergic rhinitis, interleukin 25, interleukin 33, thymic stromal lymphopoietin, phosphatidylinositol 3-kinase δ, idelalisib

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Влияние иделалисиба на продукцию цитокинов мононуклеарными клетками крови пациентов с аллергическим ринитом

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РЕЗЮМЕ

Цель. Оценить способность ингибитора фосфатидилинозитол-3-киназы δ (иделалисиба) подавлять продукцию цитокинов мононуклеарными клетками (МН-клетками) крови пациентов с аллергическим ринитом.

Материалы и методы. Мононуклеарные клетки пациентов с аллергическим ринитом (n = 17) инкубировали с иделалисибом (0,5 мкМ) и рекомбинантными белками для индукции 2-го типа иммунного ответа (ИО). Секрецию цитокинов МН-клетками определяли методом иммуноферментного анализа. Внутриклеточную продукцию цитокинов в Т-хелперах (CD4+) и цитотоксических (CD8+) Т-лимфоцитах крови анализировали методом проточной цитометрии.

Результаты. Иделалисиб существенно супрессировал секрецию интерлейкинов (ИЛ) 4, 8, 9, 13, 17A, интерферона γ, фактора некроза опухоли α МН-клетками крови пациентов с аллергическим ринитом, подвергшимся воздействию рекомбинантных белков (ИЛ-2, ИЛ-25, ИЛ-33, тимического стромального лимфопоэтина), индуцирующих ИО 2-го типа. Данный препарат также значительно подавлял внутриклеточную продукцию ИЛ-4, ИЛ-5, ИЛ-13, ИЛ-17A CD4+ и CD8+ Т-лимфоцитами крови, активированными по ИО 2-го типа.

Заключение. Полученные данные обосновывают необходимость интенсификации клинических исследований с применением иделалисиба для лечения аллергического ринита.

Ключевые слова: аллергический ринит, интерлейкин 25, интерлейкин 33, тимический стромальный лимфопоэтин, фосфатидилинозитол-3-киназа δ, иделалисиб

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Работа выполнена в рамках совместного задания Белорусского республиканского фонда фундаментальных исследований и Российского научного фонда (договор № М23РНФ-021, государственная регистрация № 20230281).

Соответствие принципам этики. Перед забором крови все испытуемые дали письменное добровольное согласие на участие в исследовании. Исследование одобрено комитетом по биомедицинской этике БГМУ (протокол № 1 от 31.08.2023).

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INTRODUCTION

Allergic rhinitis affects 10–30% of the population in different regions worldwide and is characterized by inflammation of the nasal mucosa, which forms as a result of immunoglobulin (Ig) E-mediated hypersensitivity reaction to various allergens. The use of currently available treatment methods does not lead to the complete relief of the allergic rhinitis symptoms [1]. In addition, approximately 11% of patients, when treating this disease, experience side effects of drugs [2]. Therefore, research aimed at finding alternative approaches to the treatment of allergic rhinitis remains relevant.

When exposed to allergens, epithelial cells of the nasal mucosa secrete cytokines, such as thymic stromal lymphopoietin (TSLP), interleukins (IL) 25 and 33, which upon entering the systemic circulation activate dendritic cells, type 2 innate lymphoid cells (ILC2 cells), and type 2 T-helper cells (Th2-lymphocytes). These cells, in turn, produce IL-4, IL-5, IL-13, and other mediators, which leads to the attraction of eosinophils to the nasal mucosa, their activation, and degranulation, synthesis of IgE by B lymphocytes, and mucus hyperproduction by the epithelium [3].

It was proposed that phosphatidylinositol 3-kinase (PI3K) δ may play a central role in the production of the above-mentioned cytokines. The results of experimental studies indicate that the signaling pathways mediated by this enzyme are involved in the inflammatory process in allergic rhinitis [4, 5]. In this case, the use of a PI3Kδ inhibitor in combination with a serine proteinase inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride led to weakening of the type 2 immune response (IR) in mice sensitized to cockroach allergens [6]. Results of phase I clinical trial of idelalisib (a PI3Kδ inhibitor) for the treatment of allergic rhinitis demonstrated its ability to reduce the severity of disease symptoms, CCL17 and CCL22 concentration in blood plasma, and the percentage of *ex vivo* activated (using grass pollen allergen) blood basophils [4]. However, the ability of PI3Kδ inhibitors to suppress the production of proinflammatory cytokines by blood cells in patients with allergic rhinitis has not been previously studied.

Thus, the aim of this study was to evaluate the ability of idelalisib (a PI3Kδ inhibitor) to suppress the production of proinflammatory cytokines by peripheral blood mononuclear cells (PBMCs) of patients with allergic rhinitis.

MATERIALS AND METHODS

Patient Characteristics

The study involved 17 patients aged 18 to 23 years who were diagnosed with allergic rhinitis at least 1 year before inclusion in the study (Table). The overwhelming majority (10; 58.8%) of patients suffered from perennial allergic rhinitis, while a smaller proportion (7; 41.2%) of patients suffered from the seasonal form of allergic rhinitis.

Table

Characteristics of Patients with Allergic Rhinitis who Participated in the Study

Indicator		Patients, $n = 17$
Age, years, $M \pm m$		20.0 ± 0.3
Sex, male/female		8/9
BMI, kg/m², $M \pm m$		21.5 ± 0.7
Form of allergic rhinitis (PAR/SAR)		10/7
Disease duration, years, $M \pm m$		12.5 ± 1.1
SNOT-22 questionnaire score, $M \pm m$		42.9 ± 4.4
TNSS questionnaire score, $M \pm m$		7.3 ± 0.4
Drugs used, *n* (%)	Oral antihistamines	15 (88.24%)
	Endonasal antihistamines	5 (29.41%)
	Intranasal corticosteroids	6 (35.29%)
	Leukotriene receptor antagonists	1 (5.88%)
	Endonasal saline solution	6 (35.29%)
	Decongestants	4 (23.53%)

Note. BMI – body mass index; PAR – perennial allergic rhinitis; SAR – seasonal allergic rhinitis; TNSS – total nasal symptom score; SNOT-22 (sino-nasal outcome test) – a test for assessing the quality of life and therapeutic results of treatment of patients with diseases of the nose and paranasal sinuses.

We excluded from the study patients with anatomical abnormalities of the nasal septum, with concomitant diseases (asthma, tuberculosis, diabetes mellitus, chronic rhinosinusitis, arterial hypertension, cardiovascular, and oncological diseases), other concomitant diseases requiring medication; immunodeficiency states, including those caused by HIV, or blood coagulation disorders; those who underwent a course of allergen-specific immunotherapy; had infectious diseases of the respiratory tract or gastrointestinal tract or took antibiotics during the last 6 weeks before enrollment in the study, and pregnant women. Patients refrained 3 days before the study from taking antihistamines, and 2 weeks before the study from taking oral and nasal corticosteroids. The patients refrained from taking antihistamines for three days before the study. They refrained from oral and nasal corticosteroids for two weeks before the study and from dietary supplements for one week before inclusion in the study.

Isolation and Incubation of Peripheral Blood Mononuclear Cells

PBMCs were isolated from heparinized blood of patients by 1.077 density gradient centrifugation using Lymphosep solution (Biowest, France). Cells were resuspended at a concentration of 1×10^6/ml in RPMI 1640 culture medium (Capricorn Scientific, Ebsdorfergrund, Germany) supplemented with 10% fetal bovine serum (FBS, Capricorn Scientific, collected in South America), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Capricorn Scientific).

Then 2×10^5 PBMCs were placed in wells of a 96-well plate and cultured in the presence or absence of 0.5 μM idelalisib (p110δ PI3K inhibitor) (Cayman Chemicals, USA). Subsequently, to induce type 2 IR, the cells were activated by adding recombinant proteins produced by Biolegend (USA): IL-2 (20 U/ml), IL-25 (50 ng/ml), IL-33 (50 ng/ml), and TSLP (50 ng/ml). After three days of cell incubation, the supernatants were collected and stored at –80°C. The concentrations of IL-4, IL-6, IL-8, tumor necrosis factor (TNF) α, interferon (IFN) γ (Vector Best, Russian Federation), IL-9, IL-13, and IL-17A (Biolegend, USA) were determined in the supernatants using enzyme-linked immunosorbent assay according to the manufacturer's instructions.

Evaluation of Intracellular Cytokine Production by Blood T Lymphocytes

PBMCs were adjusted to 0.8×10^6 cells per well in the 24-well plate and cultured in the presence or absence of idelalisib and recombinant proteins (in the similar concentrations as described above). To accumulate cytokines inside the cell (to prevent their secretion outside the cell), 10 μg/ml brefeldin A (Biolegend, USA) was added before the last 16 hours of incubation. The total incubation time of the cells with recombinant proteins was three days.

Next, 0.4×10^6 cells were transferred into new tubes and washed twice with wash buffer (phosphate-buffered saline containing FBS and sodium azide (Biolegend, USA). A cocktail of monoclonal antibodies to surface antigens (CD45, CD3, CD4, and CD8, Biolegend, USA; Exbio, Prague, Czech Republic) was added, and the cells were incubated for 20 min in the dark at room temperature. The cells were then washed with wash buffer (centrifugation conditions: 500g, 5 min). After fixation of leukocytes (using fixation buffer, Biolegend, USA), the cells were permeabilized using permeabilization buffer (Biolegend, USA), and monoclonal antibodies to IL-4 APC, IL-5 PE, IL-13 APC, and IL-17A PE (Biolegend, USA; BD Biosciences, USA) were added for 20 minutes in the dark at room temperature. In our experiments, we also used unstimulated and isotype controls to ensure proper fluorescence compensation and confirm antibody specificity.

Next, 3 ml of wash buffer was added, and the tubes were centrifuged at 500g for 5 minutes. After removing the supernatant, 500 μl of 1% paraformaldehyde in phosphate-buffered saline was added to the tubes, and the cells were analyzed no later than 24 hours on a CytoFLEX flow cytometer (Beckman Coulter). The results of the study were further evaluated using CytExpert 2.3 software (Beckman Coulter). Samples were analyzed by gating lymphocytes using CD45 antibody and side scatter signal. T-helper cells were identified as $CD45^+CD3^+CD4^+$ events, and cytotoxic T lymphocytes were defined as $CD45^+CD3^+CD8^+$ cells. Then, the intracellular synthesis of IL-4, IL-5, IL-13, and IL-17A by T-helper cells and cytotoxic T lymphocytes was analyzed.

Statistical Data Processing

Statistical data processing was performed using the GraphPad Prism 7 statistical data analysis package (GraphPad Software, USA). The results of the study are presented as the mean and the standard error of the mean $M \pm m$ from the total number of observations with normal data distribution, which was confirmed by constructing distribution histograms and applying the Shapiro–Wilk test. The study results were assessed using the one-way analysis of variance (ANOVA) method with post-hoc pairwise comparison of values using the Tukey test. For all types of statistical analysis, the critical value of the significance level was taken as equal to 5%.

RESULTS

The results of the study showed an increase in the secretion of IL-4, IL-8, IL-9, IL-13, IL-17A, TNFα, and IFNγ upon induction of type 2 IR (by adding IL-2, IL-25, IL-33, and TSLP to the culture medium) (Fig. 1). The analysis of intracellular production of cytokines by blood $CD4^+$ and $CD8^+$ T lymphocytes demonstrated a rise in the synthesis of IL-4, IL-5, and IL-13 by both subpopulations of T lymphocytes, as well as IL-17A by blood cytotoxic T lymphocytes of patients with allergic rhinitis upon the influence of IL-2, IL-25, IL-33, and TSLP combination (compared to cells cultured without these recombinant proteins) (Fig. 2).

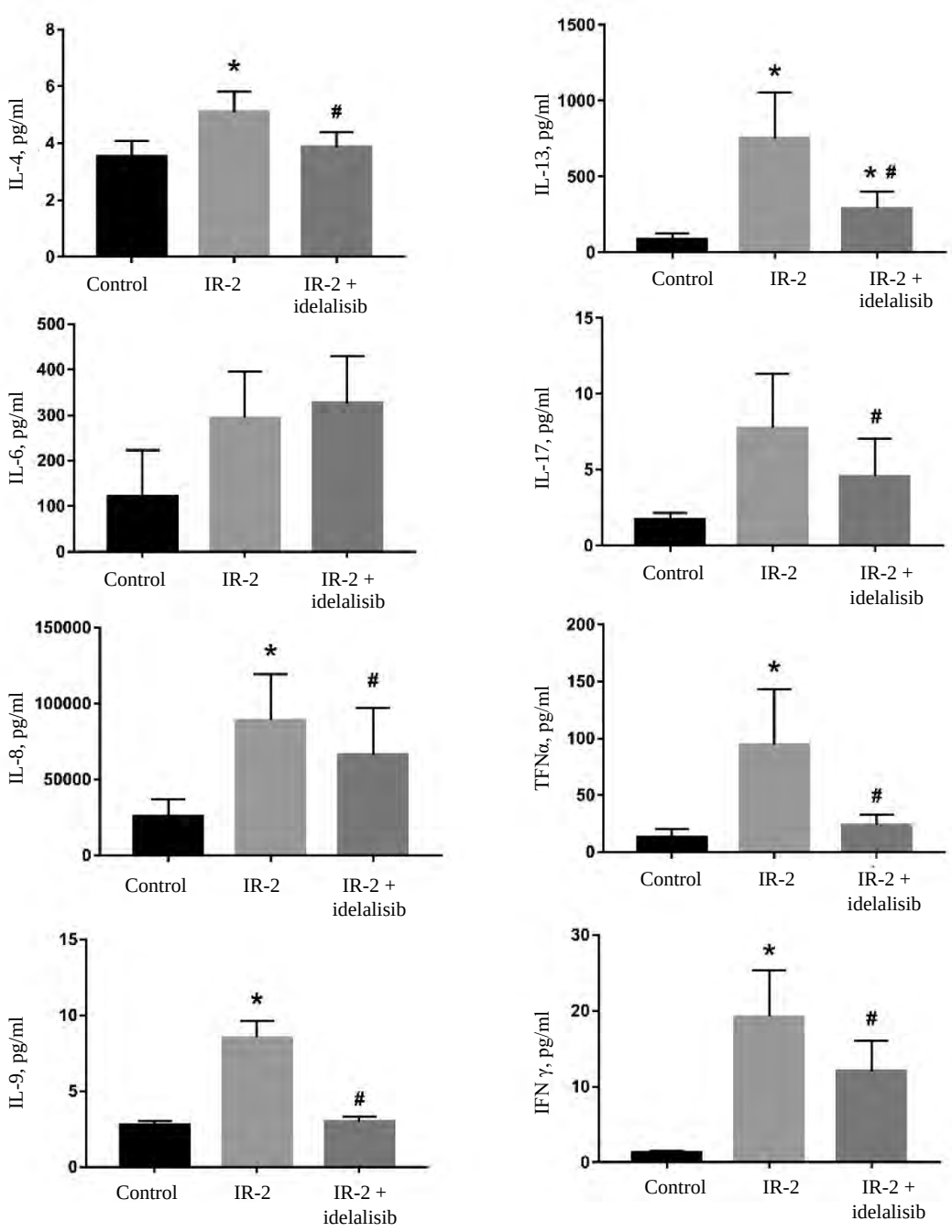

Fig. 1. Effect of idelalisib on cytokine (IL-4, IL-6, IL-8, IL-9, IL-13, IL-17A, TNFα, and IFNγ)production by peripheral blood mononuclear cells (PBMCs) in patients with allergic rhinitis; $M \pm m$, $n = 6$, $p < 0.05$; * compared with control (cells incubated in the absence of recombinant proteins); # compared with cells activated by type 2 immune response (without idelalisib). PBMCs were incubated with idelalisib (0.5 μM) and recombinant proteins to induce type 2 immune response (IR-2). Secretion of cytokines was determined by enzyme-linked immunosorbent assay

Next, we assessed the ability of the p110δ phosphatidylinositol 3-kinase inhibitor to affect the production of inflammatory mediators by PBMCs of patients with allergic rhinitis (Fig. 1, 2).

It turned out that idelalisib is able to suppress the secretion of IL-4, IL-8, IL-9, IL-13, IL-17A, TNFα, and IFNγ by PBMCs upon induction of type 2 IR. In addition, upon stimulation of type 2 IR, idelalisib inhibited the production of IL-4, IL-5, IL-13, and IL-17A by blood T-helper cells and cytotoxic T lymphocytes of patients with allergic rhinitis.

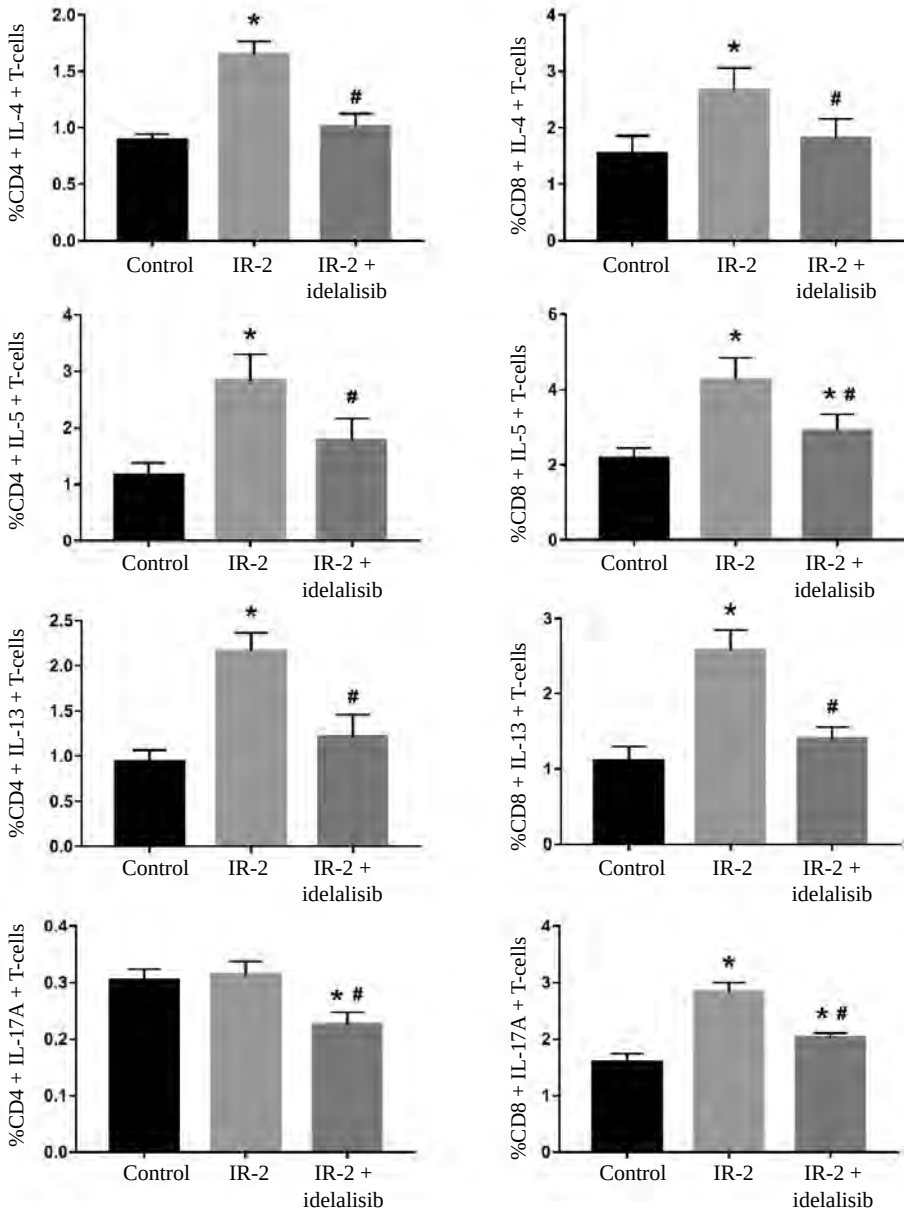

Fig. 2. Effect of idelalisib on intracellular cytokine production (IL-4, IL-5, IL-13 and IL-17A) by blood T-helper cells ($CD4^+$) and cytotoxic T lymphocytes ($CD8^+$)in patients with allergic rhinitis: $M \pm m$, $n = 5–6$, $p < 0.05$; * compared with control (cells incubated in the absence of recombinant proteins); # compared with cells activated by type 2 immune response (without idelalisib). Peripheral blood cells were incubated with idelalisib (0.5 μM) and recombinant proteins to induce type 2 immune response (IR-2). The level of IL-4 synthesis by T lymphocytes as determined by flow cytometry

DISCUSSION

PI3K are a group of enzymes involved in signal transduction into the cell through the production of secondary messengers. Under the action of PI3K, phosphatidylinositol-3,4,5-triphosphate is formed, which phosphorylates (activates) Akt kinase. The latter activates many effector molecules that control cell growth, metabolism, viability, and chemotaxis. In leukocyte cells, the p110δ PI3K isoform is predominantly found [7], which is involved in the activation and differentiation of T lymphocytes, their migration to the site of inflammation, and production of cytokines [8]. The expression of phosphoribosomal protein S6, which reflects PI3K activity, has been found to be increased in bronchial epithelial cells of patients with asthma after exposure to an allergen, indicating the importance of PI3K in allergic diseases [9].

Allergic rhinitis is a disease characterized by the formation of type 2 IR. Previously, it has been

shown that such an IR develops under the influence of IL-2, IL-25, IL-33, and TSLP on epithelial and immune cells and is accompanied by an increase in the concentration of IL-4, IL-5, IL-9, and IL-13 in the blood serum and nasal washes of patients with allergic rhinitis [10]. In the present study, idelalisib (p110δ PI3K inhibitor) reduced the production of IL-4, IL-9, and IL-13 by PBMCs and suppressed the intracellular synthesis of IL-4, IL-5, and IL-13 by blood T-helper cells and cytotoxic T lymphocytes of patients with allergic rhinitis upon the induction of type 2 IR. The data obtained demonstrate the ability of idelalisib to significantly inhibit the inflammatory response in patients with allergic rhinitis.

In our study, we also assessed the production of IL-8 and IL-17A in response to idelalisib action and stimulation of PBMCs by type 2 IR. IL-17A belongs to the group of cytokines produced by type 17 T-helper cells, while IL-8 is produced by numerous cell populations, including alveolar macrophages, T lymphocytes, mast cells, fibroblasts, epithelial and endothelial cells, platelets, and neutrophils [11, 12]. It is noteworthy that IL-17A can increase IL-8 expression in epithelial and endothelial cells of patients with allergic rhinitis, thereby enhancing neutrophil migration to the site of allergic inflammation [13, 14]. It is known that IL-8 and IL-17A concentration is increased in the blood serum of patients with allergic rhinitis compared with healthy individuals [11, 15]. Moreover, endonasal provocation test with an allergen in patients with allergic rhinitis led to an increase in IL-8 and IL-17A level in nasal lavage [16, 17]. Our experiments demonstrated the inhibitory effect of idelalisib on the secretion of IL-8 and IL-17A by PBMCs of patients with allergic rhinitis upon stimulation of type 2 IR. In addition, idelalisib reduced the production of IL-17A by blood $CD4^+$ and $CD8^+$ T lymphocytes of patients with allergic rhinitis. These results suggest the ability of idelalisib (due to the suppression of IL-8 and IL-17A) to inhibit neutrophil chemotaxis in patients with allergic rhinitis.

TNFα induces the production of antigen-specific IgE, Th2 cytokines and chemokines, as well as adhesion molecules involved in attracting eosinophils to the site of allergic inflammation. Moreover, TNFα deficiency slows down the development of allergic rhinitis [18]. An association between the polymorphic variant of the rs769178 locus of the *TNFα* gene, haplotypes (C-G-A-T and C-G-C-T) of the *TNFα* gene, and an increased risk of allergic rhinitis has been reported [19]. In a mouse model of allergic rhinitis, the TNFα inhibitor infliximab demonstrated antiallergic action by reducing the production of IL-4 and IgE, expression of adhesion molecules (E-selectin), and migration of eosinophils into the nasal mucosa [20]. In this study, suppression of TNFα production by PBMCs of patients with allergic rhinitis was achieved by adding idelalisib to the culture medium, which indicates the ability of this drug to effectively reduce the expression and synthesis of TNFα.

Another proinflammatory mediator whose production was assessed in this study is IFNγ, a key Th1 cytokine (Fig. 1). It is known for its ability to induce the production of chemokines CXCL9, CXCL10, CXCL11, which attract T lymphocytes expressing CXCR3 receptors to the site of inflammation. An increase in IFNγ production by blood $CD4^+$ T cells of patients with allergic rhinitis upon contact with allergens has been demonstrated [21], along with an increase in CXCL9 and CXCL10 concentration in nasal washes of patients with allergic rhinitis 30 minutes after exposure to the allergen [22], which indicates the importance of IFNγ and IFNγ-induced chemokines in the allergic inflammation. In our work, in response to stimulation of PBMCs of patients with allergic rhinitis according to type 2 IR, IFNγ production was increased. Addition of idelalisib to the culture medium resulted in the decreased synthesis of this cytokine. Thus, in the present study, under type 2 IR conditions, idelalisib suppressed the synthesis of Th2 cytokines, as well as Th1 and Th17 cytokines.

To date, only a single phase I clinical trial has been conducted on a small sample of patients evaluating the use of idelalisib for the treatment of allergic rhinitis, demonstrating its good tolerability, clinical efficacy, and ability to reduce the level of several inflammatory mediators in the blood of patients [4]. The data obtained in this study confirm high anti-inflammatory efficacy of this drug and indicate the need for further clinical trials using idelalisib for the treatment of patients with allergic rhinitis.

CONCLUSION

The results of the study showed that idelalisib (an inhibitor of the p110δ isoform of PI3K) can significantly suppress the secretion of cytokines (IL-4, IL-8, IL-9, IL-13, IL-17A, TNFα, and IFNγ) by PBMCs of patients with allergic rhinitis exposed to recombinant proteins (IL-2, IL-25, IL-33, and TSLP) that induce type 2 IR. This drug also significantly suppressed the intracellular production of IL-4, IL-5,

IL-13, and IL-17A in blood cytotoxic T lymphocytes and T-helper cells activated by type 2 IR. The data obtained justify the need to intensify clinical trials using idelalisib for the treatment of allergic rhinitis.

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Effectiveness of Differential Diagnosis of Primary Neck Masses in Children

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ABSTRACT

Aim. To assess the diagnostic features and accuracy of differential diagnosis of primary neck masses in pediatric patients, emphasizing the role of a reference center.

Materials and methods. A retrospective analysis was conducted in patients (aged 1 month to 17 years) who underwent surgical treatment at the Dmitry Rogachev National Research Center (Moscow, Russia) between 2012 and 2022. Inclusion criteria were as follows: age under 18 years, presence of a primary non-visceral neck mass, and confirmed histopathological diagnosis.

Results. This study was performed using data collected from 153 patients. The study cohort included malignant neoplasms in 34.6% (53/153), benign neoplasms in 29.4% (45/153), and lymphatic malformations in 36.0% (55/153) of cases. The median age at disease onset was 1.40 years (0.01; 5.12), and at hospital admission – 2.58 years (1.02; 7.86). Lymphatic malformations were most commonly diagnosed in the prenatal and neonatal periods (52.7%, 29/55), malignant neoplasms typically presented before the age of 3 years (56.6%, 30/53), while benign neoplasms were more common in children older than 3 years (80.0%, 36/45). In 55.5% of cases, the initial outpatient diagnosis was revised upon hospitalization. Diagnostic biopsy performed outside of reference centers proved insufficiently informative: an accurate initial diagnosis was made in 23.5% (36/153) of patients in non-specialized facilities. Initial histopathological examination was carried out at the Dmitry Rogachev National Research Center in 54.9% (84/153) of cases and remained unchanged in 98.7% (151/153) of cases following an internal review.

Conclusion. Non-visceral neck masses in children are frequently misdiagnosed at the outpatient stage. This study highlights the importance of a multidisciplinary approach – including imaging, biopsy, and slide review in reference centers – to improve diagnostic accuracy and guide an optimal treatment strategy.

Keywords: children, neck, neck masses, diagnosis, histopathology, pediatric

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

Conformity with the principles of ethics. The study protocol was approved by the independent Ethics Committee at Dmitry Rogachev National Research Center (Minutes No. 5/2023 dated June 20, 2023).

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Эффективность дифференциальной диагностики новообразований внеорганной шейной локализации у детей

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РЕЗЮМЕ

Цель. Оценить особенности и эффективность дифференциальной диагностики первичных образований внеорганной шейной локализации у детей с участием референс-центра.

Материалы и методы. Проведено ретроспективное исследование пациентов (1 мес – 17 лет), перенесших хирургическое лечение в ФГБУ «НМИЦ ДГОИ им. Д. Рогачева» МЗ РФ в 2012–2022 гг. Критериями включения служили: возраст пациента до 18 лет, первичное внеорганное образование шеи и наличие патоморфологической верификации диагноза.

Результаты. Исследование выполнено на основе данных 153 пациентов. Выборочную совокупность составили: злокачественные новообразования (ЗНО) – 34,6% (53/153); доброкачественные новообразования (ДНО) – 29,4% (45/153); лимфатические мальформации – 36,0% (55/153). Медиана возраста при дебюте заболевания составила 1,40 (0,01; 5,12) года, при госпитализации – 2,58 (1,02; 7,86) года. Лимфатические мальформации диагностированы преимущественно на пре- и неонатальных периодах (52,7%; 29/55), при ЗНО – до 3 лет (56,6%; 30/53), при ДНО – старше 3 лет (80,0%; 36/45). У 55,5% пациентов первичный диагноз, установленный на амбулаторном этапе, был изменен в стационаре. Диагностическая биопсия вне референс-центров была недостаточно информативна: в условиях неспециализированного стационара инициально корректный диагноз был выставлен в 23,5% (36/153) случаев. Инициально патоморфологическое исследование биоптата или операционного материала проводилось преимущественно в НМИЦ ДГОИ – в 54,9% (84/153) случаев и не подвергалось изменению в дальнейшем в 98,7% (151/153) (пересмотр проходил внутри центра).

Заключение. Внеорганные новообразования и мальформации шеи у детей часто диагностируются с ошибками на амбулаторном этапе. Исследование подчеркивает важность мультидисциплинарного подхода, включающего визуализацию, биопсию и пересмотр материала в референс-центрах для повышения точности диагностики и выбора оптимальной тактики лечения.

Ключевые слова: дети, шея, внеорганное новообразование, диагностика, патоморфологическое исследование

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

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

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INTRODUCTION

The differential diagnosis of pediatric neck masses is broad, encompassing a wide range of congenital, inflammatory, and neoplastic lesions. Notably, up to 80–90% of head and neck masses in children are benign [1–5]. In most cases, these patients present with lymphadenopathy that either regresses spontaneously or resolves with antibiotic therapy within 2 to 6 weeks. Persistence beyond this period may require biopsy [6–9].

The age at onset, duration, and nature of symptoms, as well as the anatomical location and size of the

lesion, are key factors in identifying the most likely malignant neoplasms to include in the differential diagnosis. However, these clinical parameters alone are often insufficient to distinguish between benign and malignant processes. In such cases, the diagnosis should be based on imaging studies and, when indicated, histopathological examination, including immunohistochemical and molecular genetic analyses. Current literature does not provide evidence regarding the role or degree of involvement of reference centers within multimodal diagnostic approaches for neoplastic conditions.

The aim of the study was to evaluate the diagnostic features and the effectiveness of differential diagnosis for primary neck masses in pediatric patients, specifically in cases involving a reference center.

MATERIALS AND METHODS

The article presents the results of a retrospective quantitative study involving non-random sampling of patients. The study was approved by the independent Ethics Committee of Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology (Minutes No. 5/2023 dated June 20, 2023). The approval was granted taking into consideration the Declaration of Helsinki.

The study included patients who underwent surgical treatment at Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology (Moscow, Russia) between January 2012 and December 2022 (a 10-year period). Inclusion criteria were as follows: 1) age under 18 years at the time of hospitalization to Dmitry Rogachev Center; 2) presence of a primary non-visceral cervical mass; 3) histopathologically confirmed diagnosis of a lymphatic malformation, benign tumor, or malignant neoplasm of the neck. Lymphatic malformation was selected as the representative entity among congenital developmental anomalies, based on its high prevalence in the study cohort and to reduce the risk of potential systematic bias associated with the inclusion of other malformations.

All statistical analyses were preceded by verification of the assumptions required for the application of each test. A conventional significance threshold of 0.05 was used for comparisons between two groups. For pairwise comparisons among three groups, the significance level was adjusted to 0.017 using the Bonferroni correction. The Kruskal–Wallis test was used to compare three independent groups. The Mann–Whitney *U* test adjusted for multiple comparisons was applied for comparisons between two independent groups and for post hoc pairwise analyses.

To assess differences in categorical variables across three clinical subgroups, the Pearson χ^2 test was used when expected frequencies were evenly distributed and no cells had expected counts of less than 5. In all other cases, the likelihood ratio χ^2 test (G^2), which accounts for the logarithmic relationship between observed and expected frequencies, was employed. The coefficient of variation of expected frequencies was used as a numerical indicator of distribution uniformity.The use of the G^2 test was based on current methodological research demonstrating its superior accuracy and statistical power in contingency table analysis involving small sample sizes and uneven distributions [10]. In addition, the G^2 test demonstrated greater sensitivity to deviations from independence in contingency tables larger than 2×2, making it a statistically preferable method for analyzing frequency data in pediatric studies with limited sample sizes. All statistical analyses were performed using IBM SPSS Statistics version 26. The data are presented as absolute and relative values *n* (%), the median, the interquartile range, and maximum and minimum values *Me (Q1; Q3;* min–max).

RESULTS

From January 2012 to December 2022, surgical treatment for non-visceral cervical lesions was performed in 370 patients. During the selection process, 277 patients were excluded: 217 due to lymphadenopathy of various etiologies (predominantly secondary metastatic involvement) and 60 due to the absence of histopathological confirmation of lymphatic malformation, as these patients were treated exclusively with sclerotherapy.

As a result, data from 153 patients (78 girls and 75 boys) with tumors and lymphatic malformations were included in the analysis. The sample consisted of malignant neoplasms in 34.6% of cases (53/153), benign neoplasms in 29.4% (45/153), and lymphatic malformations in 36.0% (55/153) of cases. Detailed information is presented in Fig. 1.

Among malignant neoplasms, the most frequently identified entities were poorly differentiated neuroblastoma (34.0%, 18/53), extrarenal extracranial rhabdoid tumor (13.2%, 7/53), and alveolar rhabdomyosarcoma (13.2%, 7/53).

Among benign neoplasms, the most common were schwannoma (15.2%, 7/46), paraganglioma (8.7%,

4/46), and lipoma (8.7%, 4/46). Additional benign lesions included venous malformation (8.7%, 4/46), congenital non-involuting hemangioma (NICH) (4.3%, 2/46), and small vessel–rich arteriovenous malformation (2.2%, 1/46).

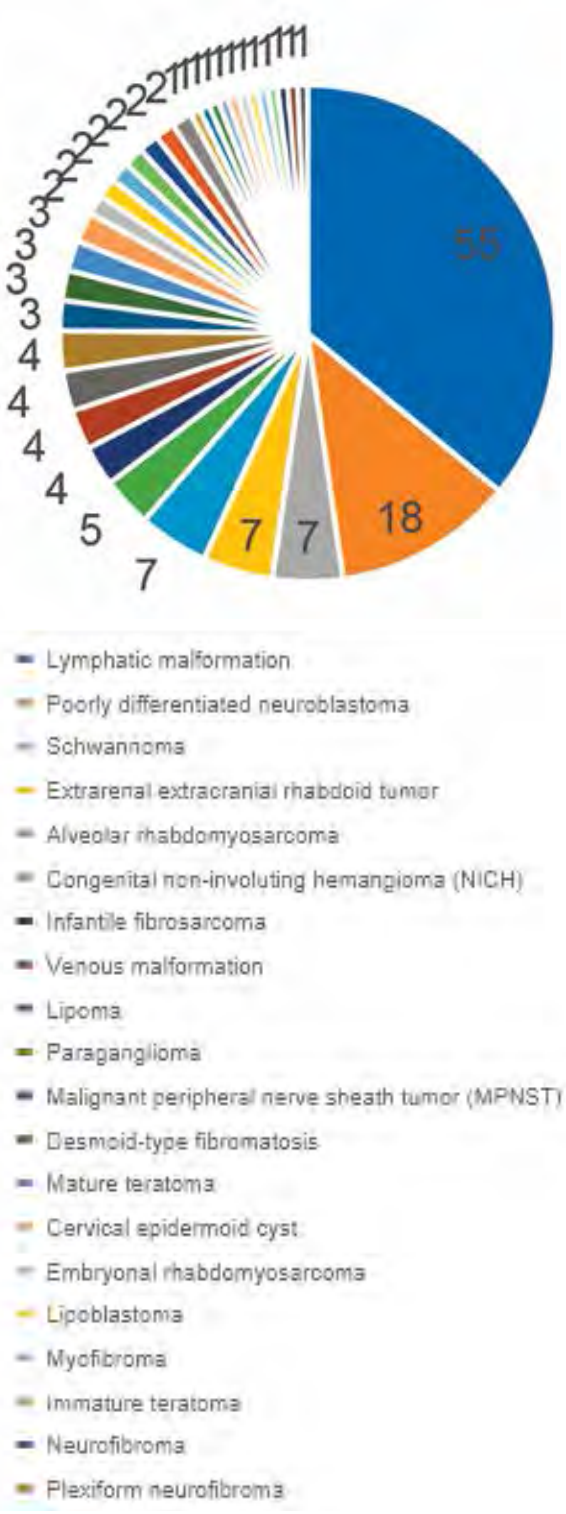

Fig. 1. Distribution of neoplastic and malformative lesions of non-visceral cervical localization

The median age at initial clinical presentation was 1.40 years (0.01; 5.12), and the median age at admission to Dmitry Rogachev National Medical Research Center was 2.58 years (1.02; –7.86). Prenatal diagnosis was reported in 9.2% of cases (14/153). Detailed data are presented in Table 1.

As shown in Table 1, lymphatic malformations were most commonly diagnosed during the prenatal and neonatal periods, accounting for 52.7% (29/55) of cases. Malignant neoplasms typically manifested before the age of 3 years (56.6%; 30/53), whereas benign neoplasms were significantly more likely to present after 3 years of age (80.0%; 36/45), with a statistically significant difference observed ($\chi^2(2) = 13.292$; $p = 0.001$). This distribution was also reflected in the median age at symptom onset, diagnostic verification, and first admission to the reference center: 1) patients with lymphatic malformations were diagnosed significantly earlier; 2) the greatest diagnostic delay was observed in patients with benign neoplasms, with a median time to diagnosis of 7.43 months (*Me* 3.33–18.98). However, despite a later onset, patients with malignant neoplasms were more frequently hospitalized and diagnosed at an earlier stage at the reference center.

At the time of initial clinical presentation, the most common primary complaint was the presence of a palpable neck mass. This symptom was reported in 92.7% (51/55) of patients with lymphatic malformations and in 100.0% (45/45) of those with benign neoplasms. In contrast, it was less frequently observed in patients with malignant neoplasms – 64.2% (34/53) – a statistically and clinically significant difference ($\chi^2_{(2)} = 31.682$; $p < 0.001$). Symptoms of respiratory distress were reported predominantly in the malignant neoplasm group (6/53; 11.3%). Other presenting complaints included swelling of the neck, face, supraclavicular area, or upper limb (6/153; 3.9%), neurologic symptoms (5/153; 3.3%), and nonspecific signs such as low-grade fever, lethargy, and reduced appetite (2/153; 1.3%).

At the time of initial admission to Dmitry Rogachev National Medical Research Center, an exacerbation of clinical symptoms was observed in association with rapid tumor growth. Neurological deficits were documented in 20.3% of patients (31/153), respiratory insufficiency of varying severity - in 14.4% (22/153), and dysphagia - in 10.5% (16/153). Respiratory insufficiency was more frequently observed in patients with malignant neoplasms: 24.5% (13/53) compared to 14.5% (8/55) in patients with lymphatic

malformations and 2.2% (1/45) in those with benign neoplasms, a statistically significant difference ($\chi^2_{(2)}$ = 11.742; p = 0.003).

Among 22 patients with respiratory insufficiency: 50.0% (11/22) were tracheostomy-dependent at the time of admission; 13.6% (3/22) required mechanical ventilation, with tracheostomy performed at the Center; in 4.5% (1/22), tracheostomy was not feasible due to extensive tumor infiltration; 9.1% (2/22) received supplemental oxygen therapy; and 22.3% (5/22) did not require any respiratory support. Neurological symptoms were also more commonly associated with malignant neoplasms, occurring in 37.7% (20/53) of cases compared to 10.9% (6/55) in patients with lymphatic malformations and 11.1% (5/45) with benign neoplasms ($\chi^2_{(2)}$ = 14.671; p = 0.001).

Dysphagia was more frequently observed in the lymphatic malformation group and was absent in patients with benign neoplasms. This symptom was documented in 21.8% (12/55) of patients with lymphatic malformations compared to 7.5% (4/53) in the malignant neoplasm group and 0% in the benign neoplasm group.

Further details regarding the diagnostic verification ("roadmap") of neoplastic lesions are presented in Table 2. For the purpose of comparative analysis, patients were divided into three clinical groups: group A – lymphatic malformations (n = 55); group B – benign neoplasms (n = 45); group C – malignant neoplasms (n = 53). A change in the initial diagnosis established at the outpatient stage was documented in 55.5% (75/135) of cases.

Diagnostic fine-needle aspiration biopsy (FNAB) performed at the local clinic was recorded in 10.9% (6/55) of cases in group A, 2.2% (1/45) in group B, and 17.0% (9/53) in group C ($\chi^2_{(2)}$ = 6.726; p = 0.035). In all such cases, the biopsy results were either non-informative or contained diagnostic inaccuracies. Notably, none of these procedures were performed at reference centers, including Dmitry Rogachev National Medical Research Center.

Pre-hospital surgical attempts to remove the lesion prior to admission to Dmitry Rogachev National Research Center were recorded in 25.5% (14/55), 35.6% (16/45), and 20.8% (11/53) of patients in groups A, B, and C, respectively ($\chi^2_{(2)}$ = 2.751; p = 0.253).

Initial histopathological examination of biopsy or surgical specimens was performed at Dmitry Rogachev National Research Center in 54.9% of cases (84/153). The group-wise distribution was as follows: 67.3% (37/55) in group A, 53.3% (24/45) in group B, and 30.2% (16/53) in group C ($\chi^2_{(2)}$ = 11.570; p = 0.003). These differences are likely attributable to the inclusion criterion requiring histological confirmation for lymphatic malformations, as well as the lack of indication for biopsy in cases with characteristic imaging findings during the preoperative workup.

A histopathological review was not performed in 24.8% of patients (38/153). When a pathology report from a local facility was available, the original slides were forwarded to Dmitry Rogachev National Research Center in 11.1% (5/45) of group B cases and in 34.0% (18/53) of group C cases ($\chi^2_{(2)}$ = 28.103; p < 0.001). In the absence of a prior pathology report, histological specimens were referred to the reference center in 18.9% (10/53) of patients in Group C. Direct re-examination of histological material upon admission to Dmitry Rogachev National Research Center was performed in 2.2% (1/45) of patients in group B and in 7.5% (4/53) in group C. Thus, in most cases, histopathological evaluation was initially performed at Dmitry Rogachev National Medical Research Center.

The highest rate of diagnostic errors prior to admission to Dmitry Rogachev Center was observed in group A (60.0%), compared to 8.9% and 24.5% in groups B and C, respectively ($\chi^2(2)$ = 33.278; p < 0.001). A correct diagnosis established at the local level was documented in 23.5% of patients (36/153), primarily among those with lymphatic malformations, which may reflect a tendency toward overdiagnosis of this condition at the outpatient stage. The greatest diagnostic challenges outside of reference centers were associated with the verification of malignant tumors. At Dmitry Rogachev National Research Center, the correct initial diagnosis was established in 75.8% of cases (116/153). In other reference centers, a correct diagnosis was reported only in group B (2.2%). Taken together, these findings underscore the critical role of reference centers in establishing accurate histopathological diagnoses of neck masses.

At Dmitry Rogachev National Research Center, the initial diagnosis was accurate in 75.8% of cases (116/153). A primary diagnosis consistent with the final histopathological verification and issued by other reference centers was documented exclusively in group B (2.2%; 1/45).

Correction of the initial diagnosis following the review of histological materials obtained at local facilities was required in 1.8% (1/55) of cases in group A, 8.9% (4/45) in group B, and 18.9% (10/53) in group

Table 1

Primary Clinical and Diagnostic Characteristics of the Study Cohort

Clinical and Diagnostic Parameters		Group A: lymphatic malformations, *n* = 55	Group B: benign neoplasms, *n* = 45	Group C: malignant neoplasms, *n* = 53	Statistical analysis results
Sex ratio, *n* (%)	Female	24 (43.6)	25 (55.6)	29 (54.7)	$\chi2_{(2)} = 1.860$; $p = 0.395$
	Male	31 (56.4)	20 (44.4)	24 (45.3)	
Timing of initial presentation, *n* (%)	Prenatal	11 (20.0)	1 (2.2)	2 (3,8)	**$\chi2_{(2)} = 11.969$; $p = 0.003$**
	Neonatal	18 (32.7)	8 (17.8)	5 (9.4)	**$\chi2_{(2)} = 9.438$; $p = 0.009$**
	1 month to 3 years	15 (27.3)	12 (26.7)	23 (43.4)	$\chi2_{(2)} = 4.164$; $p = 0.125$
	Older than 3 years	11 (20.0)	24 (53.3)	23 (43.4)	**$\chi2_{(2)} = 12.721$; $p = 0.002$**
Age at onset of clinical presentation, years [1] *Me* (*Q1*; *Q3*; min–max)		0.03 (0.00; 2.38; –0.39; 10.87)	3.54 (0.36; 11.27; –0.56; 17.06)	2.18 (0.67; 6.34; -0.78; 20.43)	**H= 22.528; $p < 0.001$** **$U_{(A-B)} = 645.000$; $p < 0.001$** **$U_{(A-C)} = 813.500$; $p < 0.001$** $U_{(B-C)} = 1086.500$; $p = 0.450$
Age at initial admission to the reference center, years *Me* (*Q1*; *Q3*; min–max)		2.10 (0.54; 5.83; 0.09; 17.72)	6.96 (1.89; 13.68; 0.12; 17.90)	1.86 (0.85; 5.49; 0.13; 17.94)	**H= 15.699; $p < 0.001$** **$U_{(A-B)} = 742.500$; $p = 0.001$** $U_{(A-C)} = 1453.500$; $p = 0.980$ **$U_{(B-C)} = 698.000$; $p < 0.001$**
Age at diagnosis confirmation, years *Me* (*Q1*; *Q3*; min–max)		3.12 (1.03; 6.77; 0.00; 17.21)	7.15 (2.07; 13.52; 0.13; 18.01)	1.86 (0.54; 4.56; 0.07; 17.96)	**H= 16.424; $p < 0.001$** **$U_{(A-B)} = 828.000$; $p = 0.005$** $U_{(A-C)} = 1248.000$; $p = 0.198$ **$U_{(B-C)} = 640.000$; $p < 0.001$**
Time from symptom onset to diagnosis confirmation, months *Me* (*Q1*; *Q3*; min–max)		1.23 (0.13; 16.40; 0.03; 147.63)	7.43 (3.33; 18.98; 0.10; 211.07)	2.50 (0.92; 6.27; 0.07; 62.07)	**H= 16.327; $p < 0.001$** **$U_{(A-B)} = 759.000$; $p = 0.001$** $U_{(A-C)} = 1295.000$; p = 0.319 **$U_{(B-C)} = 672.500$; $p < 0.001$**
Presenting complaints at onset, *n* (%)	Palpable neck mass	51 (92.7)	45 (100.0)	34 (64.2)	**$\chi2_{(2)} = 28.535$; $p < 0.001$**
	Lymph node enlargement and/or tenderness	3 (5.5)	4 (8.9)	5 (9.4)	$\chi2_{(2)} = 0.723$; $p = 0.697$
	Signs of respiratory distress	1 (1.8)	none	6 (11.3)	Not applicable
	Neurological symptoms	none	1 (2.2)	4 (7.5)	Not applicable
	Dysphagia	none	none	1 (1.9)	Not applicable
Presenting complaints at admission, *n* (%)	Palpable neck mass	53 (96.4)	45 (100.0)	39 (73.6)	**$\chi2_{(2)} = 22.401$; $p < 0.001$**
	Lymph node enlargement and/or tenderness	2 (3.6)	1 (2.2)	7 (13.2)	$\chi2_{(2)} = 5.741$; $p = 0.057$
	Signs of respiratory distress	8 (14.5)	1 (2.2)	13 (24.5)	**$\chi2_{(2)} = 11.742$; $p = 0.00$**3
	Neurological symptoms	6 (10.9)	5 (11.1)	20 (37.7)	**$\chi2_{(2)} = 14.671$; $p = 0.00$**1
	Dysphagia	12 (21.8)	none	4 (7.5)	**$\chi2_{(2)} = 16.449$; $p < 0.001$**
Maximum Lesion Size. mm *Me*(*Q1*; *Q3*; min–max)		69.50 (58.3; 93.0; 20.0; 173.0)	42.50 (35.25; 59.25; 14.0; 194.0)	43.50 (33.75; 57.25; 20.0; 90.30)	**H= 25.628; $p < 0.001$** **$U_{(A-B)} = 312.000$; $p < 0.001$** **$U_{(A-C)} = 411.500$; $p < 0.001$** $U_{(B-C)} = 607.500$; $p = 0.684$

[1] Prenatal diagnosis was established in 9.2% of cases (14/153): in group A – 20.0% (11/55), in group B – 2.2% (1/45), and in group C – 3.8% (2/53).

C ($\chi^2_{(2)} = 9.822$; $p = 0.007$). Re-evaluation of a diagnosis previously established at another reference center led to diagnostic revision after admission to Dmitry Rogachev National Research Center in 1.8% (1/55), 2.2% (1/45), and 5.7% (3/53) of cases in the respective groups.

These cumulative findings emphasize the critical role of reference centers in ensuring accurate histopathological verification of non-visceral neck masses. The diagnostic algorithm for non-visceral cervical masses is presented in Figure 2.

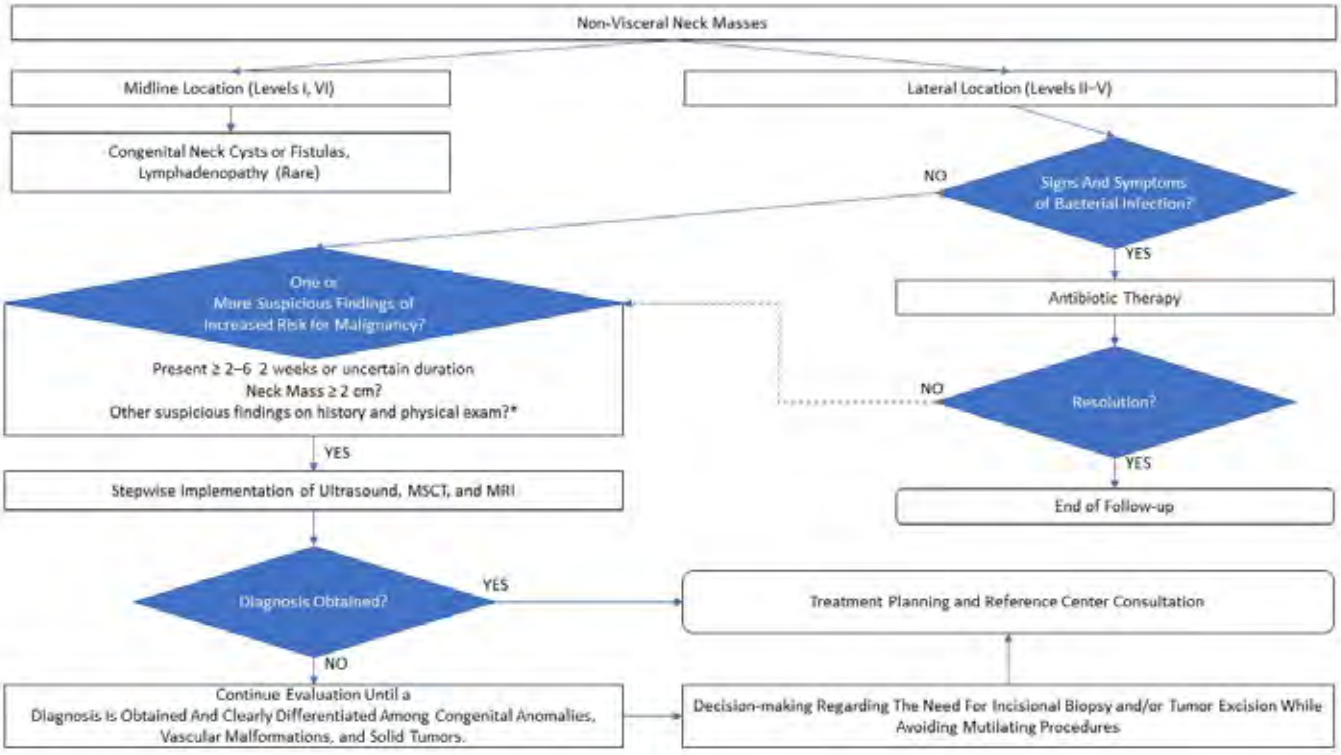

Fig. 2. Diagnostic Algorithm for Non-Visceral Neck Masses in Children.: * Other features suggestive of malignancy include the presence of a firm, painless mass, respiratory distress, and neurological symptoms

DISCUSSION

Non-visceral neck masses in children are commonly encountered in pediatric clinical practice and often pose a diagnostic challenge for clinicians. Several classification approaches have been proposed for pediatric neck masses, including age at presentation, anatomical location (taking into account fascial spaces and cervical compartments), imaging characteristics, and underlying etiology. Non-visceral neck tumors account for only a small proportion of all head and neck neoplasms [1]. This study evaluated the characteristics of initial diagnostic workup, referral pathways, and the effectiveness of histopathological examination in the verification of non-visceral cervical neoplasms and lymphatic malformations in children.

The results of the present study indicate that clinical history and physical examination alone are insufficient for accurate diagnostic verification. Notably, the initial diagnosis established in outpatient settings was revised in 55.5% of cases (75/135). These findings are consistent with previously published literature [4].

When imaging non-visceral neck masses in children, the potential risk of radiation exposure must be considered. Therefore, ultrasound (US) is typically used as the first-line diagnostic modality. US demonstrates a sensitivity of 79–95% and a specificity of 83–84% in differentiating neoplastic from reactive lymph nodes [11, 12]. In addition, elastography may improve diagnostic accuracy, achieving a sensitivity of 85.9% and a specificity of 100% in differentiating malignant lymphadenopahy from benign one [13, 14]. We previously published a study on the imaging characteristics of secondary cervical lymph node involvement in children, presenting prognostic models for predicting the likelihood of malignant lymphadenopathy [15].

When necessary, additional imaging modalities, such as magnetic resonance imaging (MRI) and multidetector computed tomography (MDCT)

Table 2

Comparative Analysis of Histopathological Verification in Non-Visceral Neck Masses by Nosological Group

Clinical and diagnostic parameters		Group A: lymphatic malformations, *n* = 55	Group B: benign neoplasms, *n* = 45	Group C: malignant neoplasms, *n* = 53	Statistical analysis results
Change of initial diagnosis made at a local outpatient facility, *n* (%)[1]		8 (14.5)	28 (87.5)	39 (81.3)	$\chi2_{(2)} = 69.417$; $p < 0.001$
Diagnostic aspiration performed, *n* (%)	Local facility	6 (10.9)	1 (2.2)	9 (17.0)	$\chi2_{(2)} = 6.726$; $p = 0.035$
	Other reference center[2]	none	none	none	Not applicable
	Dmitry Rogachev National Research Center	none	none	none	
Diagnostic biopsy performed, *n* (%)	Local facility	4 (7.3)	9 (20.0)	30 (56.6)	$\chi2_{(2)} = 35.492$; $p < 0.001$
	Another reference center[2]	none	none	5 (9.4)	Not applicable
	Dmitry Rogachev National Research Center	none	2	2	Not applicable
	Total	4 (7.3)	11 (24.4)	37 (69.8)	$\chi2_{(2)} = 52.487$; $p < 0.001$
Attempt to remove the malformation prior to hospitalization to Dmitry Rogachev Center, *n* (%)		14 (25.5)	16 (35.6)	11 (20.8)	$\chi2_{(2)} = 2.751$; $p = 0.253$
Biopsy/surgical specimen sent to a reference center					
No review performed at Dmitry Rogachev National Research Center		18 (32.7)	15 (33.3)	5 (9.4)	$\chi2_{(2)} = 11.570$; $p = 0.003$
Referred to Dmitry Rogachev National Research Center (with a prior histopathology report available)		none	5 (11.1)	18 (34.0)	$\chi2_{(2)} = 30.204$; $p < 0.001$
Referred to Dmitry Rogachev National Research Center (without a prior histopathological diagnosis)		none	none	10 (18.9)	Not applicable
Review of a specimen from local facility at Dmitry Rogachev Center (on admission)		none	1 (2.2)	4 (7.5)	Not applicable
Initial histopathological examination performed at a reference center[1]		37 (67.3)	24 (53.3)	16 (30.2)	$\chi2_{(2)} = 15.448$; $p < 0.001$
Diagnostic accuracy of histopathological evaluation					
No diagnosis established at a local facility or another reference center, *n* (%)		33 (60.0)	4 (8.9)	13 (24.5)	$\chi2_{(2)} = 33.278$; $p < 0.001$
No change in diagnosis	Accurate initial diagnosis established at a local facility	17 (30.9)	5 (11.1)	14 (26.4)	$\chi2_{(2)} = 6.337$; $p = 0.042$
	Accurate initial diagnosis established at Dmitry Rogachev National Research Center	38 (69.1)	39 (86.7)	39 (73.6)	$\chi2_{(2)} = 4.390$; $p = 0.111$
	Accurate initial diagnosis established at another reference center[1]	none	1 (2.2)	none	Not applicable
Change in diagnosis	Revision of diagnosis initially made at local facility following histopathological review at a reference center[1]	1 (1.8)	4 (8.9)	10 (18.9)	$\chi2_{(2)} = 9.822$; $p = 0.007$
	Diagnosis revised after review at Dmitry Rogachev National Research Center (originally made at another reference center)	1 (1.8)	1 (2.2)	3 (5.7)	Not applicable

1. In the analysis of diagnosis changes between outpatient and inpatient stages, data on the initial (outpatient) diagnosis were unavailable for 11.8% of patients (18/153). 2. Throughout this study, the term «reference center» refers to specialized institutions in the Russian Federation officially designated as reference centers in accordance with Order No. 1372 of the Ministry of Health of the Russian Federation dated December 25, 2020.

are employed. These methods enhance diagnostic confidence and provide important topographic and anatomical information, such as involvement of critical vascular structures and adjacent organs of the neck. This is particularly relevant given the limited penetration depth of ultrasound, which relies primarily on high-frequency probes for the assessment of superficial lesions [3].

A review of biopsy or surgical specimens obtained at local facilities was performed at a reference center in 75.2% of cases (115/153). An accurate histopathological diagnosis initially established outside a specialized institution was observed in only 23.5% of patients (36/153), the majority of which were cases of lymphatic malformation.

The greatest need for diagnostic re-evaluation was observed among patients with malignant tumors – 90.6% of cases (48/53), compared to 66.7% (30/45) among those with benign neoplasms. Revision of the initial diagnosis following the histopathological review was required in 24.5% of malignant cases (13/53) and in 11.1% of benign cases (5/45).

These findings underscore the critical role of reference centers in ensuring accurate histopathological verification of non-visceral cervical neoplasms in children.

Pediatric surgical oncologists play a critical role in the diagnosis, staging, and treatment of malignant solid tumors. Over time, many treatment protocols for solid tumors have increasingly advocated for an individualized surgical approach to both the primary tumor and metastatic lesions [1]. The decision to proceed with primary tumor resection at the diagnostic stage – or, conversely, to perform a biopsy followed by neoadjuvant therapy – is critically important and must be made by a multidisciplinary pediatric oncology team. This decision should be based on the clinical presentation, radiological findings, and results of histopathological evaluation. Inappropriate or untimely surgical intervention during the diagnostic stage of malignant neck tumors in children may result in both short- and long-term complications, incomplete tumor resection, and delays in the initiation of adjuvant therapy [16, 17]. This underscores the importance of timely and appropriate surgical intervention during the diagnostic phase to improve outcomes in children with malignant non-visceral cervical tumors.

CONCLUSION

Non-visceral neck masses in children present significant diagnostic variability and a high risk of misdiagnosis at the outpatient stage: accurate verification outside reference centers was achieved in less than a quarter of cases. These findings underscore the need for a comprehensive multidisciplinary approach involving imaging, biopsy, and mandatory slide review in specialized centers. The implementation of standardized diagnostic algorithms may substantially improve diagnostic accuracy and patient management strategies.

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Galectin-1 and Galectin-3 as Modulators of Systemic CD4+ T-lymphocyte Balance in Colorectal Cancer

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ABSTRACT

Aim. To evaluate the relationships between plasma levels of galectin-1 and galectin-3 and the composition of $CD4^+$ T-lymphocyte subpopulations (Th1, Th17, and Treg) in patients with colorectal cancer (CRC) and to determine a direct *in vitro* modulatory effect of tumor-associated galectin-1 and galectin-3 on the expression of key T-lymphocyte transcriptional factors (T-bet, RORC2, and Foxp3).

Materials and methods. The study included 26 patients with CRC and 17 healthy donors. Plasma concentrations of galectin-1 and galectin-3 were measured by enzyme-linked immunosorbent assay. Lymphocyte subpopulations were analyzed by flow cytometry. To assess the *in vitro* immunomodulatory effects of galectin-1 and galectin-3, a Transwell co-culture model of the colon adenocarcinoma cell line COLO 201 and peripheral blood mononuclear cells (PBMCs) from CRC patients was used, employing the selective galectin-1 inhibitor OTX008 and the galectin-3 inhibitor GB1107. The mRNA expression of target genes was evaluated by quantitative real-time polymerase chain reaction.

Results. Patients with CRC exhibited a decreased proportion of circulating Th1 and Th17 lymphocytes and an increased frequency of Treg cells, which is most pronounced in advanced disease stages. Plasma levels of galectin-1 and galectin-3 were also elevated. Galectin-1 concentration correlated negatively with Th1 and Th17 levels and positively with Treg levels. In contrast, the galectin-3 level was inversely associated only with the Th1 lymphocyte pool. Inhibition of galectin-1 and galectin-3 in the *in vitro* COLO 201/PBMC co-culture system induced increased mRNA expression of T-bet and RORC2 and decreased expression of Foxp3.

Conclusion. High concentrations of galectin-1 and galectin-3 in the blood of CRC patients are associated with systemic suppression of circulating $CD4^+$ T-lymphocytes. We demonstrated a direct *in vitro* modulatory effect of tumor-associated galectin-1 and galectin-3 on the differentiation of CRC patients' blood $CD4^+$ T-lymphocytes. These findings support the prospective use of targeted blockade of galectin-1 and galectin-3 in combination with existing immunotherapies for colorectal cancer.

Keywords: galectins, T-lymphocytes, immunosuppression, colorectal cancer, immunophenotype

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

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Conformity with the principles of ethics. All patients and donors signed an informed consenf to parficipafe in the study.

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Галектин-1 и галектин-3 как модуляторы системного баланса CD4+ Т-лимфоцитов при колоректальном раке

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РЕЗЮМЕ

Цель. Оценка взаимосвязей между уровнем галектина-1 и галектина-3 в плазме крови и субпопуляционным составом $CD4^+$ Т-лимфоцитов (Th1, Th17 и Treg) у больных колоректальным раком (КРР), а также определение *in vitro* прямого модулирующего влияния опухоль-ассоциированных галектинов 1 и 3 на экспрессию ключевых транскрипционных факторов (T-bet, RORC2, Foxp3) Т-лимфоцитов.

Материалы и методы. В исследование включены 26 пациентов с КРР и 17 здоровых доноров. Концентрацию галектинов 1 и 3 в плазме крови определяли с помощью иммуноферментного анализа. Субпопуляции лимфоцитов анализировали методом проточной цитофлуориметрии. Для оценки *in vitro* иммуномодулирующего действия галектинов 1 и 3 использовали модель Transwell-сокультивирования клеточной линии аденокарциномы толстой кишки COLO 201 и мононуклеарных лейкоцитов крови больных КРР с применением селективных ингибиторов галектина-1 (OTX008) и галектина-3 (GB1107). Экспрессию мРНК изучаемых генов оценивали методом полимеразной цепной реакции в реальном времени.

Результаты. У больных КРР выявлено снижение доли циркулирующих в крови Th1- и Th17-лимфоцитов и увеличение уровня Treg-клеток, наиболее выраженных на поздних стадиях заболевания, а также повышение содержания галектина-1 и галектина-3 в плазме крови. Концентрация галектина-1 отрицательно коррелировала с содержанием Th1 и Th17 и положительно – с долей Treg, в то время как уровень галектина-3 был обратно взаимосвязан с содержанием Th1-лимфоцитов. Ингибирование галектина-1 и галектина-3 в *in vitro* совместной культуре клеток аденокарциномы толстого кишечника COLO 201 и мононуклеарных лейкоцитов больных КРР индуцировало повышение экспрессии мРНК T-bet и RORC2 и снижение экспрессии Foxp3.

Заключение. Высокие концентрации галектина-1 и галектина-3 в крови больных КРР ассоциированы с системной супрессией циркулирующих в крови $CD4^+$ Т-лимфоцитов. Показано прямое *in vitro* модулирующее влияние опухоль-ассоциированных галектинов 1 и 3 на дифференцировку $CD4^+$ Т-лимфоцитов крови пациентов с КРР. Полученные результаты обосновывают перспективы таргетного блокирования галектина-1 и галектина-3 в комбинации с существующими методами иммунотерапии колоректального рака.

Ключевые слова: галектины, Т-лимфоциты, иммуносупрессия, колоректальный рак, иммунофенотип

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

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INTRODUCTION

Colorectal cancer (CRC) remains one of the most socially sensitive oncological diseases [1]. Despite active development of modern anti-tumor therapies, including immune checkpoint inhibitors and other immunotherapeutic drugs, their clinical efficacy in CRC remains underwhelming [2]. Among the factors limiting the success of immunotherapy in CRC patients, tumor-induced systemic immunosuppression plays a key role [3].

Although the most significant alterations in the composition and functional state of immune cells occur within the tumor microenvironment, the immunosuppressive influence of the tumor extends far beyond its boundaries, affecting lymphoid organs and immune cells circulating in the peripheral blood. The latter leads to an imbalance of pro- and anti-inflammatory mediators, exhaustion of effector T-lymphocytes, and accumulation of myeloid-derived suppressor cells (MDSCs) and regulatory T-cells (Tregs) [4, 5]. This not only contributes to tumor progression but also significantly reduces the efficacy of immunotherapeutic drugs, as effector lymphocytes are rendered incapable of mounting a robust response even after immune checkpoint blockade [5, 6].

Alongside tumor-associated metabolic disorders and chronic inflammation, soluble mediators (modulators) secreted by malignant cells are considered to play a substantial role in the pathogenesis of systemic immunosuppression in CRC [7, 8]. Among these modulators are the β-galactoside-binding lectins galectin-1 and galectin-3, whose overexpression is characteristic of CRC cells [9, 10]. Galectins-1 and -3 are known to possess immunomodulatory potential towards cells of the adaptive immunity; however, the vast majority of studies on the immunotropic effects of these lectins have focused on their local action within the tumor microenvironment [11–13]. Despite significant progress in understanding the biology of carbohydrate–protein interactions, the question of how galectins-1 and -3 affect the subpopulations of circulating $CD4^+$ T-lymphocytes in CRC remains open. Elucidating the contribution of galectin-1 and galectin-3 to the development of systemic immunosuppression is of fundamental importance for developing new combined therapeutic strategies for CRC aimed at restoring immune homeostasis.

The aim of this study was to assess the relationships between the plasma levels of galectin-1 and galectin-3 and the subpopulation composition of $CD4^+$ T-lymphocytes (Th1, Th17, and Treg) in patients with CRC, as well as to determine the direct modulating influence of tumor-associated galectins 1 and 3 on the expression of key transcriptional factors (T-bet, RORC2, and Foxp3) in T-lymphocytes *in vitro*.

MATERIALS AND METHODS

The study included 26 patients with a histologically verified diagnosis of CRC (14 men and 12 women, mean age 62.9 ± 6.7 years). CRC staging was performed according to the international TNM classification (8th Edition, AJCC 2017). Of these patients, 15 were diagnosed with stage 0–II CRC (T0-4 N0 M0), and 11 patients had stage III–IV disease (T1-4 N1-2 M0-1). The control group consisted of 17 apparently healthy donors (11 men and 6 women, mean age 58.2 ± 3.1 years). Exclusion criteria were as follows: prior neoadjuvant chemotherapy or radiotherapy, the presence of other malignant neoplasms, and acute or chronic inflammatory diseases (in the acute phase).

Peripheral venous blood (20 ml) was collected from the cubital vein of all participants in the morning after an overnight fast. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation on Ficoll. The plasma concentrations of galectin-1 and galectin-3 were determined by enzyme-linked immunosorbent assay (ELISA) using commercial reagent kits (BosterBio, USA).

The immunophenotyping of $CD4^+$ T-lymphocyte subpopulations (Th1, Th17, and Treg) in the PBMC suspension was performed using flow cytometry. Cells were stained with fluorochrome-conjugated monoclonal antibodies (Alexa Fluor 488, PerCP-Cy5.5, APC, PE; BD Biosciences, USA; RnD Systems, USA) against the surface antigen CD4 and the intracellular transcription factors T-bet, RORC2, and Foxp3. Analysis was conducted on an Accuri C6 flow cytometer (BD Biosciences, USA). Results were expressed as a percentage of the total lymphocyte count.

To determine the *in vitro* influence of tumor-associated galectin-1 and -3 on the expression of regulatory genes for T-lymphocyte differentiation, we developed a Transwell co-culture model using the human colon adenocarcinoma cell line COLO 201 (ATCC, USA) and PBMCs from CRC patients. Cells were cultured in complete RPMI-1640 medium (Elabscience, USA) supplemented with fetal bovine serum (Thermo Fisher Scientific, USA) and gentamicin (PanEco, Russia). The experiment utilized 24-well Transwell plates with semi-permeable membranes (0.4 μm, Sigma-Aldrich, USA). Tumor cells were seeded in

the lower chambers, while PBMCs isolated from patient blood, supplemented with phytohemagglutinin-P (10 μg/ml, PanEco, Russia), were placed in the upper chambers. Culturing was performed under the following conditions: an intact (control) co-culture and co-cultures with the addition of a galectin-1 inhibitor (OTX008, 2 μM) and a galectin-3 inhibitor (GB1107, 1 μM). Incubation lasted for 72 hours under standard conditions (37°C, 5% CO_2).

Following incubation, cells from the upper chambers were collected for molecular genetic analysis. Total RNA was extracted using the RNeasy Plus Mini Kit (QIAGEN, Germany), and RNA quality was assessed on a Multiskan Ex spectrophotometer (Thermo Fisher Scientific, USA); cDNA was synthesized by reverse transcription using the REVERTA-L kit (AmpliSens, Russia). The quantitative evaluation of mRNA expression for the target genes was performed by real-time polymerase chain reaction (RT-PCR) using the 5X qPCRmix-HS SYBR reaction mix (Eurogen, Russia) and specific primers (*tbx21*: F: 5'-CAGAATGCCGAGATTACTC-3'; R: 5'-AGGATACTGGTTGGGTAGGA-3'; *rorc*: F: 5'-CTGCTGAGAAGGACAGGGAG-3'; R: 5'-AGTTCTGCTGACGGGTGC-3'; *foxp3:* F: 5'-GCACATTCCCAGAGTTCCTC-3'; R: 5'-CAGTGGTAGATCTCATTGAGTGTC-3'; *β-actin*: F: 5'-TCGAGCAAGAGATGGCCAC-3'; R: 5'-AGGAAGGAAGGCTGGAAG-3'). The mRNA expression level of the *β-actin* gene was used for reference normalization. The relative quantity of cDNA in the samples was calculated using the ΔΔCt method and expressed in relative units (RU).

Statistical analysis was performed using IBM SPSS Statistics 27 software (IBM, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Quantitative measures were presented as the median and the interquartile range, *Me* (Q_1; Q_3). The Mann–Whitney *U* test was used for comparing two independent samples, and the Wilcoxon test was used for two dependent samples. Correlation analysis was performed by calculating the Spearman's rank correlation coefficient. Results were considered statistically significant at $p < 0.05$.

RESULTS

Flow cytometry analysis revealed significant alterations in the $CD4^+$ T-cell compartment in the peripheral blood of CRC patients. The percentage of Th1 lymphocytes ($CD4^+T\text{-}bet^+$) was 0.82% (0.24; 0.94), demonstrating a 1.5-fold decrease (p = 0.045) compared to the control group – 1.24% (0.48; 2.43). Furthermore, patients with CRC showed a 2.4-fold reduction (p = 0.005) in the proportion of Th17 lymphocytes ($CD4^+RORC2^+$) – 1.44% (0.19; 2.13) versus 3.51% (1.56; 4.79) in healthy donors. Conversely, the level of regulatory T-cells (Tregs, $CD4^+Foxp3^+$) in the blood of CRC patients was 1.19% (0.8; 1.48), which was 2.2 times higher (p = 0.011) than the corresponding value in the control group – 0.55% (0.23; 0.98) (Fig. 1).

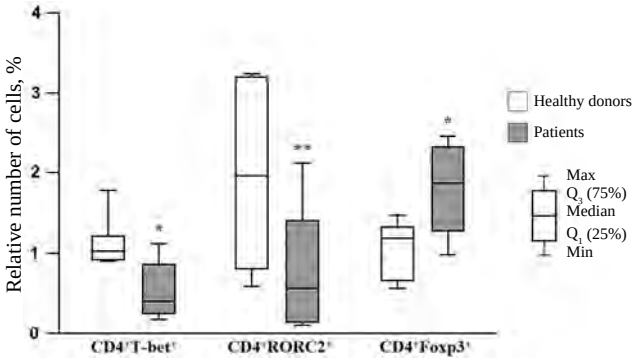

Fig. 1. Relative number of Th1, Th17, and Treg lymphocytes in the peripheral blood in patients with colorectal cancer and healthy donors, % of total lymphocytes: * $p < 0.05$; ** – $p <$ 0.01 compared to a similar indicator in healthy donors.

Stratification of CRC patients based on the tumor stage revealed that the relative content of Th1 1.03% (0.91; 1.23) and Th17 lymphocytes 1.96% (0.8; 3.21) was higher in patients with stage 0–II disease compared to those with advanced stages (III–IV) 0.4% (0.24; 0.86), p = 0.011 and 0.56% (0.13; 1.42), p = 0.038, respectively. In contrast, the proportion of Treg cells was 1.6 times lower (p = 0.017) in patients with stage 0–II CRC than in those with stage III–IV disease 1.19% (0.65; 1.34) vs. 1.87% (1.27; 2.34) (Fig. 2).

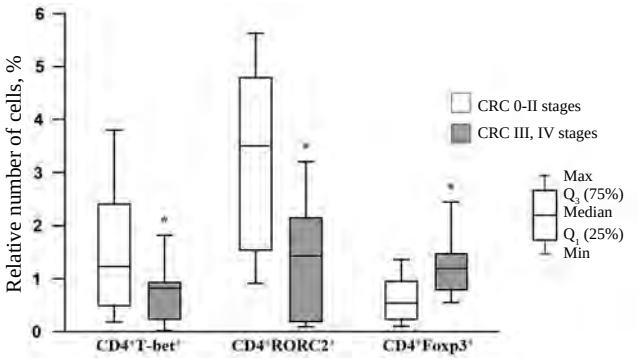

Fig. 2.– Relative number of Th1, Th17, and Treg lymphocytes in the peripheral blood in patients with colorectal cancer stratified by the disease stage, % of total lymphocytes: * $p <$ 0.05 compared to patients with stage 0–II CRC

According to ELISA, the plasma concentration of galectin-1 in CRC patients was 1.2 times higher (p = 0.003) than in the healthy donor group 16.17 (15.31; 17.10) vs. 13.74 (12.23; 14.79) ng/ml, respectively. Furthermore, the plasma level of galectin-1 in CRC patients correlated negatively with the content of Th1 (r = –0.56; p = 0.035) and Th17 lymphocytes (r = –0.59; p = 0.033) and correlated positively with the proportion of Tregs (r = 0.55; p = 0.035). The plasma concentration of galectin-3 was also elevated in CRC patients: 3.28 (2.30; 5.71) ng/ml compared to the control group 1.56 (1.19; 2.17) ng/ml in the control group (p = 0.006), but correlated only with the relative content of Th1 lymphocytes (r = –0.81; p = 0.001).

To test the hypothesis of a direct modulatory effect of tumor-derived galectins-1 and -3 on $CD4^+$ T-lymphocyte differentiation, we conducted an *in vitro* co-culture of the human colon adenocarcinoma cell line COLO 201 with PBMCs from CRC patients in the presence or absence of selective galectin-1 and galectin-3 inhibitors.

Blocking galectin-1 with the selective inhibitor OTX008 in *in vitro* co-cultures of COLO 201 cells and patient PBMCs led to a statistically significant increase in the mRNA expression of the key Th1 transcriptional factor T-bet from 1.23 (0.88; 1.60) to 2.28 (1.81; 2.58) RU, p = 0.012 and the Th17 marker RORC2 from 0.28 (0.23; 0.39) to 1.71 (1.22; 1.83) RU, p = 0.012 compared to control co-cultures. Conversely, the mRNA expression level of Foxp3, which regulates Treg differentiation, decreased from 6.25 (5.67; 7.45) to 3.48 (2.86; 4.11) RU, p = 0.012 (Fig. 3). The addition of the selective galectin-3 inhibitor GB1107 to the *in vitro* co-cultures induced unidirectional changes in the studied transcription factors: an increase in T-bet mRNA expression from 1.23 (0.88; 1.60) to 2.97 (2.83; 3.4) RU, p = 0.012 and RORC2 mRNA expression from 0.28 (0.23; 0.39) to 0.79 (0.57; 0.99) RU, p = 0.012, alongside a decrease in Foxp3 mRNA expression from 6.25 (5.67; 7.45) to 4.86 (4.26; 5.35) RU, p = 0.012 relative to the control co-cultures without inhibitors (Fig. 3).

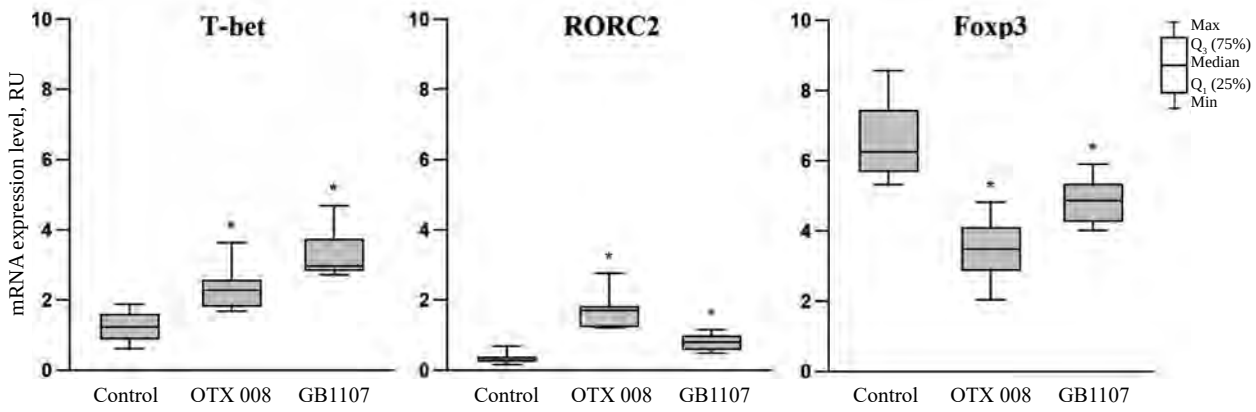

Fig. 3. mRNA expression level of the transcriptional factors T-bet, RORC2, and Foxp3 in *in vitro* cell cultures, RU: * $p < 0.05$ compared to the control co-culture

DISCUSSION

The results of our study confirm the presence of an imbalance in circulating $CD4^+$ T-lymphocytes in patients with CRC characterized by a reduction in effector subpopulations (Th1 and Th17) and an expansion of the immunosuppressive Treg cell pool.

The observed deficit in circulating $CD4^+$T-bet^+ Th1 lymphocytes, most pronounced in advanced-stage CRC, serves as a key indicator of systemic immune dysfunction and may contribute to immune evasion by malignant cells and tumor progression. Type 1 T-helper cells play a central role in orchestrating an effective anti-tumor immune response. Through the production of interferon (IFN)γ, as well as direct cell-to-cell contact, Th1 cells not only enhance the tumoricidal potential of tumor-infiltrating $CD8^+$ cytotoxic T-lymphocytes (CTLs) but also facilitate the presentation of tumor antigens by macrophages and dendritic cells and directly suppress cancer cell proliferation and tumor-associated angiogenesis [14, 15].

The relative content of $CD4^+$$RORC2^+$ Th17 lymphocytes in the blood of CRC patients was also reduced, particularly in patients with regional and distant metastases. Interpreting these results is complicated by the functional plasticity of the Th17

lymphocyte subset. Some researchers point to a predominantly pro-tumorigenic role for these cells (mediated by IL-17-dependent induction of tumor-associated inflammation and neoangiogenesis) in CRC pathogenesis [16–18]. However, under certain conditions, Th17 cells can exert anti-tumor effects by recruiting $CD8^+$ CTLs and neutrophils to the tumor site [19]. In the context of our study, the significant reduction in the Th17 pool in patients with metastatic CRC may indicate general insufficiency of the T-cell arm of adaptive immunity, despite the potentially pro-tumorigenic properties of these cells within the local tumor microenvironment.

Parallel to the deficit in effector Th1 and Th17 lymphocytes, we recorded an increase in the proportion of $CD4^+Foxp3^+$ Treg cells in the blood of CRC patients, reaching maximum values in patients with stage III and IV disease. According to current literature, Tregs are the primary inducers of immunological tolerance to tumor antigens. The tolerogenic potential of Treg cells is mediated by several complementary mechanisms, including the secretion of immunosuppressive cytokines (IL (interleukin)-10 and TGF (transforming growth factor)-β), direct cytolytic action on effector lymphocytes, expression of inhibitory molecules, such as PD (programmed cell death protein)-L1 and CTLA (cytotoxic T-lymphocyte-associated protein)-4, and the suppression of antigen-presenting cells [20, 21]. The expansion of Tregs in the peripheral circulation creates a barrier to an effective anti-tumor immune response and is a negative prognostic factor in CRC and other malignancies [20].

The mechanisms of systemic T-cell immune dysregulation in malignant colon tumors are multifaceted. Competitive consumption of glucose and amino acids by tumor cells disrupts the energy homeostasis and proliferative potential of effector T-lymphocytes [22], while lactate accumulation resulting from the Warburg effect in tumor cells suppresses type 1 and type 17 T-helpers while simultaneously stimulating Treg function [23]. Tumor-associated chronic inflammation, accompanied by elevated levels of circulating cytokines IL-1β, IL-6, and TNF (tumor necrosis factor)-α, leads to the expansion of myeloid-derived suppressor cells and Tregs against a background of CTL suppression and dysfunction of antigen-presenting cells, characterized by reduced MHC (major histocompatibility complex) II and co-stimulatory molecule (CD (cluster of differentiation)80/CD86) expression [24, 25]. Furthermore, malignant cells and elements of the tumor microenvironment secrete a wide spectrum of soluble mediators, including prostaglandin E2 (PGE2), adenosine, IL-10, and TGFβ, which modulate immune cell activity [26, 27]. The results of our study substantiate the involvement of galectin-1 and galectin-3, produced by malignant cells, in the development of tumor-induced immunosuppression in CRC.

The negative correlation between the plasma concentration of galectin-1 and the relative number of $CD4^+T\text{-}bet^+$ Th1 and $CD4^+RORC2^+$ Th17 lymphocytes and its positive correlation with the content of $CD4^+Foxp3^+$ Treg cells in the peripheral blood of CRC patients suggests a possible systemic tolerogenic influence of this lectin. Immunotropic action of galectin-1 is mediated by its binding to β-galactoside residues of membrane glycoproteins. For instance, the interaction of galectin-1 with CD45, CD43, CD7, and components of the T-cell receptor can induce apoptosis in activated lymphocytes [28]. Th1 and Th17 lymphocytes are most sensitive to the pro-apoptotic effect of galectin-1, which is associated with the specific glycosylation patterns of their membrane glycoconjugates that serve as ligands for this lectin [28, 29]. *In vitro* experiments have demonstrated other mechanisms for the immunomodulatory action of galectin-1, including the regulation of cytokine secretory activity, clonal expansion, and antigen-dependent differentiation of target lymphocytes [30]. The latter point is supported by our results, which show that selective inhibition of galectin-1 in a co-culture of PBMCs from CRC patients and COLO 201 colon adenocarcinoma cells led to increased expression of mRNA for the transcriptional factors controlling Th1 and Th17 development (T-bet and RORC2, respectively), and decreased expression of the Foxp3, which determines Treg cell differentiation.

In contrast to galectin-1, whose plasma level in CRC patients correlated with the relative content of all studied T-lymphocyte subpopulations, the concentration of galectin-3 correlated only with the number of $CD4^+T\text{-}bet^+$ type 1 T-helpers. Simultaneously, in co-cultures of COLO 201 cells and PBMCs from CRC patients, selective blockade of galectin-3 induced changes similar to those observed with galectin-1 inhibition (increased T-bet and RORC2 mRNA expression and suppressed Foxp3 expression). According to the literature, the biological activity of recombinant galectin-3 varies significantly depending on its local concentration [31, 32]. Furthermore, galectin-3-mediated

regulation of T-lymphocyte viability, function, and polarization is mediated not only through direct contact with the target cell but also via modulation of antigen-presenting cell activity in peripheral tissues [33, 34]. The relatively low level of galectin-3 (4.9 times lower than the concentration of galectin-1) in the blood of CRC patients appears insufficient for exerting a direct tolerogenic effect on the circulating pool of Th17 and Treg lymphocytes. On the other hand, our *in vitro* Transwell co-culture system replicated conditions approximating the tumor microenvironment, where the immunoregulatory activity of galectin-3 is more pronounced [35, 36].

CONCLUSION

This study demonstrates that in patients with colorectal cancer, elevated blood concentrations of galectin-1 and galectin-3 are associated with systemic suppression of T-cell immunity, manifesting as a reduced number of circulating Th1 and Th17 lymphocytes alongside a concomitant increase in Treg cells. The results of selectively inhibiting galectin-1 and galectin-3 in an *in vitro* co-culture of COLO 201 colon adenocarcinoma cells and peripheral blood mononuclear cells from CRC patients confirm the direct modulatory influence of soluble forms of galectins 1 and 3 on the expression of genes controlling the differentiation of $CD4^+$ T-lymphocytes towards either effector (Th1, Th17) or regulatory (Treg) phenotypes.

These findings contribute to the existing body of knowledge on the mechanisms of immune evasion in malignant colon tumors and substantiate the prospects of targeted galectin-1 and galectin-3 blockade in combination with existing immunotherapeutic strategies for colorectal cancer.

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Author Contribution

Reingardt G.V., Kurnosenko A.V. – conducting research, analysis and interpretation of data. Poletika V.S., Kolobovnikova Yu.V. – conception and design, justification of the aim, main provisions and conclusions of the manuscript. Urazova O.I. – critical revision for important intellectual content and final approval of the manuscript for publication. All members of the group meet the criteria and requirements for authors.

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Lung Cancer Diagnosis Based on the Analysis of Volatile Markers in Exhaled Breath

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ABSTRACT

Aim. To evaluate the diagnostic accuracy of a developed gas analysis sensor system combined with neural network algorithms for detecting lung cancer based on volatile organic compounds in exhaled breath.

Materials and methods. The study group included 53 exhaled breath samples from patients with morphologically confirmed stage I–IV lung cancer. The control group (n = 47) consisted of individuals with no history or prior diagnostic findings of cancers at the time of enrollment. The study was conducted using the developed Multisensory Gas Analysis System, comprising an array of semiconductor sensors and implementing neural network data processing algorithms.

Results. The experimental results of classifying lung cancer patients and healthy volunteers demonstrated distinct differences in the exhaled breath samples. The system achieved the accuracy of 95.8%, sensitivity of 98.1%, and specificity of 93.6%. In a series of experiments with balanced stage distribution (stages I–II vs. stages III–IV), the mean classification accuracy was 75%, with sensitivity and specificity ranging from 65 to 80%. Both prepped and non-prepped patients showed comparable results, confirming the reproducibility of the method. The accuracy level of 75% allowed for the differentiation between early- and late-stage disease samples.

Conclusion. The developed system demonstrates high diagnostic performance, surpassing existing methods, including low-dose computed tomography. The findings support the potential of this technology for both early detection and staging of lung cancer.

Keywords: lung cancer, exhaled breath, volatile organic compounds, sensory gas analysis system, non-invasive diagnosis, neural network

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

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Диагностика рака легкого на основе анализа летучих маркеров в выдыхаемом воздухе

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РЕЗЮМЕ

Цель. Определить диагностическую эффективность разработанного газоаналитического сенсорного комплекса в сочетании с алгоритмами искусственной нейронной сети для выявления рака легкого по маркерным летучим органическим соединениям в выдыхаемом воздухе.

Материалы и методы. В исследуемую группу включены 53 пробы выдыхаемого воздуха от пациентов с морфологически подтвержденным раком легкого I–IV стадий. Контрольная группа (n = 47) состояла из лиц, не имеющих на момент включения в исследование признаков онкологических заболеваний по данным анамнеза и (или) предшествующих диагностических мероприятий. Исследование проводилось с помощью разработанного мультисенсорного газоаналитического комплекса, состоящего из набора полупроводниковых сенсоров и реализующего алгоритмы нейро-сетевой обработки данных.

Результаты. Полученные при проведении экспериментов по классификации пациентов с раком легкого и здоровых добровольцев результаты показывают наличие явных признаков различия в пробах выдыхаемого воздуха. Точность составила 95,8 %, чувствительность – 98,1% и специфичность – 93,6%. В серии экспериментов с равным распределением стадий (I–II и III–IV) средняя точность классификации составила 75%, чувствительность и специфичность – 65–80%. Подготовленные и неподготовленные пациенты демонстрировали сопоставимые результаты, что подтверждает воспроизводимость метода. Уровень точности 75% позволяет различать пробы от пациентов с ранними и поздними стадиями заболевания.

Заключение. Разработанный комплекс демонстрирует высокую диагностическую эффективность, превосходящую существующие методы, включая низкодозную компьютерную томографию. Полученные данные подтверждают перспективность технологии как для раннего выявления, так и для стадирования рака легкого.

Ключевые слова: рак легкого, выдыхаемый воздух, летучие органические соединения, сенсорный газоаналитический комплекс, неинвазивная диагностика, нейронная сеть

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

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INTRODUCTION

Lung cancer remains a critical global public health burden, accounting for some of the highest incidence and mortality rates among all malignancies. These unfavorable outcomes are largely attributable to late-stage diagnosis, when curative treatment options are limited and prognosis is poor [1, 2].

Conventional imaging modalities, although indispensable, often lack the sensitivity required for the detection of early-stage disease. This limitation underscores the pressing need for non-invasive, rapid, and cost-effective screening strategies capable of facilitating earlier diagnosis and, ultimately, improving clinical outcomes.

Exhaled breath analysis has emerged as a promising non-invasive diagnostic modality. It is based on the qualitative and quantitative characterization of volatile organic compounds (VOCs) present in exhaled breath, which collectively reflect the underlying metabolic state of the body. The rationale for this approach lies in the premise that a wide range of VOCs are generated during physiological and pathological metabolic processes, and that alterations in their composition and concentration may serve as surrogate markers of disease [3]. In lung cancer, these metabolic perturbations are predominantly driven by heightened oxidative stress, chronic inflammation, and lipid peroxidation, which result in elevated levels of alkanes, aldehydes, ketones, and alcohols. Such compounds are considered to be potential biomarkers that may not only enable differentiation between malignant and benign processes but also contribute to disease staging [4, 5]. Nonetheless, robust analytical and clinical validation of these candidate biomarkers remains essential prior to their incorporation into routine clinical practice, particularly given the influence of genetic, environmental, and behavioral variables on VOC profiles across populations.

Established analytical techniques, including gas chromatography and mass spectrometry, offer high specificity for VOC detection but are constrained by prohibitive costs and lengthy processing times. By contrast, electronic nose (eNose) technologies allow for a real-time and pattern-recognition-based assessment of composite VOC signatures, thereby providing a practical and scalable solution for clinical deployment. This technology is particularly suited for point-of-care use, as it integrates rapid analysis with the capacity to detect early-stage pathological changes through unique “metabolic fingerprints” of exhaled breath [6].

The aim of the present study was to evaluate the diagnostic accuracy of a novel gas analysis sensor platform combined with artificial neural network-based algorithms for the detection of lung cancer through the analysis of VOC biomarkers in exhaled breath.

MATERIALS AND METHODS

This prospective study was conducted between 2023 and 2025 and aimed to classify individuals with lung cancer and healthy volunteers using exhaled breath analysis. A total of 100 validated breath samples were analyzed, selected from an initial pool of more than 250 samples. All participants (aged 35–80 years) were stratified into two groups: the study (lung cancer) group and the control group.

The study protocol was reviewed and approved by the Bioethics Committee of the Cancer Research Institute, a branch of Tomsk National Research Medical Center of the Russian Academy of Sciences (Minutes No. 3a dated March 25, 2020). A written informed consent was obtained from all participants.

The study group comprised 53 samples obtained from patients with morphologically confirmed

primary lung cancer, spanning clinical stages I–IV (T1–4N0–3M0–1). The control group (n = 47) consisted of individuals with no prior, clinical, and laboratory evidence of malignant disease at the time of inclusion. Both groups contained samples from prepared and unprepared patients in approximately equal proportions (50:50). Efforts were made to ensure balanced distribution of samples by age, sex, preparation status, and tumor stage (in the study group).

Inclusion criteria were: lung cancer in the medical history (for the study group), absence of decompensated somatic-symptom comorbidities, and age ≥ 18 years. Exclusion criteria were: refusal to participate in the study, prior history of malignant disease (for the control group), age < 18 years, acute infectious disease, antibiotic therapy within the preceding 30 days, decompensated comorbidities, pregnancy, or lactation.

All patients in the study group underwent comprehensive cancer staging in accordance with the national clinical guidelines for lung cancer [7]. Exhaled breath was collected as the primary biological sample using previously validated standardized protocols [8]. The samples were obtained in sterile 5-l polymer collection bags using two modalities. For prepared samples, participants abstained from food (except water), smoking, oral hygiene, and the use of perfumes or personal care products for at least 6 hours prior to sample collection. These samples were collected in the morning immediately after awakening. Unprepared samples were collected in the afternoon without any restrictions to diet, hygiene, or activity.

Breath analysis was performed using a multisensory gas analysis platform comprising 24 semiconductor gas sensors and a humidity sensor. The platform incorporated artificial neural network (ANN) algorithms capable of recognizing molecular signatures in exhaled breath from individuals with lung cancer and distinguishing them from those of healthy individuals.

A multilayer perceptron ANN architecture was employed. Input data consisted of digitized signals from the gas sensors, categorized according to the participant groups. Raw sensor outputs were initially stored in the XML (eXtensible Markup Language) format, with each file representing one exhaled breath sample. Each XML file contained integer analog-to-digital converter (ADC) values (0–1023) for all sensors.

Given the large volume of raw data, preprocessing was performed to optimize computational efficiency without compromising classification accuracy. XML data were converted into consolidated text files containing metadata (group composition, ANN input and output layer dimensions) and signal matrices. Signals were expressed as the ratio of the tenth thermal cycle to the first thermal cycle for each sensor, repeated across all sensors and participants. Larger datasets prolonged ANN training and testing times; therefore, input arrays were downsampled five-fold, reducing the input layer dimensionality without loss of performance.

The final ANN input layer comprised 432 nodes (18 values per sensor for 24 sensors), and the output layer contained two nodes corresponding to the two classification outcomes: (1, 0) indicating a healthy volunteer and (0, 1) indicating a participant with lung cancer [8]. Each ANN configuration underwent at least 10 independent training experiments with parameter optimization.

Multidimensional data visualization and clustering were performed using the t-distributed stochastic neighbor embedding (t-SNE) algorithm [9], which projects high-dimensional data into a two-dimensional space while preserving topological relationships: similar samples were projected as proximate clusters, whereas dissimilar samples were separated by greater distances.

The diagnostic performance of the ANN classifier was evaluated by the receiver operating characteristic (ROC) analysis, providing an objective measure of the discriminative ability of the proposed approach.

RESULTS

During neural network training experiments, we identified an architecture and set of hyperparameters that enabled classification of exhaled breath samples across two datasets with the mean accuracy of 92%. In selected experiments, the model achieved sensitivity and specificity values of 98 and 96%, respectively. Preliminary analysis of the datasets using the t-SNE algorithm revealed significant differences between the two subgroups (lung cancer and healthy volunteers), as visualized by scatter plots (Fig. 1).

In a series of 50 independent experiments aimed at differentiating the two subgroups, the mean area under the ROC curve (AUC) exceeded 0.9, with ROC curves approaching 1.0 (Fig. 2), indicating robust discriminative performance of the ANN-based classifier.

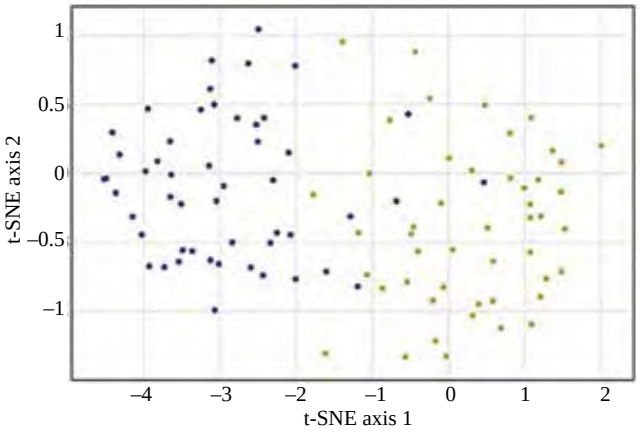

Fig. 1. The t-SNE distribution plot of exhaled breath samples from healthy volunteers and patients with lung cancer, illustrating subgroup differentiation based on dimensionality reduction

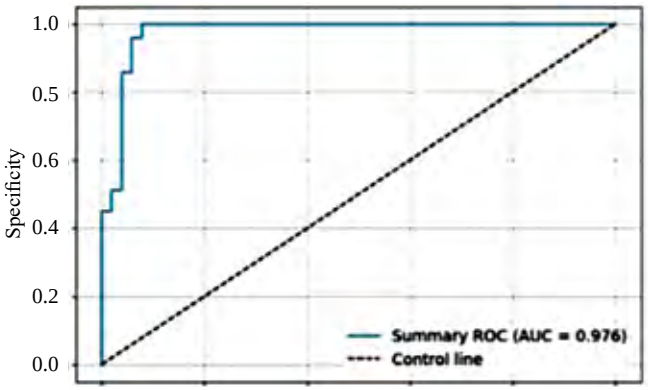

Fig. 2. ROC curve for neural network classifier performance in differentiating exhaled breath samples from healthy volunteers and patients with lung cancer.

Cross-validation analysis determined an optimal classification threshold of 0.24, which provided a balanced trade-off between false-positive and false-negative results across varying subgroup sample sizes. The corresponding distribution of exhaled breath samples after cross-validation is presented in Fig. 3.

Overall, classification of exhaled breath samples from patients with lung cancer versus healthy volunteers yielded diagnostic accuracy of 95.8%, sensitivity of 98.1%, and specificity of 93.6%. These results demonstrate strong evidence of subgroup differentiation and underscore the potential of this approach for clinical application in lung cancer diagnosis.

We further investigated the ability of the model to differentiate between prepared ($n = 55$) and unprepared ($n = 54$) breath samples from patients with lung cancer. In 50 experiments, the mean AUC reached 0.7, with ROC curves tending toward 1.0 (Fig. 4). The mean classification accuracy for this subgroup analysis was 65%, with sensitivity and specificity ranging from 60 to 72%. These findings suggest only minor compositional differences in VOC profiles between prepared and unprepared samples from patients with lung cancer. While patient preparation may marginally improve classification accuracy, its implementation would inevitably complicate the sampling procedure.

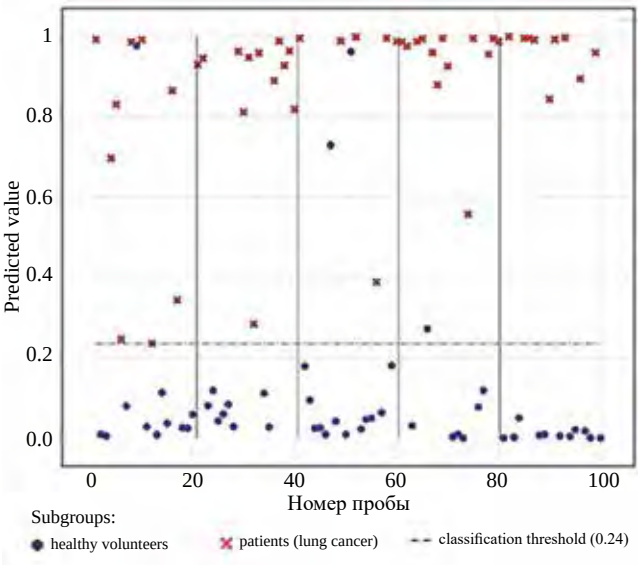

Fig. 3. Distribution plot of exhaled breath samples from the study subgroups following cross-validation

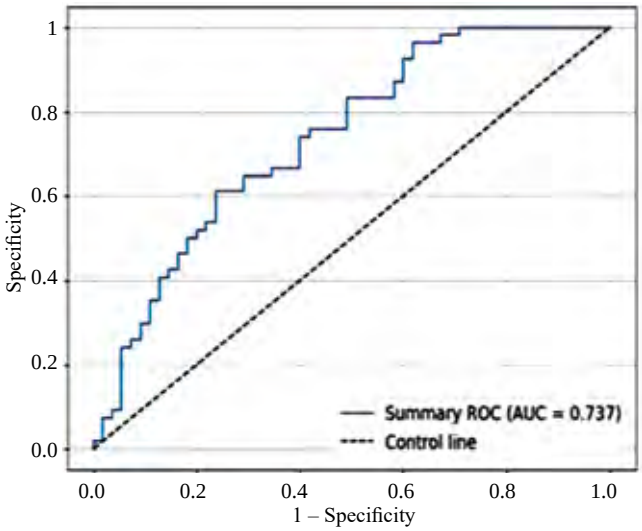

Fig. 4. ROC curve for ANN classifier performance in differentiating exhaled breath samples from prepared versus unprepared lung cancer patients.

Finally, we assessed the classifier's ability to differentiate exhaled breath samples from patients with early-stage (I–II) versus advanced-stage (III–IV)

lung cancer. This analysis was conducted separately for prepared and unprepared patients, with the same sample ratio in each subgroup (31:24). Across 50 experiments, the mean classification accuracy reached 75%, with sensitivity and specificity values ranging from 65 to 80%. Comparable results were observed for both prepared and unprepared cohorts. Achieving the mean accuracy of 75% highlights significant VOC profile differences between early- and advanced-stage disease, suggesting that the ANN-based approach may eventually enable not only early detection of lung cancer but also disease staging when applied sequentially with multiple classifiers.

DISCUSSION

Standard methods for early detection of lung cancer include chest radiography and low-dose computed tomography (LDCT). However, chest radiography — often employed for screening — has low sensitivity and a high false-negative rate due to its limited image resolution. LDCT provides superior anatomical detail and has proven high diagnostic value [10]. Current American Cancer Society guidelines recommend annual LDCT screening for high-risk individuals (current or former smokers aged 50–80 years with a $\geq$ 20 pack-year history), a strategy associated with a 20% reduction in lung cancer-related mortality compared to radiography [11].

Despite its proven efficacy, LDCT carries important limitations, including overdiagnosis (detection of clinically indolent lesions), high false-positive rates leading to unnecessary invasive procedures, and the need for multidisciplinary teams to interpret findings. Moreover, limited equipment availability, high cost, and the requirement for highly trained personnel restrict the scalability of LDCT-based screening programs, even in well-resourced health care systems [12].

Exhaled breath analysis of VOCs has emerged as a promising, non-invasive alternative for early lung cancer detection. Electronic nose (eNose) technologies have demonstrated consistently favorable results, even in populations with low to intermediate disease prevalence (5.4–22%) [13]. Across studies of non-small-cell and small-cell lung cancer, reported sensitivity and specificity have varied greatly, ranging from 71 to 99% and from 13 to 100%, respectively [6]. Integration of artificial intelligence (AI) has further enhanced eNose performance, enabling real-time data analysis and predictive assessment of tumor presence [14]. Nevertheless, the combination of AI and biosensory platforms for lung cancer screening remains underexplored, with issues of reproducibility, sensor drift, and environmental interference limiting their clinical application.

Our multisensory gas analysis platform, which integrates semiconductor-based biosensors with AI-driven data processing modules, demonstrated diagnostic performance that surpassed existing eNose systems and LDCT. In this study, the platform achieved sensitivity and specificity of 98.1 and 93.6%, respectively, outperforming LDCT (sensitivity ~93%, specificity ~73%).

Compared with LDCT, the platform offers several clinical advantages: it is entirely non-invasive, free from ionizing radiation exposure, rapid, low-cost per test, and suitable for large-scale population screening. Its portability and ease of use further allow for deployment in outpatient and primary care settings, including resource-limited regions.

A key strength of the platform lies in high selectivity of its multilayer sensor array, which can discriminate subtle variations in VOC composition. AI-driven data processing ensures robustness against noise and artifacts and adapts to inter-individual variability in breath signatures related to age, smoking status, or comorbid conditions. Validation experiments evaluating prepared and unprepared samples confirmed the reproducibility and low inter-series variability of the system.

CONCLUSION

Exhaled breath analysis is emerging as a powerful diagnostic modality for the early detection and monitoring of lung cancer, particularly at its initial stages. The newly developed multisensory gas analysis platform demonstrated clinically meaningful superiority over existing screening approaches, including LDCT.

The combination of high diagnostic accuracy, scalability, and operational simplicity positions this platform as a promising tool for integration into lung cancer screening programs and personalized diagnostic pathways. Its ability to provide rapid, non-invasive, and reproducible results could transform current approaches to early lung cancer detection and population-level screening.

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Author Contribution

Rodionov E.O. – analysis and interpretation of the data, processing of the materials, drafting of the manuscript. Podolko D.V., Kulbakin D.E., Miller S.V. – collection of the material, processing of the results. Obkhodskaya E.V., Obkhodskiy A.V. – hardware platform development and technical design, interpretation of the results. Sachkov V.I., Chernov V.I. – scientific analysis, critical revision of the manuscript for important intellectual content. Popov A.S. – hardware platform development and technical design, interpretation of the results. Lakonkin V.S. – analysis and interpretation of the data.

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Effects of Peat Humic Acids on Phagocytic Activity of Innate Immunity Cells and Humoral Immune Response

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ABSTRACT

Aim. To study the effect of a course administration of peat humic acids from bogs of the Tomsk Region on the phagocytic activity of peritoneal macrophages and blood neutrophils in mice, and the thymus-dependent humoral immune response induced by the administration of sheep erythrocytes.

Materials and methods. The following immunological methods were used: study of the phagocytic activity of peritoneal macrophages and blood neutrophils of mice, determination of the number of antibody-forming cells (AFC) in the spleen of mice, measurement of the serum level of antibodies to sheep erythrocytes by the hemagglutination assay after the course administration of three samples of peat humic acids. The experiment involved 70 C57Bl/6J mice (females), aged 7–8 weeks.

Results. It was found that the course administration of all studied samples of humic acids resulted in an increase in the number of phagocytes and the intensity of particle engulfment. Two out of three samples enhanced the humoral immune response induced by the administration of sheep erythrocytes, which was manifested by an increase in the number of AFCs in the spleen and the hemagglutination assay titer in the blood serum.

Conclusion. The samples of humic acids that influence the phagocytic function of macrophages and neutrophils and enhance the humoral immune response may serve as a basis for the development of new therapeutic agents for the treatment of immunodeficiency states.

Keywords: humic acids, phagocytosis, peritoneal macrophages, neutrophils, humoral immune response, antibody-forming cells, hemagglutinins

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Влияние гуминовых кислот торфа на фагоцитарную активность клеток врожденного иммунитета и гуморальный иммунный ответ

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РЕЗЮМЕ

Цель. Изучение влияния курсового введения гуминовых кислот торфа болот Томской области на фагоцитарную активность перитонеальных макрофагов и нейтрофилов крови мышей и тимусзависимый гуморальный иммунный ответ, вызванный введением эритроцитов барана.

Материалы и методы. Использовались следующие иммунологические методы: изучение фагоцитарной активности перитонеальных макрофагов и нейтрофилов крови мышей, определение количества антителообразующих клеток (АОК) в селезенках мышей, измерение уровня антител к эритроцитам барана в сыворотке крови мышей с помощью реакции гемагглютинации после курсового введения образцов торфяных гуминовых кислот. В эксперименте использовали 70 самок мышей линии C57BL/6J в возрасте 7–8 нед.

Результаты. Курсовое введение всех исследуемых образцов гуминовых кислот приводило к увеличению числа фагоцитов и интенсивности поглощения ими частиц. Два из трех образцов усиливали гуморальный иммунный ответ, индуцированный введением эритроцитов барана, который проявлялся увеличением числа АОК в селезенках и титра гемагглютининов в сыворотке крови.

Заключение. Образцы гуминовых кислот, влияющие на фагоцитарную функцию макрофагов и нейтрофилов и усиливающие гуморальный иммунный ответ, могут послужить основой для разработки новых терапевтических средств для лечения иммунодефицитных состояний.

Ключевые слова: гуминовые кислоты, фагоцитоз, перитонеальные макрофаги, нейтрофилы, гуморальный иммунный ответ, антителообразующие клетки, гемагглютинины

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

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INTRODUCTION

Phagocytosis is the process by which eukaryotic cells, called phagocytes, absorb microorganisms, damaged and destroyed host cells, and foreign particles. Blood monocytes, tissue macrophages, microglia cells, osteoclasts, dendritic cells, and neutrophilic leukocytes can absorb foreign agents. Phagocytes ensure the functioning of innate immunity and represent the body's first line of defense when interacting with infectious agents. Neutrophils and macrophages are typical phagocytes that can destroy both extracellular and intracellular microorganisms. These cells produce cytokines and

growth factors that regulate the development and resolution of inflammation. They also stimulate the activation and proliferation of lymphocytes and other immunocompetent cells, which are necessary for an effective adaptive immune response [1, 2].

In addition, an essential condition for activating acquired immunity is presenting the antigen to cells of adaptive immunity, which is carried out by phagocytes due to their ability to phagocytose and present foreign molecular patterns to T lymphocytes [3]. Thus, phagocytosis is a link between innate and acquired immune responses. It plays a crucial role when the innate immune system is ineffective at eliminating infectious agents.

The humoral immune response is part of the adaptive immune system. It is based on the induction of antigen-specific B lymphocytes, which results in the production and secretion of antigen-specific antibodies. Humoral immune responses can be divided into T-cell-dependent (thymus-dependent) and T-cell-independent (thymus-independent) responses. In thymus-independent reactions, the production of antibodies occurs immediately after the activation of B lymphocytes by antigens. The thymus-dependent humoral immune response is triggered when B lymphocytes are activated by T helper cells, which is preceded by antigen presentation by dendritic cells and macrophages to T lymphocytes [4]. Sheep red blood cells (SRBC), a well-known T-cell antigen, are considered as the gold standard for the formation of a thymus-dependent humoral immune response [5].

Currently, a significant number of means for correcting immune system disorders are known. However, most of them are products of chemical synthesis with a wide range of side effects. Among the immunomodulatory drugs of natural origin, only echinacea extract, drugs based on bacterial lysates, and immunomodulators of endogenous origin are known, which at the present time are usually represented by recombinant analogs [6]. A topical area of experimental medicine is the study of natural compounds, such as humic substances extracted from peat and brown coal, which are formed during the humification of biological remains of plants and animals [7]. The main component of humic substances is humic acids (HAs), which are high-molecular-weight heteropolymers that exhibit similar chemical and biological properties, but their exact molecular structure has not been determined [8]. HAs are widely used in agriculture and veterinary medicine and demonstrate numerous biological effects in experiments, namely wound healing, adaptogenic, hepatoprotective effects, as well as anti-inflammatory, antibacterial, and antioxidant activity [9, 10]. Studies have confirmed the effect of HAs on immune processes, including regulation of cytokine production with proinflammatory (TNFα, IFNγ, IL-1β, IL-12, IL-6, IL-2) and anti-inflammatory (IL-10, IL-4) properties, polarization of macrophages, and intracellular signaling processes [11–14]. Their involvement in the regulation of both humoral and cellular immunity has also been shown [15–17].

Considering the above, studying the influence of peat HAs from bogs located in the Tomsk region on such aspects of the immune system as phagocytic reactions of innate immune cells (macrophages and neutrophils) and indicators of the adaptive humoral immune response is of particular interest. Thus, the aim of this study was to investigate the effects of the course application of peat HAs on the phagocytic activity of peritoneal macrophages and peripheral blood neutrophils of laboratory mice, as well as the thymus-dependent humoral immune response induced by immunization of animals with sheep erythrocytes.

MATERIALS AND METHODS

Obtaining Humic Acids

The objects of the study were HAs isolated from peat by mass-exchange extraction. The extraction of humic substances from peat was carried out at room temperature for 8 hours using a sodium pyrophosphate solution (0.1 mol / l, ratio 1:100). HAs were precipitated from the samples by adding hydrochloric acid to pH = 1–2, then they were centrifuged, washed with water to pH = 7.0, and dried. Three types of high-moor peat obtained from the Vasyuganskoye peat deposit of the Tomsk region were used as raw materials.

The HAsphagn sample was extracted from the sphagnum peat (sampled at a depth of 20–70 cm) with a degree of decomposition of plant residues (R) of 5–10% and an ash content (A) of 2.8%. The HApine-cott sample was obtained from the pine-cotton peat (sampled at a depth of 10–50 cm, R = 30–35%, A = 7.3%), and the HAmagellan sample was isolated from the magellanic peat (sampled at a depth of 100–120 cm, R = 10–15%, A = 2.7%).

Chemical Description of Humic Acids

The elemental composition of each HA sample was studied, and its molecular weight was determined (Mw – weight average molecular weight, Mn – number average molecular weight, Mp – peak molecular weight). The elemental composition (carbon,

hydrogen, and nitrogen content) was determined using a C,H,N analyzer (Carlo Erba Strumentazione 1106, Milan, Italy) and expressed as the atomic fractions of each element. The oxygen content was determined by differences. For the HAsphagn sample: C 41.7 ± 0.2%, H 40.1 ± 0.2%, N 1.9 ± 0.01%, O 16.3 ± 0.1%. For the HApine-cott sample: C 38.6 ± 0.5%, H 42.5 ± 0.4%, N 1.40 ± 0.02%, O 17.6 ± 0.2%. For the HAmagellan sample: C 38.4 ± 0.5%, H 42.47 ± 0.4%, N 2.2 ± 0.03%, O 16.9 ± 0.2%. For the HAsphagn sample: *Mw* = 39.7 kDa, *Mn* = 7.7 kDa, and *Mp* = 17.5 kDa. For the HApine-cott sample: *Mw* = 22.8 kDa, *Mn* = 6.1 kDa, and *Mp* = 11.8 kDa. For the HAmagellan sample: *Mw* = 18.8 kDa, *Mn* = 4.9 kDa. and *Mp* = 9.6 kDa.

Experimental Animal Groups and Substance Administration Schemes. Female C57Bl/6J mice (*n* = 70) aged 7–8 weeks purchased from the Department of Experimental Biological Models of the E. D. Goldberg Research Institute of Physiology and Pharmacology were used in this study (approval of the Bioethics Committee, Minutes No. 227012024 dated February 01, 2024). The HA course lasted 10 days and involved daily intraperitoneal injections. The HA preparations were prepared at a dose of 1 mg / kg of animal body weight (0.9% sodium chloride solution was used as a solvent) and administered in a volume of 100 μl. The control group received 100 μl of normal saline intraperitoneally, the comparison group received 100 μl of glucosaminylmuramyl dipeptide (Lycopid, Skopinfarm, Russia) at a dose of 2 mg / kg for 10 days. The dosing regimen and the scheme for using the studied compounds had been previously optimized in a series of preparatory experiments.

To induce a thymus-dependent humoral immune response, sheep red blood cells (SRBC) were administered intraperitoneally to animals at a dose of 5×10^6 cells on day 5 of administration of the test substances. One day after the end of the course, the number of antibody-forming cells (AFCs) in the spleen of the animals was assessed, and blood was collected to obtain serum and determine the titers of specific antibodies to SRBC (hemagglutinins) [18].

Study of the Phagocytic Capacity of Peripheral Blood Neutrophils. Phagocytosis of peripheral blood neutrophils was stimulated by adding latex particles to them. For this purpose, heparin (500 U / ml) in a volume of 3 μl was mixed with 10 μl of a mouse peripheral blood taken from the tail vein 24 h after the last HA injection. This mixture was then placed in the wells of a round-bottom plate along with 10 μl of latex particles ($60–80 \times 10^3$ / μl) and incubated for 30 minutes at 37 °C on a shaker. Next, the plate was centrifuged for 5 min (1,000 rpm), 10 μl of the supernatant was removed, the sediment was resuspended and used to prepare smears on glass slides. The smears were fixed with May – Grünwald stain and then stained with azure II-eosin. Microscopic analysis of the stained smears was performed to determine the phagocytic index (percentage of neutrophils that phagocytized latex particles) and the phagocytic number (average number of latex particles absorbed by one neutrophil) [18].

Study of the Phagocytic Activity of Peritoneal Macrophages

The phagocytic activity of peritoneal macrophages was stimulated using a 0.05% ink solution, which was administered to mice intraperitoneally at a volume of 2 ml one day after the final administration of HAs. Ten minutes later, the abdominal cavity was washed with normal saline. The cell suspension was precipitated twice by centrifugation, then the total number of peritoneal exudate cells and the number of macrophages that had absorbed the ink were counted. Then the cell suspension was precipitated again, the supernatant was discarded, and the sediment was removed with distilled water. The optical density of the resulting solution was measured spectrophotometrically (λ = 620 nm). The optical density values reflected the volume of ink absorbed by macrophages. Based on the data, the phagocytic index (percentage of macrophages that captured ink particles) and the phagocytic number (the average amount of ink absorbed by one macrophage) were calculated [18].

Determination of the Number of Antibody-Producing Cells in Mouse Spleens. The spleens extracted from mice were homogenized together with a 0.9% sodium chloride solution, the resulting homogenate was filtered through a mesh, and the number of cells in the suspension was determined. In a water bath (50 °C), 900 μl of agarose solution containing 0.7% agar (Difco, USA) in medium 199 (Sigma, USA) was mixed with 200 μl of 20% SRBC suspension, 200 μl splenocytes suspension, and 100 μl of complement (Microgen, Russia). The resulting mixture was poured into Goryaev chambers and incubated in a humid atmosphere at 37 °C for 2 hours. The number of hemolysis zones formed in the erythrocyte monolayer, which corresponded to the number of AFCs, was counted using a light microscope [18].

Determination of the Level of Antibodies to Sheep Erythrocytes (Hemagglutinins) in the Blood Serum of Mice

The serum was inactivated at 56 °C for 30 min. After that, the samples were sequentially diluted in an immunological round-bottom plate, using a volume of 25 µl and a 1:2 dilution step. Then, 25 µl of a 1% SRBC suspension was added to each dilution, and the mixture was incubated at 37 °C for 2 hours The maximum serum dilution at which antigen agglutination was visually observed was considered as the hemagglutinin titer and expressed in logarithmic form of T to the base 2 ($\log_2$T) [18].

Statistical analysis of the data was performed using the Statistica 10 (StatSoft) for Windows. Due to the small sample size, the nonparametric Kruskal – Wallis test was used to assess the statistical significance of differences in quantitative variables of three or more groups. The parameters under study were described using the median (*Me*) and the interquartile range (Q_1; Q_3). Differences were considered statistically significant at $p < 0.05$.

RESULTS

The following results were obtained after the course administration of HAs and the comparison drug Lycopid to mice. All the HA samples and Lycopid had a stimulating effect on the phagocytic capacity of neutrophils. The phagocytic index (the percentage of neutrophils that absorbed latex) increased significantly in all groups that received HAs and Lycopid, the effect of Lycopid was the most pronounced (Table 1). Samples HAsphagn and HAmagellan increased the phagocytic index to a lesser extent.

Table 1

Indicators of Phagocytic Capacity of Peripheral Blood Neutrophils of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Phagocytic index	Phagocytic number
1. Control (*n* = 7)	9.00 (6.00; 10.00)	2.17 (1.50; 2.50)
2. Lycopid, 2 mg / kg (*n* = 7)	23.00 (19.00; 28.00); 1–2 *p* = 0.001	2.17 (1.74; 2.80)
3. HAsphagn, 1 mg / kg (*n* = 7)	14.00 (12.00; 15.00); 1–3 *p* = 0.02; 2–3 *p* = 0.001	5.21 (4.14; 7.00); 1–3 *p* = 0.001; 2–3 *p* = 0.001
4. HApine-cott, 1 mg / kg (*n* = 7)	18.00 (18.00; 23.00); 1–4 *p* = 0.001	2.00 (1.72; 3.22)
5. HAmagellan, 1 mg / kg (*n* = 7)	16.00 (13.00; 18.00); 1–5 *p* = 0.009; 2–5 *p* = 0.002	3.69 (3.41; 4.38); 1–5 *p* = 0.002; 2–5 *p* = 0.01

In contrast to Lycopid and HApine-cott, HAsphagn and HAmagellan samples significantly increased the phagocytic number of neutrophils (the number of latex particles per cell). The HApine-cott sample did not affect the phagocytic number, but significantly increased the phagocytic index, approaching Lycopid in efficiency.

It was also found that the administration of Lycopid and HA preparations increased cell population in the peritoneal fluid of the experimental mice, as well as the number of phagocytic cells in the peritoneal exudate (Table 2).

It was also shown that the use of HAs *in vivo* resulted in an increase in the relative number of macrophages that engulfed ink (phagocytic index) in both the comparison drug and experimental groups (Table 3).

Table 2

Quantitative Composition of Peritoneal Exudate Cells of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Number of cells in peritoneal exudate, $\times 10^6$	Number of phagocytic cells in peritoneal exudate, $\times 10^6$
1. Control (*n* = 7)	0.78 (0.58; 1.33)	0.20 (0.13; 0.30)
2. Lycopid, 2 mg / kg (*n* = 7)	2.05 (2.03; 2.85); 1–2 *p* = 0.002	0.68 (0.60; 0.90); 1–2 *p* = 0.002
3. HAsphagn, 1 mg / kg (*n* = 7)	3.88 (3.63; 4.18); 1–3 *p* = 0.001; 2–3 *p* = 0.005	1.03 (0.93; 1.30); 1–3 *p* = 0.001; 2–3 *p* = 0.008
4. HApine-cott, 1 mg / kg (*n* = 7)	3.70 (3.43; 4.38); 1–4 *p* = 0.001; 2–4 *p* = 0.009	1.18 (0.98; 1.28); 1–4 *p* = 0.001; 2–4 *p* = 0.01
5. HAmagellan, 1 mg / kg (*n* = 7)	4.93(4.83; 7.00); 1–5 *p* = 0.001; 2–5 *p* = 0.001	1.73 (1.48; 2.13); 1–5 *p* = 0.001; 2–5 *p* = 0.001

All experimental groups showed an increase in the amount of ink absorbed by peritoneal exudate cells compared to control values. However, in all study groups, a significant decrease in the average amount of ink absorbed by one macrophage (phagocytic number) was observed compared to the controls. We associate this decrease with a significant increase in the number of phagocytic cells in the peritoneal exudate.

During the study, we also investigated the parameters of the thymus-dependent humoral immune response induced by the administration of SRBCs to mice receiving a course of HAs. The number of AFCs in the spleens of the mice and the level of antibodies to SRBC (hemagglutinins) in the blood serum were assessed. In the experimental group of animals that received a course of the HAsphagn sample, a decrease in the number of AFCs was recorded relative to the control group and the group that received Lycopid. The level of antibodies produced to SRBC (hemagglutinin titer) also decreased in this group of mice (Table 4).

Table 3

Indicators of Phagocytic Capacity of Peritoneal Macrophages of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Phagocytic index	The amount of ink in the cells of peritoneal exudate, OD	Phagocytic number, $\times 10^{-6}$
1. Control (*n* = 7)	22.58 (20.00; 24.53)	0.17 (0.13; 0.21)	0.91 (0.85; 1.08)
2. Lycopid, 2 mg / kg (*n* = 7)	29.63 (27.22; 33.33) 1–2 *p* = 0.02	0.42 (0.39; 0.47) 1–2 *p* = 0.001	0.59 (0.50; 0.65) 1–2 *p* = 0.001
3. HAsphagn, 1 mg / kg (*n* = 7)	27.56 (24.34; 30.10) 1–3 *p* = 0.008	0.47 (0.43; 0.51) 1–3 *p* = 0.001	0.46 (0.40; 0.48) 1–3 *p* = 0.001; 2–3 *p* = 0.04
4. HApine-cott, 1 mg / kg (*n* = 7)	31.45 (26.86; 37.23) 1–4 *p* = 0.006	0.43 (0.39; 0.48) 1–4 *p* = 0.001	0.36 (0.34; 0.39) 1–4 *p* = 0.001; 2–4 *p* = 0.002
5. HAmagellan, 1 mg / kg (*n* = 7)	30.57 (30.00; 31.51) 1–5 *p* = 0.01	0.55 (0.49; 0.64) 1–5 *p* = 0.001; 2–5 *p* = 0.005	0.33 (0.30; 0.37) 1–5 *p* = 0.001; 2–5 *p* = 0.001

Table 4

Indicators of the Humoral Thymus-Dependent Immune Response of C57Bl/6J Mice after a Course of Treatment with Peat Humic Acid Preparations, *Me* (Q_1; Q_3)

Observation group, dose, number of animals (*n*)	Number of antibody-forming cells, $\times 10^3$ / spleen	Hemagglutinin titer, $\log_2$T
1. Control (*n* = 7)	24.29 (16.43; 31.43)	4.00 (3.00; 4.50)
2. Lycopid, 2 mg / kg (*n* = 7)	70.00 (58.57; 82.86); 1–2 *p* = 0.001	4.00 (3.00; 4.50)
3. HAsphagn, 1 mg / kg (*n* = 7)	5.71 (4.29; 8.57); 1–3 *p* = 0.002; 2–3 *p* = 0.004	2.00 (1.50; 2.50); 1–3 *p* = 0.02; 2–3 *p* = 0.01
4. HApine-cott, 1 mg / kg (*n* = 7)	98.57 (82.86; 150.00); 1–4 *p* = 0.001; 2–4 *p* = 0.002	6.00 (4.50; 7.00); 1–4 *p* = 0.01; 2–4 *p* = 0.03
5. HAmagellan, 1 mg / kg (*n* = 7)	91.43 (62.86; 120.71); 1–5 *p* = 0.001; 2–5 *p* = 0.001	600 (5.50; 7.00); 1–5 *p* = 0.001; 2–5 *p* = 0.005

In the group of mice that were administered the HApine-cott sample, an increase in the number of AFCs in the spleens and the titer of antibodies produced by them was observed. The same trend was observed in the animals that received the HAmagellan sample. Administration of the comparison drug Lycopid to the mice increased the number of AFCs in the spleens, but did not affect their production of hemagglutinins.

DISCUSSION

Natural products, such as mumiyo, peat, and sapropel, rich in humic substances, are widely used in traditional medicine. In addition to the experimentally proven adaptogenic, antioxidant, hepatoprotective, wound-healing, antibacterial, and anti-inflammatory properties [9, 10], it has been found that HAs can influence the immune system. D. Mudroňová et al. [16] demonstrated that a food supplement containing HAs stimulated the phagocytic activity of peripheral blood phagocytes of broiler chickens and also increased the proportion of CD4+ and decreased the proportion of CD8+ lymphocytes. R. Habibian et al. [19] obtained results using the Farmagulator Dry preparation containing HAs and administered to rats ad libitum. These results showed a dose-dependent enhancement of the humoral immune response to the B. melitensis vaccine, as well as an increase in the phagocytosis of yeast particles by rat blood mononuclear leukocytes. Another example is the work by A.V. Vucskits et al. [20], who showed that adding humic and fulvic acids extracted from brown coal to the diet of rats leads to an increase in humoral immunity, manifested in an increase in the titer of antibodies to ovalbumin. Similar observations were made in a broiler chicken model [21], where the use of a feed supplement containing HAs or a mixture of HAs with organic acids increased

antibody titers against infectious bronchitis and Newcastle disease viruses, without affecting blood biochemical parameters.

In our work, we studied the effect of HAs isolated by sodium pyrophosphate extraction from three different types of peat from the Tomsk region bogs (the Vasyuganskoye peat deposit) on the phagocytic activity of peripheral blood neutrophils and peritoneal macrophages of laboratory mice, as well as indicators of the thymus-dependent humoral immune response. HA preparations were administered to animals for 10 days. The comparison group received glucosaminylmuramyl dipeptide (Lycopid), an analog of bacterial peptidoglycan that stimulates innate and acquired immunity, including the bactericidal function of phagocytes and the humoral immune response [22].

Impaired ability of innate immune effector cells – neutrophils and macrophages – to absorb pathogens can lead to uncontrolled spread of infection, accumulation of cellular debris, chronic inflammation, and disruptions in the formation of the adaptive immune response [3]. Thus, phagocytosis plays a key role in suppressing inflammatory processes and restoring homeostasis in the body. Compounds that can increase the phagocytic activity of immune cells are therefore considered to be promising immunomodulatory drugs.

The results of the study demonstrated that the course application of peat HAs and Lycopid in mice stimulated the phagocytic activity of peripheral blood neutrophilic leukocytes – an increase in neutrophils that had absorbed latex (phagocytic index) was observed in blood samples of animals that received a course of all the tested HA preparations. It should be noted that the effect of the HApine-cott sample was comparable to that of Lycopid (Table 1), and the introduction of the HAsphagn and HAmagellan samples to the animals resulted in a higher average number of particles absorbed by one cell (phagocytic number), compared to the group receiving Lycopid. In addition, the course administration of the samples caused an increase in the total number of peritoneal exudate cells, including phagocytic elements (Table 2), which was reflected in an increase in the phagocytic index of peritoneal macrophages (Table 3).

It should be noted that the total number of peritoneal exudate cells and the number of phagocytes in the groups receiving HAs exceeded the corresponding indicators for the Lycopid group. We also observed an increase in the amount of ink absorbed by peritoneal phagocytes in all experimental groups compared to the control group receiving normal saline.

During phagocytosis of foreign agents, antigen-presenting cells present foreign molecular structures to lymphocytes. This process activates the mechanisms of adaptive immunity. This study investigated the effect of a course of peat HA application on the indicators of the humoral immune response formed in animals following immunization with SRBC. The study showed that the introduction of the HAsphagn sample suppressed the activity of AFCs in the spleen and reduced the level of hemagglutinins in the blood serum of experimental animals compared to the control group (Table 4). By contrast, the use of the HApine-cott and HAmagellan compounds stimulated the growth of these parameters in mice. Moreover, after a course of HApine-cott and HAmagellan, the number of AFCs and the antibody titer (hemagglutinins) were higher than those recorded for Lycopid. The HApine-cott and HAmagellan samples demonstrated immunostimulatory activity, manifested in the enhancement of the phagocytic function of peripheral blood neutrophils and peritoneal macrophages in mice, as well as in the activation of the humoral immune response to sheep erythrocytes. According to the results of the studies, their effectiveness was comparable to that of the reference drug Lycopid and in some cases even exceeded its performance.

CONCLUSION

Our experiments revealed that course therapy with all tested peat humic acid preparations stimulated the proliferation of phagocytic cells and increased the effectiveness of their interaction with foreign particles. Moreover, the use of HApine-cott and HAmagellan preparations enhanced the humoral immune response caused by the introduction of sheep erythrocytes into experimental animals. This was manifested in an increase in the number of antibody-forming cells in the spleen tissues and an increase in the hemagglutinin titer in the blood serum. Thus, the HApine-cott and HAmagellan samples, isolated from the bogs of the Tomsk region and capable of stimulating the phagocytic activity of macrophages and neutrophils, as well as enhancing the effectiveness of the humoral immune response, represent a promising basis for the creation of innovative drugs of natural origin that can be used in treating immunodeficiency-related diseases.

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Author Contribution

Sherstoboev E.Yu. and Danilets M.G. developed the concept and design of the experiments. Zykova M.V. derived the samples and standardized them. Trofimova E.S., Ligacheva A.A., Selivanova N.S., Karnaukhova E.A. assessed the biological activity of the studied substances using immunological methods. Danilets M.G., Ligacheva A.A., Karnaukhova E.A. analyzed and interpreted the data. Trofimova E.S., Zykova M.V. participated in drafting of the article and critical revision of the manuscript for important intellectual content. Sherstoboev E.Yu. and Belousov M.V. approved the manuscript for publication.

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Extended Diagnosis of Cervical Lesions

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ABSTRACT

Aim. To improve the efficiency of diagnosing cervical pathologies using cytology and polymerase chain reaction (PCR) for human papillomavirus (HPV), taking into account the detection of HPV in upper parts of the endocervix.

Materials and methods. The study involved 60 patients with cervical pathology. The results of the cytological studies were verified according to The Bethesda system; the patients were divided into groups based on the results: Group I (n = 22) – negative for intraepithelial lesion or malignancy (NILM), Group II (n = 18) – low-grade squamous intraepithelial lesion (L-SIL), Group III (n = 12) – high-grade squamous intraepithelial lesion (H-SIL), Group IV (n = 8) – atypical squamous cells of undetermined significance (ASC-US). Standard PCR testing for HPV and PCR of the endocervical homogenate were conducted using the Hybrid Capture Digene test (RF Patent No. 2833119 dated December 14, 2023).

Results. Persistence of HPV in the upper endocervix was detected in 45 (75%) of patients. HPV was diagnosed significantly more often (p = 0.0157) in patients with L-SIL and H-SIL cytology – in 89% (16/18) и 100% (12/12) cases, respectively. Oncogenic HPV serotypes were found in 59% (13/22) of patients with NILM and in 50% (4/8) of patients with ASC-US. High frequency of discrepancies in the profile of the detected HPV strains between standard PCR and homogenate PCR testing was observed and was comparable across all groups: NILM 64% (14/22); L-SIL 61% (11/18); H-SIL 58% (7/12); ASC-US 75% (6/8), p > 0,05. Persistence of HPV in the upper parts of the cervix with negative standard PCR results was detected in 41% (9/22) of patients with NILM. A high viral load in the homogenate was detected more frequently in patients of the H-SIL group (p = 0.0374).

Conclusion. Extended diagnosis allows for a comprehensive assessment of the degree of cervical involvement in the pathology and helps determine the optimal management strategy for women at high risk (H-SIL, recurrent L-SIL, HPV persistence with high viral load).

Keywords: cervical pathology, human papillomavirus, persistence, diagnosis, PCR, cytology, homogenate

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at SibSMU (Minutes No. 9344 dated January 30, 2023).

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Расширенная диагностика патологий шейки матки

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РЕЗЮМЕ

Цель исследования: повышение эффективности диагностики патологий шейки матки с использованием цитологической диагностики и полимеразной цепной реакции (ПЦР) на вирус папилломы человека (ВПЧ), с учетом выявления персистенции ВПЧ в верхних отделах цервикального канала.

Материалы и методы. В группу исследования включены пациентки (n = 60) с патологией шейки матки. Выполнены цитологические исследования с верификацией диагноза по классификации The Bethesda system и распределены пациентки на группы в соответствии с результатами: I (n = 22) – negative for intraepithelial lesion or malignancy (NILM), II (n = 18) – low-grade squamous intraepithelial lesion (L-SIL), III (n = 12) high-grade squamous intraepithelial lesion (H-SIL), IV (n = 8) atypical squamous cells of undetermined significance (Asc-Us). Проведены стандартная ПЦР-диагностика ВПЧ и ПЦР гомогената эндоцервикального компонента методами Hybrid Capture Digene test (панель 14 онкосеротипов) для выявления ВПЧ в верхних отделах цервикального канала (патент № 2833119 от 14.12.2023).

Результаты. Персистенция ВПЧ в верхних отделах цервикального канала выявлена у 45 (75%) из 60 пациенток. ВПЧ диагностирован достоверно (p = 0,0157) чаще у пациенток с результатами онкоцитологии L-SIL и H-SIL – в 89% (16/18) и 100% (12/12) случаях соответственно. ВПЧ в верхних отделах цервикального канала обнаружен у 59% (13/22) пациенток группы NILM и 50% (4/8) пациенток группы Asc-US. Высока частота расхождений в структуре выявленных штаммов ВПЧ при проведении стандартного ПЦР и ПЦР гомогената, сопоставима для всех групп: NILM 64% (14/22); L-SIL 61% (11/18); H-SIL 58% (7/12); Asc-Us 75% (6/8), p > 0,05. Персистенция ВПЧ в верхних отделах цервикального канала при негативных результатах стандартной ПЦР выявлена у 41% (9/22) пациенток с NILM. Высокая вирусная нагрузка в гомогенате определялась чаще у пациенток группы H-SIL (p = 0,0374).

Заключение. Расширение диагностики позволяет в полной мере оценить степень вовлечения шейки матки в патологический процесс и определить оптимальную тактику ведения у женщин с высокой степенью риска (H-SIL, рецидивы L-SIL, высокая вирусная нагрузка ВПЧ).

Ключевые слова: патология шейки матки, вирус папилломы человека, персистенция, диагностика, ПЦР, цитология, гомогенат

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 добровольное информированное согласие. Исследование одобрено этическим комитетом СибГМУ (регистрационный № 9344, дата заседания 30.01.2023).

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INTRODUCTION

Squamous intraepithelial and glandular lesions of the cervix are the most common pathologies among women of reproductive age. The leading etiological factor in these cases is human papillomavirus (HPV), followed by integration of viral deoxyribonucleic acid (DNA) into the nuclei of epithelial cells, which is pathogenetically associated with the subsequent cervical cancer development [1]. Cervical cancer remains a major global health concern, as evidenced by the projected increase in new cases to 700,000 by 2030 [2].

HPV affects not only the transformation zone but also the endocervical crypts (in 82.6% of cases). Anatomical features of the cervix, such as length of

up to 4 cm and crypt penetration of up to 4 mm, may be major factors contributing to incomplete excision (80.8% in cases with endocervical involvement) [3, 4]. For this reason, cytology has limited potential in diagnosing precancerous lesions and cervical adenocarcinoma: atypia in the collected material may be minimal or absent, since the process is often located deep within the crypts, while squamous epithelium remains practically unchanged.

There is ongoing debate regarding the optimal timing and methods of screening in women of reproductive age [5], as well as concerning the opportunity of HPV clearance in this population. T. Feng et al. (2023) reported a decrease in frequency and an increase in viral clearance time with age [6]. In contrast, S.N. Adebamowo et al. (2022) argued that both the rate and timing of viral clearance are comparable across all age groups [7]. Meanwhile, K. Louvanto et al. (2010) demonstrated reduction in clearance time with advancing age [8].

The U.S. Preventive Services Task Force guidelines do not recommend HPV screening in women under 30 years due to the high risk of unnecessary medical interventions [9]. However, it is known that HPV testing detects precancerous lesions significantly more often than cytology, and, according to O. Feldstein et al. (2023), it should be considered as the primary diagnostic method [10]. Since most low-grade squamous intraepithelial lesions (L-SIL) regress spontaneously, both the World Health Organization and the clinical guidelines of the Russian Society of Obstetricians and Gynecologists ("Cervical Intraepithelial Neoplasia, Erosion, and Ectropion of the Cervix," 2024) do not recommend active treatment in such cases [11]. Nevertheless, Y.J. Tai et al. (2017) reported that cryotherapy and excisional procedures significantly reduce the risk of lesion progression in women with L-SIL, suggesting an active management approach [12]. At the same time, other studies indicate no necessity for active screening and management of L-SIL among young women [13, 14].

Therefore, there are no clear criteria for predicting the course of squamous intraepithelial lesions and choosing the most appropriate management approach, including the optimal extent of surgical treatment.

The aim of the study was to improve the efficiency of diagnosis of cervical pathologies using cytological screening and polymerase chain reaction (PCR) for HPV, taking into account the detection of HPV in upper parts of the endocervix.

MATERIALS AND METHODS

The study involved 60 patients with cervical pathology, mean age 34.4 ± 8.6 years. The study was approved by the Ethics Committee at Siberian State Medical University (Minutes No. 9344 dated January 30, 2023) and conducted in accordance with the Declaration of Helsinki and the Rules for Clinical Practice in the Russian Federation approved by the Order of the Russian Ministry of Health (No. 266, dated June 19, 2003). All patients gave their informed consent to participate in the study.

A standard cytological examination with verification using the Bethesda system was performed. The material for the cytological examination was obtained in accordance with the clinical guidelines of the Russian Society of Obstetricians and Gynecologists "Cervical intraepithelial neoplasia, erosion, and ectropion of the cervix," 2024.

All patients were divided into groups based on the cytology results. Group 1 (n = 22) – negative for intraepithelial lesion or malignancy (NILM), mean age 37 ± 5.4 years. Group 2 (n = 18) – low-grade squamous intraepithelial lesion (L-SIL), mean age 33.7 ± 6.8 years. Group 3 (n = 12) – high-grade squamous intraepithelial lesion (H-SIL), mean age 41.4 ± 9.2 years. Group 4 (n = 8) – atypical squamous cells of undetermined significance (ASC-US), mean age 43 ± 7.5 years.

A mandatory part of the examination was a PCR test (Hybrid Capture Digene test) for high-oncogenic-risk HPV with quantitative determination of viral DNA (a panel of 14 oncoserotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). The viral load was considered to be low at < 3.0 LgDNA / 10^5 cells, moderate – at 3.0–5.0 LgDNA / 10^5 cells, high at ≥ 5.0 LgDNA / 10^5 cells. The material for PCR test was obtained in accordance with the clinical guidelines of the Russian Society of Obstetricians and Gynecologists "Cervical intraepithelial neoplasia, erosion, and ectropion of the cervix," 2024.

To detect HPV persistence in the upper parts of the endocervix, fragments of the cervix obtained during the following procedures were also used for HPV testing:

Excisional biopsy in women with cervical ectopia and NILM cytology, with or without HPV infection.

Targeted mono-/multifocal biopsy in the initial detection of H-SIL.

Excisional biopsy in L-SIL, benign hyperplastic lesions, recurrent ASC-US, and infection with high-risk HPV with a high viral load.

Diathermoelectroexcision as the procedure of choice for H-SIL, as well as recurrences due to HPV infection with a high viral load.

Detection of HPV persistence in the upper parts of the cervical canal (RF Patent No. 2833119, December 14, 2023). A tissue specimen, necessarily including the upper parts of the endocervix, was placed in a sterile test tube with saline (0.9% NaCl). Over the first 2–4 hours, the specimen was homogenized in saline using a rotary homogenizer, followed by centrifugation for 15 minutes at 3,000 rpm. Supernatant was used for subsequent PCR analysis, which made it possible to perform PCR test of the distal parts of the cervix, inaccessible for analysis during traditional PCR material sampling.

Statistical analysis was performed using the Statistica 10.0 software based on contingency table analysis. Qualitative variables were presented as the absolute values and percentages (*n*, %). The McNemar test was used to compare paired binary data (standard PCR and PCR homogenate results from the same patients in the same groups). The Fisher's exact test was used to assess the statistical significance of differences in the frequency of detected HPV strains, as well as PCR diagnostic results between independent groups. The significance level p for all analytical procedures was 0.05.

RESULTS

Standard PCR testing detected HPV in 30 (50%) out of 60 patients. HPV was detected more frequently in patients of the L-SIL group ($p < 0.001$). PCR of the homogenate revealed HPV with greater frequency ($p = 0.0098$), and persistence of HPV in the upper parts of the endocervix was detected in 45 (75%) out of 60 patients. HPV was detected more often in patients with L-SIL and H-SIL ($p = 0.0157$). HPV was detected prevalently by homogenate PCR than by standard PCR in patients of the NILM group ($p = 0.046$). The frequency of HPV detection in PCR studies is presented in Table 1.

Discrepancies in the profile of HPV strains detected by standard PCR testing and PCR testing of the homogenate were observed in 38 (63%) out of 60 patients.

Table 1

HPV Detection by Standard PCR Testing and PCR Testing of the Homogenate in Patients with Different Cytology Results, *n* (%)

Method	Groups			
	I ($n = 22$)	II ($n = 18$)	III ($n = 12$)	IV ($n = 8$)
Standard PCR	5 (23%)	18 (100%)*	7 (58%)	0 (0%)
PCR of the homogenate	13 (59%)#	16 (89%)*	12 (100%)*	4 (50%)

* statistically significant differences within the method, # statistically significant differences within a group (here and in Table 2)

Discrepancies in the detected HPV strains were observed in 14 (64%) out of 22 patients in the NILM group, 11 (61%) out of 18 patients in the L-SIL group, 7 (58%) out of 12 patients in the H-SIL group, and 6 (75%) out of 8 patients in the ASC-US group ($p > 0.05$). Persistence of HPV in the upper cervical canal with a negative standard PCR result was detected in 18 (30%) out of 60 patients ($p = 0.0074$): in 9 (42%) out of 22 patients in the NILM group, in no patients in the L-SIL group (since all patients in this group had a positive standard PCR result), in 3 (25%) out of 12 patients in the H-SIL group, and in 6 (75%) out of 8 patients in the ASC-US group.

Persistence of HPV with high viral load in the homogenate was detected in 3 out of 22 patients (14%) in the NILM group, in 7 out of 18 patients (39%) in the L-SIL group, in 7 out of 12 patients (58%) in the H-SIL group, and in 2 out of 8 patients (25%) in the ASC-US group. The frequency of HPV persistence with high viral load in the homogenate was significantly higher in the H-SIL group than in the other groups ($p = 0.0374$). No differences in the frequency of multiple HPV detection in the homogenate were found between the groups (NILM 23%, 5/22; L-SIL 39%, 7/18; H-SIL 25%, 3/12; ASC-US 0%, $p > 0.05$). Serotype 16 was significantly prevalent in the L-SIL group (Table 2) according to both standard PCR and homogenate PCR results ($p < 0.001$). Serotypes 35, 45, 51, and 68 were detected in single cases.

Table 2

Frequency of Detecting High-Risk HPV Strains by Standard PCR Testing and PCR Testing of the Homogenate in Patients with Different Cytology Results, *n* (%)

HPV strains	Groups							
	I ($n = 22$)		II ($n = 18$)		III ($n = 12$)		IV ($n = 8$)	
	Stand.	Hom.	Stand.	Hom.	Stand.	Hom.	Stand.	Hom.
16	3 (14%)	8 (36%)	13 (72%)*#	16 (89%)*#	3 (25%)	8 (67%)	0	4 (50%)
18	0	0	0	0	2 (17%)	3 (25%)	0	0

End of table 2

HPV strains	Groups							
	I (n = 22)		II (n = 18)		III (n = 12)		IV (n = 8)	
	Stand.	Hom.	Stand.	Hom.	Stand.	Hom.	Stand.	Hom.
31	0	5 (23%)	4 (22%)	4 (22%)	0	0	0	0
33	2 (9%)	2 (9%)	4 (22%)	5 (28%)	2 (17%)	3 (25%)	0	0

Note. Stand. – frequency of detecting HPV strains by standard PCR testing; Hom. – frequency of detecting HPV strains by PCR testing of the homogenate

DISCUSSION

The results demonstrate the potential for improving the effectiveness of extended diagnosis of cervical pathology by assessing the involvement of the upper parts of the cervical canal in the pathological process. The high frequency of HPV persistence in the upper parts of the cervical canal in all groups, particularly in patients with negative standard PCR results, demonstrates limitations of traditional screening methods. Thus, 59% of patients with NILM cytology and 50% patients with ASC-US were found to have HPV persistence in the upper parts of the endocervix, which is consistent with the data from T. Malagón et al. (2020), indicating a high risk of developing precancerous lesions even with normal cytology [15].

We found that HPV 16, 31, and 33 are the most frequently diagnosed strains in homogenate samples, regardless of cytology results. According to W.D. Kang et al. (2024), the detection of these HPV strains may require closer monitoring in women with L-SIL due to a higher risk of dysplastic progression [16]. The choice of the management strategy for patients with L-SIL remains controversial. On the one hand, according to C. Buick et al. (2020), the high probability of spontaneous HPV elimination in young women justifies a wait-and-see approach [13], on the other hand, the detection of high-risk strains, particularly HPV 16, 31, and 33, may justify the use of excisional treatment, which is confirmed by the studies of Y.J. Tai et al. (2017) and C. Firnhaber et al. (2017) [12, 17].

Multiple HPV infection was detected in approximately one in three patients in the NILM, L-SIL, and H-SIL groups following homogenate testing. According to D. Zhou et al. (2024), single-type infection (particularly with HPV16) predominates in H-SIL lesions, whereas multiple HPV infection is more characteristic of L-SIL [18]. A retrospective study by X. Tao et al. (2022) including women with L-SIL found that the proportion of histologically confirmed H-SIL was significantly greater in the presence of multiple HPV infection [19]. In contrast, data from X. Ni et al. (2023) suggested that multiple-type infection was associated with a lower risk of H-SIL and, simultaneously, a higher rate of spontaneous viral clearance [20]. These findings support the concept of complex interactions between different HPV types, which requires further investigation. It seems perspective to investigate the role of impaired immune barriers and their impact on antigen-presenting cells and macrophage subpopulations in the cervical canal, as they may play a key role in the pathogenesis of endocervical HPV persistence.

The results indicate high frequency of pathological endocervical involvement which is particularly important in planning organ-preserving treatment and determining the extent of excision, as margin status combined with HPV persistence after surgery are key risk factors for recurrence [21]. Based on the obtained data, the conventional approach of determining the excision volume based solely on the type of the transformation zone cannot be considered optimal due to the high rate of latent endocervical HPV persistence.

CONCLUSION

The results of the study demonstrate high frequency of HPV persistence in the upper parts of the endocervix among patients with cervical pathology and underscore the necessity of a personalized treatment approach. This approach should account for the patient's age, reproductive plans, and HPV infection type when planning organ-preserving treatment, especially in high-risk groups (with H-SIL, recurrent L-SIL, or a high HPV viral load). Further data collection is warranted to establish statistically significant patterns and to develop personalized algorithms for the diagnosis and management of cervical pathology. These algorithms should integrate cytology findings and HPV status with immunohistochemical detection of cellular proliferation markers (p16/INK4a) and potentially include the assessment of local cervical immunological barrier impairments, including evaluating dendritic cell activity and macrophage subpopulations.

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Author Contribution

Chernov D.Y., Tikhonovskaya O.A. – conception and design. Tikhonovskaya O.A., Logvinov S.V. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Chernov D.Y., Potapov A.V., Gerasimov A.V., Gereng E.A., Akbasheva O.E., Lasukova T.V. – acquisition and processing of the data.

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Modern Methods of DNA Probe Synthesis for Fluorescence *in situ* Hybridization (FISH): Technologies and Applications

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ABSTRACT

Fluorescent *in situ* hybridization (FISH) remains an indispensable tool for molecular diagnostics, which makes it possible to detect chromosomal abnormalities underlying many hereditary and oncological diseases with high accuracy. The advancement of medicine towards personalized approaches and the expansion of the spectrum of diagnosed pathologies require constant improvement of methods for synthesizing DNA probes. Despite existing limitations, such as the cost and complexity of synthesis, the future of FISH diagnostics is linked to the development of highly specific, multiplex, and affordable probes that will enable the transition to complex genome and transcriptome analysis. The aim of this article was to reflect the evolution of probe production methods from classical to high-tech ones, including SABER-FISH, CRISPR/Cas9 (CASFISH), and smFISH technologies.

Keywords: fluorescence *in situ* hybridization, molecular cytogenetics, DNA probe synthesis, chromosomal aberrations

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Современные методы синтеза ДНК-зондов для флуоресцентной гибридизации *in situ* (FISH): технологии и применение

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РЕЗЮМЕ

Метод флуоресцентной гибридизации *in situ* (FISH) остается незаменимым инструментом молекулярной диагностики, позволяющим с высокой точностью выявлять хромосомные аномалии, лежащие в основе многих наследственных и онкологических заболеваний. Движение медицины в сторону персонализированных подходов и расширение спектра диагностируемых патологий требуют постоянного совершенствования методов синтеза ДНК-зондов. Несмотря на существующие ограничения, такие как стоимость и сложность синтеза, будущее диагностики с помощью FISH связано с разработкой высокоспецифичных, мультиплексных и доступных зондов, которые позволят перейти к комплексному анализу генома и транскриптома. Данная работа написана с целью отразить эволюцию методов получения зондов от классических к высокотехнологичным, включая SABER-FISH, технологии на основе CRISPR/Cas9 (CASFISH), smFISH.

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Ключевые слова: флуоресцентная гибридизация *in situ*, молекулярная цитогенетика, синтез ДНК-проб, хромосомные аберрации

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INTRODUCTION

Fluorescence *in situ* hybridization (FISH) remains one of the most reliable and sensitive cytogenetic techniques for detecting major chromosomal aberrations, including deletions, amplifications, translocations, and inversions, thereby enabling highly accurate quantification of cells harboring these abnormalities [1]. This powerful cytological method facilitates the detection and precise localization of specific nucleic acid sequences directly on metaphase chromosomes or within interphase nuclei through the use of fluorescently labeled DNA probes. Within clinical diagnostics, FISH has become an indispensable tool for identifying chromosomal abnormalities, such as aneuploidies, translocations, deletions, and amplifications that underpin numerous hereditary disorders and oncological conditions. In basic research, FISH enables the investigation of genome architecture, spatial chromosome organization, gene expression patterns, and chromatin dynamics.

The evolution of the FISH methodology is inextricably linked to advancements in DNA probe technology. This progression has transitioned from the initial use of radioactive labels, which were limited by low resolution and associated health hazards, to contemporary fluorescent detection systems. The adoption of fluorescent probes has provided superior spatial resolution, multiplexing capabilities, enhanced safety, and the potential for quantitative analysis. Consequently, the quality, specificity, and accessibility of DNA probes are critical determinants of the efficacy and broad applicability of the FISH technique.

In light of the rapid advancements in molecular biology and genomics, which demand increasingly sophisticated and specialized probes, the systematization of modern synthesis methods is becoming a task of critical importance. The aim of this review is to conduct a comprehensive analysis of current technologies for synthesizing DNA probes for FISH, to compare their respective efficiencies, and to identify the most promising directions for future development in this rapidly evolving field.

CLASSICAL APPROACHES TO CREATING DNA PROBES FOR FISH

DNA probes utilized for fluorescence *in situ* hybridization (FISH) are classified into three principal groups: (1) whole-chromosome painting probes, (2) repetitive sequence probes, and (3) locus-specific probes. Each group fulfills distinct experimental and diagnostic objectives, necessitating divergent methodological approaches for their synthesis.

Whole-chromosome painting probes are used to detect complex interchromosomal rearrangements, which are used in both scientific research and clinical practice [2]. The conventional methodology for their generation involves the microdissection of target metaphase chromosomes or chromosomal regions via laser capture or mechanical scraping with a glass micropipette. The isolated genetic material is subsequently subjected to amplification, followed by labeling with fluorochromes [3]. This technique is notably labor-intensive and requires a high degree of technical expertise. For commercial-scale production, flow cytometric sorting is the predominant method [4]. This approach enables the isolation of all human chromosomes, with the exception of chromosomes 9–12 due to their analogous size and morphological characteristics. Approximately 300 chromosomes are required to generate a single probe. The resultant DNA is fragmented and amplified enzymatically by polymerase chain reaction (PCR), a process that increases template quantity and enhances hybridization specificity by suppressing non-specific binding [5].

For the construction of both whole-chromosome and locus-specific probes, cloning strategies utilizing large-insert genomic libraries, particularly those housed in artificial chromosome vectors, are frequently employed. Among these, bacterial artificial chromosomes (BACs) are accorded significant prominence due to their advantageous properties, which include facile DNA purification,

low incidence of chimeric clones, and high genomic stability [6]. Although the derivation of probes from BAC or plasmid clones necessitates additional procedures involving microbial culture and nucleic acid extraction, this method remains indispensable. It is particularly critical for the development of probes targeting pericentromeric and subtelomeric regions, as the highly repetitive DNA content in these loci presents a substantial limitation for *de novo* enzymatic or chemical oligonucleotide synthesis.

A fundamentally distinct class of FISH probes is represented by oligonucleotide-based probes. Such probes can be engineered to target both repetitive genomic elements and unique, single-copy DNA sequences. For the former, a complete reference genome sequence is not a prerequisite [7]. Signal amplification is achieved intrinsically through the hybridization to multiple identical repeat elements, and the probe length typically ranges from 15 to 30 base pairs (bp), produced via chemical synthesis. While shorter probes exhibit enhanced accessibility to their target sites, their capacity to incorporate fluorescent reporter molecules is proportionally limited [8]. For oligonucleotide probes designed against unique sequences, a complex pool of oligonucleotides must be synthesized. The collective length of this pool must exceed 10–30 kilobase (kb) to ensure the resultant DNA probe generates a signal of sufficient intensity for clear visualization at the specific chromosomal locus during FISH analysis.

Probes for FISH analysis have become an indispensable component of cytogenetic diagnostics for determining the level of genetic mosaicism, identifying marker chromosomes, and elucidating their origin and compositional architecture [9]. Comprehensive, chromosome-specific DNA libraries have been constructed and are continuously refined for the diagnosis of prevalent aneuploidy syndromes, including Patau, Edwards, and Down syndromes (trisomies of autosomes 13, 18, and 21, respectively), as well as Klinefelter syndrome and Turner syndrome (which are associated with numerical abnormalities of the X and Y sex chromosomes).

Locus-specific probes have been developed to identify Robertsonian translocations, Prader–Willi syndrome, and Angelman syndrome among others, and are furthermore employed to detect specific microdeletions and chromosomal rearrangements [10]. Additionally, gene-specific probes are utilized to detect gene amplification events, a common characteristic of neoplastic cells. A prominent example is the assessment of the copy number variation of the human epidermal growth factor receptor 2 (*HER2*) gene, which serves as a critical prognostic biomarker in breast cancer [11] and ovarian cancer [12].

A distinctive category of probes is constituted by PNA (peptide nucleic acid) probes. A PNA molecule is structurally analogous to DNA, with the fundamental distinction that its sugar-phosphate backbone is replaced by an uncharged N-(2-aminoethyl)-glycine pseudopeptide polymer. This neutral charge confers upon the PNA probe a significantly higher binding affinity for complementary DNA or RNA sequences compared to traditional DNA-DNA or RNA-RNA duplexes [13]. PNA was originally developed by the research group of Peter Nielsen in 1991 [14]. The atypical structure of PNA probes is not a substrate for nucleases or proteases, thereby substantially extending their half-life in both *in vivo* and *in vitro* applications [15], while still permitting direct fluorescent labeling. The implementation of PNA FISH has enabled the precise detection of signals at chromatid ends and the accurate measurement of telomere length in metaphase chromosomes [16, 17].

Methods for Synthesizing DNA Probes

As previously indicated, enzymatic and chemical methodologies for the *de novo* synthesis of oligonucleotide probes have achieved widespread adoption. Enzymatic strategies for DNA probe synthesis encompass techniques such as degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) and long-range PCR.

The PCR necessitates the presence of a target sequence, which serves as the template for probe synthesis. In DOP-PCR, templates most frequently consist of BAC clones or microdissected chromosomal material, as these provide the researcher with an isolated genomic region of interest. The fundamental principle of DOP-PCR involves the utilization of partially degenerate primers. These primers incorporate a core random hexamer sequence flanked on both the 3’ and 5’ ends by defined nucleotide sequences.

The protocol mandates two consecutive PCR amplifications. The second amplification stage employs primers complementary to the fixed sequences introduced during the initial cycle and is conducted at an elevated annealing temperature to enhance specificity [18]. The employment of high-fidelity proofreading polymerases, such as *Pwo*, in conjunction with extended annealing and elongation durations, optimizes assay performance. This results

in superior fidelity, the generation of longer amplicons, and increased genomic coverage [19, 20].

The synthesis of FISH DNA probes via long-range PCR entails a multi-stage process: identification of the target genomic locus; subsequent design and selection of specific oligonucleotide primers for the designated sites; generation of long DNA amplicons (up to 15 kb in length) via long-range PCR; creation of a DNA fragment library through enzymatic or non-enzymatic fragmentation methods; amplification of this library using the pre-designed target-specific primers; and ultimately, the incorporation of a fluorophore into the amplified genomic library. This synthesis paradigm is predominantly employed for the generation of locus-specific DNA probes.

Chemical synthesis of oligonucleotides for *in situ* hybridization applications is categorized into solid-phase and microarray-based synthesis. Contemporary oligonucleotide production is dominated by automated instrumentation utilizing the solid-phase phosphoramidite method, a technology pioneered by Marvin Caruthers in the 1980s [21, 22]. Oligonucleotide synthesis proceeds via a cyclic four-step reaction. In this process, nucleotide phosphoramidites are sequentially added to a nascent oligonucleotide chain that is covalently attached to an insoluble solid support. The cycle initiates with a deprotection step, removing an acid-labile dimethoxytrityl (DMT) group to reveal a reactive 5'-hydroxyl moiety. Subsequently, an activated phosphoramidite monomer is coupled to this hydroxyl group, forming an unstable phosphite triester linkage. Any unreacted 5'-hydroxyl groups are then rendered inert through a capping step with acetic anhydride, a critical measure to prevent the formation of deletion sequences. The cycle concludes with an oxidation step, converting the phosphite triester into a more stable phosphate triester. This iterative cycle is repeated until the desired oligonucleotide length is attained. Upon completion of the synthesis, the full-length oligonucleotide is cleaved from the solid support and subjected to deprotection [23]. Final purification of the crude oligomers is achieved through techniques such as high-performance liquid chromatography (HPLC) or polyacrylamide gel electrophoresis (PAGE).

A logical technological progression from conventional solid-phase synthesis is microarray oligonucleotide synthesis. In the early 1990s, the American company Affymetrix developed one of the first synthesizers for oligonucleotide microarrays utilizing photolabile protecting groups, an innovation that ultimately catalyzed the entire DNA microarray industry [24]. This method is essentially a modification of the standard phosphoramidite chemistry, wherein the acid-labile DMT group is substituted for a photolabile protecting group, allowing for light-directed spatial control of synthesis [25]. The synthesizer from Agilent Technologies (USA) is a leader in terms of both the multiplexing capacity (simultaneous synthesis of up to 1 million unique oligonucleotides on a single substrate) and the achievable oligonucleotide length, which can extend to 200 nucleotides [24].

Enzymatic methodologies for the synthesis of DNA probes are increasingly being used in laboratory practice, a trend partially attributable to the labor-intensive nature of conventional chemical synthesis protocols. Conversely, chemical synthesis affords a superior degree of structural customization: standard nucleotides can be substituted with synthetic analogues, the stoichiometry of incorporated fluorochromes can be precisely controlled, among other modifications. Oligonucleotides generated via chemical synthesis are typically shorter in length (up to 100 nucleotides), a characteristic that facilitates enhanced penetration and accessibility to their target sequences, particularly when hybridization is performed on complex tissue sections rather than individual cell preparations. In contrast, elongated probes synthesized via PCR amplification demonstrate heightened hybridization specificity due to their increased complexity; however, the presence of amplification byproducts can potentially contribute to elevated non-specific background signal. Probes intended for clinical applications – specifically the diagnosis of substantial chromosomal rearrangements associated with various pathologies, which are in high demand – are predominantly manufactured utilizing enzymatic synthesis techniques. The principal consumers of commercially available, chemically synthesized probes are research institutions. These probes command a higher cost but possess a greater multiplexing potential, thereby enabling more sophisticated experimental applications, including the quantitative analysis of individual transcript molecules.

Probe Labeling Methods

Probe labeling methodologies for fluorescence *in situ* hybridization are conventionally categorized into two principal classes: direct labeling, wherein fluorophores are covalently conjugated to the nucleotide chain, and indirect labeling, where a fluorescent signal is produced by a secondary complex that is bound to the probe via an intermediary molecule.

One of the simplest techniques for the direct labeling of DNA/RNA oligonucleotides involves the incorporation of fluorophores during automated chemical synthesis. In this approach, fluorescently tagged phosphoramidite monomers are directly integrated into the growing oligonucleotide chain. This chemical method is highly reproducible as it is independent of the specific activity of labile enzymatic components.

Classical enzymatic strategies for direct labeling exploit the inherent ability of DNA polymerases to extend primer templates. Direct enzymatic labeling is predominantly achieved through techniques such as DOP-PCR, primer extension, and nick translation.

The primer extension method entails the hybridization of a short primer, complementary to a specific genomic locus, to a single DNA strand, followed by its extension by a DNA polymerase. In contrast to PCR-based amplification, this is a linear process with no exponential amplification of the template and, consequently, no concomitant amplification of the fluorescent signal [26].

The nick translation method utilizes a synergistic enzymatic combination: deoxyribonuclease I (DNase I), which introduces single-strand nicks into double-stranded DNA, thereby generating free 3'-hydroxyl groups, and DNA polymerase I, which incorporates nucleotides at these 3' ends. Concurrently, the 5'–3' exonuclease activity of DNA polymerase I degrades the DNA strand ahead of the nick. During this repair synthesis, labeled and unlaneled nucleotides are incorporated, resulting in the generation of double-stranded, uniformly labeled probes [27]. A significant limitation of this technique is that the introduction of nicks can potentially compromise the probe's specificity for its target sequence. Furthermore, the method necessitates a relatively substantial quantity of input DNA (approximately 1 μg).

Direct labeling is more ubiquitously employed due to its procedural simplicity and convenience. Post-hybridization, the protocol merely requires washing steps to remove excess unbound probe.

In indirect labeling schemes, a hapten-modified nucleotide (e.g., deoxyuridine monophosphate conjugated to a reporter molecule) is incorporated into the probe. This reporter molecule subsequently forms a high-affinity complex with a fluorescently labeled ligand. The most prevalent reporter-ligand pairs include biotin–avidin/streptavidin, digoxigenin with anti-digoxigenin antibodies, and estrogen with specific anti-estrogen antibodies [28]. The primary disadvantage of indirect labeling is that it requires additional incubation steps subsequent to the hybridization reaction. However, this is frequently counterbalanced by a significant amplification of the final fluorescent signal, a particular advantage when working with targets of low abundance.

For further amplification of the signal generated via indirect labeling, tyramide signal amplification (TSA) can be employed. TSA is predicated on the catalytic activity of horseradish peroxidase (HRP), which, in the presence of low concentrations of hydrogen peroxide, converts fluorophore-conjugated tyramide substrates into highly reactive radical species. These radicals form covalent bonds with electron-rich tyrosine residues on proteins located in immediate proximity to the enzyme [29]. The TSA protocol consists of sequential steps: fixation of the cell or tissue sample; incubation with a primary biotinylated antibody; incubation with a secondary antibody conjugated to horseradish peroxidase; and finally, incubation with the fluorophore-tyramide substrate. As the tyramide deposition is covalent, multiple sequential rounds of TSA, employing tyramides conjugated to distinct fluorophores, can be performed on a single sample for the multiplexed detection of numerous targets.

Methods for Increasing the Signal-to-Background Ratio

A fundamental prerequisite for successful signal detection in FISH is that the intensity of the specific hybridization signal must significantly exceed the level of background autofluorescence inherent to the sample. Enhancement of the signal-to-noise ratio can be achieved through two principal strategies: suppression of the signal emanating from dispersed repetitive genomic sequences or augmentation of the signal intensity derived from the unique target sequences. The former strategy typically employs methods to competitively inhibit the hybridization of repetitive elements, while the latter focuses on enriching the proportion of unique sequences within the DNA probe preparation [30].

One of the seminal methodologies for suppressing the hybridization of repetitive sequences is chromosomal *in situ* suppression (CISS) hybridization. This technique is based on the pre-hybridization of the labeled DNA probe with a substantial excess of unlabeled C_0t-1 DNA, which is derived from the same species and is enriched for high-copy repetitive elements. This pre-annealing step allows the repetitive sequences within the probe to form duplexes with excess C_0t-1 DNA, thereby preventing them from

hybridizing to the chromosomal target and reducing non-specific background [31]. However, CISS hybridization often proves ineffective for organisms with very large genomes, as the extreme complexity and abundance of repetitive sequences impedes their complete and efficient suppression [32].

A contrasting line of research involves the development of techniques for the physical elimination of repetitive sequences from pre-existing DNA clone libraries prior to probe labeling. One such method employs magnetic separation of macromolecules for this purpose [33]. In this protocol, magnetic nano- or microparticles are utilized to form complexes with specific DNA fragments. In a standard magnetic separation procedure, the material from the original DNA library is first hybridized with biotin-labeled C_0t-1 DNA. The resultant duplexes containing repetitive sequences are then removed from the solution via affinity chromatography using streptavidin-coated magnetic beads. The supernatant now enriched for unique sequences is subsequently amplified by PCR. This process yields DNA samples with a significantly increased proportion of unique sequences, which are then used for labeling [30]. The net effect of this purification is analogous to that of CISS hybridization. Nevertheless, the utilization of C_0t-1 DNA for generating low-repetition DNA probes carries an inherent risk of co-eliminating some adjacent unique sequences through their association with repetitive elements, potentially leading to a loss of genomic coverage in the final probe.

ADVANCED TECHNOLOGIES FOR DNA PROBE SYNTHESIS

Probes synthesized utilizing the classical methodologies previously delineated remain the most extensively employed in both laboratory practice and diagnosis. Despite their widespread adoption, research consortia globally persist in refining existing technologies and pioneering novel approaches for FISH probe generation.

• SABER-FISH

This methodology is predicated on primer exchange reaction (PER) technology, initially described by J.Y.Kishi et al. in 2017 [34]. A subsequent modification termed SABER-FISH (signal amplification by exchange reaction) was advanced by the same research group in 2019 [35]. Within this paradigm, the foundational probe oligonucleotide incorporates a sequence complementary to the target molecule (30–50 nucleotides) and an auxiliary 9-nucleotide sequence at the 3’ end, which functions as a primer for the PER cascade. This primer anneals to a catalytic hairpin structure, which subsequently serves as a template for the enzymatic synthesis of concatemers. Polymerases possessing strand-displacement activity, such as *Bst* LF, mediate this synthesis without hydrolyzing the DNA backbone, elongating the chain. The amplification cycle is terminated by thermal denaturation at 80 °C. Each catalytic cycle appends approximately 10 nucleotides; iterative cycling enables the concatemer to attain lengths of several hundred nucleotides, a parameter dictating the ultimate fluorescence signal intensity. Following hybridization of these concatemerized probes to their chromosomal targets, a secondary hybridization with fluorescently labeled oligonucleotide ‘imagers’ (20–30 nucleotides complementary to the concatemer) is requisite for signal detection. This technology facilitates a 5 to 450-fold signal amplification and enables highly multiplexed analyses on a single sample.

• LNA Probes

Locked nucleic acids (LNAs) represent structural analogs of RNA wherein the ribofuranose ring is constrained in a C3’-endo conformation via a methylene bridge linking the 2’-oxygen and the 4’-carbon atoms. LNA nucleotides obey the Watson–Crick base-pairing rules and are integrated into oligonucleotides via a standard phosphodiester backbone, permitting the chemical synthesis of chimeric LNA-DNA or LNA-RNA oligonucleotides using conventional phosphoramidite chemistry.

The conformational rigidity imparted by the bicyclic structure enhances the thermal stability of duplexes formed with complementary DNA or RNA sequences, significantly increasing binding affinity [36, 37]. Analogous to PNA probes, the atypical structure of LNA oligonucleotides confers considerable resistance to degradation by nucleases.

• smFISH

Single-molecule FISH (smFISH) is a quantitative methodology that enables the formulation of precise mathematical models, thereby facilitating the investigation of biological processes at an exceptionally high resolution. The quantitative visualization of RNA, for instance, permits the exact enumeration of transcript copies within individual cells, subcellular compartments, and distinct ribonucleoprotein complexes [38].

A considerable diversity of commercial, ready-to-use *in situ* hybridization solutions is now available from numerous entities, including Thermo Fisher Scientific (Affymetrix) with its ViewRNA and PrimeFlow platforms, Bio-Techne (Advanced Cell Diagnostics, ACD) with the RNAscope system, and LGC Biosearch Technologies with Stellaris FISH probes. Despite this commercial availability, the development of novel probe labeling strategies, frequently aimed at cost reduction, remains an active area of research.

An exemplar of a comparatively cost-effective and efficacious technology is the $3P^3$ (three-pot probe production assay), delineated by its authors in 2018 [39]. This methodology comprises three principal stages: (1) conjugation of the fluorophore to a modified terminator nucleotide (NH2-ddUTP); (2) enzymatic labeling of oligonucleotide-DNA probes utilizing terminal deoxynucleotidyl transferase (TdT); (3) purification of the final labeled probe. The foundational principle relies on the template-independent addition of nucleotides to the 3'-hydroxyl end of DNA oligonucleotides catalyzed by TdT. Although this enzyme possesses the capacity for indefinite elongation, the strategic incorporation of dideoxynucleotides ensures the incorporation of precisely a single labeled nucleotide, thereby terminating further extension. While TdT activity has been historically exploited for labeling, these authors pioneered the application for labeling complex pools of standard oligonucleotides via PCR, employing a custom-synthesized biotin-conjugated ddUTP. This reaction achieves a high yield, exceeding 90%, of probes bearing a single label.

A more intricate protocol for smFISH probe generation was detailed by a separate research consortium in 2022 [40]. The initial phase involves the *in silico* design of a substantial pool of primary oligonucleotides complementary to discrete regions of the target RNA molecule. These oligonucleotides subsequently undergo two rounds of PCR amplification followed by *in vitro* transcription. The resultant oligonucleotide-RNA products are then labeled via hybridization with short LNA oligonucleotides that are directly conjugated to fluorophores. A significant advantage of this platform is its inherent capacity for multiplexed, simultaneous detection of numerous distinct RNA targets.

• Quantum Dot Methodology

Conventional organic fluorophores, while widely employed, exhibit photophysical limitations, such as photobleaching and blinking, that can constrain their utility in FISH applications. Inorganic nanocrystals, or quantum dots (QDs), represent a next-generation alternative, offering exceptional photostability and enabling more reliable transcript quantification due to their superior brightness (high signal intensity). A primary challenge in deploying QDs as FISH labels pertains to their substantially larger hydrodynamic diameter (~25–35 nm) compared to organic dyes (~1 nm) [41]. Contemporary research efforts are consequently directed toward the synthesis of more compact QD constructs [42]. The second challenge is the propensity for non-specific adsorption of QDs, inherent to their solid-state colloidal nature, onto cellular structures. This phenomenon can be effectively overcome by the inclusion of blocking agents such as bovine serum albumin (BSA) and polyanions, within the hybridization buffer.

• CASFISH

In 2015, W. Deng and colleagues [43] pioneered the adaptation of the CRISPR/Cas9 system for FISH, designating the technology CASFISH. In their approach, a HaloTag ligand covalently conjugated to a fluorophore was attached to a recombinant catalytically inactive Cas9 protein (dCas9). Concurrently, the guide RNA (sgRNA) designed to target a specific satellite DNA sequence was itself fluorescently labeled. Initial hybridization experiments on mouse embryonic fibroblasts required merely 30 minutes, successfully yielding detectable signals at pericentromeric regions via fluorescence microscopy. Subsequent investigations have demonstrated the exceptional thermodynamic stability of the resultant dCas9/sgRNA/target DNA ternary complex; fluorescence intensity remains unaltered even in the presence of a hundredfold molar excess of competing non-target DNA. These properties – speed and high specificity – endow the CASFISH platform with significant potential for performing highly multiplexed analyses, allowing for sequential or simultaneous hybridizations with multiple distinct probes on a single sample.

CONCLUSION

The analysis of modern methods for synthesizing DNA probes for FISH demonstrates a variety of technologies with unique advantages. Classical enzymatic approaches, such as nick translation and PCR incorporating labeled nucleotides, maintain their relevance for the generation of long-range probes essential for the comprehensive mapping of extensive genomic loci. Both chemical and enzymatic labeling strategies provide significant flexibility

for the structural modification of oligonucleotides. The most transformative advancement has emerged from the field of oligonucleotide synthesis, enabling the engineering of sophisticated constructs with set properties, enhanced specificity, and superior multiplexing capabilities. These are critical for the analysis of complex genomic rearrangements and for applications in spatial transcriptomics.

Despite these advancements, considerable limitations persist. The synthesis of long, highly specific probes remains a labor-intensive and costly endeavor. Although oligonucleotide-based methods are becoming increasingly accessible, they necessitate sophisticated bioinformatic design and extensive empirical optimization to mitigate non-specific background hybridization, a challenge particularly acute in regions rich in repetitive sequences.

Future prospects for the field are anticipated to evolve along several pivotal trajectories: 1) the enhanced automation and standardization of probe synthesis and purification processes to reduce costs and improve inter-laboratory reproducibility; 2) the development of novel fluorophores with improved brightness and photostability, coupled with advanced detection systems; 3) the deeper integration of computational biology and bioinformatic tools to optimize probe design *in silico* and accurately predict hybridization kinetics and specificity; 4) the creation of innovative hybrid methodologies that synergistically combine the strengths of disparate synthesis technologies. It is a reasonable projection that the continued refinement of DNA probe synthesis methodologies will serve as a primary catalyst for the evolution of FISH technology, thereby substantially expanding its diagnostic and research applications.

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Regulatory Proteins of Epithelial-Mesenchymal Transition in Colorectal Cancer: From Biology to Clinical Application

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ABSTRACT

This lecture presents current data on the role of regulatory proteins of epithelial-mesenchymal transition (EMT) in colorectal cancer (CRC) in the context of tumor molecular stratification and personalized treatment selection. EMT is a key biological process associated with tumor invasion, metastasis, chemoresistance, and immune evasion. Special attention is given to the characterization of CRC molecular subtypes according to the Consensus Molecular Subtypes (CMS) classification, particularly the CMS4 subtype with mesenchymal features, which is marked by high EMT activity and poor prognosis.

The lecture discusses molecular and immunohistochemical markers indicative of EMT activation, including CDX2, ZEB1, HTR2B, FRMD6, BMI-1, and ROR1. The expression patterns and associated signaling pathways of these proteins are examined along with their influence on tumor aggressiveness, therapy resistance, and prospects for clinical application. Based on the literature analysis, the potential of these proteins as prognostic and possibly predictive biomarkers, as well as therapeutic targets, is discussed.

Keywords: colorectal cancer, molecular subtypes, CDX2, ZEB1, HTR2B, ROR1, BMI-1, FRMD6, epithelial-mesenchymal transition, prognosis

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Регуляторные белки эпителиально-мезенхимального перехода в колоректальном раке: от биологии к клиническому применению

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РЕЗЮМЕ

Представлены современные данные о роли регуляторных белков эпителиально-мезенхимального перехода (ЭМП) при колоректальном раке (КРР) в контексте молекулярной стратификации опухолей и персонализированного подбора терапии. Эпителиально-мезенхимальный переход представляет собой ключевой биологический процесс, связанный с инвазией, метастазированием, химиорезистентностью и иммунным уклонением опухоли. Особое внимание уделено характеристике молекулярных подтипов КРР согласно консенсусной классификации (CMS), в частности, CMS4-подтипу с мезенхимальными признаками, для которого характерен высокий уровень ЭМП и неблагоприятный прогноз.

В лекции обсуждаются молекулярные и иммуногистохимические маркеры, отражающие активацию ЭМП, включая CDX2, ZEB1, HTR2B, FRMD6, BMI-1 и ROR1. Рассматриваются особенности экспрессии и сигнальные каскады, с которыми ассоциированы указанные белки, их влияние на опухолевую агрессивность, устойчивость к терапии и перспективы клинического применения. На основании анализа литературных данных рассматриваются возможности использования этих белков в качестве прогностических и потенциальных предиктивных маркеров, а также терапевтических мишеней.

Ключевые слова: колоректальный рак, молекулярные подтипы, CDX2, ZEB1, HTR2B, ROR1, BMI-1, FRMD6, эпителиально-мезенхимальный переход, прогноз

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant neoplasms globally and within Russia, being a major contributor to both morbidity and mortality rates. According to the World Health Organization, CRC is one of the three most common cancers, second only to lung and breast cancer [1]. In 2020, more than 1.9 million new cases of CRC and about 935,000 related deaths were reported worldwide [2]. In the Russian Federation in 2023, over 74,000 new cases of malignant tumors of the colon and rectum were detected, and the number of patients registered with this pathology exceeded 437,000. At the same time, during the first year after diagnosis verification, death is observed in 20.6% and 18.2% of patients with colon and rectal cancer, respectively [3]. Despite the advances in modern oncology (the development of high-tech imaging methods, screening, and targeted therapy), the 5-year survival rate in CRC remains at about 60–65%, and the prognosis significantly depends on the stage of the disease at the time of diagnosis [4].

The problem of assessing the risk of recurrence and stratification of patients with stage II–III tumors is particularly relevant, when the need for adjuvant chemotherapy often becomes the subject of discussion [4].

In recent years, considerable attention has been paid to the epithelial–mesenchymal transition (EMT) as a key mechanism of tumor invasion, metastasis, chemoresistance, and immune evasion. The study of EMT regulatory proteins is a promising area of molecular oncology that can enrich personalized medicine.

The aim of this lecture is to examine current understanding of the role of EMT regulatory proteins in CRC with an emphasis on their relationship with molecular subtypes of the tumor, the biological characteristics of the aggressive course of the disease, and their potential significance in translational oncology.

MOLECULAR HETEROGENEITY OF COLORECTAL CANCER AND THE SIGNIFICANCE OF THE EPITHELIAL–MESENCHYMAL TRANSITION

CRC is a molecularly and clinically heterogeneous disease caused by differences in the embryonic development of the intestinal tract, multiple pathways of carcinogenesis, and features of the tumor microenvironment. This heterogeneity leads to marked

differences in a response to therapy and a clinical outcome in different patients. Based on studies of biological features of CRC, several molecular genetic classifications have been proposed [5].

Currently, the Consensus Molecular Subtypes (CMS) classification is the most widely used system. This classification is considered as the most informative and convenient, since four molecular subtypes of colorectal carcinoma are distinguished based on the similarities and differences in the molecular genetic characteristics of tumors, as well as the features of their clinical course:

– CMS1 (immune, ~14%) is characterized by high microsatellite instability (MSI-H), accompanied by intensive infiltration of tumor tissue by T-lymphocytes ($CD4^+/CD8^+$) and natural killer cells (NK cells), activation of the antitumor immune microenvironment and global hypermethylation of promoters of a number of genes;

– CMS2 (canonical, ~37%) is associated with chromosomal instability (CIN), frequent mutations in APC, TP53, and KRAS, as well as activation of the Wnt/β-catenin and MYC cascades, which provide the proliferative potential of tumor cells; it is located mainly on the left;

– CMS3 (metabolic) demonstrates pronounced metabolic shifts, including activation of glutaminolysis and lipogenesis, which is often combined with mutations in the *KRAS* and *PIK3CA* genes, as well as with dysregulation of Wnt signaling, occurs in about 13% of cases, has no clear connection with a specific localization;

–CMS4 (mesenchymal, ~23%) is characterized by the predominance of tumor-associated fibroblasts in the stroma, activation of the TGFβ signaling cascade, formation of a developed vascular network and manifestation of signs of EMT, reflecting its high invasive potential and resistance to many standard therapies [6].

CMS4 subtype is associated with the most aggressive course of the disease, poorer overall and relapse-free survival rates, reduced sensitivity to chemotherapy, and marked activation of the immunosuppressive microenvironment [7, 8]. It is in this subtype that EMT plays the most significant role forming the invasive phenotype of the tumor and contributing to the progression of the disease [9].

EMT is a fundamental phenotypic transformation during which tumor cells lose epithelial polarity, intercellular adhesion bonds, and expression of typical epithelial proteins such as E-cadherin. These changes are accompanied by the acquisition of a mesenchymal phenotype, including increased mobility, invasiveness, as well as resistance to apoptosis and antitumor therapy. The EMT is controlled by various signaling pathways (TGFβ, Wnt/β-catenin, Notch, Hippo, PI3K/Akt, etc.), transcriptomic factors (Snail, Slug, Twist, and ZEB1/2), as well as microRNA and epigenetic mechanisms [10–15].

In the context of modern CMS classification, EMT is considered as a critical process underlying aggressive CRC subtypes, as well as a potential target for therapy and risk stratification. EMT regulatory proteins are actively studied in order to assess their role as prognostic markers and possible therapeutic targets [16, 17]. The key characteristics of CMS subtypes and their relationship to EMT and immune mechanisms are shown in Fig. 1.

CDX2: A MARKER OF INTESTINAL DIFFERENTIATION AND EMT NEGATIVE REGULATOR WITH A ROLE IN SUPPRESSING TUMOR GROWTH, PROGNOSTIC VALUE, AND EFFECT ON CHEMOSENSITIVITY

CDX2 (Caudal-related homeobox 2) is a transcription factor containing a homeobox domain that plays a key role in differentiation and morphogenesis of the intestinal epithelium [18]. Due to the presence of a homeodomain, CDX2 is able to bind to specific DNA regions, regulating the activity of the target set of genes. It belongs to the ParaHox family evolutionarily related to the Hox cluster and along with CDX2 includes the *CDX1* and *CDX4* genes.

It is known that CDX2 is one of the first to be activated in embryogenesis and retains expression in the pericecal region of the intestine, including the ileocecal valve [19]. CDX2 is actively used in clinical practice as an immunohistochemical marker of intestinal differentiation, but its specificity is limited – high CDX2 expression can be observed in both ovarian mucinous carcinoma and bladder adenocarcinoma [20].

Loss of CDX2 expression in patients with CRC is associated with an unfavorable clinical course, including a decrease in both relapse-free and overall survival, especially in stage II–III tumors [21]. In addition, decreased CDX2 expression is often accompanied by vascular invasion, low differentiation, right-sided tumor location, CIMP phenotype (global hypermethylation of CpG islands; CpG island methylator phenotype), and BRAF mutation [22]. It is important to note that

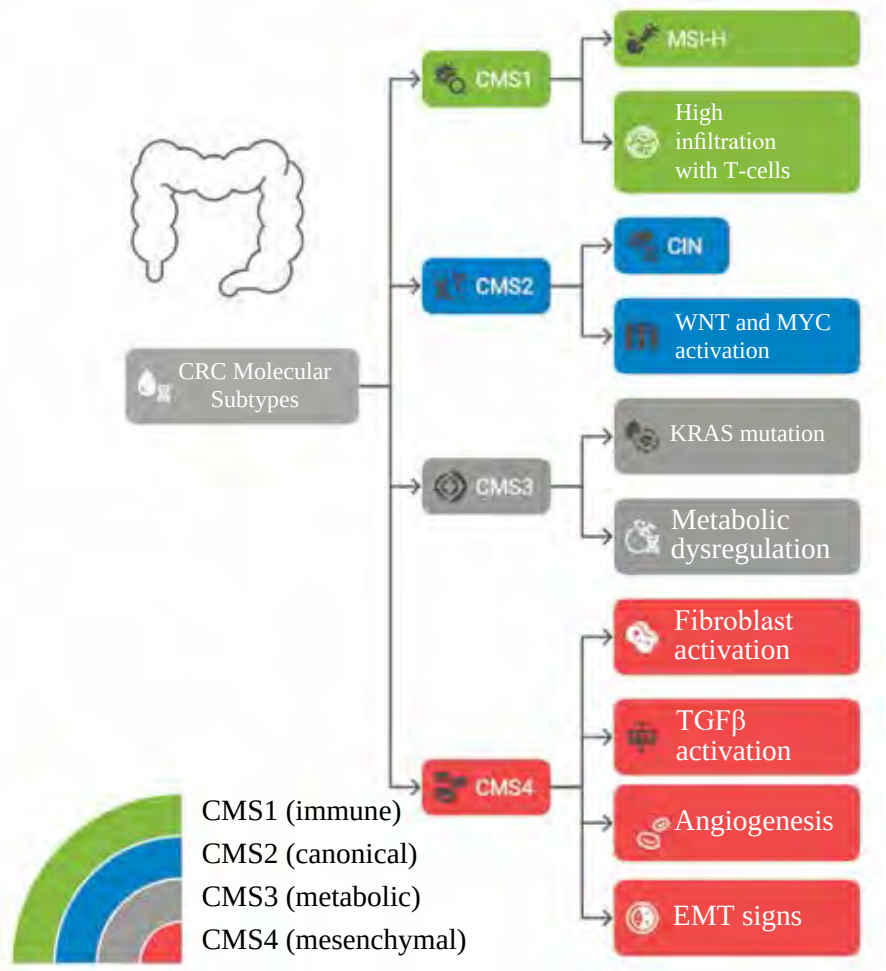

Fig. 1. Key characteristics of CRC (CMS) molecular subtypes: CMS1 (immune) – MSI-H, pronounced tumor infiltration by T-lymphocytes ($CD4^+/CD8^+$) and NK cells; more often found in the right half of the colon. CMS2 (canonical) – chromosomal instability (CIN), mutations in APC, TP53, and KRAS, activation of Wnt/β-catenin and MYC signaling; more often left-sided tumors (~37%). CMS3 (metabolic) – KRAS and PIK3CA mutations, metabolic disorders and dysregulation of Wnt signaling; ~13% of cases without clear localization. CMS4 (mesenchymal) – ~23% of cases; activation of TGFβ, EMT, angiogenesis, left-sided localization, and pronounced drug resistance

in some cases, heterogeneity of staining is revealed by immunohistochemical profile: high CDX2 expression is observed in the central areas of the tumor, while expression weakens in the periphery, especially in the area of invasive growth [23].

CDX2 exhibits antitumor activity by suppressing EMT. The mechanism is described through the activation of the tumor suppressor let-7b, which leads to a decrease in the expression of collagen XI α1 (COL11A1) associated with cell migration, infiltration of immune elements, and chemoresistance [24, 25]. The research results show that a decrease in CDX2 expression is significantly associated with the proximal location of the tumor, a low degree of differentiation, mucinous phenotype, microsatellite instability, CIMP phenotype, and the presence of mutations in the *BRAF* gene. Such tumors are also characterized by increased cytokeratin CK7 expression and decreased CK20 expression [26].

CDX2-negative tumors are more common in the metastatic stage of CRC – up to 19% of cases [27]. In the study by Y. Shigematsu et al., low CDX2 expression was associated with a deterioration in relapse-free survival (245 vs. 420 days) and overall survival (1,024 vs. 3,145 days) after resection of liver metastases; it was also shown that such patients did not significantly benefit from adjuvant or neoadjuvant therapy [28].

Interesting results were obtained in the 2023 study: in 14 patients with metastatic MSI-H CRC who

received immunotherapy, the CDX2-positive group had a 1-year relapse-free survival of 81%, while in the CDX2-negative group, there was not a single relapse-free case [29]. This indicates the potential of CDX2 as a predictive marker of the immunotherapy response. In another study, P. Dalerba et al. showed that adjuvant chemotherapy was beneficial only in patients with CDX2 loss: their 5-year relapse-free survival rate was significantly higher, whereas in CDX2-positive patients, chemotherapy did not improve survival [30].

ZEB1: TRANSCRIPTIONAL EMT INDUCER AND MEDIATOR OF DRUG RESISTANCE AFFECTING INVASIVENESS, IMMUNOSUPPRESSION, AND TUMOR STEM PHENOTYPE FORMATION

ZEB1 (Zinc finger E-box binding homeobox 1) is a transcription factor that plays a key role in EMT regulation. Under normal physiological conditions, ZEB1 is involved in the regulation of cellular differentiation, homeostasis, and the development of nervous, smooth muscle, and bone tissues [31].

In the context of malignant growth, ZEB1 acts as an EMT activator, inducing the expression of mesenchymal markers and suppressing the expression of epithelial markers, in particular E-cadherin. ZEB1 induction occurs under the influence of several signaling pathways: WNT, TGFβ, COX2, HIF, etc. [32]. One of the mechanisms involves the interaction of ZEB1 with the ELK3 transcription factor, resulting in the suppression of the E-cadherin gene and activation of the migration phenotype of tumor cells [33].

According to data obtained by Y. Guo et al., activation of TGFβ leads to an increase in ZEB1 expression on the invasive tumor front, which initializes the EMT cascade [34]. Moreover, hypermethylation of the *ZEB1* gene promoter is associated with its reduced expression and a more favorable prognosis in CRC. This epigenetic phenomenon is more often observed in patients with CMS1 subtype and correlates with higher relapse-free and overall survival, regardless of the stage and other clinical factors [35].

ZEB1 is also associated with immune evasion mechanisms. According to data obtained by M.Z. Noman et al., ZEB1 induces PD-L1 expression, which contributes to the suppression of the T-cell immune response in the tumor [36]. This makes ZEB1 a potential predictor of sensitivity to immune checkpoint inhibitors.

ZEB1 is involved in the development of chemoresistance, in particular, it is associated with increased expression of Bcl-xL and cyclin D1, key factors of resistance to antitumor drugs [37]. In studies on breast cancer models, high ZEB1 expression correlated with a decrease in relapse-free survival and the frequency of pathologic complete response (pCR) in patients receiving neoadjuvant therapy [38].

The results of two meta-analyses published in 2017 and 2019 confirmed the negative prognostic role of high ZEB1 expression, as the most pronounced decrease in overall survival with high ZEB1 levels was found in patients with CRC, gastric, and pancreatic cancers [39].

FRMD6: CONTEXT-DEPENDENT EMT SUPPRESSOR THROUGH ACTIVATION OF THE HIPPO PATHWAY

FRMD6 (FERM domain-containing protein 6) is a protein from the family of FERM domain–containing proteins (4.1/ezrin/radixin/moesin) involved in a number of processes: regulation of cell proliferation, differentiation, apoptosis, and cell-extracellular matrix interaction [40–43]. It plays a role in neurophysiological processes, and is also involved in Hippo, ERK/MAPK, c-MYC, and mTOR signaling pathways. In CRC, the FRMD6 protein exhibits the properties of a tumor suppressor, primarily due to its involvement in the Hippo signaling pathway, which regulates cell proliferation, apoptosis, and growth restriction. The main components of the Hippo cascade are kinases MST1/2 and LATS1/2, which inhibit nuclear translocation and the activity of transcriptional coactivators YAP/TAZ. Phosphorylation of YAP by serine-127 prevents its transfer to the nucleus and thereby suppresses transcription of genes responsible for growth and EMT [44–47]. FRMD6 promotes the activation of LATS kinases and YAP phosphorylation, which leads to a restriction of the transcriptional activity of the latter, resulting in a decrease in the expression of EMT-inducing factors, including Snail, Slug, and ZEB1 [48].

In prostate cancer research, decreased FRMD6 expression was associated with overactivation of the YAP and MYC pathways, as well as with an increased risk of biochemical relapse after prostatectomy [49].

Similar protective properties of FRMD6 were also revealed in CRC. According to the results of a major study led by A.Von Koskull et al. (2024), which included 538 patients with CRC, a high level of FRMD6 expression was associated with improved survival rates, and therefore the protein was considered as an independent prognostic marker

in a multifactorial analysis model [50]. However, in some other malignant tumors, this molecule, on the contrary, demonstrates pro-tumor activity.

Thus, in adenocarcinoma and squamous cell lung cancer, high FRMD6 expression is associated with an unfavorable prognosis. In these cases, FRMD6 activates the mTOR pathway, which leads to increased proliferation and drug resistance of tumor cells [51]. Thus, FRMD6 is a molecule with a context-dependent function. In CRC, it acts as a suppressor of tumor growth and EMT, but its role in tumors of other locations requires further study.

HTR2B: SEROTONIN-DEPENDENT EMT ACTIVATOR AND MEDIATOR OF INTERCELLULAR COMMUNICATION – INVOLVED IN GROWTH AND MIGRATION CASCADES, ASSOCIATED WITH CRC PROGRESSION AND IMMUNE EVASION

The HTR2B serotonin receptor (5-HT_2B), which is actively involved in the regulation of neurohumoral and metabolic processes, is expressed in the tissues of the nervous system, heart, and gastrointestinal tract [52]. It is known that the serum serotonin (5-HT) level in patients with CRC is significantly higher than in healthy individuals, which contributes to tumor progression through activation of the 5-HT-Wnt and 5-HT-YAP signaling axes [53].

High expression of HTR2B has been detected in various malignant neoplasms: melanoma, gastrointestinal stromal tumors, breast, ovarian, kidney, and pancreatic carcinomas, as well as in CRC [54]. Activation of HTR2B triggers the Akt/mTOR cascade, which leads to phosphorylation and activation of the transcription factor CREB1, which stimulates ZEB1 expression, which is known to play a key role in triggering EMT [55]. Suppression of HTR2B activity is accompanied by a decrease in the migration potential of tumor cells and partial blockade of EMT processes, which confirms the role of this receptor in maintaining an aggressive phenotype. Additionally, it was found that stimulation of HTR1B and HTR2B activates the MAPK/ERK signaling cascade, which plays a key role in cell proliferation and differentiation [55–57].

In a 2023 study, it was shown that HTR2B expression is associated with lipid metabolism in gastric adenocarcinoma. Thus, an increased level of HTR2B was associated with decreased survival, which indicates the role of the molecule in maintaining the viability of tumor cells and the possibility of its use as a prognostic marker [58]. In hepatocellular cancer, the role of serotonin HTR2B with its involvement in the process of tumor growth was similarly noted [59]. In contrast, the results of another study showed a correlation between high HTR2B expression and lower tumor spread in ovarian cancer, which demonstrates the ambiguity of its prognostic value [60].

Thus, despite convincing experimental data on the role of HTR2B in the induction of EMT and the metastatic potential of tumors, its clinical significance in CRC has not been definitively determined and requires further study. Nevertheless, HTR2B blockade is considered as a promising area of antitumor therapy in translational oncology.

BMI-1: EPIGENETIC REGULATOR OF TUMOR STEMMING AND THERAPY RESISTANCE, PARTICIPATING IN THE MAINTENANCE OF PROGENITOR CELLS AND THE INDUCTION OF EMT THROUGH THE AKT/SNAIL CASCADE

BMI-1 (B-cell-specific Moloney murine leukemia virus integration site 1), also known as RNF51 or PCGF4, is a transcription factor that plays a key role in maintaining stem cell functions, especially in the nervous and hematopoietic systems. In experiments on animal models, switching off this gene led to impaired embryonic development, and its overexpression led to the development of lymphomas [61].

BMI-1 belongs to the Polycomb (PcG) family of epigenetic transcription regulators, it is part of Polycomb repressive complex 1 (PRC1) and, together with the Ring1B/Rnf2 component, ubiquitinates histone H2A by lysine–119 (H2AK119ub), which suppresses the transcription of genes regulating apoptosis, differentiation, and aging [62]. BMI-1 promotes EMT induction through activation of the Akt/GSK-3b/Snail pathway, as well as through stimulation of the TGF-β/SMAD cascade [63, 64].

The study by Z.Y.Jiang et al. showed on experimental CRC models that BMI-1 activates peritumoral stellate liver cells, contributing to the formation of a pre-metastatic niche and increased migration of tumor cells, while inhibition of BMI-1 restored the expression of E-cadherin and disrupted the processes of migration, invasion, and drug resistance [65]. High expression of BMI-1 has been observed in many malignant neoplasms, including tumors of the stomach, breast, ovaries, prostate, lung, and pancreas.

The correlation of the marker expression with more intensive proliferation, high metastatic potential, and drug resistance of tumors was noted [66, 67].

The meta-analysis by M. Pourjafar et al. (2022), which included nine studies (seven Asian and two European), revealed population differences in the prognostic value of BMI-1 expression in CRC. It was found that high expression was associated with an unfavorable prognosis (decreased overall and relapse–free survival) in the Asian population, whereas in the European population, on the contrary, more favourable outcomes were noted. In addition, increased BMI-1 levels were more often associated with a large tumor size, the presence of distant metastases, an older age of patients ,and a male sex. No reliable relationship was found with the parameters of TNM, the degree of differentiation, and localization of the tumor [68].

Thus, BMI-1 is a potential marker of aggressive tumor development and a prognostic biomarker, but its significance in different populations requires additional validation in multicenter studies.

ROR1 IN COLORECTAL CANCER: ONCOEMBRYONIC RECEPTOR, ITS SIGNALING PATHWAYS AND THERAPEUTIC POTENTIAL

ROR1 (Receptor tyrosine kinase-like orphan receptor 1) is a receptor tyrosine kinase from the ROR family, expressed mainly at the stage of embryogenesis and practically absent in healthy tissues in adults. Under normal conditions, the highest expression of ROR1 is observed in the pancreas and adipose tissue, whereas it is minimal in most other tissues (colon, lungs, uterus, and parathyroid glands) [69, 70].

ROR1 contains several evolutionarily conserved domains: an extracellular immunoglobulin-like domain, a Frizzled domain, a kringle domain, and an intracellular tyrosine kinase-like domain [69]. The biological role of ROR1 is related to the regulation of cell migration, polarity, and organogenesis during embryonic development [69, 70]. In oncology, ROR1 is involved in the non-canonical Wnt signaling pathway through binding to the Wnt5a ligand. This pathway regulates cell migration, apoptosis, EMT, and chemoresistance [71]. Increased expression of ROR1 has been found in various solid tumors (breast cancer, ovarian cancer, lung cancer, hepatocellular carcinoma, CRC, etc.) [71, 72].

CRC studies have revealed significantly higher ROR1 expression in tumor cells compared to healthy tissues. ROR1 expression correlates with the stage of the disease, lymph node damage, and decreased overall survival [73]. Similar data were obtained in ovarian cancer, where high ROR1 expression was also associated with low rates of relapse-free and overall survival [74]. A meta-analysis in 2022, including the results of 14 studies, showed that high ROR1 expression was significantly associated with poorer overall survival. A particularly strong association was found in endometrial cancer, ovarian cancer, and B-cell lymphoma [75].

Earlier in the 2019 meta-analysis, similar results were obtained for solid tumors, including lung cancer; there were no differences in prognostic significance between solid and hematological tumors [75]. Due to its high tumor-specific expression and lack of expression in healthy tissues, ROR1 is a promising therapeutic target. Currently, the effectiveness of a number of targeted ROR1 kinase inhibitors that cause apoptosis of tumor cells has been shown [75, 76].

Of particular interest is the use of ROR1 as an antigen for chimeric antigen receptors of T-lymphocytes (CAR-T; Chimeric antigen receptor to T-cells). CAR-T therapy, which involves the modification of patient's T cells to attack tumor cells, is already actively used in hematological malignant neoplasms [75]. Preliminary results of phase I clinical trials demonstrate encouraging efficacy of therapy using CAR-T cells directed against ROR1 in patients with triple negative breast cancer in cases of expression of this receptor [75–79]. These data open up the prospect for adapting a similar approach in the treatment of CRC with positive ROR1 expression.

Thus, ROR1 simultaneously acts as a prognostic marker and a promising target for targeted therapy, especially in the context of immuno-oncological approaches, including CAR-T.

EMT AND THE PROBLEM OF CRC STRATIFICATION OF STAGES II–III

Despite the advances in molecular diagnostics and the improvement of drug therapy, the rationale for the need and scope of adjuvant chemotherapy in stage II-III CRC remains the subject of active discussion. Current guidelines (for example, ESMO) suggest dividing stage II patients into low-, intermediate-, and high-risk groups based on a combination of clinical and morphological factors. The "significant" risk factors include the pT4 stage (regardless of the presence of intestinal wall perforation) and the examination of less than 12 lymph nodes; the "less significant" ones include a low degree of tumor differentiation, vascular and perineural

invasion, intestinal lumen obstruction, and a high level of cancerous embryonic antigen (CEA) [4].

Microsatellite instability (MSI) is recognized as an important prognostic and, in some cases, predictive factor. Patients with early-stage MSI-H tumors have a more favorable prognosis, and in the presence of a single "less significant" risk factor, it is possible to safely refrain from prescribing fluoropyrimidine chemotherapy without worsening relapse-free and overall survival [80]. Nevertheless, the existing stratification based on a combination of morphological and molecular parameters (TNM stage, MSI status, RAS/BRAF mutations, Immunoscore index, and circulating tumor DNA – ctDNA level) does not allow for predicting the risk of recurrence or a response to therapy in a particular patient with high accuracy [81–85], relapse within 5 years develops in 12–38% of stage II patients and in almost half of stage III patients.

EMT regulatory proteins represent a promising group of biomarkers potentially capable of improving the accuracy of prognosis. Loss of CDX2 expression is associated with a lower survival rate. In addition, data obtained by P. Dalerba et al. showed that adjuvant chemotherapy was effective mainly in patients with CDX2-negative tumors, whereas its administration did not improve the results in CDX2-positive patients [30].

ZEB1, in turn, can be identified as a marker of an immunosuppressive phenotype (mediating an increase in PD-L1) and a predictor of drug resistance. ROR1, FRMD6, BMI-1, and other EMT proteins are also involved in the regulation of chemosensitivity and metastatic potential. The analyzed protein molecules considered as EMT regulators and their phenotypic effects in the context of CRC are shown in Fig. 2.

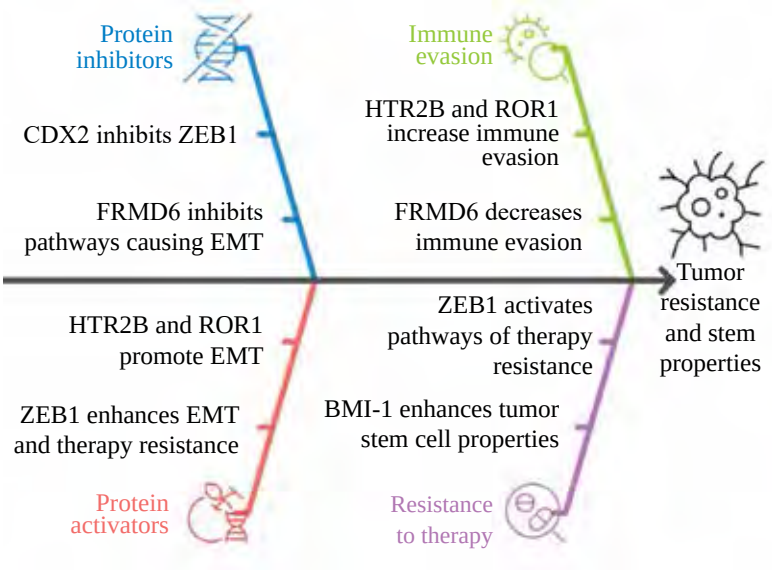

Fig. 2. Key molecular links between EMT regulators, signaling pathways, and phenotypic effects in CRC: CDX2 suppresses ZEB1 expression, reducing EMT activity and reducing tumor stemming. On the contrary, ZEB1 activates cascades that enhance EMT and the formation of drug resistance. FRMD6 blocks the signaling pathways that induce EMT, which leads to a decrease in the immune evasion. HTR2B and ROR1 contribute to the activation of EMT, accompanied by increased therapy resistance and avoidance of immune elimination. BMI-1 enhances the stem properties of the tumor, playing an important role in the development of chemoresistance

CONCLUSION

EMT is a complex biological process that plays a key role in the progression, metastasis, formation of chemoresistance, and immunosuppressive microenvironment in CRC. Modern molecular studies show that EMT is not a binary phenomenon, but rather a spectrum of transitional phenotypes with both epithelial and mesenchymal features, reflecting the plasticity of tumor cells and their adaptive capabilities. This phenotypic shift is closely related to the activation of the Wnt/β-catenin, Notch, TGFβ, and Hippo-YAP/TAZ signaling pathways, as well as the expression of transcriptional regulators, including ZEB1, SNAIL, and TWIST.

Of particular interest is the interaction of EMT with components of the tumor microenvironment, including macrophages, fibroblasts, and neurotransmitter signaling pathways (for example, serotonergic HTR2B receptors) that contribute to the maintenance of stem cells and invasiveness of tumor cells. In addition, PD-L1 expression during EMT activation may contribute to evading immune surveillance, which underscores the importance of a comprehensive analysis of the CRC immune landscape, including the use of immunoscoring and circulating tumor DNA.

The markers CDX2, ZEB1, FRMD6, BMI1, ROR1, and HTR2B presented in the review demonstrate a wide range of biological functions

from regulation of differentiation and invasiveness to involvement in the formation of therapy resistance. The potential of their use as prognostic and predictive biomarkers is confirmed by both meta-analyses and data on expression in primary tumors and metastases. Moreover, a number of these molecules are being considered as promising therapeutic targets for the development of targeted drugs and CAR-T-cell therapy.

Despite significant advances, the translation of knowledge about the mechanisms of EMT and its regulators into routine clinical practice requires standardization of assessment methods, validation on large cohort samples, and integration into existing prognostic models. Particular attention should be paid to the phenotypic heterogeneity of the tumor and the dynamics of expression of key markers during treatment. Progress in this area may provide the basis for a personalized approach to CRC therapy and enhance the effectiveness of existing strategies, including immunotherapy and signaling pathway inhibitors.

Thus, a deep understanding of the role of EMT and its regulators in the pathogenesis of CRC opens up new horizons for diagnosis, risk stratification, and the development of innovative therapeutic approaches aimed at overcoming the metastatic potential and resistance of the tumor to therapy.

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Terms Used to Characterize the Course of Chronic Heart Failure: Are All the Points on the Board?

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ABSTRACT

The natural course of almost any chronic pathology, if its latent forms are excluded, is cyclic with alternating periods of exacerbation and remission. Chronic heart failure (CHF) is no exception – periods of stable (sometimes seeming stable) course are followed by episodes of worsening of clinical symptoms, leading to a decrease in quality of life and an increased risk of premature death. In turn, in a patient with decompensated heart failure, various changes in the clinical severity of CHF are possible: resolution of symptoms (including remission), persistence of heart failure, and, unfortunately, further worsening. The characteristic of CHF course should become an integral part of the clinical diagnosis based on the appropriate classification. The latter can be an effective instrument in clinical practice only if the terms it provides have an unambiguous meaning and clearly delineated boundaries of their correct usage. The authors of the lecture reviewed the main terms used to characterize the course of CHF. Unfortunately, despite the almost permanent discussion of the problem of concept demarcation and repeated attempts to formulate agreed positions, experts from reputable cardiological communities in the Old and New Worlds cannot reach consensus, and the definitions of terms used to describe the CHF course differ in a number of guidelines.

Keywords: chronic heart failure, classification, worsening, exacerbation, decompensation, resolution of symptoms and signs, remission, persistence, hospitalized heart failure

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Термины, применяющиеся для характеристики течения хронической сердечной недостаточности: все ли точки над i расставлены?

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РЕЗЮМЕ

Естественное течение практически любой хронической патологии, если исключить ее латентные формы, характеризуется цикличностью со сменой периодов обострения и ремиссии. Хроническая сердечная недостаточность (ХСН) не является исключением, периоды стабильного (иногда кажущегося) течения сменяются эпизодами нарастания клинической симптоматики, приводящими к снижению качества жизни и повышению риска преждевременной смерти. В свою очередь, у пациента с декомпенсированной сердечной недостаточностью возможны различные траектории изменений клинической выраженности ХСН: разрешение симптомов (в том числе ремиссия), персистирование сердечной недостаточности и, к сожалению, дальнейшее ухудшение. Характеристика течения ХСН должна стать неотъемлемой частью клинического диагноза, основанного на соответствующей классификации. Последняя может быть эффективным инструментом клинической практики только в случае, если предусмотренные в ней термины имеют однозначное значение и четко очерченные границы их корректного применения. Авторы лекции рассмотрели основные термины, применяющиеся для характеристики течения ХСН. Несмотря на практически перманентную дискуссию по проблеме демаркации понятий и неоднократные попытки сформулировать согласованные позиции, эксперты авторитетных кардиологических сообществ в Старом и Новом Свете не могут прийти к полному консенсусу, и определения терминов, используемых для описания течения ХСН, в ряде рекомендаций отличаются.

Ключевые слова: хроническая сердечная недостаточность, классификация, ухудшение, обострение, декомпенсация, разрешение симптомов и признаков, ремиссия, персистирование, сердечная недостаточность, требующая госпитализации

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

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

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INTRODUCTION

A comprehensive clinical diagnosis is used to solve a wide range of fundamental problems: from an expanded description of a disease in a particular patient and justification of the choice of methods of personalized treatment/prevention and rehabilitation to the assistance in the assessment of working capacity and fitness for military service, as well as occupational health check and medical monitoring in sports [1-3]. It is no coincidence that people say: “His Majesty the Diagnosis” [4]. In this case, in accordance with paragraph 5 of Article 70 of the Federal Law dated November 21, 2011 No. 323-FZ “On the Foundations of Healthcare for Citizens in the Russian Federation” (revised edition of December 28, 2024 amended on March 1, 2025), a medical report based on the results of a thorough examination of the patient’s health status, their diseases (injuries) or the cause of death is expressed in terms provided by the current classifications [5].

The less primitive the classification used, the better the diagnosis will be and the more information it will contain for clinical practice[6]. The thoughts reflected in 1993 by Chazov in the lead article in the journal Therapeutic Archive [7] remain fully relevant [7]. It says that the high need for detailed grouping is dictated not by theoretical calculations and ambitions of some scientists or individual clinical schools, and not even by the desire to unite patients by the nature and degree of pathologic changes, but by the desire to create the most effective differentiated therapy and determine the prognosis of the disease. Only the logical rigor of the classification features taken as a basis, on the one hand, and the use of terms with unambiguous meaning and clearly delineated boundaries of their correct usage, on the other hand, can guarantee the success of effective fulfillment of the key functions of clinical diagnosis, which is a product of the classification procedure, outlined by Chazov [8-10].

Many people probably remember the axiom “Logic is never odd: it is either there or it is not” expressed in the feature film “The Oligarch” (directed by Lungin, 2002), and a good scientific classification that obviously fulfills the given functions is not conceivable without observing the rules that are formed in logic [11]. Nevertheless, clinical classifications often deviate from the ideal described by the theory, in particular, from the observance of strict rules of division, when, for example, a single feature (or a set of features) chosen at the beginning as a basis is replaced by another classification criterion in the course of division. For example, when determining the first two stages of chronic heart failure (CHF), Strajesko and Vasilenko suggested relying on the presence and severity of hemodynamic disorders, and when justifying the third stage - on the identified severe (irreversible) structural changes of target organs [12]. We have to accept this because establishing a diagnosis, a doctor pursues certain goals (one of the most important ones is to justify the choice of methods of personalized treatment/prevention and rehabilitation), and, based on practical expediency, the choice or change of the classification basis is dictated by these goals.

It is clear that not everything that is used in everyday life corresponds to the requirements of high theory and meets the standards of perfect logic [11]. In everyday life, it is quite acceptable to divide shoes into men’s, women’s and rubber (children’s) shoes because, despite the fact that from the point of view of logic such classification is not good (the rules are violated: “division should be carried out only on one basis” and “the members of the division should mutually exclude each other”), it can nevertheless satisfactorily serve practical purposes in a shoe store [11]. However, allowing in clinical classification some deviation from the rules of logical operation, when instead of strict division simple grouping is applied, examples of which are numerous, we cannot tolerate a violation of common sense. It is necessary to proceed from the fact that the diagnosis is the basis for the choice of therapeutic tactics and not to allow in clinical classification the reverse situation when abuse of discretion by doctors associated with a decision on hospitalization, prescription, continuation or discontinuation of any therapy, etc. is taken into account as a basis for determining the type (form), stage, and phase of the disease or syndrome.

It may seem that the latter statement does not need special argumentation, but in clinical classifications, when determining the basis for division, everything is often put “upside down” and when justifying the diagnosis doctors begin to rely not only on important

characteristics of the disease, but also largely on subjective criteria. For example, the refusal (maybe erroneous) to conduct cardioversion by a doctor who convinced the patient of this fact may allow to justify the diagnosis of permanent (in Russia it is referred to as constant) form of atrial fibrillation [13–15], ongoing antimicrobial chemotherapy – active infective endocarditis [16, 17], and the decision on emergency hospitalization of a patient with CHF and intravenous administration of loop diuretics – acute decompensated heart failure (ADHF) [18]. Here is one example of the opposite situation, when sometimes misplaced persistence in repeated restoration of sinus rhythm turns permanent atrial fibrillation into long-term persistent atrial fibrillation [19]. Due to the lack of clear demarcation of concepts, perhaps the most critical issue regarding terminology arises in the classification of CHF, since the definitions of terms used to describe this syndrome differ in a number of guidelines [20, 21].

The aim of this lecture is to discuss the terms used to characterize the course of chronic heart failure.

HEART FAILURE EXACERBATION

The natural course of almost any chronic pathology, if we exclude its latent forms, is cyclic with periods of exacerbation and remission. CHF is not an exception - periods of a stable (sometimes only seeming stable) course are followed by episodes of worsening of clinical symptoms, leading to a decrease in the quality of life and an increase in the risk of premature death [21-23].

Despite the fact that the description of the syndrome phase in the clinical diagnosis was not provided in Russian CHF classifications of the late 20th - early 21st century, they say the idea was in the air. So, Sidorenko during the roundtable discussion "Issues of Classification of Chronic Heart Failure", held in 1993 at the Cardiology Research Center as part of the scientific session of the Russian Academy of Medical Sciences, made a proposal, which is recorded in the transcript as follows: "the classification should also somehow provide for the possibility of temporary exacerbation and aggravation of heart failure"[1]. This proposal, the fairness of which no one disputed, is very logical because the exacerbation of CHF caused by the progression of the underlying disease and/or so-called immediate causes of decompensation (in particular, infections, anemia, arrhythmia, and instability of blood pressure) requires the activation of etiotropic, pathogenetic, and symptomatic therapeutic actions. On the other hand, fortunately, optimal drug therapy is often able to achieve cardiac compensation that justifies treatment de-escalation.

The discussed idea is still in the air, as the classification of CHF proposed by the Russian Society of Cardiology in 2023 did not include the term "exacerbation", and this term is not used in examples of diagnosis formulation: "Major[2]: CHD: postinfarction cardiosclerosis (myocardial infarction in 2019). Complications: HFmrEF stage 1. 2 FC. Pulmonary hypertension 1 FC WHO." (CHD - coronary heart disease; HFmrEF – heart failure with mildly reduced ejection fraction; FC - functional class; WHO - World Health Organization). The 2024 version excluded the description of the risk of heart failure development and replaced the term "pre-heart failure" with "pre-stage of heart failure" [18].

Apparently, the exacerbation of CHF in the diagnostic report can be described using the equivalent term "decompensation", an example of the application of which is presented by Boytsov in the leading article "Chronic Heart Failure: Evolution of Etiology, Prevalence and Mortality over the Past 20 Years" in the journal "Therapeutic Archive" [26]: "CHD. PICS (I25.2[3]); CHF IV FC, decompensation (I50.0)" (PICS stands for postinfarction cardiosclerosis). As the author rightly emphasizes, such a diagnosis well explains the reason for hospitalization or patient's appeal to the polyclinic, on the one hand, and the fact that decompensation of cardiac activity will be taken as a subject of diagnosis and treatment, on the other hand. Nevertheless, the 2023 classification of CHF by experts of the Russian Society of Cardiology

1 Classification Issues of Chronic Heart Failure. Therapeutic Archive. 1993; 65(9): 7–18 (In Russ.).

[2] Correct title of the diagnosis section - "Underlying disease".

[3] The term "old myocardial infarction" (I25.2) is used by experts from the World Health Organization only to describe cases of myocardial infarction that were detected accidentally, retrospectively, and had no clinical manifestations at the time of detection and observation of the patient, and should be distinguished from the term "postinfarction cardiosclerosis" ("Other forms of chronic ischemic heart disease") (I25.8) [25].

[18] does not provide the term “decompensation” and does not use it in the examples of diagnosis formulation.

Experts of the Heart Failure Association of the European Society of Cardiology in 2023 announced that a consensus was reached on the key issues (definition, classification, pathogenesis, epidemiology and outcomes, and treatment and prevention) of exacerbation (worsening) of CHF [27]. According to the latter, deterioration of CHF can be defined as an increase in symptoms and signs of previously diagnosed syndrome, which requires intensification of treatment, most often diuretic therapy. Unfortunately, the authors of the consensus refrained from describing clear criteria for an increase in symptoms and signs, in particular, the severity of congestion, as well as a decrease in exercise tolerance. Obviously, the magnitude of exacerbation may vary. We can only guess what the authors suppose, speaking about an increase in clinical severity of CHF.Within one functional class (FC) or one FC higher, or when CHF symptoms appear at rest, the severity of symptoms associated with hypervolemia even within the IV FC can vary significantly, ranging from complaints about swelling of the back of the feet to complaints of massive generalized swelling of subcutaneous fatty tissue. It should be added that the increasing severity of symptoms and signs of CHF is described without any reference to time frames (the rate of progression of heart failure): does the patient feel worse than a day, a week, a month or a year ago? However, symptoms and signs of decompensation in any case require optimization of CHF drug therapy aimed at achieving euvolemia [27-30].

The experts of the Heart Failure Association of the European Society of Cardiology believe that rather than providing an exhaustive description of CHF characteristics that leaves no questions unanswered and allows one to outline the boundaries of correctly applying the term “worsening of chronic heart failure”[1] it is sufficient to indicate the need to intensify therapy in this situation (an essential component of the concept definition) [27]. Considering the significant variability declared in the cited document, both in patient routing (ranging from an unconditional need for emergency hospitalization to recognizing the possibility of therapy in outpatient settings or emergency departments) and in choosing the optimal diuretic administration method (the cornerstone of therapy for patients with decompensated CHF, either intravenously or through intensified oral diuretic therapy), it can be said that the essential component of the term definition is described without particular details.

The discussed definition does not include patients with a newly diagnosed CHF [27]. This exclusion is logical since any exacerbation implies decompensation in a patient with an established CHF diagnosis. However, it is more difficult to understand why episodes of CHF deterioration associated with comorbid factors (including comorbid diseases) and noncompliance with therapy recommendations are also excluded. The direct causes of CHF decompensation (regardless of the underlying cardiovascular lesion) can be various conditions, such as infection, systemic arterial hypertension, pregnancy, anemia, heart rhythm disorders, or noncompliance with treatment or diet [23, 31]. In any case, exacerbation is exacerbation! Identifying the direct cause of CHF is important because timely diagnosis and adequate treatment can save the patient’s life. Some of the aforementioned conditions usually do not lead to heart failure, but their development in persons with cardiovascular diseases can figuratively be called the last straw contributing to the clinical manifestation of systolic and/or diastolic cardiac dysfunction [31-34].

Finally, the Heart Failure Association of the European Society of Cardiology introduces another exception: the definition of “worsening of chronic heart failure” does not include cases of exacerbation that do not require changes in heart failure treatment [27]. Once again, we note the assumption of medical arbitrariness, to which we have already expressed our attitude. In a clinical situation with increasing symptoms and signs of CHF, the diagnostic conclusion will depend on the doctor’s decision to correct the heart failure therapy. For us, it is axiomatic that, in such a situation, the doctor cannot refrain from doing something but must change the treatment in the hope of improving the patient’s condition, at least clinically.

[1] In our opinion, the best translation into Russian is “exacerbation of heart failure”.

REMISSION AND PERSISTENCE OF HEART FAILURE

The 2022 treatment guidelines for heart failure from the American College of Cardiology, the American Heart Association, and the American Heart Failure Society [35] detail the main development patterns of clinically significant heart failure, aligning closely with real clinical practice. In addition to worsening symptoms and signs of the syndrome, American experts distinguished variants with symptom resolution (including remission) and heart failure persistence. As the terms imply, a patient with resolution of symptoms and signs of CHF is said to have an absence of clinical manifestations of the syndrome [35]. The authors rightly pointed out that complete elimination of structural and functional cardiac abnormalities, labeled as remission, is rarely observed. Accordingly, the term "persistent heart failure" is proposed to denote a clinical situation in which symptoms and signs of the syndrome are preserved, as well as those of a functional activity limit.

This approach should be rated well and the Russian CHF classification should include a description of the development pattern. We believe that the severity of heart failure should be considered in the context of ongoing treatment and assessment of its effectiveness. Clearly, the probability of improvement in clinical status is much higher for a patient with the same severity of symptoms who did not receive optimal drug therapy or medical assistance in implant arrhythmology and cardiac surgery than for a treated patient [37].

ACUTE DECOMPENSATOIN OF HEART FAILURE

The course of decompensation of CHF can vary. In most cases, the clinical picture develops gradually over a few weeks. However, it can also have a rapid onset, with progression of symptoms and signs within a few hours [38]. In the latter case, when the rapid increase in severity of heart failure symptoms becomes the reason for urgent medical attention and emergency hospitalization of a CHF patient, it is recommended to use the term ADHF [18, 39].

ADHF is considered as the most common form of acute heart failure (50–70%), and it should be distinguished from pulmonary edema, cardiogenic shock, and acute right ventricular failure [18, 39]. However, this approach contradicts the provision enshrined in the duly approved industry standard (OST 91500.11.0002-2002, "Standardization System in Healthcare of the Russian Federation. Protocol for the Management of Patients. Heart Failure (I50)"), according to which : "The terms "heart failure" and "chronic heart failure" are essentially synonymous since, when discussing acute heart failure, it is customary to specify its form, such as acute (cardiogenic) pulmonary edema or cardiogenic shock." We agree with A. Xanthopoulos et al. [40] that ADHF should not be considered a form of acute heart failure.

The term ADHF has been proposed to describe patients with mild acute heart failure symptoms that do not meet the criteria for cardiogenic shock, pulmonary edema, or hypertensive crisis [41], this raises the question of the criteria indicating the transition from stable CHF to ADHF [38]. Those who advocate for viewing ADHF as a distinct phenotype of acute heart failure syndrome argue that CHF decompensation is only part of this syndrome when it manifests as a clinical picture that poses an immediate threat to life, necessitating emergency hospitalization [38]. Given the severity of clinical manifestations of the so-called classical forms of acute heart failure, it is not surprising that even senior medical students know how to diagnose pulmonary edema or cardiogenic shock, which justifies the need for emergency hospitalization [23], however, it is difficult to understand what specific clinical manifestations are hidden behind the phrase "poorly expressed symptoms of acute heart failure that do not meet the criteria for cardiogenic shock or pulmonary edema".

Russian experts rightly raise questions: Where is the boundary between progressive CHF and ADHF? Is a patient's transition from class II to class III or class III to class IV heart failure a proof of the development of ADHF? These questions would be inevitably asked by emergency room internists when deciding how to route patients with decompensation [38]. The authors of the agreed position acknowledge the absence of definitive answers to the posed questions and stress the importance of defining the criteria for ADHF development as precisely as possible (these criteria include clinical symptoms and signs of stasis

and/or hypoperfusion, as well as laboratory and instrumental markers of decompensation suitable for differential diagnosis).

Considering that the term ADHF does not always accurately reflect the timing of this condition's development [42], it is difficult to disagree with S.N. Tereshchenko et al. [20] and V.Yu. Mareev et al. [38] about the uncertain boundary between CHF, CHF exacerbation (progressive CHF), and ADHF. What basis should an emergency room physician use to decide whether to hospitalize or manage on an outpatient basis a patient with symptoms and signs of CHF decompensation? Clearly, a significant proportion of patients with CHF decompensation should be treated in outpatient settings [38, 43], with the aim of achieving and maintaining euvolemia through the use of diuretics alongside combined therapy with neurohormonal modulators and sodium-glucose cotransporter type 2 inhibitors [44-47].

What are the criteria for a life-threatening condition that requires immediate hospitalization? This question is relevant unless we are talking about pulmonary edema or cardiogenic shock, conditions that cannot be replaced by the term ADHF in the diagnostic report. We searched for the answer to this question in the ADHF registry materials, including large registries with more than 10,000 patients and smaller ones [48-53]. Despite the description of the design invariably emphasizing the need for emergency hospitalization due to the intensity of heart failure symptoms and signs, the protocols of the discussed registries often allowed for the inclusion of data on patients with heart failure whose severity at hospitalization corresponded to functional class III (up to one third of cases) or even II according to the New York Heart Association (NYHA) classification. Many people may not doubt that all CHF FC IV patients require immediate hospitalization, but one should try to convince oneself that heart failure with a severity corresponding to classes II-III according to the New York Heart Association classification (remember, patients have no symptoms at rest) is an indication for emergency hospitalization.

Since the need for emergency hospitalization is unclear for many reasons and is the only substantial criterion for recognizing ADHF, attempts are being made to replace the term ADHF as one of the form of acute heart failure with "heart failure requiring hospitalization". However, leading cardiologists have reasonable objections to the introduction of this term not only because of the aforementioned lack of clear indications for hospitalization, but also because the latter depends on clinical practice and the capabilities of medical institutions in different regions [20, 54]. The decision to hospitalize a patient with heart failure depends on the characteristics of the patient, physician, hospital, and insurance policy, but the practice of hospitalization differs by region and is gradually changing; recently, the provision of medical care has become increasingly common in alternative settings, such as outpatient or emergency departments [54, 55].

The same arguments can be used against the use of the term ADHF itself or, at the very least, against using the location as an essential criterion for recognizing it [55]. The decision to hospitalize should be based on a clinical diagnosis and an evaluation of important characteristics of heart failure. Again, the decision to hospitalize patients, prescribe, continue, or discontinue therapy should not be considered as a basis for determining the type, stage, or phase of the disease or syndrome. Heart failure will not fundamentally change; an exacerbation will remain an exacerbation, even if the physician manages decompensation on an outpatient basis or optimization of therapy with diuretics, without the use of intravenous drugs, is sufficient [27].

In any case, patients should be evaluated after therapy initiation for ADHF to determine decompensation trajectory (improvement, worsening, or persistence), which affects therapeutic tactics and prognosis [36, 42]. Due to the absence of consistent guidelines for hospitalization and/or emergency care, as well as precise time criteria for distinguishing between scenarios with rapid and gradual CHF progression, the authors of the universal heart failure definition and classification system appropriately opt for the term "decompensated heart failure" (without the term "acute") to describe a condition characterized by escalating symptoms and/or signs of heart failure, regardless of CHF progression rate [56].

CONCLUSION

The characterization of CHF should be an integral part of clinical diagnosis based on appropriate classification. This classification can be an effective

clinical tool only if its terms have unambiguous meanings and clearly defined application boundaries. Despite the ongoing discussion about the demarcation of concepts and repeated attempts to reach an agreement, experts from authoritative cardiology communities in the Old and New Worlds cannot reach a consensus, and the definitions of terms used to describe CHF differ in a number of guidelines.

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Constitutional and Biological Predictors of the Risk of Suicidal Behavior in Mental Disorders

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ABSTRACT

The prevention of suicidal behavior is an extremely important issue in modern psychiatry and is of high social importance due to high prevalence of this phenomenon. Despite the availability of a number of psychometric scales for assessing suicide risk, their use may be limited, since due to the mental state of patients, it is not always possible to assess the risk of suicide.

Thus, the search for potential structural and peripheral biomarkers of a suicidal behavior risk is a pressing issue in psychiatry. Existing studies are usually limited to searching for one or several markers or factors and do not take into account the integrity of the human body with its inherent complementarity of both pathogenic and sanogenic factors, including social and environmental, compensatory mechanisms, adaptation threshold, and reversible and irreversible decompensation.

To date, there is no single point of view that fully explains the genesis of suicidal behavior, and the potential biological factors vary greatly depending on the methods used. Based on data collected from recent studies examining a variety of biological markers associated with suicide, it can be confirmed that suicidal behavior in individuals with mental disorders is a complex, multifactorial, and polygenic mental state.

Keywords: suicidal risk, suicide, parasuicide, predictors, risk factors, mental disorders

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Конституционально-биологические предикторы риска суицидального поведения при психических расстройствах

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РЕЗЮМЕ

Профилактика суицидального поведения является чрезвычайно важным вопросом современной психиатрии и имеет высокую социальную значимость в связи с большой распространенностью данного феномена.

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Несмотря на наличие ряда психометрических шкал для оценки суицидального риска, их применение может быть ограничено, поскольку в силу психического состояния пациентов далеко не всегда удается провести оценку риска самоубийства.

Таким образом, поиск потенциальных структурных и периферических биомаркеров риска суицидального поведения является актуальным направлением в психиатрии. Имеющиеся исследования, как правило, ограничены поисками одного или нескольких маркеров или факторов и не учитывают целостности человеческого организма со свойственными ей комплементарностью как патогенных, так и саногенных факторов, включая социально-средовые, компенсаторными механизмами, порогом адаптации, обратимой и необратимой декомпенсацией.

На сегодняшний день не существует единой точки зрения, полностью объясняющей генез суицидального поведения, а потенциальные биологические факторы сильно различаются в зависимости от используемых методов. На основе данных, собранных в ходе недавних исследований, изучающих разнообразные биологические маркеры, связанные с суицидом, можно подтвердить, что суицидальное поведение у лиц с психическими расстройствами является сложным, многофакторным и полигенным психическим состоянием.

Ключевые слова: суицидальный риск, суицид, парасуициды, предикторы, факторы риска, психические расстройства

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

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INTRODUCTION

Suicidal behavior remains one of the most pressing issues in clinical psychiatry due to its high prevalence among patients [1, 2]. According to numerous studies, approximately 90% of individuals who have ever attempted suicide have a mental disorder [2]. The risk of suicide is 20 times higher in patients with depressive and bipolar disorder and 10–13 times higher in patients with schizophrenia compared to the general population [2, 3].

Suicidal behavior is a complex and multifactorial process encompassing various forms and manifestations of mental activity aimed at taking one's own life intentionally. The study of suicidal phenotypes within psychopathology is crucial for elucidating the nature of risk factors, predicting, and preventing suicide.

Previously, suicide risk factors in patients with mental disorders were proposed, including previous suicide attempts, early age of disease onset, substance abuse, pronounced delusions and hallucinations, severe neurocognitive deficits, patient's illness perception, etc. [4–7]. However, predicting suicidal behavior based on these factors remains unreliable, since demographic factors are not universal, and clinical factors are subject to change, depending on both the course of the pathological process itself and the implemented treatment and rehabilitation measures.

In this regard, the study of the constitutional and morphological type (morphological phenotype, morphophenotype, constitutional type) of patients, which is a structural biomarker of reactivity that is not subject to significant changes in adults, is a promising direction in the search of predictors of the suicidal behavior risk. The most comprehensive studies in this area have been conducted on a schizophrenia model. Based on the results of these studies, N.A. Kornetov [8–10] formulated an anthropological paradigm that showed the role of constitution in the course and outcome of the

disease, opening up possibilities for predicting pathokinesis.

The pathogenesis of many mental disorders is believed to begin long before the onset of the main symptoms, during critical periods of brain development [11]. Adverse conditions during prenatal development can lead to changes in the brain structures responsible for perception and emotional regulation. In addition, abnormalities in the development of the nervous system, including dysfunctions of the neurotransmitter systems (dopaminergic, serotonergic, glutamatergic, etc.), have been associated with both the risk of developing many mental disorders and suicidal behavior [11, 12]. It is assumed that peripheral biomarkers characterizing brain neuroplasticity, damage to neuronal structures and nervous tissue, neurotoxicity, immune inflammation, breakdown of monoamines and catecholamines, and carriage of certain polymorphisms of the genes encoding these biomarkers also contribute to the genesis of suicidal behavior to some extent [13, 14]. Thus, ignoring the biological mechanisms underlying suicidal behavior in patients with mental disorders may decrease the quality of this prognosis.

In light of the above, we have summarized and systematized data on the constitutional and biological factors involved in the genesis of suicidal behavior in individuals with mental disorders. The data presented will form the basis for the development of predictive models of suicide risk, which will ultimately contribute to reducing the burden of suicide among patients.

We classified constitutional and biological predictors of the risk of suicidal behavior as constitutional and morphological, structural and functional, genetic, and molecular.

THE ROLE OF CONSTITUTIONAL AND MORPHOLOGICAL PREDICTORS IN THE SUICIDAL BEHAVIOR GENESIS

The theories of body types proposed by E. Kretschmer [15] and W.H. Sheldon [16] are historical concepts that attempted to correlate somatotypes and mental disorders. Accordingly, E. Kretschmer distinguished four constitutional and morphological types of people: 1) the asthenic type, which has a slender build and is more prone to developing schizophrenia; 2) the pyknic type, which has a round and soft physical build and is probably more prone to bipolar disorder; 3) the athletic type which is more prone to epilepsy; and 4) the dysplastic type, which cannot be classified as any of the other three types [15].

W.H. Sheldon had similar criteria, classifying body types into ectomorphic (asthenic type), endomorphic (pyknic type), and mesomorphic (athletic type). He also believed that there is a deeper, genetically determined association between the somatotype and personality traits [16]. However, these classifications were based only on observational studies at the beginning of the XX century.

Later, the concept of anthropometry and the role of constitution in the development and course of mental disorders was continued by Russian authors [8, 17, 18]. The accumulated research and theoretical experience has proven the feasibility of identifying the main constitutional and morphological types in the clinical analysis of somatic-symptom and mental disorders [8]. With regard to the role of anthropometric characteristics of suicidal individuals, it was established that the asthenic type is associated with unfavorable clinical dynamics of schizophrenia and suicidal behavior [17].

The predominance of the asthenic body type in patients with pronounced hypochondriacal symptoms also supports the constitutional and morphological predisposition of suicidal behavior in schizophrenia [18]. In the study by A.A. Zalivin [19], the role of constitutional features of suicidal individuals with mental disorders in the post-suicide period was established.

The analysis of the distribution of somatic sexual differentiation of suicidal individuals by cohorts of the post-suicide period showed that suicide ideation in the post-suicide period was associated with the dysplastic body type. The remaining cohorts were primarily characterized by the normosthenic body type, secondarily – by the hypersthenic body type in the critical period, and by the hypersthenic and asthenic body types in the manipulative period.

A number of studies on individual anthropometric parameters have also been conducted in this context. E. Laakso et al. [20] examined excess weight as a risk factor for suicidal behavior. The authors found

that girls with suicide attempts were more likely to be overweight and to have affective and eating disorders and anxiety. A similar conclusion was reached by M.Z. Zhang et al. [21] – overweight and obesity were associated with an increased risk of suicidal ideation (for overweight, odds ratio (OR) = 1.10; 95% confidence interval (95% CI) 1.01–1.20; for obesity: OR = 1.17; 95% CI: 1.01–1.35) and suicide attempts (for overweight: OR = 1.12; 95% CI = 1.02–1.23; for obesity: OR = 1.12; 95% CI: 1.00–1.25). Subgroup analysis showed that the associations between overweight/obesity and suicide attempts were significant only for girls.

The study including an older age group with adjustments for covariates revealed that overweight and obese young adults (19–44 years) (OR = 1.18, $p < 0.01$), underweight and thin middle-aged adults (45–64 years) (OR = 1.32, $p < 0.05$), and obese elderly people (65 years and older) (OR = 1.19, $p < 0.05$) were more likely to have suicidal ideation compared to age-matched healthy-weight individuals [22].

The results of this study show that the association between body weight and suicidal ideation varies by age group. An increase in the body roundness index (BRI), proposed by D.M. Thomas et al. [23], led to an increased likelihood of suicidal ideation; individuals with the highest BRI had suicidal ideation 1.52 times more often, regardless of sociodemographic features [24].

In an analytical review, J. Zhang et al. [25] indicated an inverse relationship between body mass index (BMI) and the risk of completed suicide, regardless of the region of residence and gender of the study participants. In general, among men, high BMI was associated with a low risk of suicidal behavior, whereas among women, high BMI was associated with an increased risk of unsuccessful suicide attempts.

Therefore, the discrepancies in the results obtained from studies of individual anthropometric parameters indicate the need for a comprehensive approach to identifying structural biomarkers of suicidal behavior, especially since body weight fluctuates over a lifetime both up and down. The use of integrated body type parameters based on skeletal muscle measurements (constitutional and morphological type and somatic sexual differentiation) is a more promising and reliable tool for assessing the risk of suicidal behavior, as they are relatively stable during ontogenesis and are genetically determined.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE BRAIN IN PATIENTS WITH SUICIDAL BEHAVIOR

The study of neurophysiological changes underlying suicidal behavior has some limitations due to difficulties in analyzing brain structure and function. Nevertheless, such studies are widely represented in the literature. Most neuroimaging studies in suicidal individuals focus on the prefrontal cortex. This region is involved in stress response, executive functions, and psychomotor skills [26].

Studies of the prefrontal cortex in patients with suicide attempts have shown changes in activation patterns, leading to social maladjustment and impaired decision-making related to reward [3, 27]. Structural magnetic resonance imaging (MRI) also clearly demonstrates a decrease in the volume (thickness) of gray matter in the ventromedial prefrontal cortex in patients with suicide attempts compared to healthy controls [3, 28, 29], which confirms the role of prefrontal cortex dysfunction in the genesis of suicidal behavior. In addition, multimodal neuroimaging studies combining structural and functional imaging methods (MRI and positron emission tomography (PET)) showed a significantly smaller volume of gray matter in the cerebellum, right orbitofrontal cortex, and hippocampus in young patients with bipolar disorder with suicide attempts compared to similar patients without suicide attempts. What is more, in the uncinate fasciculus and ventral and right cerebellar areas, a decrease in white matter integrity and a decline in functional connectivity between the amygdala, right rostral prefrontal cortex, and left ventral prefrontal area were observed [30].

Impaired functioning of neural networks responsible for behavior and emotion regulation may be associated with alterations in thalamocortical pathways, potentially increasing the risk of suicidal behavior in patients [31]. The analysis of brain activity patterns using electroencephalography (EEG) indicated associations between the degree

of suicidal intent and decreased cortical function in patients with depression [32, 33].

Similar results were obtained in one of our studies on patients with affective disorders, where lower alpha and theta power and pronounced interhemispheric asymmetry with a predominance of the right hemisphere were found in patients with a history of suicide attempts [34]. An increased risk of suicide in patients with depression may be associated with relatively low mean (0.5–5 Hz) EEG coherence in the frontal and occipital regions, as well as a decrease in the amplitude of changes in the mean coherence (0.5–45 Hz) in the prefrontal cortex in response to emotional stress [35, 36]. These data also indicate a decline in brain resources in suicidal individuals and are consistent with the results of evoked potential tests [37, 38].

In relation to patients with schizophrenia, who also have a relatively high risk of suicidal behavior, we previously found lower baseline beta power and a weak activation response (Berger effect) in those with a history of suicide attempts [39]. These parameters were significant factors in a model for predicting suicide attempts in patients with schizophrenia. Lower amplitude values and increased latency of evoked potentials were also observed in patients with schizophrenia and suicide attempts [40].

Thus, the results of the studies reviewed above suggest that patients at high risk for suicidal behavior exhibit reduced activity in brain structures, which may be responsible for reduced adaptive responses to stress. However, the structural and functional characteristics identified in suicidal individuals do not serve as screening tools and merely complement the clinician's assessment of a suicide risk.

GENETIC PREDICTORS OF THE SUICIDE RISK

The constitutional and morphological as well as structural and functional factors discussed above are determined by the genotype. In terms of the biological basis of suicidal behavior, there is currently a growing body of research examining the role of genetic factors. These studies demonstrate that suicidal behavior is determined both by genetic and hereditary factors, with the heritability of suicidal behavior accounting for approximately 43% [41]. Furthermore, genetic studies demonstrate a high association between mental disorders and suicidal behavior [42–44].

To date, more than 2,500 genes associated with suicidal behavior have been identified, 40 of which are linked to the cell cycle and DNA repair [45]. In a large study of the European population, two significant loci were found, including six single-nucleotide polymorphisms (rs34399104, rs35518298, rs34053895, rs66828456, rs35502061, and rs35256367), associated with the risk of suicide [42]. According to the results of another study, including 122,935 participants, three more polymorphisms were found to increase the risk of suicide (rs62535711, rs598046, rs7989250) [43]. The role of other polymorphisms in the genesis of suicidal behavior in mental disorders is also suggested, for example, rs4809706, rs4810824, and rs6019297 [44].

Associations of genes involved in various signaling pathways with suicidal behavior in patients have also been established [46–48]. A 20% increase in CD68 mRNA levels was found in the prefrontal and anterior cingulate cortex of individuals with completed suicide, which in turn explains the increase in cerebral cytokines, including tumor necrosis factor α and interleukin-1β (IL-1β) [49]. A potential role in the genesis of suicidal behavior is also attributed to changes in the expression of genes involved in the biosynthesis of gamma-aminobutyric acid (GABA) and adenosine triphosphate (ATP) [50]. Additionally, increased DNA-dependent ATPase activity has been found in suicidal individuals, and changes in the expression of polyamines (involved in immunity, oxidative stress, cell proliferation / apoptosis) and their metabolic enzymes suggest their potential role in suicidal behavior in patients [51]. Changes in the expression of genes encoding catechol-O-methyltransferase (COMT), brain-derived neurotrophic factor (BDNF), monoamine oxidase A (MAOA), serotonin (5-HTTLPR, HTR2A), and adrenergic receptors (ADRA2A, ADRA2B) are associated with an increased risk of suicide in patients with mental disorders [52].

Modern data confirm the heritability and genetic predisposition of the suicide risk. At the same time, accumulated data suggest a polygenic nature of

suicidal behavior. Thus, the heritability of suicide attempts may be caused by the accumulation of relevant genes, an example of pleiotropic interaction and/or epistasis. Therefore, the underlying causes of suicide can be diagnosed early for preventive treatment.

MOLECULAR MARKERS OF A SUICIDE RISK

Attempts to identify peripheral biomarkers that can predict suicidal behavior have long been made by assessing serotonin metabolism disorders [53–55]. Low serotonin activity has been associated with suicide in the general population [53]. One early study demonstrated significantly lower concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid of individuals with major depression who had committed suicide [55].

Clearly, the pathophysiological changes in suicidal behavior are not limited to the functioning of monoamine metabolism. Using high-throughput technologies, in particular microarray-based gene expression profiling, made it possible to identify molecular pathways that were previously not suspected of involvement in suicidal behavior: GABAergic, glutamatergic, and polyamine neurotransmission [56].

The role of inflammation in suicidal behavior was proposed as early as in 1993, when elevated levels of IL-2 were found in individuals who had attempted suicide [57], and this was later confirmed in other studies, backing the links between immune system imbalance and the pathophysiology of suicide [58, 59]. Some authors point to a potential role of IL-6 in the genesis of suicidal behavior [59–61]. The presence of IL-6 receptors on brain cells confirms its effect on neurons, thereby causing aggressive / helpless behavior by regulating monoaminergic neurotransmitters and their metabolites in the central nervous system, in addition to synaptic transmission and regulation of neuroplasticity [59, 60]. Furthermore, studies have shown that patients with suicide attempts have elevated concentrations of tumor necrosis factor α, transforming growth factor β1, vascular endothelial growth factor, kynurenic acid, IL-1β, and IL-6 and lower levels of interferon γ, IL-2, and IL-4 [61].

Apparently, suicidal behavior is associated with dysfunction of the hypothalamic – pituitary – adrenal and hypothalamic – pituitary – thyroid axes [62–67]. Elevated cortisol levels activate microglia and cause neuroinflammation, disrupting BDNF function, and then induce neurotoxicity, leading to neuronal death [62]. Suicidal individuals were found to have higher levels of cortisol in saliva, cerebrospinal fluid, and plasma than healthy volunteers [63, 64]. Patients with depression and suicide attempts were found to have elevated levels of corticotropin-releasing hormone in the paraventricular nucleus of the hypothalamus, forebrain, and locus coeruleus [65]. Suicide attempts are also significantly more common in patients with hypothyroidism [66, 67]. There is a suggestion that low thyroid-stimulating hormone levels may be associated with a predisposition to depression and suicidal behavior [67].

Some studies suggest that cholesterol levels may be a potential marker of depression and suicide risk [68]. A meta-analysis of primary prevention trials of statins (cholesterol-lowering drugs) showed that they reduce the risk of cardiovascular mortality but increase the risk of suicide [69]. On the one hand, studies show that patients at high risk of suicidal behavior had lower total cholesterol levels than the control group [68, 69]. On the other hand, Y. Molero et al. [70] did not find any significant associations between cholesterol levels and suicidal behavior.

CONCLUSION

To date, there is no unified perspective that fully explains the genesis of suicidal behavior, and potential biological factors in its development vary significantly across studies, depending on the methods and approaches used. Existing studies are typically limited to searching for one or a few markers or factors and do not consider the integrity of the human body, with its inherent complementarity of both pathogenic and sanogenic factors, including socio-environmental factors, compensatory mechanisms, adaptation thresholds, and reversible and irreversible decompensation.

Based on data collected in recent studies examining a variety of biological markers associated with suicide, it can be confirmed that suicidal

behavior in individuals with mental disorders is a complex, multifactorial, and polygenic mental state, and a relevant area of research.

Understanding the genesis of suicidal behavior provides the basis for its prevention. Further improvement of molecular genetic methods, neuroimaging technologies, and brain function is necessary to uncover neural networks and their molecular and biochemical associations with the risk of developing suicidal behavior in patients in order to develop new pathogenetically based prediction models of suicidal tendencies.

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Brain-derived Neurotrophic Factor: Significance in the Physiology and Pathology of the Cardiovascular System

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ABSTRACT

The lecture provides an analysis of literature data on the role of brain-derived neurotrophic factor (BDNF) in the development and functioning of the cardiovascular system and its involvement in the heart and blood vessels pathogenesis. The information is structured according to the multifunctional properties and effects of BDNF allowing for the BDNF to be considered as a therapeutic target for attenuating myocardial dysfunction and restoring cardiac function during ischemia/reperfusion.

The lecture contains data on the ability of neurokine to exert a cardioprotective effect by activating angiogenesis and neovascularization of ischemic myocardial tissue via increasing endotheliocyte viability. It is known that vegetative tone is the most important indicator of the state of the cardiovascular system. The nature of BDNF affecting the activity of sympathetic and parasympathetic neurons is yet to be determined. However, the current prevailing view is that BDNF regulates heart rate by enhancing parasympathetic activity of the brainstem structures. Based on experimental and clinical data, the prospects for the use of neurokine analogs in cardiology practice are considered.

Keywords: brain-derived neurotrophic factor, heart, blood vessels, angiogenesis, cardioprotection, autonomic regulation of heart

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Нейротрофический фактор мозга: значение в физиологии и патологии сердечно-сосудистой системы

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РЕЗЮМЕ

В лекции проведен анализ литературных данных о роли нейротрофического фактора мозга (BDNF) в развитии и функционировании сердечно-сосудистой системы и его участии в патогенезе сердца и сосу-

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дов. Информация структурирована в соответствии с многофункциональными свойствами и эффектами BDNF, позволяющими рассматривать нейротрофический фактор мозга в качестве терапевтической мишени для ослабления миокардиальной дисфункции и восстановления деятельности сердца при ишемии/реперфузии.

Приведены данные о способности нейрокина оказывать кардиопротекторное действие посредством активации ангиогенеза и неоваскуляризации ишемизированной ткани миокарда путем повышения жизнеспособности эндотелиоцитов. Известно, что вегетативный тонус является важнейшим показателем состояния сердечно-сосудистой системы. Вопрос о характере влияния нейротрофического фактора мозга на активность симпатических и парасимпатических нейронов остается открытым. Однако в настоящее время преобладает мнение о том, что BDNF регулирует частоту сердечных сокращений, усиливая парасимпатическую активность стволовых структур головного мозга. На основании экспериментальных и клинических сведений рассматриваются перспективы применения аналогов нейрокина в кардиологической практике.

Ключевые слова: нейротрофический фактор мозга, сердце, сосуды, ангиогенез, кардиопротекция, вегетативная регуляция сердца

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

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

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INTRODUCTION

The key representative of the neurotrophin/neurokine family is brain-derived neurotrophic factor (BDNF) [1, 2]. The scientific literature provides extensive information on the neuroprotective effects of BDNF including its positive influence on the growth, development, and regeneration of the nervous system. There is substantial evidence that, in addition to regulating neuroplasticity, BDNF is involved in the pathogenesis of many immune, inflammatory, and metabolic reactions in the body [3–5]. Neurotrophin acts through tyrosine kinase receptors associated with tropomyosin-related kinase (TrkB-type) receptors, which are also synthesized in non-neuronal tissues [3–5], including the heart and blood vessels [6].

Studying the role of BDNF in the physiology and pathology of the cardiovascular system is of particular interest due to its involvement in nervous regulation of heart function [7–10]. It is known that TrkB-type BDNF receptors are localized in neurons of the hypothalamus and brainstem, where the control centers of the cardiovascular system are situated [11, 12]. Neurokine also influences the development and metabolism of the sympathetic nervous system (SNS), acting as a trophic factor and regulator of cardiac nerve growth and axon branches [13]. Accordingly, alterations in sympathetic regulation of cardiac activity may be associated with dysfunctions in BDNF signaling mechanisms [6]. Evidence suggests that BDNF modulates heart rate by enhancing parasympathetic activity within brainstem structures [14].

In many cases, disorders or alterations in BDNF synthesis are associated with cardiovascular diseases, such as high blood pressure (BP), arrhythmias, myocardial infarction (MI), and atherogenesis [6]. An analysis of recent scientific data indicates that BDNF plays a fundamental role in assessing the risk of cardiovascular diseases, since lower concentrations of BDNF are often linked to these conditions [15].

The lecture focuses on the analysis of data regarding the role of BDNF in the physiology and pathology of the cardiovascular system, highlighting its potential as a promising therapeutic target for reducing myocardial dysfunction and restoring cardiac activity.

THE ROLE OF THE BDNF/TRKB AXIS IN THE PHYSIOLOGY OF THE HEART AND BLOOD VESSELS

BDNF is directly involved in the formation and development of the cardiovascular system during the prenatal period [1, 16]. Primarily, this pertains to BDNF's role in angiogenesis: increased expression of BDNF/TrkB receptors occurs in the coronary artery endothelium [1], contributing to capillary development in heart tissue during late pregnancy [17–19]. During embryogenesis, neurotrophin participates in forming the coronary vessel wall through direct angiogenic action on endothelial cells expressing tropomyosin receptor kinase B (TrkB) [1]. BDNF deficiency leads to endotheliocyte apoptosis, a lack of significant intramyocardial blood vessels, ventricular wall hemorrhage, atrial septal defects, decreased cardiac contractility, and early postnatal death in mice [20–23].

The critical role of neurotrophin in cardiac physiology is confirmed by pioneering experiments conducted by a large group of Chinese researchers [24]. These studies involved cardiomyocytes from the developing mouse heart with suppressed BDNF expression under the control of the myosin heavy chain 6 (MYH6) promoter. It was found that removing BDNF from cardiomyocytes did not affect heart growth and development. However, subsequent pathological changes were observed in young animals, including cardiomyocyte death, myocardial degeneration, thrombosis of the left atrial appendage, reduced cardiac function, increased inflammation, and reactive oxygen species (ROS) generation, as well as metabolic disturbances [24].

Furthermore, suppression of BDNF expression at the stage of cardiomyocyte development impaired regenerative processes after MI in hearts of adult animals. The authors concluded that BDNF synthesized in cardiomyocytes is essential for maintaining structural and functional integrity of adult cardiac muscle and for regeneration following MI [24].

During embryogenesis, BDNF stimulates the development of the cholinergic phenotype in autonomic brainstem neurons and enhances their viability [14]. Its involvement in the neurogenesis of sensory and sympathetic neurons has also been demonstrated [25, 26].

In adult mammals, BDNF participates in the autonomic regulation of cardiac activity and exhibits significant angiogenic and angioprotective effects [1, 22, 23]. M. Cefis et al. provided evidence that endothelial-derived BDNF functions as a nitric oxide-dependent autocrine factor produced by endothelium that influences the vessel wall condition [27]. Research by B.L.Wang et al. showed that rats engaged in regular physical activity exhibited increased myocardial angiogenesis and improved cardiac function; these effects were attenuated by the BDNF K252a blocker [28].

Experiments involving BDNF microinjections into the subfornical organ of rats revealed a significant decrease in blood pressure without notable changes in heart rate [7], suggesting that this brain region is a site where circulating BDNF can influence the cardiovascular system state [7]. However, direct involvement of BDNF in blood pressure regulation has been demonstrated [29] at the level of catecholaminergic signaling between neurons of the nucleus tractus solitarius and the paraventricular hypothalamic nuclei (PVN).

The authors observed decreased sensitivity of PVN neurons to inhibitory beta-adrenergic hypotensive input from the nucleus tractus solitarius — a phenomenon attributed to BDNF-mediated downregulation of β1-adrenergic receptor expression in PVN, resulting in increased blood pressure [29]. Research by N. Feng et al. highlighted BDNF's role in calcium ion (Ca^{2+}) circulation within cardiomyocytes [23]. It was established that myocardial contraction and relaxation mediated by Ca2+/calmodulin-dependent protein kinase II involve this neurokine and TrkB receptors [23].

Experiments on TrkB knockout mice revealed impaired inotropic processes within the heart [23]. The authors believe that the BDNF/TrkB signaling pathway includes a previously unrecognized mechanism whereby the peripheral nervous system directly influences myocardial function alongside beta-adrenergic control. In hippocampal neuron cultures, activation of the BDNF–TrkB complex via PLCγ/IP3 signaling led to increased intracellular calcium levels [30]. This calcium rise promotes myosin II activation and facilitates translocation of cytoplasmic protein Drp1 (dynamin-related protein 1) from cytoplasm to mitochondria, accelerating mitochondrial fission and ATP synthesis [30].

Furthermore, binding of BDNF to its TrkB receptor activates PI3K and Akt kinases within the brain [31], leading to mechanistic target of rapamycin (mTOR) activation — a key regulator of cell growth and metabolism. The latter stimulates translation of mRNAs encoding glucose transporter GLUT3 and monocarboxylate transporter 2, thereby enhancing cellular uptake of glucose and lactate [31]. Based on these findings, the authors assume that a similar mechanism may operate in cardiomyocytes, where activation of downstream signaling pathways by the BDNF–TrkB complex promotes mitochondrial fission and ATP production, supporting energy supply to cardiomyocytes and exerting a protective effect on the heart [30].

Thus, these data support the notion that neurokine BDNF largely governs processes related to heart and vascular system development and functioning. Its key effects include angiogenic and angioprotective actions, improved energy supply to cardiomyocytes, participation in maintaining intracellular homeostasis of calcium ions, ultimately contributing to enhanced cardiac contractility.

The main aspects of BDNF's multifunctional activity under cardiovascular pathology are discussed further in subsequent sections.

THE ROLE OF BDNF IN THE PATHOLOGY OF THE CARDIOVASCULAR SYSTEM

Numerous data indicate that BDNF plays a significant role not only in physiological processes but also in the pathology of the cardiovascular system [2, 30]. The BDNF/TrkB complex is known to be expressed within the cardiovascular system and is closely associated with the development and outcomes of cardiovascular diseases (CVD) including coronary heart disease, heart failure, cardiomyopathy, hypertension, and metabolic disorders [32]. In this regard, considerable efforts by researchers are focused on studying the contribution of BDNF to the pathogenesis of heart diseases and exploring the potential use of neurokine analogs in cardiological practice [2, 30].

CORONARY HEART DISEASE

Endothelial dysfunction is considered as an initiating factor in the formation of atherosclerotic lesions and is associated with all stages of atherosclerosis. The vascular endothelium lines the luminal surface of blood vessels and functions as a physical barrier that regulates the movement of plasma proteins and circulating cells through the blood vessel. Dysfunction of this barrier leads to lipoprotein leakage and extravasation of monocytes into the vascular walls, thereby accelerating atherosclerosis [33]. ROS play a crucial role in the pathogenesis of coronary artery disease and plaque instability [34].

Studies by J. Ejiri et al. have shown that macrophages, smooth muscle cells, and fibroblasts are the main sources of BDNF within human atherosclerotic plaques [34]. In this context, BDNF can contribute to plaque instability due to its ability to induce oxidative stress and promote superoxide radical formation [34–36] by activating the NAD(P)H oxidase system in coronary vessels [34]. Elevated BDNF levels in coronary vessels are also associated with platelet activation and an inflammatory response [2, 37]. Consequently, increased neurotrophin levels may exacerbate this pathology under these conditions.

However, other studies have found that plasma BDNF concentrations are inversely correlated with levels of triglycerides, LDL cholesterol, and fibrinogen [2, 38]. Interestingly, plasma BDNF levels have been identified as an independent predictor of both coronary and overall mortality [2]. Furthermore, serum BDNF concentrations in patients with coronary heart disease have been linked to inflammatory biomarkers, such as soluble P-selectin and procoagulant platelets [37].

Experimental studies demonstrate that mice lacking BDNF exhibit impaired survival of coronary artery and capillary endotheliocytes, whereas overexpression of BDNF in cardiac tissues promotes increased capillary density [19]. There are reports indicating that BDNF levels decrease in blood samples from patients with acute coronary syndrome [2, 39].

Finally, according to H. Jiang et al., activation of TrkB receptors can stimulate vascular endothelial cadherin synthesis and restore endothelial barrier integrity during atherogenesis in coronary heart disease [20].

Thus, most authors agree about the protective role of BDNF in coronary heart disease, while elevated serum BDNF levels are associated with a

reduced risk of coronary heart disease and mortality [36, 38, 40, 41].

ISCHEMIC INJURIES AND MYOCARDIAL INFARCTION

It is well established that myocardial ischemia/reperfusion (I/R) injury manifests primarily through cardiomyocyte necrosis and apoptosis, reperfusion-induced contractile dysfunction of the heart, arrhythmias, and endothelial dysfunction of the coronary artery, which can lead to incomplete restoration of coronary perfusion [42, 43]. Signaling pathways associated with BDNF play a crucial role in cardioprotection mechanisms during the development of myocardial infarction or hypoxia accompanied by reoxygenation [44].

P. Hang et al. demonstrated that BDNF significantly inhibited cardiomyocyte apoptosis by upregulating the expression and activity of Bcl-2 and decreasing the expression and activity of caspase-3 in ischemic myocardium [45]. In another study, P. Hang et al. also demonstrated that BDNF exerted a cardioprotective effect by reducing the pro-apoptotic influence of miRNA-195 in rat cardiomyocytes following myocardial ischemia/reoxygenation [46].

Recent studies indicate that TrkB receptor expression in myocardial cells decreases after cardiac ischemia, with BDNF binding to another subtype of NT receptors, p75NTR [30]. Under hypoxic conditions, BDNF activates p75NTR and converts it into the TrkB receptor, thereby promoting myocardial cell proliferation. Reactivation of p75NTR after hypoxia enhances BDNF activity. Consequently, increased BDNF expression under hypoxic conditions can be achieved through p75NTR activation [30]. The authors conclude that BDNF protects the heart, likely by suppressing apoptosis through reduced expression of caspase-3 and cleaved caspase-9 [30].

The anti-apoptotic effect of the neurotrophic factor was confirmed by other Chinese researchers in experiments involving a model of left coronary artery occlusion in rats [47]. These researchers succeeded in stimulating BDNF synthesis by upregulating sirtuin deacetylase 1 (SIRT1), which ultimately improved cardiac inotropic function and decreased cardiomyocyte apoptosis [47].

It has been established that BDNF promotes neovascularization in ischemic tissue by recruiting endotheliocytes [48]. Mice lacking BDNF exhibit high mortality in postnatal ontogenesis due to impaired endothelial adhesion, accompanied by numerous hemorrhages in cardiomyocytes [3], which indicates BDNF's involvement in angiogenic processes [21]. Some authors suggest that BDNF's pro-angiogenic role is realized through two mechanisms: (a) local activation of TrkB receptors expressed on endotheliocytes; and (b) involvement of bone marrow-derived cells that facilitate neovascularization [1, 21, 48].

Thus, BDNF activates factors that stimulate cardiomyocyte survival and angiogenesis following MI [21, 22]. Both *in vitro* and *in vivo* models have demonstrated that BDNF triggers anti-ischemic protective mechanisms in the myocardium via signaling pathways involving vascular endothelial growth factor [21, 49], protein kinase B (Akt) [50], transient receptor potential channels (TRPC) [42, 51], and macrophage activation [19, 52].

Studies have shown that exogenous delivery of BDNF improves angiogenesis and enhances contractile function of the left ventricle [22]. It has been observed that myocardial BDNF levels decrease in models of heart failure in mice and humans with heart failure [53]. According to these researchers, mice with TrkB receptor knockouts exhibit a reduced adaptive cardiac response to exercise, accompanied by diminished activation of transcription factor networks that regulate mitochondrial biogenesis and metabolism, including the coactivator of the 1-alpha gamma receptor PGC-1a [53].

Following pathological stress, such as transaortic constriction (TAC), mice with the *cTrkB* gene knockout experienced progression of heart failure. Additionally, these scientists observed a decrease in PGC-1α levels in *cTrkB* knockout mice, which is one of the key regulators of mitochondrial biogenesis in striated muscles [53]. Consequently, under conditions of physical exertion or stress (TAC), there is a significant reduction in energy supply processes to the heart in experimental animals lacking the *cTrkB* gene. Furthermore, these researchers identified that BDNF induced an increase in PGC-1α and bioenergetic levels via a novel signaling pathway involving the pleiotropic transcription factor Yin Yang 1 [53].

Further studies confirm that BDNF plays a crucial role in regulating cellular energy in an ischemic heart [53, 54]. The findings of another research team also suggest that neurotrophic factor can improve the condition of ischemic myocardium by reducing mitochondrial dysfunction in cardiomyocytes and thereby increasing ATP production [54]. As an *in vitro* model of mitochondrial dysfunction, P. Hang et al. employed rotenone (Rot), a specific inhibitor of mitochondrial respiratory complexes.

They found that the neurotrophic factor mimetic 7,8-dihydroxyflavone (7,8-DHF) dose-dependently prevented Rot-induced cell death [54]. In this context, treatment with 7,8-DHF resulted in decreased lactate dehydrogenase release and mitochondrial ROS production, as well as restoration of mitochondrial membrane potential [54]. The authors suggest that one possible molecular mechanism underlying the mitoprotective effect of 7,8-DHF involves a signaling pathway mediated by the cardiomyocyte protein p-STAT3 [54]. In experiments conducted by the same team of Chinese scientists, the mitoprotective effects of neurotrophic factor analogs – 7,8-DHF and 7,8,3'-trihydroxyflavone (THF) – were demonstrated on another model of mitochondrial dysfunction [55]. Collectively, these data suggest that BDNF plays a vital role in regulating cellular energy in an ischemic heart [53–55].

Italian researchers conducted experiments to investigate the effects of ischemia on cardiomyocytes in wild-type mice knocked out by both the β_3-adrenergic receptor gene and the *BDNF* gene. They found that in wild-type hearts, BDNF levels sharply decreased four weeks after MI, coinciding with the development of left ventricular (LV) dysfunction and impaired angiogenesis. The administration of the LM22A-4 TrkB receptor agonist in BDNF knockout animals attenuated the progression of LV dysfunction and impaired angiogenesis [56]. The authors also observed that the β_3-adrenergic receptor agonist BRL-37344 increased BDNF content in cardiomyocytes.

Therefore, the use of TrkB receptor agonists may mitigate LV ischemic dysfunction by restoring BDNF levels in the myocardium, and stimulation of the heart's β_3-adrenergic receptors represents a potential strategy to prevent chronic post-ischemic heart failure through upregulation of BDNF [56].

There are isolated reports indicating the antiarrhythmic effect of neurotrophin [57, 58]. The authors observed a significant reduction in the average monthly duration of atrial fibrillation (AF) episodes – by more than sixfold – following administration of a low dose of BDNF [58]. In the study by F. Rahman et al., a correlation was identified between low BDNF concentrations and risk factors for AF [57].

Thus, a substantial body of evidence suggests that BDNF plays a protective and beneficial role in ischemia–reperfusion injury and/or MI. However, the opposite activity of neurokine in some experimental models remains unexplained [51].

THE ROLE OF BDNF IN SYMPATHETIC AND PARASYMPATHETIC REGULATION OF HEART RHYTHM

The rostral ventrolateral medulla (RVLM) is a key integrative region involved in heart rate regulation, containing sympatho-excitatory neurons that play a crucial role in modulating sympathetic nerve activity [59]. These sympatho-excitatory neurons tonically regulate the activity of sympathetic neurons by transmitting excitatory signals to preganglionic sympathetic neurons located in the intermediolateral cell column of the spinal cord [59].

It has been demonstrated that BDNF is expressed in several neural groups within this pathway, indicating its potential role in cardiovascular regulation [60]. The neurotrophic factor is involved in the development and functioning of the arterial baroreceptor system [61], and its injection into the RVLM results in increased blood pressure [60]. Additionally, BDNF and its TrkB receptors are localized in neurons within the hypothalamus and brainstem, regions that house autonomic control centers of the cardiovascular system [11, 12, 60].

BDNF is an unusual neurotrophin that acts not only as a classical neurotrophic factor promoting neuronal survival and differentiation but also as a neurotransmitter [60]. Two lines of evidence have been proposed to explain BDNF-dependent synaptic transmission as a key component of heart rate regulation:

Physical exercise and intermittent fasting, which increase BDNF expression in various brain regions

[14, 62], can reduce resting heart rate by enhancing parasympathetic activity [14, 63];

BDNF induces the expression of choline acetyltransferase and promotes the synthesis and release of acetylcholine (ACh) in developing autonomic neurons cultured *in vitro* [14].

Vagal cardioinhibitory preganglionic cholinergic neurons of the brainstem project their axons via the vagus nerve to the heart where they release ACh onto cardiac ganglion cells, thereby reducing heart rate [14]. Vagal preganglionic neurons in the brainstem express the high-affinity TrkB receptor [14] and produce BDNF [14]. A study by R. Wan et al. demonstrated that intracerebroventricular administration of BDNF to haplon-deficient (BDNF+/-) mice enhanced the activity of parasympathetic nuclei in the nucleus ambiguus, resulting in a decreased heart rate. Collectively, these findings suggest that BDNF signaling is essential for normal cardioinhibitory parasympathetic regulation of the heart at rest [14].

Research on bimodal neonatal sympathetic neurons capable of maintaining both adrenergic and cholinergic neurotransmitter status in co-culture with cardiomyocytes [64] has shown that BDNF acting through the panneurotrophin receptor p75NTR induces a rapid switch toward ACh release [64, 65], leading to a slowdown in spontaneous cardiomyocyte contractions [14]. Sympathetic neurons express TrkA and Trkc receptors, which are not activated by BDNF and do not express BDNF-specific TrkB; instead, they express p75NTR [66]. It appears that BDNF functions as an agonist for p75NTR in sympathetic neurons [66].

It is also noteworthy that BDNF likely influences neurons providing glutamatergic or GABAergic input to the CNS. Indeed, BDNF enhances glutamate release from presynaptic terminals of hippocampal and visual cortex neurons [14], and modulates activity in GABAergic synapses [14]. These findings highlight a novel background and potential role for altered BDNF signaling in disorders associated with autonomic dysregulation. Accordingly, mice with Huntington's disease mutations exhibit increased heart rates associated with a significant decrease in brainstem BDNF levels [14]. Elevated BDNF levels have also been observed in patients with Chagas disease, a phenomenon attributed to both inflammatory processes and cardiac autonomic dysfunction [67].

A group of researchers evaluated the effect of BDNF on the autonomic tone of the heart using heart rate variability (HRV) [68]. A comparative analysis of HRV parameters and serum BDNF levels was performed in patients diagnosed with generalized anxiety disorder (GAD) and healthy individuals. The authors observed a significant decrease in HRV in these patients compared to the control group. Additionally, significantly higher levels of BDNF in blood plasma were detected in healthy individuals relative to patients with GAD at the initial stage of the study [68]. Following pharmacological treatment with paroxetine, an increase in HRV and BDNF levels was noted [68].

Based on our own studies involving 28 healthy volunteers aged 20 to 22 years, HRV indicators also demonstrate a close relationship with blood plasma levels of BDNF. A statistically significant negative correlation was established between BDNF content and the absolute power of the VLF parameters. This finding may serve as evidence of the cerebral ergotropic effects of neurotrophin on underlying autonomic regulation and suggests a relationship between BDNF content in blood plasma, psychoemotional stress, and the functional state of the cerebral cortex.

The NT content in tissues innervated by the SNS changes with age, and these changes are associated with altered sympathetic function during heart diseases [66]. There is evidence that nerve growth factor (NGF) and BDNF exert functionally antagonistic effects on sympathetic neuron growth. BDNF has been shown to inhibit sympathetic nerve growth via p75NTR [69] and is necessary for normal programmed cell death and regulation of neuronal numbers during development [70]. Additionally, BDNF promotes local axonal degeneration and suppresses NGF-stimulated TrkA signaling *in vitro* [70].

CONCLUSION

Neurotrophins have been extensively studied in relation to their effects on the development and functioning of the nervous system and have historically been investigated exclusively within the field of neuroscience. In the lecture, we focused on highlighting the significance of the most well-studied representative of this class of neurokines, BDNF, in maintaining a cardiovascular phenotype and homeostasis. We discussed the multifunctional

properties of BDNF and its potential role in conditions characterized by resistance or heart failure.

Beyond its critical role in neurobiology, increasing evidence suggests that BDNF is also involved in the development and pathophysiology of the cardiovascular system. It is known that BDNF promotes cardioprotection by activating angiogenesis and neovascularization in ischemic tissue through the recruitment of endotheliocytes and regulation of their survival. Studies have demonstrated that BDNF and its receptors are expressed in various tissues, including the heart, endothelium, macrophages, vascular smooth muscle cells, and atherosclerotic coronary arteries [6, 53, 71, 72].

According to R. Samal et al., BDNF-mediated effects are not limited solely to neurons or endotheliocytes but can also exert regulatory influence on cardiac progenitor cells, promote cardiac recovery, and mitigate myocardial dysfunction [16]. Over the past decade, cell therapy has emerged as a potential alternative approach. Data indicate that a subset of undifferentiated progenitor cells resides in the adult heart and can stimulate regeneration of damaged myocardium, thereby offering new opportunities for endogenous heart repair mechanisms [16]. Additionally, circulating BDNF has been identified as a promising biomarker for both the diagnosis and prognosis of cardiovascular disease (CVD) [32].

Therefore, further research on neurotrophins is essential to develop new effective therapeutic strategies for the treatment and prevention of cardiovascular diseases.

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Artificial Intelligence in the Diagnosis and Prognosis of Multimorbidity in the Elderly

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ABSTRACT

Aim. To evaluate the effectiveness of artificial intelligence in diagnosing and predicting multimorbidity in people over 65 years based on current literature data.

Materials and methods. A systematic review of 153 studies from January 1, 2020 to March 1, 2025 was conducted following PRISMA 2020 guidelines. The PICO model was applied: population – elderly people with multimorbidity (two or more chronic conditions), intervention – artificial intelligence tools (machine learning, deep learning), outcomes – diagnostic accuracy and prognostic performance. Keyword searches were performed in PubMed, Scopus, Web of Science, and Google Scholar databases. Data were synthesized narratively and quantitatively via meta-analysis using the R software version 4.3.2. The method excels in detecting hidden patterns compared to clinical scales.

Results. Artificial intelligence demonstrated high diagnostic accuracy for dementia (AUC = 0.833), stroke (AUC = 0.91), cardiovascular diseases (AUC = 0.986–0.991), and osteoporosis (AUC= 0.972). Prognostic performance reached AUC ≈ 0.87 (95% confidence interval: 0.83–0.91) for mortality and hospitalizations. However, for multimorbidity, accuracy was lower (AUC = 0.787– 0.93) due to data heterogeneity and the complexity of disease interactions.

Conclusion. Artificial intelligence enhances diagnostic and prognostic capabilities in geriatrics, particularly for individual conditions, but requires data standardization and dynamic models for multimorbidity. Challenges, such as digital ageism and data quality, still hinder its implementation.

Keywords: artificial intelligence, multimorbidity, the elderly, diagnosis, prognosis, machine learning, deep learning, geriatrics

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Искусственный интеллект в диагностике и прогнозе полиморбидности у пожилых

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РЕЗЮМЕ

Цель: оценить эффективность искусственного интеллекта в диагностике и прогнозировании полиморбидности у пожилых людей старше 65 лет на основе актуальной литературы.

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Материалы и методы. Проведен систематический обзор 153 исследований за период с 1 января 2020 г. по 1 марта 2025 г. по стандартам PRISMA 2020. Использован фреймворк PICOS: популяция – пожилые с полиморбидностью (два и более хронических заболевания), вмешательство – инструменты искусственного интеллекта (машинное обучение, глубокое обучение), исходы – точность диагностики и прогностическая эффективность. Поиск выполнен в PubMed, Scopus, Web of Science и Google Scholar. Данные синтезированы нарративно и количественно с помощью метаанализа в программном обеспечении R v. 4.3.2. Преимущество метода – способность выявлять скрытые закономерности по сравнению с клиническими шкалами.

Результаты. Искусственный интеллект показал высокую точность в диагностике деменции (AUC = 0,833), инсульта (AUC = 0,91), сердечно-сосудистых заболеваний (AUC = 0,986–0,991) и остеопороза (AUC = 0,972). Прогностическая эффективность составила AUC ≈ 0,87 (95%-й доверительный интервал: 0,83–0,91) для смертности и госпитализаций. Однако при полиморбидности точность ниже (AUC = 0,787–0,93), что связано с гетерогенностью данных и сложностью взаимодействия патологий.

Заключение. Искусственный интеллект улучшает диагностику и прогноз в гериатрии, особенно для отдельных заболеваний, но требует стандартизации данных и динамических моделей для полиморбидности. Цифровой эйджизм и качество данных остаются вызовами для внедрения.

Ключевые слова: искусственный интеллект, полиморбидность, пожилые люди, диагностика, прогнозирование, машинное обучение, глубокое обучение, гериатрия

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

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

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INTRODUCTION

Population ageing is one of the most pressing global challenges, especially in regions where the proportion of people over 65 is increasing rapidly. According to the United Nations, the number of people aged 65 years and older will reach 1.5 billion by 2050, which will significantly increase the burden on health systems worldwide [1]. Multimorbidity, defined as the coexistence of two or more chronic diseases in one person, affects 60–80% of the elderly and poses significant challenges for timely diagnosis and treatment [2]. Traditional approaches, such as clinical assessment scales, are not often sufficiently accurate due to complex interactions between pathologies and individual patient characteristics, which highlights the urgent need to develop innovative solutions to improve care for this population group [3].

Artificial intelligence (AI), including machine learning and neural networks, has become a revolutionary tool in healthcare, demonstrating outstanding results in analyzing large and complex datasets [4]. International studies show that AI can improve the accuracy of diagnosis and prediction of complications in chronic diseases, outperforming traditional methods by 15–20% in various applications [5].

In geriatric practice, AI offers opportunities for the management of multimorbidity due to its ability to identify hidden patterns in patient data, such as electronic health records or parameters of wearable devices, enabling the development of personalized treatment strategies [6]. For example, AI-based models have proven efficient in improving cardiovascular risk prediction in the elderly based on data from various clinical sources [7]. However, the use of AI to manage multimorbidity in the elderly remains understudied, especially in regions with limited technological infrastructure [8].

This review is motivated by the need to summarize the current evidence on the role of AI in the diagnosis and prognosis of multimorbidity in the elderly, which is becoming increasingly important in the face of growing health care needs [9]. Although individual examples of AI applications, such as health monitoring and risk assessment, have been documented, comprehensive studies of their effectiveness and scalability remain rare [10].

The aim of this systematic review was to assess the effectiveness and feasibility of AI in the diagnosis and prognosis of multimorbidity in elderly people (≥ 65 years) based on current evidence from the literature.

MATERIALS AND METHODS

A systematic review was conducted according to PRISMA 2020 guidelines to analyze the role of AI in the diagnosis and prognosis of multimorbidity (≥ 2 chronic diseases) in the elderly. Literature from January 1, 2020 to March 1, 2025 focusing on AI methods (machine learning, deep learning, etc.) was included [11, 12].

Inclusion criteria according to the PICO model:

– population: elderly (≥ 65 years, elderly/older adults/geriatric) or studies with relevant conditions (multimorbidity);

– intervention: AI tools for the diagnosis or prognosis of multimorbidity;

– outcome: diagnostic accuracy (sensitivity, specificity, AUC), prognostic performance (mortality, hospitalization);

– design: original research, systematic reviews, randomized controlled trials (RCTs); non- peer-reviewed sources excluded;

– time frame: 2020–2025, language – English (or annotated in English)

Studies dated earlier than 2020, without a focus on the elderly, AI or multimorbidity were excluded.

The literature search was conducted in PubMed, Scopus, Web of Science, and Google Scholar databases using keywords and MeSH terms: “artificial intelligence”, “machine learning”, “deep learning”, “multimorbidity”, “comorbidity”, “elderly”, “older adults”, “geriatric”, “diagnosis”, and “prognosis”. An example query for PubMed: (“artificial intelligence”[MeSH Terms] or “machine learning” or “deep learning”) and (“multimorbidity”[MeSH Terms] or “comorbidity”) and (“aged”[MeSH Terms] or “elderly” or “older adults”) and (“diagnosis” or “prognosis”) and (“2020/01/01”[Date - Publication]: “2025/03/01”[Date - Publication]). The search was performed in March 2025, covering the period from January 1, 2020 to March 1, 2025.

Initially, 199 records were identified: 194 from the main databases (PubMed, Scopus, and Web of Science) and 5 additional records from Google Scholar and reference lists of relevant articles. After removing 2 duplicates (e.g., Alsaleh M.M. et al., 2023 [13]), 197 records remained. At the title and abstract screening stage, 44 studies were excluded: 20 did not match the population criteria (e.g. Gupta R. et al., 2021 [14] did not describe the elderly), 10 did not match the time range, 8 did not match the AI focus, and 6 were unreviewable. Full-text analysis of the 153 remaining records confirmed they met the inclusion criteria. The selection process is summarized in the PRISMA flow diagram (Figure).

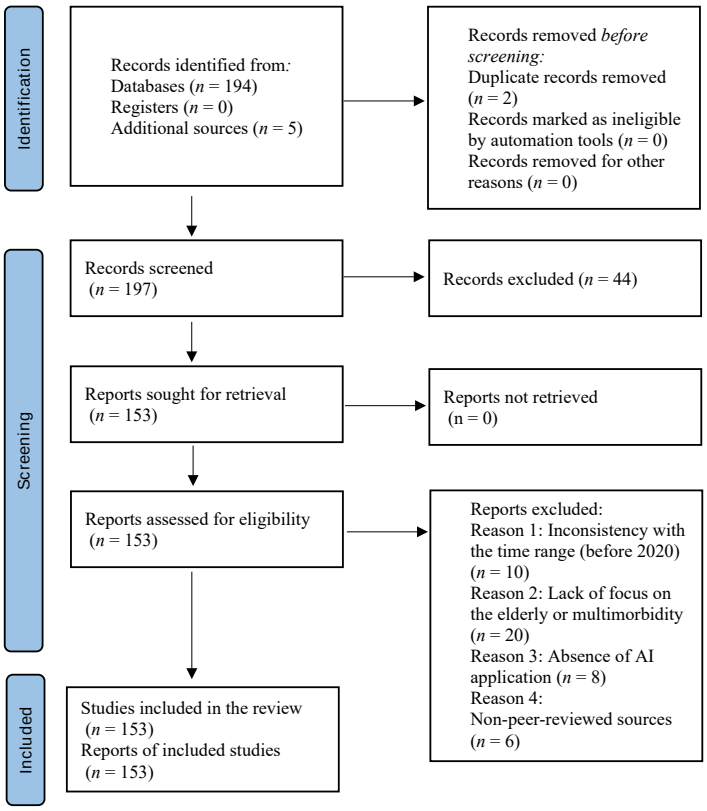

Figure. PRISMA flow diagram

The following data were retrieved: authors, year of publication, study design, age of participants, AI methods (e.g. Random Forest), outcomes (AUC, HR/OR), limitations. Data were collected manually.

The risk of bias was assessed using the ROBINS-I, RoB 2, and AMSTAR 2 (selection bias, confounding, reporting) tools. Most studies showed low to moderate risk.

Data were synthesized narratively (diagnosis, prognosis, multimorbidity) and quantitatively (meta-analysis for a subset of studies with ≥ 3 similar metrics, performed in R v. 4.3.2 with a meta-package; heterogeneity was assessed through I^2 and τ^2). GRADE assessment revealed high reliability for the diagnosis of dementia and moderate reliability for predicting mortality in the respective subgroups.

Bivariate models and HSROC were used for the diagnosis; odds ratio (OR) / hazard ratio (HR) with 95% confidence interval (CI) were used for prognosis. R v. 4.3.2 software was applied.

RESULTS

The application of AI in the diagnosis and prognosis of multimorbidity in elderly people (≥ 65 years) represents a promising area of modern geriatrics, but is accompanied by a number of challenges due to the complexity of multimorbid conditions. This systematic review, performed according to PRISMA 2020 standards, analyzed 153 studies published between January 1, 2020 and March 1, 2025. The focus was on assessing the accuracy of AI-based diagnostic models, their prognostic performance and applicability in the context of multimorbidity (the presence of two or more chronic diseases). Quantitative data synthesis was performed using the meta-analysis in the R software (version 4.3.2), allowing for aggregation of key metrics, such as area under the ROC curve (AUC), sensitivity, specificity and, where available, risk ratios (HR/OR). The risk of bias assessment of most of the included studies for the ROBINS-I, RoB 2, and AMSTAR 2 tools showed low to moderate risk of bias, confirming the validity of the results presented.

AI has demonstrated high efficiency in diagnosing certain diseases in elderly patients, which emphasizes its potential as a tool for screening and early detection of pathologies. For example, a study by S. P. Obuchi et al. applied machine learning (ML) algorithms to analyze gait to diagnose cognitive disorders, including dementia [15]. Using motion sensor data, the authors reported mean classification accuracy of 80.2%, with sensitivity of 96.1%, specificity of 64.3%, and AUC = 0.833 based on 30 test datasets.

GRADE assessment showed high confidence in these data due to the rigorous study design and low risk of bias associated with participant selection, although confidence intervals for the metrics were not provided. A similar approach was implemented by Y. Wang et al. where AI based on deep neural networks (Efficient Net, Xception, VGG, ResNet) analyzed facial images to detect acute ischemic stroke [16]. The model trained on 185 stroke patients and 551 controls using cross-validation achieved AUC = 0.91, accuracy of 86% (95% CI: 83.5–88.5%), sensitivity of 76%, and specificity of 89% at a probability threshold of 0.40. On an independent test set (38 strokes, 50 controls), the AUC was 0.82, and the accuracy was 73% (95% CI: 64.2–81.8%). This makes the model a valuable tool for emergency diagnosis in settings with limited access to MRI or CT, especially given its ability to confirm the diagnosis in the face of conflicting imaging findings. The low risk of bias assessed by RoB 2 is due to strict age and sex matching in the sample and the use of cross-validation to prevent overtraining.

In the field of cardiology, AI has shown itself to be a highly accurate method. Y. Wang et al. used deep learning to analyze cardiovascular magnetic resonance imaging (MRI) including SAX cine and 4CH cine projections for screening [17]. The screening model achieved AUC = 0.986 (95% CI: 0.984–0.988), sensitivity of 97.3% (95% CI: 96.8–97.8%), and specificity of 90% on a primary dataset (n = 7,900) to detect abnormalities covering 11 types of cardiovascular diseases, including coronary heart disease (CHD) and hypertensive heart disease (HHD), frequently occurring in the elderly.

On the external test set (n = 1,819), the AUC was 0.990 (95% CI: 0.986–0.992). A diagnostic model using SAX cine, 4CH cine, and SAX LGE achieved a weighted mean AUC = 0.991 to classify these diseases (n = 6,650). The high performance was supported by rigorous three-fold cross-validation and generalizability to external data, although potential differences in MRI protocols between centers may have affected the results, consistent with a moderate risk of bias according to ROBINS-I.

Y. Yang et al. applied chest CT data analysis to screen osteopenia and osteoporosis, achieving AUC = 0.831 for osteopenia and AUC = 0.972 for osteoporosis in the healthy group [18]. The method is based on density assessment of the thoracic vertebrae and the first lumbar vertebrae, where with an increase in CT

values by 10 HU, the risk of osteopenia was reduced by 32–44% and the risk of osteoporosis decreased by 61–80%. This approach is particularly useful in the elderly with multimorbidity, as it allows for the detection of hidden pathologies without additional examinations. Confidence intervals for AUC are not given in the article, and the pooled diagnostic performance of all thoracic vertebrae was greater than that of a single vertebra, although a specific value was not given.

However, the effectiveness of models may be reduced in the diagnosis of multimorbidity. H. Chen et al. investigated cognitive impairment in cerebral microvascular disease (CMD) with consideration of vascular risk factors, such as hypertension (81.5%) and diabetes (21.9%) using a model based on oculo-gait measurements (antisaccade accuracy, step rate, and sweep rate) [19]. In the hospital cohort (n = 194), the adjusted model achieved the AUC = 0.787 after accounting for age and education, and in the population-based cohort with early CMD, the AUC was 0.810. These values are lower than for some isolated conditions due to variability in factors, such as age and education, although confidence intervals for AUC are not specified. The limitations of the study do not include explicit social factors, but the influence of demographic characteristics is emphasized.

Y. Wang et al. in their bibliometric analysis noted that AI-based research in geriatric care has focused on the monitoring and treatment of diseases, such as Alzheimer's disease and mild cognitive impairment, as well as daily care and rehabilitation of the elderly [20]. Narrative synthesis confirms the effectiveness of AI in addressing individual pathologies, but highlights limitations, such as cost, safety in the home environment, and digital inequality, which may complicate the application of AI in more complex scenarios, including multimorbidity

Predicting clinical outcomes in the elderly using AI involves mortality estimation. C. Guo et al. developed a ML (ensemble) model to predict 28-day mortality in elderly patients with colorectal cancer in the ICU, achieving AUC = 0.86 in the training cohort (eICU, n = 693), AUC = 0.73 in the validation cohort (MIMIC-IV, n = 181) and AUC = 0.81 in the Union cohort (n = 95) [21]. The predictive value of the model is supported by the analysis of key features (vasopressors, albumin, urea nitrogen), although confidence intervals for AUC are not specified. The limited sample size, especially in the Union cohort, may affect the generalizability of the results.

Y. Song et al. investigated prediction of postoperative delirium (POD) in elderly patients with hip fractures using ML and logistic regression models [22]. The best model (Random Forest) achieved AUC = 0.81, and logistic regression model achieved AUC = 0.77 (95% CI: 0.696–0.845) in the training sample (n = 557) and 0.71 (95% CI: 0.593–0.827) in the validation sample (n = 240). These data support the ability of the models to detect complications associated with multimorbidity (renal failure, COPD) in the elderly, although CIs for ML models are not reported.

In the context of repeat hospitalizations, R. Loutati et al. developed a multimodal model predicting 30-day remissions (16.65% of 19,569 cases), achieving AUC = 0.93 with the TabNet model (sensitivity 86.7%, specificity 88.9%) [23]. Random Forest showed AUC = 0.89, gradient boosting of 0.87, with no CIs indicated. Key factors were the number of hospitalizations, heart failure (45.3%), and chronic kidney disease (47.9%), highlighting the difficulty of prediction in the elderly. Social reports were limited (n = 4,721) but were accounted for through NLP assessment. A meta-analysis of three studies (C. Guo et al., Y. Song et al., R. Loutati et al.) showed a pooled AUC ≈ 0.87 (95% CI: 0.83–0.91, $I^2 \approx 70\%$, $\tau^2 \approx 0.04$), indicating high heterogeneity due to differences in populations and outcomes [21–23].

For chronic diseases, AI shows prognostic potential. A.T. Ayers et al. applied AI to predict diabetes complications (retinopathy, nephropathy) with high accuracy [24]. R.D. Sriram et al. used wearable devices to monitor diabetes, improving glycemic control [25]. J. Yang et al. achieved AUC = 0.972 to diagnose osteoporosis (vs. normal) on chest CT, indirectly supporting fracture prevention in the elderly, although fracture risk was not directly predicted [26]. G. Voltan et al. developed a tool to detect osteoporosis in primary care, but validation of models on large cohorts considering multimorbidity remains a common challenge [27].

The application of AI in multimorbidity faces limitations, including data heterogeneity affecting the accuracy of models. R.J. Woodman et al. in a review of ML algorithms noted that differences in data from EMR and IoT devices create challenges, such as insufficient validation and transparency, which may reduce the effectiveness of AI in geriatrics [28]. For example, in the work by R. Loutati et al., incomplete data on social factors (available for 24% of the cohort) limited the analysis, although their influence was accounted for through NLP assessment. Y. Wang et

al. emphasized the need for standardization of data and algorithms to improve the quality of AI applications, which is particularly important when there is high heterogeneity in multimorbidity studies [29].

Secondly, ethical challenges, such as digital ageism, limit the availability of AI to the elderly. C.H. Chu et al. and Y. Aranda Rubio et al. note that low digital literacy and limited access to technology (especially in rural areas) deprive some patients of the benefits of AI [30, 31]. This is supported by E. Burnazovic et al., where the use of AI in geriatrics during the pandemic was limited by technical barriers [32]. T. Skuban-Eiseler et al. add that the opacity of algorithms may impair patient autonomy, increasing the risk of decision bias (moderate AMSTAR 2 score) [33].

Thirdly, the dynamics of disease interactions in multimorbidity are not adequately accounted for in current models. M.M. Alsaleh et al. note that most studies (19 out of 22) rely on static retrospective data, which may limit their applicability to the dynamic processes in multimorbidity [34]. For example, H. Chen et al. analyzed oculo-gait measurements in CMD based on one-step data without considering temporal changes, although the model achieved moderate accuracy (AUC 0.787–0.810) for screening cognitive impairment.

AI improves diagnosis and prognosis in geriatrics, especially for isolated pathologies. The accuracy for dementia (AUC 0.833) and stroke (AUC 0.91) supports its role in screening and early intervention [15, 16]. In telemedicine, as suggested by E. Burnazovic et al., AI may speed up diagnosis, which is important in the elderly with multimorbidity, but specific data are limited [32]. For complex conditions, the AUC varies: 0.787–0.810 for cerebral vegetative – vascular dystonia and 0.87–0.93 for repeat hospitalizations, suggesting that models need to be optimized [19, 23].

Prospects include the development of data standards and the potential for personalized care, although integration of wearable devices and specific approaches require further validation [25, 29, 35, 36]. Validation of models in multimorbidity remains a priority.

DISCUSSION

The application of AI in geriatrics is showing significant progress, especially in the diagnosis and prognosis of diseases in the elderly. Our systematic review covering 153 studies from 2020 to 2025 emphasizes the potential of AI as a screening and early intervention tool. The high accuracy of the models in detecting isolated pathologies, such as acute ischemic stroke or cardiovascular disease, allows for the use of AI for emergency diagnosis and resource optimization, especially in settings with limited access to conventional imaging modalities [16, 17]. This is clearly important for timely initiation of treatment, which can reduce mortality and improve quality of life in patients over 65 years of age. However, the transition to multimorbidity, a key characteristic of advanced age, decreases the effectiveness of AI due to heterogeneity of data and complexity of disease interactions [19].

Heterogeneity of data due to differences in sources (e.g., electronic medical records vs. Internet of Things devices), as well as insufficient consideration of social factors, limits the creation of universal models [23, 28]. In addition, most studies rely on static data, making it difficult to capture the dynamics of multimorbidity [34]. For example, analyses of oculo-gait measurements in CMD have shown moderate accuracy but have not accounted for progression of the condition, which reduces its prognostic value [19]. The prediction of mortality and repeat hospitalizations also demonstrates the potential of AI, but the high heterogeneity of results ($I^2 \approx 70\%$) indicates the need to adapt models to specific populations and outcomes [21–23]. For chronic conditions, such as diabetes and osteoporosis, AI offers opportunities for the prevention of complications, although it requires validation in the context of multiple comorbidities [24, 26].

Ethical and practical challenges play a key role in limiting the adoption of AI. Digital ageism associated with low digital literacy and access to technology, especially in rural areas, deprives a proportion of older patients of the benefits of AI [30, 31]. In Uzbekistan and CIS countries, where medical infrastructure is often limited, AI can optimize screening in primary care by identifying multimorbidity risks early, but requires adjustment to local conditions, including staff training and integration with existing systems. The opacity of algorithms, as noted by T. Skuban-Eiseler et al., can undermine physician and patient confidence, emphasizing the importance of developing explainable models [33].

The prospects for AI in geriatrics are related to overcoming these barriers. Standardization of data and algorithms has the potential to reduce heterogeneity and improve accuracy [29]. The integration of wearable devices promises to improve monitoring, and telemedicine, as shown by E. Burnazovic et al., can accelerate diagnosis in the elderly with

multimorbidity through remote analysis of data (e.g. gait or glycaemia), which is particularly relevant in times of crises, such as the COVID- 19 pandemic [32]. The development of dynamic models that take into account time trends and disease interactions will be the key to managing the complexity of multimorbidity, providing a personalized approach to care.

CONCLUSION

This systematic review confirms that AI significantly enhances diagnostic and prognostic capabilities in geriatrics, especially for selected diseases, such as dementia, stroke, cardiovascular pathologies, and osteoporosis. Its role in screening and early intervention makes AI a valuable tool in resource-limited settings. However, in multimorbidity, the accuracy of models is reduced due to heterogeneity of data, static approaches, and insufficient consideration of the dynamic nature of pathologies.

The prognostic potential of AI to assess mortality and hospitalizations is clear, but needs to be optimized for complex conditions. The introduction of AI into clinical practice, including Uzbekistan and CIS countries, holds promise to improve early diagnosis of multimorbidity in primary care, but faces ethical challenges (digital ageism, opacity) and technical barriers (data standardization). Future research should focus on building dynamic models, integrating wearable devices, and increasing technology accessibility to enable effective management of multimorbidity in the elderly.

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Thyroid Dysfunction as a Key Link in the Concept of Mutual Aggravation of Colorectal Cancer and Metabolic Syndrome: Review

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ABSTRACT

The mutually aggravating role of endocrine glands and metabolic disorders in the process of carcinogenesis is well known, but it is underestimated in modern oncological practice. The study of the manifestations of thyroid dysfunction, its effect on carcinogenesis in patients with metabolic syndrome and the possibility of improvement should become an important direction in refining patient outcomes in colorectal cancer (CRC).

The aim of our review was to study the issue of thyroid dysfunction as a key link in the concept of colorectal carcinogenesis in metabolic syndrome. Current research data demonstrate a link between hypothyroidism and metabolic syndrome, suggesting that they mutually exacerbate each other, thereby worsening the condition of patients. Metabolic syndrome not only contributes to the development and progression of cancer, but also affects patient outcomes.

In clinical practice, an imbalance of thyroid hormones occurs in various types of cancer and is regarded as a confounding factor. Existing data regarding the influence of thyroid hormones on tumors are inconsistent. While hypothyroidism appears to play a role in promoting cancer progression, the underlying mechanisms of this association remain poorly understood and necessitate further research. Despite conflicting evidence regarding the impact of thyroid hormones on CRC development, their significance in influencing a patient's overall condition should not be overlooked. Therefore, it is important to integrate strategies for controlling the endocrine profile and correcting its changes into standard cancer treatment protocols. Moreover, some publications report the effect of levothyroxine replacement therapy on reducing the risk of developing CRC. Investigating the interplay between metabolic syndrome and cancer, particularly through the lens of thyroid dysfunction, may contribute to the development of novel approaches to colorectal cancer management and improve patient outcomes.

Keywords: thyroid dysfunction, cancer, carcinogenesis, thyroid hormones, hyperthyroidism, hypothyroidism, metabolic syndrome

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Дисфункция щитовидной железы как ключевое звено в проблеме лечения колоректального рака в сочетании с метаболическим синдромом: обзорная статья

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РЕЗЮМЕ

Взаимоотягощающая роль желез внутренней секреции и метаболических нарушений в процессе канцерогенеза общеизвестна, но современная онкологическая практика ее недооценивает. Изучение проявлений дисфункции щитовидной железы, ее влияния на канцерогенез у больных с метаболическим синдромом и возможности коррекции должно стать важным направлением в улучшении результатов лечения больных в терапии колоректального рака (КРР).

Целью нашего обзора стало освещение вопроса дисфункции щитовидной железы как ключевого звена в концепции колоректального канцерогенеза при метаболическом синдроме и возможных путей его решения. Актуальные данные исследований демонстрируют связь между гипотиреозом и метаболическим синдромом, указывая на возможность их взаимного усиления, что ухудшает состояние пациентов. Метаболический синдром не только способствует развитию и прогрессированию рака, но и влияет на исход лечения.

В клинической практике дисбаланс тиреоидных гормонов встречается при разных видах рака и расценивается как отягощающий фактор. Существующие положения о влиянии тиреоидных гормонов на опухоль противоречивы, и, хотя гипотиреоз, вероятно, способствует раковому прогрессу, механизмы этого влияния остаются неясными и требуют дальнейших исследований. Несмотря на противоречивость данных о влиянии тиреоидных гормонов на развитие КРР, их роль в состоянии пациента не должна недооцениваться. Поэтому важно интегрировать стратегии контроля гормонального фона и коррекции его изменений в стандартные протоколы лечения колоректального рака. Тем более, что в некоторых публикациях приводится влияние заместительной терапии левотироксином на снижение риска развития КРР. Исследование взаимодействий между метаболическим синдромом и раковым процессом через призму дисфункции щитовидной железы, возможно, поможет в разработке новых стратегий борьбы с КРР и улучшении результатов лечения.

Ключевые слова: дисфункция щитовидной железы, колоректальный рак, канцерогенез, тиреоидные гормоны, гипертиреоз, гипотиреоз, метаболический синдром

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

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

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INTRODUCTION

Modern oncological practice tends to underestimate the mutually aggravating role of endocrine dysfunction and metabolic disorders in carcinogenesis. This is due to the widespread perception that the endocrine system does not play a significant pathogenetic role in tumor growth and cancer development. At the same time, tumor growth itself alters the metabolism through the shutdown or distortion of the hormonal activity of endocrine glands, particularly the thyroid gland. In the study

by J.J. Díez et al., which included 506,749 patients, 23,570 (4.7%) were diagnosed with hypothyroidism. The overall incidence of malignancy in the group of patients with hypothyroidism examined was 13.8%, which is significantly higher than the incidence in patients without hypothyroidism [1].

The lack of attention from the scientific and medical communities to the state of endocrine glands in patients with colorectal cancer (CRC) has left one of the most significant links in the pathogenesis unexplored. This, in turn, inevitably leads to an incomplete understanding of the clinical picture, ultimately leading to suboptimal or ineffective treatment strategies. In this regard, we believe that in patients with tumor activity, it is essential to take into consideration the role of the endocrine system and seek ways to address any destabilization in their function.

Thyroid dysfunction, especially hypothyroidism, is closely related to metabolic syndrome (MS), which encompasses a number of disorders, such as insulin resistance, atherogenic dyslipidemia, central obesity, and hypertension [2]. Patients with hypothyroidism frequently exhibit characteristic clinical manifestations, such as overweight or obesity. These conditions are known to be linked to inflammation and the development of metabolic and hormonal imbalances, which may contribute to the formation and growth of malignant tumors [2]. Given these associations, the detection of hypothyroidism is an important element of early diagnosis in oncology, which creates opportunities for active monitoring and, if necessary, screening for CRC.

We hypothesize that implementing this strategy would not merely enable the prompt detection of CRC at its earliest stages, but also substantially enhance treatment efficacy, thereby improving prognosis and survival rates for these patients – an imperative goal amidst contemporary efforts.

The aim of our review was to study thyroid dysfunction as a key link in the concept of carcinogenesis in patients with CRC and MS and possible ways to correct it. Given the absence of a consensus regarding the course of hormonal imbalance in different cancer stages and types, despite every bodily phenomenon adhering to inherent mechanisms, we aim to scrutinize literature guided by expert perspectives and research insights to elucidate the thyroid gland's role in colorectal carcinogenesis.

MATERIALS AND METHODS

A systematic review of the literature was conducted in accordance with the guidelines Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

To identify the literature, we conducted a search using keywords that, when searched in the title and abstract, necessarily varied, combined and included thyroid dysfunction, colon cancer, carcinogenesis, thyroid hormones, hyperthyroidism, hypothyroidism, and metabolic syndrome in the PubMed and Semantic Scientist databases from 1990 to 2024.

The search results turned out to be unexpected and ambiguous, and as this may be of some scientific value, we decided to reflect their characteristics.

Upon further examination, accounting for terminological variations revealed divergent results. Notably, the most prevalent keyword combination emerged as (metabolic syndrome) AND (colorectal cancer) AND (thyroid dysfunction) with seven publications. The next most common combination was (metabolic syndrome) AND (colorectal cancer) AND ((hyperthyroidism) OR (hypothyroidism)) with five publications. Then, in descending order of frequency, the following search results were observed ((metabolic syndrome) AND (carcinogenesis)) AND (thyroid dysfunction) with two publications, (metabolic syndrome) AND (colorectal cancer) AND (thyroid hormones), ((metabolic syndrome) AND (carcinogenesis)) AND (thyroid hormones) and (metabolic syndrome) AND (carcinogenesis) AND ((hyperthyroidism) OR (hypothyroidism)) with one publication each.

Impressive results were obtained from Semantic Scholar, another popular academic search engine. Employing the designated search criteria, the platform retrieved a comprehensive dataset comprising a total of 12,570 relevant publications. For example, a combination (metabolic syndrome) AND (colorectal cancer) AND (thyroid hormones) was found in 5,210 publications. The request (metabolic syndrome) AND (colorectal cancer) AND (thyroid dysfunction) turned out to be the second most common with 3,630 related publications. Search for the combination ((metabolic syndrome) AND (carcinogenesis)) AND (thyroid hormones) resulted in 1,600 publications, and by ((metabolic syndrome) AND (carcinogenesis)) AND (thyroid dysfunction) – 718 publications. Search for combinations such as

(metabolic syndrome) AND (colorectal cancer) AND ((hyperthyroidism) OR (hypothyroidism)) and (metabolic syndrome) AND (carcinogenesis) AND ((hyperthyroidism) OR (hypothyroidism)) resulted in 1,220 and 192 publications, respectively.

At the second stage, all the articles obtained as a result of the search were processed using Rayyan platform, which allowed us to significantly simplify the selection of necessary publications and eliminate repetitive researches.

Three independent authors read all the abstracts and applied the inclusion and exclusion criteria, which were taken from the Oxford Centre for Evidence-Based Medicine (CEBM) criteria. Two reviewers read the selected quotes in full, while the grounds for exclusion were indicated in Covidence. The discussion or participation of a third reviewer was used to resolve disagreements between the reviewers. Duplicate entries were automatically deleted from all uploaded extracted links in Covidence. The titles and abstracts were evaluated by two independent reviewers, who removed the publications that did not meet the inclusion requirements.

The publications selected this way were classified according to the level of evidence from degree I to V and recommendations from A to D within the framework of evidence-based medicine. After this, the authors thoroughly reviewed the selected studies to draw the conclusions presented in this review.

Thus, as of April 7, 2025, we identified 42 publications related to thyroid dysfunction in the context of CRC and MS.

In addition, as a reliable clinical expert of a broad profile, we also used the Jadad scale, which is sometimes known as Jadad scoring, and is a procedure for evaluating the methodological quality of a clinical trial according to objective criteria. It forms a system for assigning ratings from zero to five for different tests and is designed to more accurately reflect the methodological quality of each individual study. It is the most universally adopted method of assessment. Unpublished materials, abstracts of congressional reports, hearing materials, and reviews were excluded. The search results were checked by two current authors separately. Both quantitative and qualitative data were extracted from the studies included in the review by two independent reviewers using AGREED II. If necessary, the data extraction tools were modified to take into account the differences of each included study; our review describes the conclusions in detail. Any disagreements that arose between the reviewers were resolved through discussion or with the help of the third reviewer. If required, we contacted the authors of the articles to request any missing or additional information.

As a result, we selected reviews from the original publications. We received a total of 42 publications appropriate for analysis.

RESULTS

Thyroid hormones (thyroglobulin, TG) are key regulators of major cellular reactions, including proliferation, differentiation, apoptosis, and metabolism. Tetraiodothyronine (T4), the main hormone synthesized in the thyroid gland, is catalyzed to triiodothyronine (T3) by specific iodothyronine deiodinases. T3 acts as the main metabolic agent through the formation of complexes between T3 and the nuclear receptors of thyroid hormones alpha (TRα) and beta (TRβ). This T3 receptor complex within the cell nucleus interacts with thyroid hormone response elements on specific genes, thereby regulating their expression. Disorders associated with elevated TG levels (hyperthyroidism) or TG deficiency (hypothyroidism) are prevalent and have distinct clinical manifestations [3].

Hypothyroidism is one of the most common endocrine disorders, and even subclinical hypothyroidism (SCH) is a widespread and asymptomatic condition, which, unfortunately, often goes unnoticed not only by patients, but also by doctors. SCH is characterized by high levels of serum thyroid-stimulating hormone (TSH) and normal levels of free TG [4]. Cross-sectional studies have additionally revealed a link between hypothyroidism (or SCH) and the components of MS [5, 6]. According to S.S. Alsulami et al., a high normal TSH level is associated with a higher incidence of MS [7]. Previous studies have also demonstrated that elevated TSH levels are linked to an increased risk of MS, particularly in women. Data provided by S.S. Alsulami et al. claimed that age has a significant effect on the severity of SCH [7]. Half of the participants with markedly elevated TSH were older than 50 years.

Dyslipidemia is the most commonly observed metabolic disease associated with hypothyroidism.

Patients with dyslipidemia account for 1.4% to 13.3% of all patients with hypothyroidism [8]. A. Shinkov [6] conducted a cross-sectional study of 2,153 euthyroid subjects and found that, within the normal range, the MS prevalence increased along with TSH levels, mainly manifesting itself as an increase in dyslipidemia.

Thus, MS was more common in the group with the highest TSH levels (34.9%) than in the group with the lowest levels (27%) ($p < 0.001$), as were low HDL-C levels (32% vs. 25%, $p < 0.001$) and hypertriglyceridemia (26.8% vs. 20.4%, $p = 0.015$). Furthermore, treatment for hypothyroidism has been shown to improve lipid metabolism. Some randomized controlled trials have found that T4 replacement therapy has a positive impact on dyslipidemia improving total cholesterol and LDL-cholesterol levels [9, 10].

Most studies suggest that the more MS components a patient has, the higher the risk of developing cancer [11, 12, 54]. At the same time, MS also increases cancer mortality by up to 2.4 times [11]. Individual components of MS appear to have a different impact on carcinogenesis. Mechanisms of interest regarding cancer etiology include the roles of insulin, insulin-like growth factor 1 (IGF-1), hyperglycemia, high triglyceride (TG) levels, and low high-density lipoprotein levels [13].

In addition, increased insulin levels can stimulate the expression of vascular endothelial growth factor and promote the proliferation of vascular endothelial cells in cancer [11]. These changes in the composition and phenotype of immune cells in adipose tissue in obese individuals contribute to hormonal and metabolic alterations that, in combination with inflammation, create an ideal environment for tumor growth. This multistage involvement of metabolic disorders in carcinogenesis may explain the increased risk of severe cancers among individuals with MS [11].

Due to the lack of sufficient information regarding the direct impact of MS on carcinogenesis and patient survival, it is worthwhile considering conditions closely related to MS, specifically non-alcoholic fatty liver disease (NAFLD). The study by Z. Liu et al. included 352,911 people (37.2% with NAFLD), among whom 23,345 developed cancer. Compared with non-NAFLD, NAFLD was significantly associated with 10 of 24 cancers studied, including cancers of the uterus (hazard ratio [HR] = 2.36; 95% CI 1.99–2.80), gallbladder (2.20; 1.14–4.23), liver (1.81; 1.43–2.28), kidneys (1.77; 1.49–2.11), thyroid gland (1.69; 1.20–2.38), esophagus (1.48; 1.25–1.76), pancreas (1.31; 1.10–1.56), bladder (1.26; 1.11–1.43), breast (1.19; 1.11–1.27), and CRC and cancer of the anus (1.14; 1.06–1.23) [14]. Associations between NAFLD and cancers of the liver, esophagus, pancreas, rectum, anus, bladder, and malignant melanoma were increased in men. In contrast, associations with cancers of the kidneys, thyroid gland, and lungs were increased in women [14].

It is important to note that thyroid hormones affect liver lipid metabolism through various mechanisms, including stimulating the transport of free fatty acids into the liver for their re-esterification into triglycerides and enhancing the beta-oxidation of fatty acids, which affects the accumulation of fat in the liver [15].

In their study, A. Bano et al. showed that SCH is associated with the risk of NAFLD [16]. The study by Y. Tao et al. aimed to assess the association of NAFLD with changes in thyroid function within the euthyroid range [17]. Higher TSH levels in euthyroidism have been found in subjects with NAFLD, but they may also be associated with a slight increase in serum alanine aminotransferase (ALT) in the context of MS and insulin resistance. A large population-based study by E.H.Van den Berg et al. demonstrated that NAFLD was associated with higher FT3 levels and lower FT4 levels among severely euthyroid individuals [18]. The findings collectively support the hypothesis that elevated levels of FT3 within the range of euthyroidism may be associated with increased fat accumulation in the liver, likely as part of central obesity [18].

TSH levels are related to body mass index (BMI) and are often higher in obese people than in normal-weight people of the same age, gender, and weight. The study by B. Biondi showed that a genetically predetermined high BMI significantly increased serum TSH levels; as a result, an increase in BMI can cause an increase of FT3 [19]. Moreover, data collected from humans and a mouse model suggest that obesity causes fat accumulation in the thyroid gland. The work by L.Zhong et al. mentioned a study in obese mice that shows that obesity can affect the thyroid gland's ability to produce hormones and cause SCH [20].

As is well known TG and their derivatives play numerous roles in various tissues, including the activation and remodeling of adipose tissue. Recently, it has been demonstrated that some TG metabolites that were previously believed to be inactive products of thyroid hormone metabolism actually possess biological activity. One such example is 3,5-T2 [21]. This metabolite exhibits some TG effects within one hour after administration, and mitochondria are considered as a direct target of 3,5-T2. In addition, *in vivo* studies show that 3,5-T2 has a metabolically beneficial effect on adipose tissue [21].

J. Gómez-Izquierdo et al. concluded that SCH is associated with an increased risk of cancer, as well as mortality associated with it [22]. The study by F. Gagliardi et al. showed that carcinogenesis occurred significantly more often in patients with SCH than in people with euthyroid condition [23]. It has also been reported that CRC involves two additional nuclear receptors with opposing effects, which have been previously mentioned: TRα1 and TRβ1. The effect of TG on TRα1 leads to the activation of β-catenin, which promotes cell proliferation in the colon. Conversely, TRβ1 inhibits cell proliferation when it is activated by TG. In particular, TG can regulate the balance between proliferation and differentiation of CRC stem cells stimulating differentiation and reducing growth, thus acting as an anti-cancer agent [24].

Thus, the lack of TRβ1 expression is associated with malignant transformation in colon cancer [25]. Among other things, TG has been shown to contribute to the depletion of stem cells in the tumor site. The study by A. L'Heureux et al. demonstrates a potential feedback mechanism observed in patients with hyperthyroidism; in their xenografts and *in vitro* models, CRC stem cells treated with T3 had a significantly reduced ability to self-renew, decreased accumulation of nuclear β-catenin, and increased sensitivity to treatment, especially when type 3 deiodinase (D3) was suppressed [26]. Intracellular T3 may have antitumor properties because it induces the differentiation of CRC stem cells [26].

If we discuss T4, its non-genomic activity through binding to the αvβ3 integrin leads to an increase in nuclear β-catenin levels. T4 also promotes cell viability in CRC cell lines in a dose-dependent manner. Thus, there may be a potential mechanism in which low FT4 levels in primary hypothyroidism may protect against cancer by reducing interaction with integrin [26]. The authors also noted that TRβ1 may play a role as a tumor suppressor in the development of malignant tumors. Conversely, overexpression of TRα1 receptor appears to be linked with an accelerated appearance and progression of tumors [26].

G. Schiera et al. suggest that hypothyroidism correlates with an increased risk of CRC and hepatocellular carcinoma [24]. F. Gagliardi et al. suggested that cancer may determine the onset of the low T3 syndrome and that a decrease in the peripheral transformation of T4 to T3, which occurs under the action of type 2 deiodinase (D2), may be a pivotal moment in this process. Using the example of patients with metastatic CRC, the loss of muscle mass that occurs during cachexia and the subsequent decrease in deioding, as well as liver damage caused by tumor spread, followed by impaired deioding mediated by type 1 deiodinase (D1), can be considered as sequential pathological mechanisms. Based on the identified correlation between changes in peripheral deiodination and adverse clinical outcomes, the researchers concluded that the FT3/FT4 ratio may serve as a prognostic indicator for determining the life expectancy of individuals with metastatic CRC. A relationship between low T3 syndrome and adverse clinical outcomes was also observed in patients with hematologic tumors, lung tumors, and brain tumors [24]. -

In addition to the crucial biological importance of TG in metabolism and growth, there is evidence that they can influence the clinical outcome of cancer, as well as individual life expectancy [27]. Numerous *in vitro* and *in vivo* studies, as well as population studies, indicate the cancer-promoting effects of triiodothyronine and thyroxine. These hormones are known to be mediators of tumor growth and proliferation, as well as its progression. This hypothesis is supported by numerous clinical studies that have shown that hypothyroidism can suppress tumor growth, whereas hyperthyroidism can have the opposite effect [27, 28]. In addition, hyperthyroidism is also associated with a poorer prognosis of cancer [29].

Elevated serum TSH levels have been linked to improved treatment outcomes in patients with head and neck cancer, gliomas, and breast cancer (BC). However, these levels have also been associated with poorer outcomes in patients diagnosed with kidney

cancer [30]. This discrepancy indicates differences in oncogenesis between different types of cancer. Hypothyroidism also appears to be associated with a poor prognosis for patients with endometrial cancer (EC). A multicenter study conducted by V. Seebacher et al. [13] in 2013 was the first to study the effect of TSH on the prognosis of patients with EC. Elevated TSH levels have been shown to be independently associated with poor disease-related survival in univariate and multivariate survival analyses ($p = 0.01$ and $p = 0.03$, respectively).

Thus, TSH serum measurements can be utilized as an independent prognostic indicator for the survival of EC patients to determine recurrence during the follow-up period of EC. However, in the aforementioned study [13], no correlations were observed between elevated pre-treatment TSH levels and advanced-stage FIGO tumors, higher histological grades, unfavorable histologic subtypes, elderly patient age, or lifestyle factors, such as obesity, hypertension, or diabetes. Based on this discovery, TSH may be associated with systemic processes that interact with carcinogenesis (for example, hormonal imbalance or inflammation) rather than with local neoplastic transformation. However, the specific mechanism by which serum TSH levels affect EC remains unknown.Over the past few decades, considerable attention has focused on investigating how TG exert tumor-stimulating effect [3, 31]. TG mediate their effect on the cancer cell through several non-genomic pathways, including activation of the membrane receptor for TG integrin αvβ3 [27, 32]. It has been shown that this receptor contains two different hormone-binding sites, S1 and S2, each of which triggers unique signaling cascades. Only T3 in physiological concentrations can bind to S1, triggering phosphorylation and activation of the phosphatidylinositol-3-kinase (PI3K) pathway, which potentiates cell proliferation and inhibits apoptosis. The second site, S2, binds T4 and has lower affinity for T3, triggers oncogenic extracellular signal-regulated kinase 1/2 (ERK1/2), contributing to a similar side effect, while stimulating angiogenesis and expression of fibroblast growth factor 2, as well as components necessary for rapid oncogenesis [3, 26, 33]. Binding of integrin αvβ3 stimulates the proliferative effect of hormones on cancer cells, as well as on blood vessel cells [3, 32, 34].

This ability may be significant not only in CRC, but also in other types of cancer, as malignant cells express a greater amount of integrin avβ3 compared to normal cells [24]. The effect of TG on the expression of the P-gp gene, whether genomic or non-genomic, has also been reported, which increases the likelihood that TG in an oncological patient may contribute to chemoresistance of tumor cells. This possibility has not yet been investigated in CRC cells. However, it has been demonstrated that avß3 integrin, a carrier of the cell surface receptor for T4, plays an important role in doxorubicin resistance in metastatic BC [35]. Potential mechanisms underlying the link between TG and CRC are also reported. Since CRC can be a hormone-dependent cancer, tumor progression is inversely related to the expression of the estrogen beta receptor (ERß) [36].

P.A. Konstantinopoulos et al. reported significantly lower ERß expression in colon cancer cells compared to normal colon epithelium [37]. Decreased ERß expression in CRC may be associated with loss of differentiation and advanced stages of cancer [37]. Moreover, it is reported that the potential for CRC progression is suppressed by ERß expression [28]. Estrogen has been shown to increase ERß expression [37]. The downstream genomic protective effects of estrogen result in gene transcription associated with angiogenesis and cell adhesion. Additionally, ERβ has been reported to induce apoptosis through various mechanisms, including increased p53 signaling in LoVo colon cancer cells and increased DNA fragmentation in COLO205 colon cancer cell lines [28].

An imbalance of thyroid hormones is a possible factor influencing the development of CRC resulting from diseases, such as SCH, Hashimoto's disease, and Graves' (or Bazedow's) disease, and their treatment. In 2010, G. Rennert et al. published a study titled CRC Risk Therapy [38]. Further studies were consistent with these results and showed that high concentrations of TG (but within the acceptable range) reduced the risk of developing CRC. Despite these promising initial results, there are currently only 11 papers available analyzing this issue, both at the molecular and epidemiological levels. The human studies included in this analysis show consistent and promising results [39].

Speaking about the effect of TG on CRC during long-term use as part of hormone replacement therapy

(HRT), G. Rennert et al. in a case-control study involving 2,566 couples showed that levothyroxine use was associated with a statistically significant reduction in the relative risk of CRC (HR = 0.59; 95% CI = 0.43–0.82; $p = 0.001$) [38]. In accordance with the well-known fact that hypothyroidism is much more common in women than in men, the study showed that levothyroxine women were more likely to take dietary supplements than men in the control group (8.2% vs. 2.0%, respectively, $p < 0.0001$). After analyzing the subgroups, the study showed a reduced risk of CRC in postmenopausal women taking levothyroxine (odds ratio (OR) = 0.53; 95% CI: 0.37–0.74; $p < 0.001$). In a fully adjusted model for postmenopausal women, levothyroxine intake was associated with a significant reduction in the risk of CRC (OR = 0.60; 95% CI: 0.4–0.81; $p = 0.001$) [38].

The study by B. Boursi et al. conducted on a large UK population included 20,990 CRC patients and 82,054 matched control patients. Analysis of the relationship between the time of initiation of replacement therapy and the risk of developing CRC showed that the protective effect increased along with the duration of therapy. Moreover, patients with clinical or subclinical hypothyroidism without a history of HRT had a higher risk of developing CRC compared to patients without any thyroid dysfunction (OR = 1.16; 95% CI: 1.08–1.24; $p < 0.001$) [29].

The meta-analysis conducted by Jang et al. among women diagnosed with CRC showed that received HRT users had a reduced risk of mortality compared to women who had not previously taken TG, while the risk remained unchanged in patients who had previously used HRT, suggesting that the relationship between HRT use and survival rate may be complex and depend on the duration of hormone use.

DISCUSSION

The role of thyroid hormones in carcinogenesis is not considered or regarded as an ambiguous phenomenon today. Due to insufficient research, there is currently no unified perspective on the nature of thyroid dysfunction and its course across different stages and types of cancer. There is still a question about the effect of TG on cancer carcinogenesis, while the mechanisms are different. However, there is already evidence of the effect of TG on the clinical outcome of cancer [30]. Hormonal and metabolic alterations associated with obesity, coupled with a persistent state of low-grade chronic inflammation, create a favorable environment for the development of cancer.

Most studies indicate that the greater the number of MS components a patient has, the higher their risk of developing malignancy [11, 12]. At the same time, MS also increases cancer mortality by up to 2.4 times [11]. For example, J.H.Park et al. observed 9,890,917 adults for 7 years and found that the risk of thyroid cancer was higher in the group with MS than in the group without MS (HR = 1.15; 95% CI 1.13–1.17).

This cohort study showed that MS patients tended to have larger tumors, more invasive characteristics, including a greater number of lymph node metastases, and a later stage of AJCC. Blood pressure ≥ 130/85 mm Hg and low levels of HDL cholesterol were risk factors for the development of larger tumors and metastases to lymph nodes. BMI has been used as the main indicator in most studies. However, the distribution of body fat and impaired adipose tissue function rather than the total fat mass may be a better predictor of insulin resistance and associated complications for each patient.

NAFLD is a hepatic manifestation of MS [25]. Associations of NAFLD with cancers of the liver, esophagus, pancreas, rectum, anus, and bladder, as well as malignant melanoma, were increased in men, while associations with cancers of the kidneys, thyroid, and lungs were increased in women. Interestingly, the associations between NAFLD and the risk of liver, kidney, and thyroid cancer remained statistically significant after further adjusting for waist circumference, BMI, and the number of MS components based on the primary models. Thus, NAFLD has been linked to an increased risk of several types of cancer, although the effect varies considerably depending on the specific location. We suppose that NAFLD should be given a higher priority within the current cancer prevention strategy [14].

Associations of metabolic dysfunction with the risk of BC and CRC were observed regardless of BMI, with an increased risk in individuals with metabolically unhealthy normal weight or overweight/obese compared to people with a metabolically healthy normal weight [48]. Thus, metabolic dysfunction is a key risk factor for obesity-related cancer, regardless of obesity status.

Recently, evidence has emerged that some TG metabolites previously considered as inactive products of thyroid hormone metabolism have biological activity, such as 3,5-T2 [21]. This metabolite exhibits some TG effects within one hour after administration, and mitochondria are considered as its direct target. In addition, *in vivo* studies have shown that 3,5-T2 exerts a metabolically favorable effect on adipose tissue [21], which could certainly play an important role in the development of therapeutic approaches not only for isolated MS, but also when combined with CRC and thyroid dysfunction.

G. Schiera et al. suggest that TG can control the balance between proliferation and differentiation of CRC stem cells inducing differentiation and reducing growth, thus acting as an anti-cancer agent [24]. On the other hand, the study found that genetically predicted hyperthyroidism, TSH, and FT4 were not associated with an increased risk of CRC, and the reverse analysis failed to reveal any effect of CRC on thyroid function [42].

On the one hand, TG mediate their effect on the cancer cell through several non-genomic pathways, including activation of the membrane receptor for TG integrin αvβ3. Binding of the latter promotes the proliferation of cancer cells [3]. However, malignant cells express a higher amount of integrin αvβ3 than normal cells [24]. The most important thing is that a chain of interaction is formed, which is realized in various types of cancer, including CRC [26].

The possibility of the P-gp gene expression under the influence of TG has not yet been studied in CRC cells, but it has been shown that integrin αvβ3, which is a carrier of the cell surface receptor for T4, makes an important contribution to doxorubicin resistance in metastatic BC [34], which opens up new directions for in-depth study of the role of TG not only in colorectal cancer, but also in carcinogenesis in general.

There is also evidence of a positive effect of HRT with TG on the outcome of CRC. G. Rennert et al. established that long-term use of levothyroxine was associated with a reduced risk of CRC [38]. B. Boursi et al. in their study demonstrated a lower risk of CRC among HRT users and a higher risk among patients with hyperthyroidism or in hypothyroidism patients who did not receive HRT. The protective association of TG increased along with the duration of treatment and cumulative dose and was higher in colectomy patients and more pronounced in women. The results of the work of Jang et al. also show that current HRT use has been associated with a significant reduction in the risk of CRC and all-cause mortality in women with CRC [41]. These findings confirm our belief that in patients with CRC, special attention should be paid to the hormonal status of TG.

Therefore, strategies to combat CRC in the presence of concomitant MS and thyroid dysfunction should be followed throughout treatment period. Thus, we believe that in addition to targeted, chemotherapy and radiation therapy, it is necessary to include some measures aimed at weight loss and BMI normalization, and most importantly, correction of TG levels, which we believe will significantly reduce the risk of severe forms of CRC and mortality rates.

By avoiding the assessment of endocrine disorders, their observation and analysis, we may be underestimating the patient's condition, missing the opportunity to prescribe proper complex therapy, thereby depriving patients of a cure, inhibiting the development of oncology, and supporting existing scientific results.

The main limiting factor of this review is the scope of studies included. This is due to the limited number of series available in the literature. This circumstance does not allow us to draw clear and definitive conclusions about the mutual influence of TG, MS and CRC, but the trend can still be traced.

CONCLUSION

Currently available clinical and experimental data provide contradictory results on the ability of TG to influence the onset and progression of CRC in the presence of concomitant MS.

However, based on the findings of certain studies, cancer cells appear to be responsive to alterations in thyroid hormone concentrations, both during the process of mutagenesis and following the emergence of a tumor. Yet, the lack of sufficient information combined with the complex and multifaceted interactions between TG, MS, and CRC currently precludes us from drawing definitive conclusions regarding the role of TG in the development or suppression of colorectal tumors. We hypothesize that the discrepancies in the data are due to the intricate interactions between TG and their receptors

in both normal and malignant tissues in the context of MS.

Speaking about the isolated effect of MS components on CRC, most studies indicate their complex effect not only on various types of cancer, but also on carcinogenesis in general. MS can not only contribute to the occurrence, development, and progression of CRC and other cancers, but it also influences patient outcomes after treatment, which highlights its significance and role in understanding colorectal carcinogenesis in thyroid dysfunction.

We set ourselves the task of deciphering and studying the root cause of the pathogenetic interaction of CRC, MS, and thyroid dysfunction. Disrupting this malignant triad will bring us closer to new horizons and strategies for combating cancer. It seems to us that the inclusion of a reasonable correction of thyroid hormone levels using HRT with thyroxine preparations and the control of MS components in patients with CRC can help improve the quality of early cancer diagnosis and treatment outcomes by increasing Life expectancy.

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Galectin-1 and -3: Intracellular Pathways of Signal Transduction in Carcinogenesis (Lecture)

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ABSTRACT

The lecture was created following the analysis of experimental data and review articles presented in the PubMed database. The lecture consists of five parts summarizing the literature data on galectin-1 and -3 in terms of their modulating effect in signal transduction processes. Possible mechanisms of galectin-1 and -3 involvement in proliferation, apoptosis, angiogenesis, migration, and adhesion of tumor cells are considered. The lecture data make it possible to identify intracellular signaling molecules, whose qualitative or quantitative changes can prove the effect of candidate compounds of galectin-1 and -3 inhibitors as potential antitumor agents.

Keywords: galectin-1, galectin-3, proliferation, apoptosis, angiogenesis, migration, adhesion, carcinogenesis

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Галектин-1 и -3: внутриклеточные пути сигнальной трансдукции в канцерогенезе (лекция)

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РЕЗЮМЕ

Лекция разработана на основе анализа данных экспериментальных работ и обзорных статей, представленных в базе данных PubMed. Лекция состоит из пяти частей, обобщающих данные литературы о галектине-1 и -3 с позиции их модулирующего действия в процессах сигнальной трансдукции. Рассмотрены возможные механизмы участия галектина-1 и -3 в пролиферации, апоптозе, ангиогенезе, миграции и адгезии опухолевых клеток. Данные, представленные в лекции, позволяют обозначить внутриклеточные молекулы-посредники, качественные или количественные изменения которых способны доказать действие соединений-кандидатов ингибиторов галектинов-1 и -3 как потенциальных противоопухолевых средств.

Ключевые слова: галектин-1, галектин-3, пролиферация, апоптоз, ангиогенез, миграция, адгезия, канцерогенез

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

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INTRODUCTION

High toxicity of non-selective antitumor agents supports the need to develop new targeted antitumor drugs. The involvement of galectin-1 and -3 in the regulation of main stages of oncogenesis and successful results of international studies on development of inhibitory compounds make it possible to consider galectins as a potential target of new antitumor agents. The complexity of molecular mechanisms regulating tumor growth and modulating effects of galectins on various signaling pathways justify the need to identify intracellular targets. Changes in the activity of these intracellular targets will confirm the specific action of a potential inhibitory compound.

The aim of this study was to analyze and systematize data on the involvement of galectin-1 and -3 in the main stages of oncogenesis and identify potential intracellular indicators confirming the inhibitory effect of candidate compounds as potential antitumor agents.

GALECTINS AS MODULATORS OF SIGNAL TRANSDUCTION

Galectins constitute a family of β-galactoside-binding proteins with a highly conserved carbohydrate recognition domain (CRD) present in the cytosol, cell nucleus, and extracellular space [1–3]. Depending on the number and arrangement of CRD, galectins can be classified in three groups:

– prototype galectins (galectin-1, -2, -5, -7, -10, -11, -13, -14, -15) displaying one CRD capable of dimerization;

– tandem repeat-type galectins (galectin-4, -6, -8, -9, -12) displaying two homologous CRDs connected by a linker amino acid sequence;

– chimera-type galectins (galectin-3) displaying one CRD linked to a collagen-like N-terminal site and capable of oligomerization into a pentamer [2–7].

Galectins located inside cells interact with cytoplasmic and nuclear proteins (β-catenin, hnRNPA2B1, gemin4, NUP98, importin α, Alix, LAMP 1 and 2, N-CAM) through carbohydrate-independent protein – protein interactions [2, 8, 9]. Extracellularly, galectins bind to glycoconjugates on the cell surface and in the extracellular matrix (laminin, fibronectin, vitronectin, and elastin) containing the disaccharide *N*-acetyllactosamine (Galβ(1-4)GlcNAc; LacNAc) forming a galectin – glycan structure called a molecular lattice or cluster [2, 8, 10–12]. Binding of glycoproteins by galectin results in prolongation of residency time of receptors on the cell surface and, thus, modulation of strength or duration of signal transduction [2, 9, 10, 12].

One of the variants of atypical glycosylation of membrane glycoproteins characteristic of malignant cell transformation is multiple branching of *N*-glycans, that is the addition of β1,6-linked N-acetylglucosamine (β1,6-GlcNAc) to α1,6-linked mannose [12–18]. Remodeling of cell surface glycans results from genetic and epigenetic changes in enzymes regulating the glycome structure – Golgi glycosyltransferases, the best studied of which is β1,6-*N*-acetylglucosaminetransferase V (Mgat5) [19]. The presence of a large number of *N*-glycan fragments in the structure of glycoprotein receptors, such as growth factor receptors (FGFR, EGFR, VEGFR, TGF-βR, etc.) [17], cell adhesion molecules (integrin-α1β1, -α3β1, -α4β7, -α6β1, -αMβ1) [16, 20], and epithelial cell mucins (MUC1, MUC4) [2], makes them preferred ligands for binding to galectins [2, 16, 17, 20].

The capacity of galetins to bind to a large number of glycoproteins on the cell surface and in the extracellular matrix determines their biological role in aggregation [2], angiogenesis [1, 2, 8, 20], migration [1, 8], adhesion [2, 17, 18, 21], growth regulation [18, 21], apoptosis [2], and metastasis [2, 20, 21] of tumor cells.

THE ROLE OF GALECTINS-1 AND -3 IN PROLIFERATION AND APOPTOSIS

The involvement of galectins in stimulating proliferation and suppressing apoptosis of tumor cells is due to their ability to interact with the Ras proteins

and, thereby, modulate the strength and duration of signals regulating growth, proliferation, and differentiation [2, 22, 23]. In tumor cells, the H-Ras, K-Ras, and N-Ras proteins are constantly active, which promotes their continuous proliferation. The interaction between galectins-1 and -3 and activated H-Ras (H-Ras-GTP) plays an important role in stabilizing the H-Ras – GTP complex at the membrane level, which is necessary for cell proliferation and migration [3–5, 9]. Active GTP-binding forms of Ras proteins induce various effector molecules, such as Raf-1, phosphoinositide 3-kinase (PI3K), and Ral-stimulator of guanine nucleotide dissociation (Ral-GDS) (Ral-GDS) [11, 22].

Galectin-1 shifts initial and epidermal growth factor (EGF)-stimulated steady state of Ras-GTP/Ras-GDP toward Ras-GTP. The galectin-1 – Ras-GTP complex activates Raf-1 by exerting an allosteric effect on Ras-GTP and reducing the effect of Ras-GAP (GTPase activating factor, activating the GTPase protein) on GTP hydrolysis by the Ras protein [5, 22]. Galectin-1 stabilizes H-Ras and K-Ras in the GTP-bound state. The affinity of H-Ras-GTP for galectin-1 has been found to be higher than that of K-Ras-GTP, and more galectin-1 molecules bind to H-Ras than to K-Ras [22]. Galectin-3 binds mainly to K-Ras-GTP rather than to K-Ras-GDP and H-Ras. Formation of the K-Ras/galectin-3 complex enhances and prolongs EGF-stimulated activity of not only Ras-GTP and Raf-1, but also PI3-K [2, 14, 24].

The interaction of galectin-3 and the K-Ras protein results in the activation of the downstream signaling pathways PI3K/Akt, PLC/PKC, Raf/MEK/ERK, RalGDS/Ral, and TIAM1/Rac, which promotes proliferation, migration, and invasion of tumor cells [5]. The Ras inhibitor farnesylthiosalicylic acid and the mitogen-activated protein kinase/ERK inhibitor UO126 are known to suppress galectin-3-mediated resistance to apoptosis [23]. Using molecular docking methods and *in vitro* studies, it has been established that the disruption of the galectin-1/Ras interaction induced by the experimental compound LLS30 is accompanied by suppression of the Ras/ERK signaling pathway, inhibition of proliferation, and induction of apoptosis in malignant peripheral nerve sheath tumor (MPNST) [25].

C. Fischer et al. (2005) demonstrated that galectin-1 suppressed integrin-dependent growth of hepatocellular carcinoma (HepG2), breast carcinoma (T-47D), and ovarian adenocarcinoma (OV-90) cell lines following inhibition of the Ras-MEK-ERK signaling cascade and subsequent induction of transcription of the *p21* and *p27* genes. Inhibition of cyclin-dependent kinase 2 by the p27 and p21 proteins resulted in cell cycle arrest in the G_1 phase and suppression of cell growth. The authors demonstrated that the antiproliferative effect of galectin-1 required a functional interaction of galectin-1 with the α5 subunit of the integrin α5β1receptor [26]. Suppression of galectin-3 expression in prostate cancer cell line (PC-3) resulted in cell cycle arrest in the G_1 phase, elevated levels of the nuclear protein p21, and hypophosphorylation of retinoblastoma protein (pRb), providing no effect on levels of cyclins (D1 and E), cyclin-dependent kinases (CDK2 and CDK4), and the p27 protein [27].

The activation of telomerase is one of the most important stages of carcinogenesis. Galectin-1 (*LGALS1*) gene overexpression was associated with high telomerase (*hTERT*) mRNA expression in tumor cells in patients with multiple myeloma [11]. Suppression of galectin-3 expression reduced the expression of the telomerase reverse transcriptase gene *hTERT* in tumor cells in patients with gastric cancer [4].

The canonical Wnt/β-catenin signaling pathway plays a key role in regulation of cell proliferation and differentiation. In the absence of an activating signal, low concentrations of β-catenin in the nucleus and cytoplasm are maintained by a protein destruction complex that includes the proteins Axin, APC, and protein kinase GSK-3β. Within this complex, β-catenin undergoes phosphorylation and subsequent degradation. Activation of the canonical Wnt signaling pathway caused by formation of the Wnt-ligand/Frizzled receptor/LRP5/6 coreceptor ternary complex results in translocation to membrane of the Dvl, Axin, and GSK-3β proteins, degradation of the destruction complex, and suppression of β-catenin phosphorylation. Stabilized β-catenin translocates into the nucleus, binds to transcription factors of the TCF/Lef family, and activates specific target genes (c-Myc, CyclinD1, COX-2, MMP7, and ITF-2) that regulate cell proliferation, differentiation, and migration [28, 29]. Galectin-3 activates the PI3K-Akt-GSK-3β signaling cascade leading to the accumulation of β-catenin in the nucleus [30]. Suppression of galectin-3 gene expression reduces the β-catenin protein expression in serous epithelial ovarian cancer (SEOC) cells [31], expression of the β-catenin target gene *CyclinD1*, and proliferation of human pancreatic ductal adenocarcinoma tumor cells [32].

The involvement of galectin-1 in cell proliferation regulation is mediated by its interaction with the neuropilin receptor (NRP-1) [33]. Recombinant human galectin-1 has been shown to enhance proliferation and metastasis of the gastric adenocarcinoma cell line (GC AGS) by activating the NRP-1/c-JUN/Wee1 signaling pathway and to increase resistance to cisplatin [34]. Nuclear kinase Wee1 belongs to the cyclin-dependent protein kinase family and is a key regulator of the cell cycle. By binding to terminal phosphorylation sites, it inactivates cyclin B, resulting in cell cycle arrest in the G_2 phase as a response to DNA damage. The NRP-1 inhibitor EG00229 suppresses galectin-1-induced proliferation and metastasis in GC AGS cells and restores cell sensitivity to cisplatin [34].

It has been established that binding of galectin-3 to the T-antigen on the mucin MUC1 activates the MAPK and PI3K/Akt signaling pathway, which is accompanied by activation of cell proliferation and motility [35].

High levels of galectin-1 expression in HepG2 cells promote epithelial-mesenchymal transition by inducing expression of the transcription factor SNAIL1 and activating the Wnt/PI3K/AKT signaling pathway with subsequent repression of E-cadherin [21]. The SNAIL1 factor induces transcription of the *MDR1* gene causing drug resistance, and post-transcriptionally suppresses the expression of the p53 protein preventing apoptosis of multiple myeloma cells [3, 11]. In gastric cancer patients, overexpression of galectin-1 in tumor tissue is associated with elevated expression of the mutant p53 protein incapable of exhibiting anti-oncogenic properties [36].

Studies of the effects of galetin-3 on apoptosis have revealed that it exhibits both pro-apoptotic and anti-apoptotic activity. The determining factors include the cell type, stimulus nature, and localization of galetin-3 [37, 38]. Galectin-3 has been found to increase resistance of malignant human urothelial cells (J82) to TRAIL (TNF α-related apoptosis-inducing ligand)-induced apoptosis by enhancing activity of the Akt protein kinase in the PI3K/Akt signaling pathway. Activation of the PI3K/Akt signaling cascade results in the formation of phosphatidylinositol 3,4,5-triphosphate which binds to the pleckstrin homology domain of the Akt Ser/Thr kinase. Subsequent attachment of Akt to the cell membrane and its activation phosphorylate specific target proteins, such as Bad, procaspase-9, GSK-3 (Glycogen Synthase Kinase 3), transcription factor FKHRL1 (FKHR-like protein 1, which is a member of the Forkhead transcription factor family), thereby promoting cell survival and blocking apoptosis [39].

Galectin-3 is known to contain the anti-apoptotic motif Asp-Trp-Gly-Arg (NWGR), which is conserved in the homology domain of Bcl-2 and is critical for the anti-apoptotic function of galectin-3 [2, 4, 8, 23, 38, 39]. The antiapoptotic effects of Bcl-2 display by stabilizing the mitochondrial membrane potential and suppressing the release of the apoptosis-inducing protein cytochrome C from the mitochondria [39]. Suppression of galectin-3 gene expression increases caspase-3 expression and induces apoptosis in oral squamous cell carcinoma (OSCC) cells [40]. Galectin-3 is capable of suppressing apoptotic signals by binding to Fas/CD95 [4]. Expression of galectin-3 mRNA in leukemic B cells is associated with the expression of proliferation markers (Ki-67 and PCNA) and the anti-apoptotic protein Bcl-2 [38]. The involvement of galectin-3 in cell apoptosis regulation is also associated with the ability to interact with annexin VII, Ca^{2+} ions and phospholipid-binding protein that mediates translocation of galectin-3 to the perinuclear membrane of mitochondria to control membrane integrity and release of cytochrome C [2].

A pro-apoptotic action is characteristic of the phosphorylated form of galectin-3. In human breast carcinoma cells (BT549), phosphorylated galectin-3 has been found to promote TRAIL-induced apoptosis by activating the proapoptotic protein Bad with subsequent release of cytochrome C [37]. Proline-46 isomerization in the N-terminal region of galectin-3 has been shown to enhance T cell apoptosis via activation of the ROS/ERK cascade [41].

Extracellular galectin-1 is capable of triggering T lymphocyte apoptosis via both the receptor and mitochondrial pathways [25, 42]. It was established that the JNK/c-Jun/AP-1 signaling pathway plays a key role in galectin-1-induced apoptosis of Jurkat T cells. The pro-apoptotic effect of galectin-1 is associated with lower expression of the anti-apoptotic protein Bcl-2 and, conversely, with the induction of the pro-apoptotic protein Bad [43].

Tumor cell-secreted galectin-1 induces T cell apoptosis via interaction of the carbohydrate recognition domain with LacNAc-linked galactose residues in the CD45 receptor structure [25]. F.R. Zetterberg et al. (2024) demonstrated that the galectin-1 inhibitor GB1908 suppressed galectin-1-induced apoptosis in Jurkat T cells [44].

Galectin-1-induced cell death comes amid phosphatidylserine externalization, chromatin

condensation, DNA fragmentation, and membrane blebbing. It has been shown that apoptosis of Jurkat T cells induced by galectin-1 is associated with rapid translocation from mitochondria to the nucleus of apoptotic endonuclease G which is capable of selectively cleaving double DNA strands at poly-G sequences. Apoptosis of T lymphocytes occurs before the loss of mitochondrial membrane potential and is not accompanied by release of cytochrome C, AIF translocation, and caspase activation. Interestingly, intracellular galectin-3 inhibits galectin-1-induced Jurkat T cell death. Intracellular expression of galectin-3 stabilizes mitochondrial membranes, prevents galectin-1-induced loss of mitochondrial membrane potential, and degradation of the anti-apoptotic protein Bcl-2 [45].

On the contrary, *in vitro* assessment of the effect of recombinant galectin-3 on apoptosis has revealed a dose-dependent pro-apoptotic effect on Jurkat tumor cells and CD^{4+} T lymphocytes associated with an elevated number of cells with a depolarized mitochondrial membrane [46, 47].

THE ROLE OF GALECTINS-1 AND -3 IN ANGIOGENESIS

Angiogenesis is one of the stages of invasion and metastasis of tumor cells [2]. During angiogenesis, tumor cells secrete vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [48]. A key role of VEGF in abnormal angiogenesis is due to the activation of VEGF receptor tyrosine kinases, such as EGFR1 (Flt-1), VEGFR2 (KDR/Flk-1), and VEGFR3, on endothelial cells (Flt-4) [13]. In patients with colorectal cancer, an elevated blood level of desquamated endotheliocytes correlates with a higher concentration of galectin-1, -3, and VEGF [49].

Activation of endotheliocytes by growth factors leads to higher expression of galectin-1 and protein transfer to the surface of endothelial cells [48]. Galectins-1 and -3 secreted by tumor cells stimulate abnormal angiogenesis through VEGF-dependent and VEGF-independent mechanisms. The interaction of recombinant human galectin-1 with mouse melanoma endothelial cells (B16-F10) *in vitro* promotes transmission of H-Ras signals in the Raf/mitogen-activated protein kinase/ERK cascade, which stimulates proliferation and migration [4, 48].

By recognizing complex *N*-glycans in VEGFR2 on endothelial cells, galectin-1 triggers VEGF-like signaling including phosphorylation of VEGFR2, ERK1/2, and Akt [4, 5, 11, 13]. The involvement of galectin-1 in the stimulation of glioma angiogenesis is associated with the activation of transmembrane kinase/ribonuclease of the endoplasmic reticulum, which regulates expression of the ORP150 protein (150-kda oxygen-regulated protein) [8]. The ORP150 protein accelerates intracellular transport of the VEGF protein from endoplasmic reticulum to the Golgi apparatus for subsequent secretion [50].

Galectin-1 also interacts with neuropilin-1 (NRP-1), which serves as a VEGFR co-receptor in endothelial cells and is necessary for tumor angiogenesis [5]. In vessels localized in tumors sensitive to VEGF blockers (anti-VEGF), a high level of α2-6-bound sialic acid is reported, which prevents galectin-1 binding. Tumors resistant to anti-VEGF treatment secrete elevated amounts of galectin-1. Interruption of β1-6GlcNAc branching in endothelial cells or suppression of galectin-1 expression transforms refractory tumors into anti-VEGF sensitive ones. Elimination of α2-6-bound sialic acid increases resistance to anti-VEGF treatment. Disruption of galectin-1 interaction with *N*-glycan promotes vascular remodeling, influx of immune cells, and suppression of tumor growth [13]. The involvement of galectin-3 in tumor angiogenesis is mediated by interaction of its carbohydrate-recognizing domain with *N*-glycans on VEGFR2 and integrin-αvβ3. The formation of clusters activating FAK-mediated signaling pathways regulates VEGF expression and migration of endothelial cells and leads to an increased angiogenic response to VEGF type A (VEGF-A) [2, 4, 14, 20, 51].

THE ROLE OF GALECTINS-1 AND -3 IN CELL MIGRATION

Cell motility is based on dynamic remodeling of the actin cytoskeleton and focal adhesions. When interacting with the extracellular matrix, integrin receptors cluster in the membrane plane, activate Src, and involve paxillin, talin, and vinculin in the formation of complexes that fix microfilaments. Tyrosine kinases of cytokine receptors together with integrins activate common oncogenic signaling mediators – protein kinase Cy, PI3K, Rac/Cdc42, and the adapter proteins Grb7, Grb2, and p130cas. Cells with low activity of focal adhesion kinase (FAK) are unable to move fibronectin bound to integrins along actin filaments necessary for formation of mature fibrillar adhesions [52].

It has been established that the expression of endogenous galectin-1 in human malignant astrocytoma cells (U343MG-A, U87MG, U87MG-10)

correlates with cell migration ability and invasiveness [53]. Galectin-1 acts as the main factor inducing epithelial-mesenchymal transition and metastasis by reducing the level of E-cadherin expression, activating the Hedgehog signaling pathway, transcription of NF-kB, followed by increased expression of the genes *MMP1, S100A7* and *ankyrin-3* which are responsible for invasion and migration of tumor cells [4, 54]. Lower galectin-1 expression induced by miRNA is accompanied by a decrease in the amount of integrin-β1 on membranes of human malignant erythrocytoma cells (U87, Hs683) at cell adhesion points, accumulation of integrin-β1 inside cells, and a parallel increase in the perinuclear localization of protein kinase C and vimentin, which cause integrin recirculation [8, 55].

The role of galectin-1 in tumor cell invasion is also associated with a higher level of matrix metalloproteinases (MMP-2, MMP-9) and reorganization of actin cytoskeleton by activating Cdc42, a small GTPase of the Rho family, followed by an increase in the number and length of filopodia on tumor cells [4]. I. Camby and et al. [2002] demonstrated an increase in the motility of malignant astrocytoma cells (U87, U373) under the action of recombinant galectin-1. The authors associated enhanced migration abilities of neoplastic astrocytes with changes in organization of the actin cytoskeleton and elevated expression of the small GTPase RhoA [56].

After interaction of glioblastoma cell integrin with the extracellular matrix, RhoA attracts actin. Then more stable focal adhesion complexes are formed, which connect lamellipodia to lamellae. Rho A activity decreases, Rac1 activity increases. Focal complexes either cause migration or mature, forming focal adhesions that enhance cellular adhesion and interfere with cell mobility. At the same time, the activity of Rho A increases, while that of Rac1 decreases. Thus, switching between RhoA and Rac1 activation determines the cell ability to detach and migrate, or to lock onto and attach to the extracellular matrix [57].

The involvement of galectin-3 in regulation of invasion and motility is due to weakening of contacts between cell adhesion molecules and N-glycosylated extracellular matrix proteins, such as laminin and fibronectin [4]. Galectin-3 stimulates secretion of interleukin-6 (IL-6) and colony-stimulating factors (G-CSF, GM-CSF) by endothelial cells of human lung micro vessels (HLMVECs). Cytokines promote autocrine or paracrine metastasis by enhancing expression of adhesion molecules by endothelial cells (integrin-$\alpha_v\beta_1$, E-selectin, ICAM-1, and VCAM-1) and their migration [20, 58]. Galectin-3 activates FAK and Rac1, which are involved in reorganization of the actin skeleton and formation of lamellipodia by N-glycosylation of integrin-α3β1 [20]. The experiment run by A. Lagana et al. (2006) showed that a galectin-3-induced sequential increase in the activity of FAK and PI3K attracts conformationally active integrin-α5β1 to fibrillar adhesions and increases the turnover rate of F-actin in breast carcinoma cells [52].

The heterodimeric transcription factor HIF-1 is one of the main molecules regulating the response of cells to hypoxia. The alpha subunit of HIF-1 is stable under hypoxia, while it is destroyed under normoxia. After activation and stabilization, the HIF-1 factor moves to the nucleus and induces transcription of target genes, followed by angiogenesis stimulation. It has been found that HIF-1 activation is accompanied by elevated galectin-3 expression. The interaction of the galectin-3-integrin-α3β1 complex with a structural component of microvascular pericytes, proteoglycan NG2, stimulates endothelial cell motility and morphogenesis [8].

THE ROLE OF GALECTINS-1 AND -3 IN CELL ADHESION

Galectins are described as matrix molecules that regulate cell adhesion to the extracellular matrix by interacting with the N-acetyllactosamine sequences of *N*-glycans, integrins, and mucins [2, 8, 52]. The αv integrins (αvβ3, αvβ5, αvβ6) form an abnormal cell surface repertoire due to the high affinity of their poly-N-acetyllactosamine (LacNAc) to galectin-3. On the cell surface, integrins and the galectin-3 pentamer form clusters that regulate adhesion to the extracellular matrix and migration of tumor cells [59]. The interaction of galectins with branched *N*-glycans on integrins is accompanied by renewal and maturation of fibronectin, fibrillogenesis of fibronectin, and remodeling of actin microfilaments [60]. The interaction of galectin-3 with modified β1.6 N-acetylglucosaminyltransferase V (Mgat5) *N*-glycans in the structure of α5β1-integrin promotes its activation and formation of focal contacts, thereby regulating fibrillogenesis of the extracellular matrix and migration of mammary carcinoma cells in mice [52]. Suppression of Mgat5 activity in tumor cells reduces the number of branched *N*-glycans, number of intercellular contacts, and cell migration [60].

Mucins (MUC) are highly glycosylated high-molecular-weight proteins synthesized primarily by epithelial cells. Two subfamilies of mucins are distinguished: secreted ones (MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, and MUC19) perform a protective function, and membrane-bound ones (MUC1, MUC3A, MUC3B, MUC4, MUC11-13, MUC15–17, MUC20, MUC21, and MUC22) are involved in cell adhesion, intercellular receptor interactions, signal transduction, and growth and proliferation of epithelial cells.

One of the most studied mucins is type 1 transmembrane mucin (MUC1), which is physiologically expressed on the apical surface of secretory epithelial cells. MUC1 is considered to be the most significant target of galectin-3 [2]. The formation of hydrogen bonds between galectin-3 and T-antigen (CD176; Thomsen-Friedenreich antigen (TF)), a structural component of MUC1 associated with the protein framework, is important for interaction of galectin-3 and MUC1 [2, 4]. In normal epithelial cells, the T-antigen undergoes glycosylation and sialylation; in tumor cells, it is expressed in an open form.

Tumor cells are also characterized by abnormal glycosylation and overexpression of the aberrant mucin MUC1 containing shortened overly sialylated O-glycans and branched *N*-glycans. The interaction between MUC1 with unmodified T-antigen and galectin-3 leads to clustering of MUC1 on the surface of tumor cells and exposure of smaller adhesion molecules, such as E-cadherin. Subsequent cell aggregation prevents anoxosis, one of the main mechanisms for removing cancer cells from the bloodstream, due to inability of cells to adhere [2]. The formation of the galectin-3-MUC4 complex is accompanied by clustering of mucin and exposure of latent adhesion molecules, in particular integrins, which promotes the attachment of tumor cells to endothelial cells [2].

CONCLUSION

By interacting with atypical N-glycans in the structure of receptors regulating proliferation, apoptosis, adhesion, angiogenesis, and metastasis, galectin-1 and -3 promote oncogenesis. The receptor-mediated maintenance of tumor-transformed cells allows galectin-1 and -3 to be positioned as targets for compounds with antitumor effects. The challenge in this approach is related to polyreceptor interaction and involvement of various signaling cascades in key processes of tumor progression. It is necessary to perform a comprehensive assessment of the function of the receptor initiating a corresponding stage of oncogenesis and the signaling messenger proteins regulated by it to make it possible to confirm or refute an inhibitory effect of a potential compound with an antitumor effect.

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Protein Analysis Capabilities in the NCBI Bioinformation System

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ABSTRACT

Aim. To review and summarize information about the features of protein data storage, as well as the possibilities for their analysis using NCBI tools.

The lecture summarizes data on existing repositories of protein sequences and structures and analyzes the capabilities of bioinformatics tools for protein research on the NCBI (National Center for Biotechnology Information) platform. The primary databases contain information about proteins (records) obtained through experimental studies; in addition, databases with supplementary information added by curators after analysis are also presented. Furthermore, bioinformatics analysis of protein sequences and structures using the tools discussed in this lecture enables the identification of phylogenetic features, as well as the prediction of functions and structures. Thus, the extraction of extensive information and its analysis through specialized services facilitate insights into *in silico* research of experimentally undetected protein characteristics, providing new knowledge that forms the basis for further investigations.

Keywords: bioinformatics, protein sequence, domain, alignment, three-dimensional structure, NCBI

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Возможности анализа белков в биоинформационной системе NCBI

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РЕЗЮМЕ

Цель – рассмотреть и обобщить информацию об особенностях хранения данных о белках, а также о возможностях их анализа с помощью инструментов NCBI (National Center for Biotechnology Information, Национальный центр биотехнологической информации).

В лекции обобщены данные по существующим хранилищам белковых последовательностей и структур, проанализированы возможности биоинформационных инструментов для исследования белков на платформе NCBI. Первичные базы данных содержат информацию о белках (записи), полученную при проведении экспериментальных исследований. Помимо этого представлены базы с дополнительной информацией, добавленной кураторами после аналитики. Биоинформационный анализ белковых последовательностей и структур с помощью представленных в лекции инструментов позволяет выявить особенности филогенети-

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ческого развития, спрогнозировать функции и структуры. Таким образом, извлечение обширной информации и возможность ее анализа с помощью специализированных сервисов помогает пролить свет при исследовании *in silico* на необнаруженные экспериментально характеристики белков, получить новые знания, служащие основой для дальнейших теоретических и экспериментальных исследований.

Ключевые слова: биоинформатика, последовательность белков, домен, выравнивание, трехмерная структура, NCBI

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

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

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INTRODUCTION

Analysis of protein sequences and proteomes is a key area of bioinformatics research, serving as a means to address a wide range of medical and biological problems. Leading bioinformatics resources and platforms include tools for conducting these studies. This article reviews the most important of these tools, provided by the National Center for Biotechnology Information (NCBI) System.

NCBI is an information retrieval and integration system that encompasses numerous databases and tools for bioinformatics analysis, including those for proteins. These include data on protein sequences, protein molecule structures, as well as tools for their comparison and visualization, along with tools and databases for protein domain analysis. Overall, the system's capabilities are realized through a comprehensive set of databases and bioinformatics services. Currently, NCBI provides search and data extraction from 31 separate repositories and knowledge bases [1, 2].

One of the key features of NCBI is its search system, which allows for access not only to records within this platform but also from other repositories [3]. Access to records – specifically protein sequences – is essential for performing fundamental bioinformatics operations, such as sequence alignment (pairwise and multiple) [4, 5]. Pairwise sequence alignment involves comparing one sequence with another (by lining them up one under the other) to achieve maximum similarity, while multiple alignment compares three or more sequences simultaneously. The primary goal of alignment is to identify identical regions across different sequences, calculate the identity score, and thereby facilitate the identification of homologous protein sequences, track their evolutionary changes, and analyze functions based on the observed similarities [4, 6].

Analysis of protein sequences within the context of homologous clusters (orthologous and related groups) is crucial for functional and evolutionary genome analysis. To maximize the information extracted from the rapidly accumulating number of genome sequence records, all conserved genes must be classified according to their homologous relationships. Comparing proteins encoded in complete genomes of certain phylogenetic lineages enables the identification of sequence similarity patterns and the delineation of Clusters of Orthologous Groups (COGs). Each COG comprises individual orthologous proteins (similar proteins across different species) or sets of paralogs (similar proteins within a single species). Orthologs most often perform equivalent functions, which can facilitate successful functional predictions for genomes that are poorly characterized [7].

Since the identification of protein functions uses the domains and motifs they contain, tools capable of detecting these features are widely used in proteomics.

Working with three-dimensional protein structures enables the study and modeling of molecular interactions, which is essential for understanding cellular processes and for drug development. Tools for 3D visualization are also described in this lecture.

The examples of data extracted from bioinformatics repositories and tools presented in the lecture focus on SARS-CoV-2 proteins (Severe Acute Respiratory Syndrome-related Coronavirus 2).

In general, the study and prediction of various protein properties require modern bioinformatics approaches and tools. It is necessary to utilize databases and tools tailored to specific research tasks, whose key characteristics are described below.

NCBI DATABASES CONTAINING PROTEIN INFORMATION

The main NCBI databases that include protein-related information are BioProject, Conserved Domain Database (CDD), HIV-1, Human Protein Interaction Database, Identical Protein Groups, Protein Clusters, Protein Database, Protein Family Models, and Reference Sequence (RefSeq) [1, 2]. Below, examples of their characteristic data extraction related to SARS-CoV-2 protein information will be discussed .

The BioProject database (https://www.ncbi.nlm.nih.gov/bioproject) serves as an organizational platform for accessing information on research projects, with links to data deposited in archives maintained by members of the International Nucleotide Sequence Database Collaboration [8]. In addition to genomics and transcriptomics data, the database contains records of proteins and proteomes. Information within the repository is presented as a set of linked data, referred to as a "project". BioProject distinguishes between two types of projects: primary projects — data posted for the first time (using the NCBI submission portal) that can be registered by submitters; and umbrella projects with higher-level organizational structures for larger initiatives that provide an additional level of data tracking. These umbrella projects are created upon request [9]. Currently, regarding SARS-CoV-2 research, there are 177 umbrella projects and 3,639 primary projects posted for the first time (of which 185 contain data on proteins).

CDD (The Conserved Domain Database, CDD, https://www.ncbi.nlm.nih.gov/cd) is a resource for annotating functional modules (i.e., domains) in proteins. Its collection contains a set of NCBI-curated data, including 3D structures [10]. CDD provides well-annotated models of multiple sequence alignments for conserved domains and full-length proteins. These multiple alignments generate profiles of aligned sequences, with homologous (similar) regions arranged in columns across the length of the sequences (an example is given below). The aligned protein regions are expected to share a common origin, perform similar functions, and exhibit spatial similarity. These models are used to identify domains within protein sequences. Collections of domain alignment models are crucial for studying protein evolution and for annotating genomic sequences [11]. When searching this domain database with a query related to SARS-CoV-2, the user receives 81 results, which include families of different proteins from the corresponding species. One of these results – ORF8-Ig_SARS-CoV-2-like (Fig. 1) – represents a subfamily that includes the immunoglobulin (Ig) domain protein ORF8 (SARS-CoV-2) and related proteins from ORF8 sarbecoviruses.

The results of multiple domain alignment of these proteins demonstrate a high degree of similarity and relatedness (Fig. 2):

The CDD also includes NCBI-curated domains that utilize information about 3D structures to define domain boundaries and provide insights into the possible relationship between a protein sequence, its structure, and its function [12]. Domain curation enables users to gain insights into patterns of conservation of residues and divergence during evolution within protein families, as well as their relationship to functional properties. To enhance traditional multiple sequence alignments – the foundation of domain models – the repository incorporates additional types of information.

– Regarding 3D structures and major conserved motifs: curators extract multiple protein alignments from external resources, aligning them with 3D structural data and their superposition (spatial overlap). As a result, users are presented with aligned blocks that include all lines of the multiple alignment without gaps, along with unaligned regions between them. These blocks illustrate the conserved core regions of the corresponding domain family, and the 3D structures can be interactively visualized using the Cn3D tool [11]. Thus, for the SARS-CoV-2 ORF8 Ig family discussed above, the 3D structure of proteins can be studied in Cn3D by selecting this option.

– Regarding conserved features/sites. In addition to multiple sequence alignments of proteins, NCBI curators record locations and properties of entities within a domain family, when it is possible. This typically includes catalytic residues, binding sites, or motifs that are referenced in the literature.

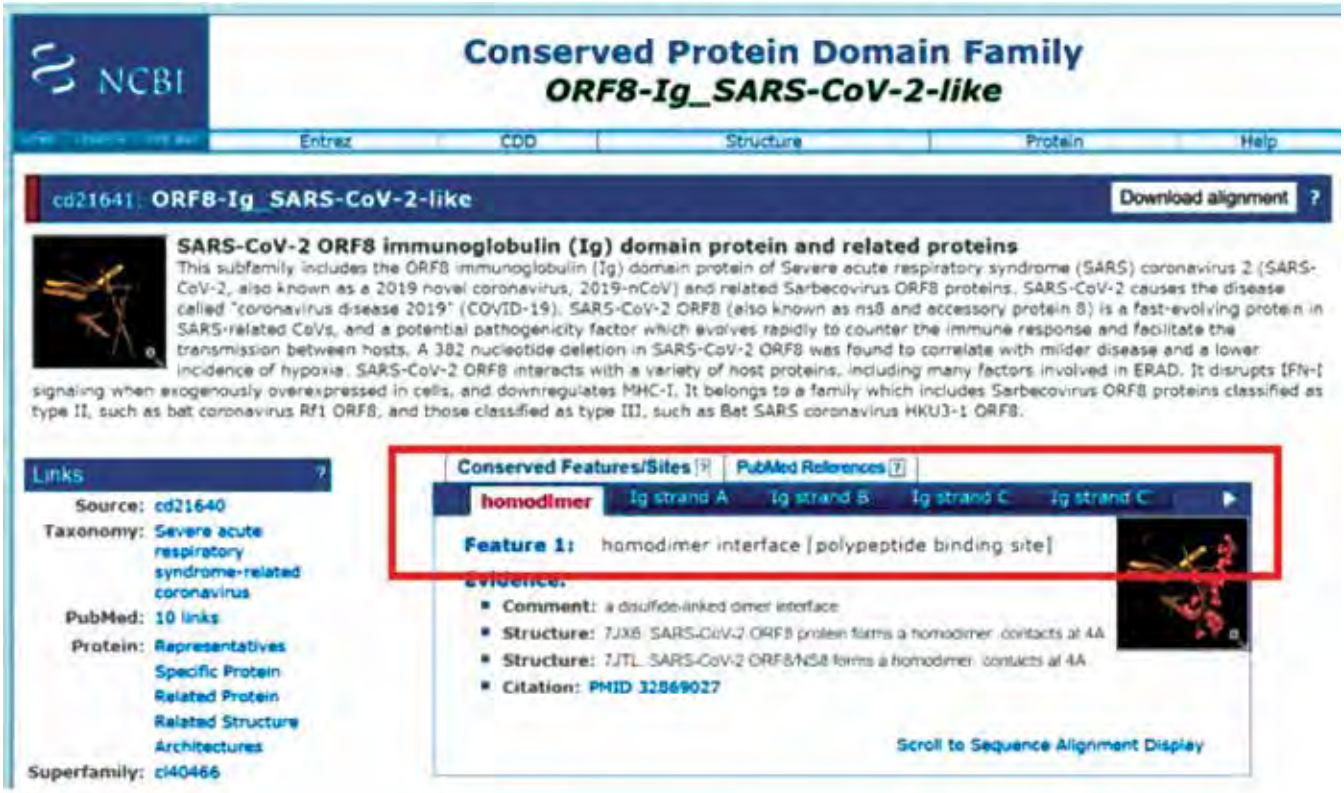

Fig. 1. The subfamily that includes the immunoglobulin (Ig) domain protein ORF8 (SARS-CoV-2) and the ORF8 proteins of sarbecoviruses. The section with data (tabs) on conserved sites identified using the NCBI-curated repository is highlighted in red.

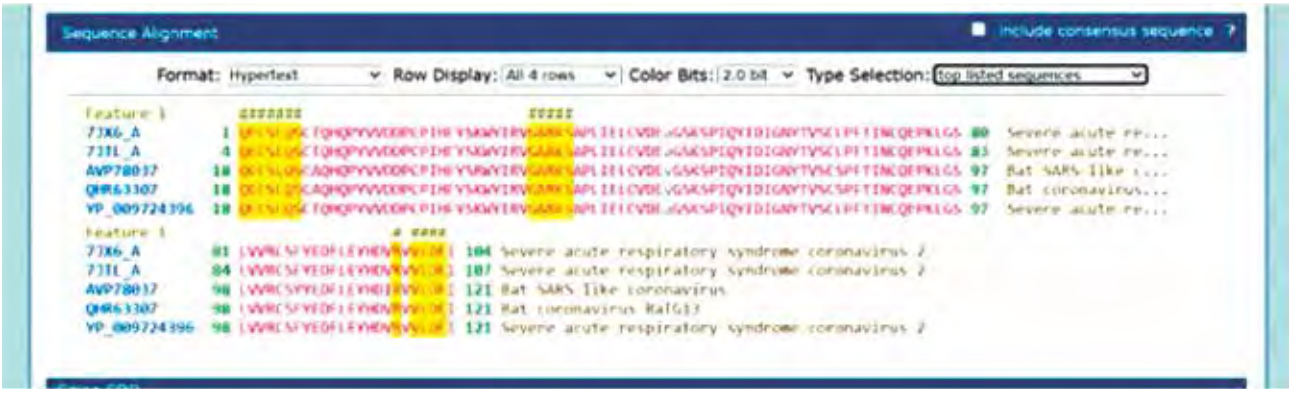

Fig. 2. Multiple protein alignment. All residues in each row of the sequence are shown, with aligned residues in uppercase and unaligned residues in lowercase. The horizontal scale indicates the number of residues in the overall sequence. The numbers at the beginning and end of each sequence row specify the range of sequence elements imported from the complete protein record

Functions that are potentially applicable to the domain family under analysis are also incorporated into the database if there is evidence linking these functions to a set of alignment elements within the family [13]. In this example, conserved features and sites are annotated within the NCBI-curated domain and are noted in the summary field at the top of the page, with a separate tab for each function (Fig. 1);

– Regarding phylogenetic organization: Based on sequence comparison data, curators organize models of related domains into a phylogenetic hierarchy of families. A domain family hierarchy consists of related domains sharing a common ancestor, a set of conserved residues, and a common function. However, these families may exhibit differences in phylogenetic features, specific functions, and additional conserved residues. Such hierarchies facilitate understanding how patterns of residue conservation and divergence within a protein family relate to their functional properties [12].

- Regarding links to electronic literature resources: NCBI-curated domains also provide active links to citations containing information — if available — about the domain's biological function, evolutionary context and classification data in PubMed (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd_help.shtml#Link_cdd_pubmed) and NCBI Bookshelf (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd_help.shtml#Link_cdd_books).

CDD also includes data imported from several external databases (Pfam, SMART, COG, PRK, TIGRFAM) [10]. As a result, the current version of CDD, v 3.20, contains 59,693 protein and protein domain models derived from Pfam (19,178 models), SMART (1,009), COGs collections (4,871), TIGFAMS (4,488), NCBI protein cluster collections, NCBIfam (1,125) and CDD's own data curation results (18,882) [10]. Although these external databases are designed for different purposes, they address specific subsets of the protein space and vary in size, together they facilitate large-scale domain analysis.

The largest collection of multiple alignments within CDD is Pfam (http://pfam.sanger.ac.uk/), which covers data on common protein domains and families. Each family is represented by multiple sequence alignments and hidden Markov models (HMMs). The diversity of existing proteins arises from various combinations of domains, and identifying these domains in proteins provides valuable insights into their functions [14]. For example, in research related to the pathogen and disease process of COVID-19, as well as in the search for treatment options, Pfam offers useful annotations for SARS-CoV-2. The models, family names, and annotations for this virus are periodically updated. Nearly all gene products encoded by SARS-CoV-2 have now been reviewed; however, Orf10 – a small protein encoded at the 3' end of the SARS-CoV-2 genome – remains unannotated by Pfam [14].

Another tool, SMART (http://smart.embl-heidelberg.de/), in addition to identifying and annotating protein domains in the studied sequences, offers functions for the comparative analysis of complex domain architectures in proteins. It contains manually curated models for more than 1,300 protein domains, with families that are thoroughly annotated in terms of phylogeny, functional classes, 3D structures, and functionally important residues of the molecules [15].

COG (https://www.ncbi.nlm.nih.gov/research/cog-project/) is a protein classification resource also curated by NCBI. The project was originally developed to provide functional annotation of common bacterial and archaeal genes, clustering their protein products based on sequence similarity, reflecting their shared evolutionary origin. By including only genes from fully sequenced genomes, COG enables accurate identification of orthologous genes (or gene groups). COG offers precise and up-to-date annotations of the most prevalent bacterial and archaeal protein families, as well as those that are poorly studied or uncharacterized [16].

TIGRFAMs (https://www.ncbi.nlm.nih.gov/Structure/cdd/docs/tigrfams.html) is a collection of manually curated protein families with a focus on prokaryotic sequences, primarily intended for researchers working in this field [17].

Protein Clusters (https://www.ncbi.nlm.nih.gov/proteinclusters) is a collection of related NCBI protein clusters composed of proteins from the RefSeq reference sequence database (https://www.ncbi.nlm.nih.gov/RefSeq/), extracted from complete genomes, organelles, and plasmids. Each protein cluster is represented by a list of protein identifiers and the genomes that encode them. Currently, Protein Clusters includes data from archaea, bacteria, plants, fungi, protozoa, and viruses; it encompasses both

curated and uncurated (automatically generated) clusters [18].

NCBI Virus (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/) is an integrated resource that offers optimized search and analysis of curated collections of virus sequences and large datasets from GenBank and other NCBI repositories. The database contains information on various viruses, including hepatitis B and C, dengue virus (DENV), enterovirus A, and influenza; a dedicated repository is allocated for SARS-CoV-2 (SARS-CoV-2 Data Hub). Currently, NCBI Virus includes sequence data from GenBank and RefSeq. The repository also features annotated bibliographies of published protein interaction reports, with links to relevant sequence records [19].

Identical Protein Groups (https://www.ncbi.nlm.nih.gov/ipg) is a collection of consolidated records describing proteins identified by annotated coding regions in GenBank and RefSeq databases, as well as SwissProt and PDB. This resource enables researchers to obtain more targeted search results and quickly identify a protein of interest. Typically, searching for a specific protein in the Protein database can be challenging due to the large number of records returned. Protein Groups simplifies this process by searching through groups of records, each associated with a single unique sequence. Each group is considered as a single hit, resulting in a smaller set of results and faster identification of the protein of interest [20].

The Protein Database (https://www.ncbi.nlm.nih.gov/protein) includes protein sequences from several sources, such as GenPept, RefSeq, SwissProt, PIR, PRF, and PDB [20]. This extensive repository provides access to sequence collections from different databases – including translation results from annotated coding regions of GenBank, RefSeq, and TPA – as well as records from other protein databases like SwissProt, PIR, PRF, and PDB. The Protein Database is an essential resource for working with proteins, since protein sequences form the basis for determining their structure and function. Sequence analysis allows for establishing homology, determining phylogenetic relationships, characterizing functions, and modeling structures. The large size of the database facilitates these tasks. For example, numerous proteins can be retrieved from the Protein Database for various studies of SARS-CoV-2, since currently, the database contains 1,461 SARS-CoV-2 Mpro protein sequences.

Protein Family Models (https://www.ncbi.nlm.nih.gov/protfam) contain a set of models representing homologous proteins with a common function. The database includes conserved domain databases (CDD), hidden Markov models, and BlastRules [21]. Families based on hidden Markov models are created by transforming multiple sequence alignments with known functions and serve as probabilistic frameworks for determining whether a particular protein belongs to a specific family. BlastRules are a type of evidence used for functional classification of proteins based on BLAST tool (Basic Local Alignment Search Tool, which is discussed in the next section). A BlastRule consists of one or more "model" proteins with known biological function, and BLAST helps identify proteins similar to a given "model" [21]. For SARS-CoV-2 proteins, the repository currently identifies 47 models, including 17 hidden Markov models and 10 conserved domain architectures.

The Reference Sequence database (RefSeq, https://www.ncbi.nlm.nih.gov/RefSeq/) contains a comprehensive, well-annotated collection of NCBI genomic DNA, transcript (RNA), and protein sequences, making it particularly popular among researchers. RefSeq provide a high-quality information base for various studies, including genome annotation, gene identification and characterization, mutation and polymorphism analysis, expression studies, and comparative analysis. The RefSeq collection can be accessed through nucleotide and protein databases [22, 23].

NCBI BIOINFORMATICS TOOLS FOR PROTEIN ANALYSIS

Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/) identifies regions of local similarity in biological sequences. The program compares a query nucleotide or protein sequence against sequences in databases (the search databases and parameters can be specified by the user) and calculates the statistical significance of the matches [24]. BLAST enables the identification of local sequence similarities, which are used to analyze functional and evolutionary relationships between sequences.

BLAST was developed in 1990 based on the k-tuple method, since then it has been integrated into

GenBank, undergoing numerous updates to enhance efficiency and accuracy. The word or k-tuple method [5, 25, 26] is a rapid heuristic pairwise alignment approach typically used as an initial step when dealing with large datasets. The similarity score S_{ij} between sequences *i* and *j* is defined as the number of matching k-tuples in the best pairwise alignment minus a fixed gap penalty. For DNA and RNA, *k* generally ranges from 2 to 4, while for amino acids, *k* is usually 1 or 2. Although this method does not guarantee an optimal alignment, it is a fast heuristic technique that can be used to initialize BLAST and facilitate multiple sequence alignments. The BLAST algorithm begins by creating a list of k-letter words from the query sequence. It then searches the database for potential matching k-letter words and scores them; all words scoring above a certain threshold are retained. Higher-scoring words are stored in a search tree. This process is extended to identify high-scoring pairs (HSPs), which involve searching for similar words (not necessarily exact matches) [27, 28]. As a fundamental tool, BLAST is used to detect, identify, or find similar sequences within a database. For example, researchers have identified coronavirus-like sequences in other organisms, such as pangolins [29] and bats [30]. BLAST has also been employed to detect SARS-CoV-2 in environmental samples [31, 32] , including wastewater [33, 34].

In work by M. Parmar et al. [35], pairwise BLAST comparisons of SARS-CoV-2 Mpro protein sequences with other Mpro sequences were performed to assess potential identity. The results allowed for evaluation of the similarity between SARS-CoV-2 Mpro and its closest known homologs (SARS- CoV , MERS- CoV , Bat-CoV-RaTG13, HCoV -HKU, HCoV-OC43, HCoV-NL63, and HCoV-229E) facilitating the identification of conserved regions. Sequence analysis revealed that most residue changes (8 out of 12) occurred in domains I and II of the Mpro β-chains – regions containing the catalytic/inhibitor region – while the remaining four residues were located in domain III [35]. In another study by R. Naderi Beni et al. [36], BLAST was also used for bioinformatic analysis of the SARS-CoV-2 Mpro structure and its ligands and inhibitors; however, this analysis utilized a database containing three-dimensional protein structures [36].

In addition, there are specialized versions of BLAST designed to address more specific issues:

SmartBLAST (https://blast.ncbi.nlm.nih.gov/smartblast/?LINK_LOC=BlastHomeLink) for searching proteins with a high degree of similarity;

IgBLAST (https://www.ncbi.nlm.nih.gov/igblast/) for searching immunoglobulin and T-cell receptor sequences,

CDART (https://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi?cmd=rps) for identifying sequences with similar architectures of conserved domains.

Another tool is Batch Entrez (https://www.ncbi.nlm.nih.gov/sites/batchentrez), which allows users to retrieve records from multiple NCBI databases by uploading a file containing sequence identifiers (individual numbers) from the relevant repositories. The search results are sequence records that can be saved to a file for further analysis.

COBALT (https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi) performs multiple alignments of protein sequences using information about conserved domains and local sequence similarity, based on tools from the BLAST family [37].

Cn3D (https://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml) is a standalone visualization application for viewing NCBI 3D structures that must be installed on your computer. Cn3D simultaneously displays various data types: structure, sequence, multiple sequence alignment, and offers alignment editing capabilities. Additionally, NCBI provides an interactive 3D visualization tool for macromolecules called iCn3D (https://www.ncbi.nlm.nih.gov/Structure/icn3d/icn3d.html), which does not require installation. It allows for visualization of interaction surfaces, binding sites, export of models for 3D printing, alignment of two structures or chains, and comparison of protein sequences with structures [38].

The Conserved Domain Search Service (CD Search, https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) is used to identify conserved domains within protein sequences. If CD Search detects a specific match, it indicates a high degree of association between the query sequence and the corresponding conserved domain. This information can serve as a basis for understanding the functional classification of the query protein [10].

E-Utilities (https://www.ncbi.nlm.nih.gov/books/NBK25501/) are tools that provide access to data within the NCBI system beyond the standard web query interface. They offer a means to automate

tasks within software applications. Each utility performs a specific search task and can be used by constructing a specially formatted URL. E-utilities utilize a fixed URL syntax that converts a standard set of input parameters into the values required by NCBI software components to search and retrieve the requested data (including nucleotide and protein sequences, gene transcripts, three-dimensional molecular structures, and literature) [39].

The ProSplign tool (https://www.ncbi.nlm.nih.gov/sutils/static/prosplign/prosplign.html) is used to align distantly related proteins with low sequence similarity, based on genomic DNA sequence data. It is built on a variation of the global alignment algorithm and specifically accounts for the presence of introns [2].

The tools described above, which employ sequence alignment methods and algorithms, are widely used in SARS-CoV-2 research. They are applied to identify mutations and compare viral sequences across different species and organisms [40, 41, 42], to elucidate mechanisms of transmission of asymptomatic COVID-19 infection [43], to study the impact of mutations on its diagnosis and treatment [44], and to compare SARS-CoV-2 sequences with other animal and human coronaviruses as well as related artificial constructs [45, 46]. The data presented demonstrate that sequence alignment is an essential approach for analyzing and modeling protein properties [40–46].

CONCLUSION

Analysis of protein sequences, evolution, structure, and functions can be more comprehensive and complete when utilizing the NCBI platform repositories and bioinformatics tools. The information provided, including the results of curatorial analysis, can help clarify the structure of proteins of interest, their domain composition, conservation, and divergence during speciation and facilitate the exploration of structure and function for a wide range of proteomics issues and beyond.

NCBI protein data mining encompasses sequence records, collections of conserved domains and protein families, 3D structures, and research project information, all designed to address diverse needs. However, all data share cross-referencing, access to external repositories, and user accessibility.

NCBI tools are designed to employ bioinformatics algorithms for analyzing protein data within the NCBI databases: to find similar protein sequences, identify conserved domains, determine function and structure, and visualize the information.

The variety of services enables researchers to apply a broad spectrum of approaches for protein data analysis. Continuous improvements in tool versions, algorithm optimization, and the addition of new sections to data repositories make these resources indispensable elements of modern research.

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Modern Methods of Diagnosis and Treatment of Severe Bronchial Asthma (Systematic Review)

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ABSTRACT

Currently, the prevalence of bronchial asthma (BA) is steadily increasing worldwide. Official statistics show that severe BA accounts for 5–10% of cases in the severity profile of this disease, and when treated with high doses of corticosteroids, uncontrolled symptoms persist in most people, which significantly reduces their quality of life. This supports the relevance of finding new strategies for the treatment of severe BA. The aim of the review was to analyze and summarize published data on modern approaches to the diagnosis and treatment of severe BA.

Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, 3,177 sources were found. Excluding publications that were unavailable for viewing allowed us to leave 578 sources, of which 120 papers were relevant to the study topic to some extent. Of these, 63 sources were selected that contained the information necessary for the study and met the selection criteria for the studies: 28 of them were review articles and 35 were original studies (randomized controlled, cohort, and case-control studies). The work presents a description of phenotypes and endotypes, as well as characteristics of modern biomarkers of severe BA.

Particular attention is paid to a new approach to the treatment of severe BA. The conducted studies, systematized in this article, indicate that a detailed description of asthma phenotypes and endotypes can help identify new biomarkers and therapeutic targets specific to each endotype. Profound knowledge of the patient's phenotype and endotype can determine a personalized approach to the treatment of severe BA.

Keywords: severe bronchial asthma, biological therapy, phenotype, endotype, biomarkers

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Современные методы диагностики и лечения бронхиальной астмы (систематический обзор)

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РЕЗЮМЕ

В настоящее время распространенность бронхиальной астмы (БА) во всем мире неуклонно растет. Данные официальной статистики свидетельствуют, что от 5 до 10% случаев в структуре тяжести данного заболевания составляет тяжелая БА и при ее лечении высокими дозами ингаляционными кортикостероидов (ИКС)

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сохраняются неконтролируемые симптомы у большинства людей, что значительно снижает качество их жизни. Это поддерживает актуальность поиска новых стратегий лечения тяжелой БА. Цель обзора заключалась в проведении анализа и обобщении опубликованных данных о современных подходах диагностики и лечения тяжелой БА.

Используя рекомендации «Предпочтительные элементы отчетности для систематических обзоров и мета-анализов» (PRISMA), были обнаружены 3177 источников. Исключение публикаций, недоступных для просмотра, позволило оставить 578 источников, из которых теме исследования в той или иной степени соответствовали 120 работ. Из них отобраны 63 источника, содержащие необходимую информацию и соответствующие критериям отбора исследования. Из них 28 обзорных и 35 оригинальных исследований (рандомизированные контролируемые, когортные и исследования «случай – контроль»). Представлено описание фенотипов и эндотипов, а также характеристика современных биомаркеров тяжелой БА. Особое внимание уделяется новым подходом лечения тяжелой БА.

Проведенные исследования, систематизированные в данной статье, свидетельствуют о том, что подробное описание фенотипов и эндотипов БА может помочь выявить новые биомаркеры и терапевтические мишени, специфичные для каждого эндотипа. Глубокое знание фенотипа и эндотипа пациента может определить персонализированный подход к терапии тяжелой БА.

Ключевые слова: тяжелая бронхиальная астма, таргетная терапия, фенотипы, эндотипы, биомаркеры

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

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INTRODUCTION

Bronchial asthma (BA) is a chronic inflammatory disease of the airways, the mechanisms of which are associated with the influence of many factors, including genetic predisposition, environmental exposure, and regulation of the immune system [1]. The prevalence of BA increased sharply in the late 20th century, and despite the advent of biological drugs, in 2019, the WHO recorded that the incidence of BA reached 260 million cases, and the mortality rate was 450 thousand per year [2]. Allergic BA is associated with increased sensitivity to allergens, and their repeated exposure to the airways leads to the activation of antigen-presenting cells [3]. The developing inflammation can be characterized by a T2 or non-T2 immune response (endotype) [4], and each of them can lead to a severe uncontrolled course of the disease. However, the non-T2 endotype is more often associated with uncontrolled asthma [5] and ineffectiveness of step-down therapy with glucocorticoids (GSs) [6, 7]. It is necessary to develop new therapeutic strategies for severe BA based on modern scientific data on inflammation and biotargets.

The aim of the work was to present modern data on the study of biotargets to improve the effectiveness of targeted therapy based on the analysis of scientific publications, including recommendations for the use of biomarkers for the diagnosis of phenotypes and endotypes of bronchial asthma.

MATERIALS AND METHODS

The study material included publications in scientific journals that to some extent touched upon the topic of the study. The search was carried out in the scientific electronic library Elibrary.ru. In the advanced search, using the keyword combination “severe bronchial asthma”, 3,177 sources were found. Further limitation by the writing time from 2015 to present and exclusion of any forms of manuscripts except for articles in scientific journals narrowed the search to 2,269 sources. Exclusion of publications unavailable for viewing allowed us to leave 578 sources. A search was also carried out in the PubMed database, where 1,094 sources were found in the advanced search using the keyword combination “severe bronchial asthma”. The keywords for selecting articles were: biological therapy or targeted

therapy or asthma biomarkers or asthma biotargets. Of these, 120 works corresponded to the research topic to one degree or another, of which 63 sources were selected containing the information necessary for the study and meeting the study selection criteria. Twenty-eight of them were review studies and 35 – original articles (randomized controlled, cohort and case-control studies). References of the found articles also contained less than 10% of sources published in 1998–2014.

PHENOTYPES AND ENDOTYPES OF BRONCHIAL ASTHMA

The 2024 Clinical Guidelines for Bronchial Asthma contain a section devoted to the characteristics of clinical phenotypes. In particular, allergic (atopic) and non-allergic (including aspirin-induced, including occupational) asthma are distinguished, as well as late-onset asthma, asthma with fixed airflow obstruction, and asthma in obese patients. This approach to diagnosis is widely used in clinical practice when determining a treatment program for most patients [8].

However, patients with severe BA symptoms require personalized treatment using biological / targeted therapy, when it is necessary to determine a specific biotarget and diagnose it using biomarkers. In this regard, a classification of BA is currently proposed based on information on the molecular and cellular mechanisms of inflammation, which makes it possible to distinguish endotypes and inflammatory phenotypes of the disease. This is a fairly new area for clinical science, where data are rapidly accumulating, allowing for more accurate selection of targeted drugs and development of new medications, but many questions remain unanswered [9, 10].

Currently, two endotypes of BA are recognized based on the type of immune response – T2 and non-T2. The T2 endotype is based on the dominance of the CD4+ T cell response, providing eosinophilic inflammation. However, evidence has recently been obtained about type 2 innate lymphoid cells (ILC-2) as the primary regulators of the type 2 immune response. ILC-2 express the main transcription factor GATA 3, which, in turn, regulates the production of type 2 cytokines. Thus, biomarkers of the T2 endotype include eosinophilia, high levels of IL-4, IL-5, and IL-13 in the blood and sputum, the presence of ILC2 in the blood and respiratory tissues, high levels of total immunoglobulin E (IgE) in the blood, increased levels of fractional exhaled nitric oxide (FeNO), and a good response to inhaled corticosteroids (ICS) and biological therapy [11, 15].

The non-T2 endotype is not clearly defined. This is an endotype without signs of T2 inflammation: there is no eosinophilia. Severe symptoms of the disease and resistance to ICS are more often recorded, and IL-6, IL-1b, IL-8, and IL-17A are involved in the immune response [16, 17]. Along with endotypes, four inflammatory phenotypes of BA are distinguished: eosinophilic (EA), neutrophilic (NA), mixed granulocytic (MG), and paucigranulocytic (PG). The gold standard for diagnosing phenotypes is the results of an induced sputum analysis of patients [18].

In the work by A. Plavsic et al., based on the study of induced sputum in 80 patients, 17 of whom received biological therapy, it was shown that EA and MG phenotypes were more common than others [13]. In the same work, the characteristics of phenotypes were presented with and without biological therapy. Thus, patients with NA were characterized by the highest level of IL-8 compared to patients with EA, MG, and PG phenotypes ($p = 0.002$, $p = 0.031$, $p = 0.021$, respectively).

Patients with an EA phenotype have significantly higher IL-17A levels in the blood compared to others ($p = 0.004$). During biological therapy, these patients have lower IL-5 levels compared to untreated patients ($p = 0.043$).

Patients with a MG phenotype after targeted therapy are characterized by lower lymphocyte and neutrophil counts than before treatment ($p = 0.003$). In contrast, IL-5, IL-6, and IL-8 levels after treatment are higher than baseline values ($p = 0.012$, $p = 0.032$, $p = 0.038$, respectively).

The lack of reduction in some inflammation indices during targeted therapy in the MG phenotype in this study, according to the author's comment, indicates the need for search for another biological target in such patients.

BIOMARKERS OF SEVERE BRONCHIAL ASTHMA

The use of biological markers for the selection of targeted drugs depending on the nature of inflammation (phenotype/endotype of BA) has significantly expanded the possibilities of achieving control over BA symptoms. Along with this, the effectiveness of severe BA treatment still remains a therapeutic problem. Therefore, the search for biotargets for the treatment of severe BA is an urgent research and clinical task [11–15].

The results of studies in recent years indicate possible expansion of a list of new biotargets, as well as biomarkers of already known biotargets. In particular, the following are discussed [11, 12, 20]: eosinophil peroxidase (EP) in the respiratory tract (sputum) [12–21], eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) [11], exhaled breath condensate (EBC), urine metabolites, microRNA, Charcot – Leyden crystals, dipeptidyl peptidase, osteopontin [11, 12, 21].

In general, the analysis of biomarkers is regularly updated in publications. In this article, we will focus on those that are valid for severe BA.

1. Blood / serum biomarkers.

1.1. Eosinophilia.

Eosinophils play a key role in the development of type T2 BA [14–16]. Currently, the determination of the number of eosinophils in the blood is used as a biomarker for BA and the choice of targeted therapy. The most important in the context of inflammation are activated eosinophils. They release mediators that damage bronchial epithelium, cause overproduction of mucus, edema, and bronchospasm, which leads to frequent exacerbations in patients [17].

1.2. Eosinophil-derived neurotoxin and eosinophil cationic protein.

The results of the study of EDN and ECP were published in the work of by T. Tsuda et al. It was shown that their level increased after the activation of eosinophils by cytokines (including IL-5, IL-1b, and IL-33), and the level of EDN in the blood serum of patients with severe BA was significantly higher [11, 12, 20, 26, 27]. Probably, the determination of the level of EDN in the blood serum can help in assessing the severity of the disease [12].

1.3. Eosinophil peroxidase.

Eosinophils have a unique set of enzymes that allow them to produce reactive oxidants that damage the respiratory tract [13]. One of these oxidants is EP, the level of which was higher in patients with severe BA than in the control group. It is proposed to use EP as a biomarker of eosinophilic inflammation in severe BA with the EA phenotype [14, 15, 28].

1.4. Periostin.

Periostin is a matricellular protein that is produced by epithelial cells and fibroblasts in response to stimulation by IL-4, -5, -13. The association of periostin levels with eosinophilic inflammation of the airways has been shown in a large number of studies. The relationship between periostin levels and lung function parameters and asthma characteristics (severity, exacerbation frequency) indicates the potential applicability of this biomarker to identify patients with severe forms of the disease [15, 28–33]. However, there are limitations to using periostin as a prognostic biomarker in children due to bone growth and its constant high levels [34].

2. Sputum biomarkers.

The number of eosinophils in sputum reflects the degree of inflammation in the airways and, therefore, is a sensitive and specific non-invasive diagnostic biomarker [35–38], which is used as the gold standard for diagnosing asthma phenotypes [39]. The eosinophilic inflammatory phenotype is the most common. It is diagnosed when 3% or more eosinophils are detected in sputum.

Normalization of sputum eosinophils is associated with better asthma control, reduced hospitalizations, and exacerbations.

4. Exhaled breath condensate.

EBC is a noninvasive method for studying the respiratory tract. In adult patients with BA, the concentration of hydrogen ions in exhaled air, nitric oxide breakdown products, hydrogen peroxide, and 8-isoprostanoids is increased and is associated with poorer lung function compared to healthy individuals [40]. Recently, there has been growing interest in metabolomic analysis of EBC [41–51].

5. Urine biomarkers.

The composition of urine metabolites changes significantly during different periods of BA. Research results indicate an increase in the content of alkanes, aldehydes, and amino acids in urine during an exacerbation episode [41]. The most studied metabolite is the amino acid bromotyrosine, high levels of which are associated with the eosinophilic phenotype of BA. In the work by A. Tiotiu et al., it was shown that its content decreased during the use of GCs in severe BA. The author suggested using bromotyrosine as a biomarker of the response to steroid treatment [42–44].

6.OMICS technologies.

Based on the analysis of the sputum transcriptome in patients with BA, three molecular phenotypes are distinguished. The first (TAC1) is associated with the T2 endotype and is characterized by eosinophilia and high levels of IL-13 and ILC2. The second (TAC2) and third (TAC3) are not associated with the T2 endotype. TAC2 is characterized by high levels of INFγ, TNFα, as well as increased expression of the *NLRP3*, caspase-1, and IL-1b genes in sputum macrophages in patients with NA phenotype of BA.

TAC3 is characterized by increased expression of genes associated with paucigranulocytic inflammation [45–49].

7.MicroRNA (miRNA).

MicroRNAs are short sequences of single-stranded RNA (19–24 nucleotides) that, by complementary binding to the 3'-untranslated end of messenger RNA (miRNA), can interfere with the proper function of a particular gene. Recent studies have shown that miR-28-3p, -16-2-3p, -210-3p, -185, -125b, -338-3p, and -125b are markers of severe BA [49–51]. MicroRNAs can also be used to predict the response to targeted therapy. Thus, in a study by J.A. Cañaset et al., miR-1246, miR-5100, and miR-338-3p were shown to be potential biomarkers for predicting the response to benralizumab [53]. In the study by M. Gil-Martínezet et al. [54], which examined changes in microRNA expression in patients with severe BA depending on systemic GC therapy, significant differences were found in the expression of eight microRNAs: hsa-miR-148b-3p, -221-5p, -618, -941, -769-5p, -144-3p, -144-5p, and -451a (the first five were determined in serum, the last three – in lung tissue). MicroRNA profiling can be used to search for new biomarkers of severe BA and predict the effectiveness of biological therapy [51].

MODERN METHODS FOR TREATING SEVERE BRONCHIAL ASTHMA

The strategy of modern asthma therapy is based on achieving and maintaining control over symptoms of the disease and reducing the risk of exacerbations. For this purpose, a stepwise approach is used, which involves the possibility of increasing or decreasing the volume of therapy in the patient. Genetically engineered biological drugs are currently used to achieve control over severe BA symptoms.

According to the 2024 clinical guidelines, monoclonal antibodies against T2 cytokines are used to treat patients with severe BA: omalizumab is a monoclonal body against IgE, mepolizumab and reslizumab – against IL-5, benralizumab – against the IL-5 receptor, dupilumab – against IL-4Rα, and tezepelumab – against thymic stromal lymphopoietin [55, 56].

Despite the obvious clinical effect in patients with severe BA taking biological drugs, questions remain open regarding the insufficiency of biomarkers for the selection and prognosis of the effectiveness of biological therapy and phenotype variability during the natural course of the disease. In some patients, there is no association between the clinical effect and the positive dynamics of biomarkers [8, 57–62].

Publications of recent years contain a large number of studies devoted to the analysis of biomarkers and their suitability for use in the process of biological therapy for BA [8, 57–62].

Thus, in a retrospective study, M. Lampalo et al. compared the effect of therapy with omalizumab, reslizumab, benralizumab, and mepolizumab in patients with severe BA (n = 74). The patients were followed up for 24 months. The response to therapy was assessed using the asthma control test (AST), forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FeNO, the number of eosinophils and IgE in the blood, the number of exacerbations, and the need for GCs. The results showed that treatment with both anti-IgE and anti-IL-5 monoclonal antibodies can reduce the frequency of exacerbations and the volume of GC therapy and increase AST values. At the same time, the authors point out the insufficient information on the dynamics of inflammation against the background of the applied therapy and associate this with the lack of prognostic biomarkers of an individual response to treatment [57].

N. Contreras et al. published the results of an 18-month follow-up of adult patients with severe BA (n = 67) during treatment with omalizumab (n = 20) and mepolizumab (n = 36). The clinical effect is confirmed by an increase in AST and FEV1 after therapy and a decrease in the number of eosinophils in the blood and the frequency of exacerbations. Proteomic and metabolomic analysis revealed a group of metabolites (arachidonic, oleic, palmitoleic, lactic acids, propionyl L-carnitine, bilirubin, CCL11, and TNFSF10) that changed in response to therapy with mepolizumab alone, in association with a clinical improvement. These results indicate different effects of omalizumab and mepolizumab on the metabolomic kinetics of inflammation in severe BA. Thus, the study confirms the need to search for multiple biomarkers of inflammation for biological therapy of BA [58].

R. Djukanović conducted a cross-sectional open study, where the clinical efficacy of omalizumab was assessed in patients with severe BA (n = 216) over 16 weeks. Using omics technologies, they studied 1,408 parameters of a number of eicosanoids and volatile organic compounds in exhaled breath, as well as proteins in sputum and urine. Following the use of covariance or quantile regression models, the authors established a list of biomarkers of volatile organic compounds and blood lipids, the use of which is able

to predict a decrease in the frequency of exacerbations by more than 50% during treatment with omalizumab ($p < 0.05$). The inclusion of such markers as eosinophil count in the blood and sputum, nitric oxide in exhaled breath, and serum IgE in the regression model did not confirm their prognostic value in this study [60].

The search for biotargets for adequate therapy for severe BA is also carried out using genomic technologies. Thus, the results of a 12-month retrospective observational cohort study, including patients with severe eosinophilic asthma (n = 72), revealed that after treatment with mepolizumab and benralizumab, the volume of GC therapy and the frequency of exacerbations decreased, and FEV1 increased. An association was established between the alleles ZNF415 rs1054485-T, IL1RL1 rs1420101-T, and FCER1B rs569108-AA with severe BA.

At the same time, carriers of the ZNF415 rs1054485-T allele showed a decrease in the frequency of exacerbations after completion of treatment with mepolizumab ($p = 0.042$), and carriers of the IL1RL1 rs1420101-T allele exhibited an improvement in FEV1 ($p = 0.023$).

The use of benralizumab led to a decrease in the number of exacerbations in patients who were carriers of the ZNF415 rs1054485-T allele (p = 0.073) and FCER1B rs569108-AA ($p = 0.050$) [61].

The publication by S. Harada et al. is devoted to the study of changes in single nucleotide polymorphisms when using benralizumab in patients with severe BA (n = 72). The results of the work show that after 12 months of treatment with benralizumab, patients showed positive clinical dynamics: FEV1 increased, the volume of GC therapy and the frequency of exacerbations decreased, and the expression of key genes involved in non-T2 inflammation (IL-8, IL-17RA, CXCR1, and CXCR2) decreased. An important conclusion of the work was that benralizumab affects T2 and non-T2 endotypes of BA. However, further research in this area is needed to confirm the role of pharmacogenetics in the search for prognostic biomarkers of severe BA [62].

Biological therapy is widely used in severe BA in pediatric practice, although the list of drugs for children has age restrictions. According to the 2024 clinical guidelines, drugs such as omalizumab and mepolizumab are approved for use in children aged 6 years, dupilumab – for children aged 12 years, and reslizumab and benralizumab – for children aged 18 years [8]. Given the lack of research on prognostic biomarkers in children, it is currently difficult to assess the effectiveness of biological therapy in a pediatric cohort [8, 34].

CONCLUSION

Biological therapy is an expensive treatment strategy, so there is a high demand for personalized approaches when prescribing targeted therapy for BA. In this regard, new data on prognostic biomarkers and disease biotargets are needed, which are an important part of scientific research in pulmonology.

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Clinical Features of Urolithiasis in Patients With Comorbid Conditions

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ABSTRACT

Urolithiasis is a common disorder of the urinary system. The disease often becomes recurrent, characterized by rapid calculus growth, a trend toward staghorn and multiple stone formation, emergency complications, and disability – particularly in patients with metabolic disorders.

This article presents a clinical case of a patient with a long history of recurrent urolithiasis that developed against the background of comorbid pathology associated with metabolic syndrome and complicated by chronic kidney disease. The case analysis highlights the crucial role of metabolic disorders in the progression of nephrolithiasis and renal dysfunction. The paper describes specific features of surgical treatment in comorbid patients, emphasizing adherence to the "golden hour" principle and risk minimization. It underscores the necessity of a multidisciplinary approach, early metabolic correction, and systemic metaphylactic measures to prevent stone recurrence and reduce the risk of chronic kidney disease development in this patient category.

Keywords: urolithiasis, metabolic syndrome, comorbidity, chronic kidney disease

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Особенности течения уролитиаза на фоне коморбидной патологии

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РЕЗЮМЕ

Мочекаменная болезнь является распространенным заболеванием мочевыделительной системы. Нередко заболевание приобретает рецидивирующий характер с быстрым ростом конкрементов, склонностью к образованию коралловидных и множественных камней, возникновением неотложных состояний и инвалидизации особенно у пациентов на фоне метаболических нарушений.

Представлен клинический случай пациента с длительным анамнезом рецидивирующей мочекаменной болезни, развившейся на фоне коморбидной патологии, ассоциированной с метаболическим синдромом, осложненными хронической болезнью почек. Анализ случая подчеркивает ключевую роль метаболических нарушений в прогрессировании нефролитиаза и почечной дисфункции. Описаны особенности хирургиче-

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ского лечения у коморбидного пациента с акцентом на соблюдение принципа «золотого часа» и минимизации рисков. Подчеркивается необходимость междисциплинарного подхода, ранней метаболической коррекции и системной метафилактики для предотвращения рецидивов камнеобразования и снижения риска развития хронической болезни почек у данной категории пациентов.

Ключевые слова: мочекаменная болезнь, метаболический синдром, коморбидная патология, хроническая болезнь почек

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

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

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INTRODUCTION

Urolithiasis (UL) remains one of the most prevalent diseases of the urinary system, affecting approximately 10% of the global population [1]. In the Russian Federation, the incidence of UL reaches 5–7%, with an annual increase of 1–2%. The high prevalence of the disease among the working-age population [2] positions it not only as a medical but also as a socio-economic problem. The disease often follows a recurrent course characterized by rapid calculus growth, a propensity for staghorn and multiple stone formation, the onset of emergency conditions, and disability, particularly in patients with underlying metabolic disorders. Furthermore, existing methods for calculus removal do not prevent the recurrence of UL [3].

Currently, most researchers are convinced that UL should be considered as a condition associated with metabolic syndrome (MetS), with proven involvement of its components in the mechanisms of stone formation [4].

The components of MetS (insulin resistance, abdominal obesity, dyslipidemia, arterial hypertension (AH), hyperuricemia, etc.) are now recognized as determinants of the severe course of associated pathologies. They impact the rates of disability and mortality, contributing to a significant decline in the quality of life of the population [5, 6].

The role of UL as an independent risk factor for chronic kidney disease (CKD) deserves particular attention. Recurrent stone formation, urinary tract obstruction, and chronic inflammation lead to renal parenchymal damage, interstitial fibrosis, and a progressive decline in kidney function. This progression, in combination with MetS components and other associated pathologies (atherosclerosis, diabetes mellitus (DM)), increases the risk of developing CKD.

A clinical case of a patient with recurrent UL against the background of MetS-associated conditions demonstrates the complex interplay of comorbid states. This interplay is characterized by mutual exacerbation, which aggravates the adverse outcome.

CLINICAL CASE

Patient K., 69 years old, was admitted to the urology department on 02.06.2025 for elective surgical treatment with a diagnosis of Urolithiasis. Stones in both kidneys. Right-sided staghorn calculus, K4.

The medical history for UL was significant. The first episode was recorded 30 years ago with spontaneous passage of a stone from the right kidney. In subsequent years, the disease relapsed with episodes of stone passage from both sides up to 2–3 times per year. In 2017, percutaneous nephrolithotomy (PNL) was performed on the right side, achieving 100% effectiveness according to the stone-free rate (SFR) criterion. Stone composition analysis revealed calcium oxalate (90%) and carbonate apatite (10%). In 2019, a follow-up examination again detected a stone in the right kidney. However, an attempt at its dissolution in the outpatient setting for one month using a preparation of potassium citrate + sodium citrate + citric acid yielded no results. A surgical intervention was recommended and was performed during the current hospitalization.

From the life history, it is known that the patient has been under long-term follow-up by a therapist for

chronic conditions: coronary artery disease: stable angina, FC I; post-infarction cardiosclerosis (PICS) since 2016, state after stenting of the right coronary artery (RCA) in 2017. Stage 3 hypertension, controlled. Grade 2 obesity. Dyslipidemia. Hyperuricemia. Cardiovascular risk category 4 (very high). Stage I chronic heart failure with preserved ejection fraction (EF 63%). Type 2 diabetes mellitus (HbA1c – 9.25%). Nephropathy of mixed genesis. Stage C4 CKD (eGFR – 25 ml / min).

The patient has been receiving continuous medication: dapagliflozin 10 mg, insulin detemir 12–12 units daily, insulin glulisine 8–10–8 units, losartan 50 mg, bisoprolol 5 mg, allopurinol 300 mg.

Objective findings: in addition to a standard physical examination, extended anthropometric measurements were performed to assess the degree of obesity and the nature of fat distribution. Height – 164 cm, body weight – 102 kg, calculated body mass index (BMI) – 37.9 kg / m², corresponding to grade 2 obesity; waist circumference – 115 cm, hip circumference – 100 cm, and sagittal abdominal diameter – 28 cm, indicative of abdominal obesity.

Results of the blood biochemistry dated 26.05.2025: creatinine – 150.5 μmol / l (eGFR 25 ml / min), glucose – 11.0 mmol / l, uric acid – 0.38 mmol / l, triglycerides – 2.6 mmol / l, low-density lipoproteins (LDL) – 2.08 mmol / l, total calcium – 2.7 mmol / l, magnesium – 0.56 mmol / l.

The acidity of the 24-hour urine sample (pH) dated 03.06.2025 was 5.5; microalbuminuria – 300 mg / day. No bacterial flora was detected.

According to the results of spiral computed tomography (CT) of the retroperitoneal organs from 14.02.2025: on the right, a staghorn calculus filling its shape; 39 × 35 × 20 mm, 460 HU. On the left, in the upper group of calyces, a calculus was seen measuring 5 × 7 mm, 300 HU (Fig. 1).

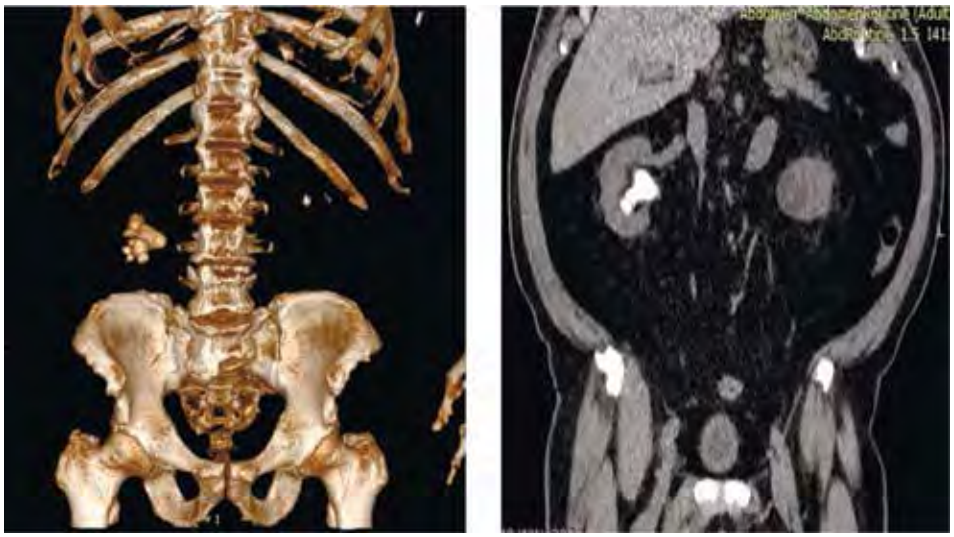

Fig. 1. Results of spiral computed tomography of the retroperitoneal organs dated 14.02.2025: on the right — a staghorn calculus (39 × 35 × 20 mm, 460 HU) filling its shape; on the left, in the upper group of calyces — a calculus of 5 × 7 mm, 300 HU

According to the STONE nephrolithometry scale, the case complexity was assessed at 11 points (high level).

To assess the cardiometabolic status, indirect calorimetry was performed using the COSMED equipment (Italy). The following parameters were determined at rest: oxygen consumption (VO_2) – 354 ml / min, carbon dioxide production (VCO_2) – 266 ml / min, respiratory quotient (RQ) – 0.75 (VCO_2 / VO_2), resting energy expenditure (REE) – 2,405 kcal / day. The obtained data demonstrate a pronounced reduction in aerobic capacity, impaired metabolic flexibility, and signs of a hypermetabolic state, which is characteristic of obesity and insulin resistance and indicates significantly reduced cardiorespiratory endurance.

The patient underwent right PNL in the prone position. Dilation of the percutaneous tract up to 24 CH was performed (Fig. 2).

A combined method using a thulium fiber laser FiberLase U2 and an electrohydraulic lithotripter Urolit 105M was employed for stone fragmentation; disintegration of the staghorn calculus into fragments was performed, and fragments were extracted with forceps.

During revision of the upper calyceal group, a residual stone fragment of up to 6 mm in diameter was found. Complete visualization and removal were impossible due to anatomical features of the pelvicalyceal system, limited instrument mobility, and a high risk of renal parenchyma injury. Given the operation duration of over two hours, high surgical risk (ASA III), reduced renal function, and high risk of infectious complications, a decision was made to terminate the surgical procedure with the placement of a nephrostomy drain. In the early postoperative period, the patient was prescribed prolonged antibiotic prophylaxis to prevent infectious complications. The postoperative period was uneventful. The nephrostomy drain was removed on day 2 with restoration of normal urine passage. The patient was discharged in satisfactory condition on day 5 after the surgery.

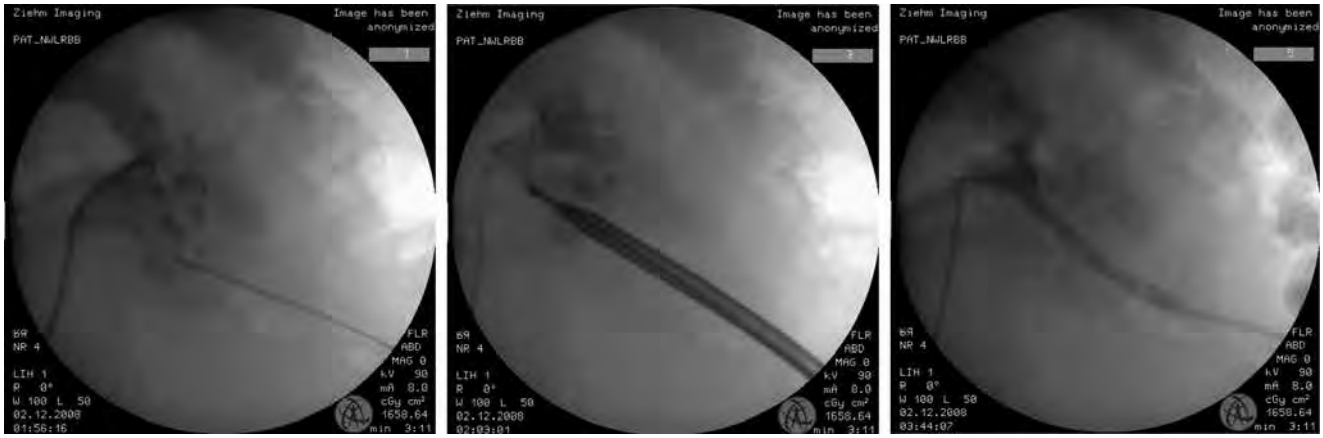

Fig. 2. Intraoperative radiograph (C-arm) during right percutaneous nephrolithotomy.

DISCUSSION

This clinical case presents a classic example of severe comorbid pathology, where UL develops against the background of a full-blown clinical presentation of MetS. The patient exhibited all key components of MetS: abdominal obesity, type 2 DM, AH, dyslipidemia, and hyperuricemia. This combination created ideal conditions for the formation of recurrent nephrolithiasis with the development of CKD [3].

A key aspect of this observation is the pronounced mutual exacerbation of comorbid conditions. The systemic nature of the damage, manifested by the formation of type 2 cardiorenal syndrome (a combination of CAD, stage I CHF, and stage C4 CKD) and metabolic disorders (type 2 DM, dyslipidemia, and hyperuricemia), deserves special attention.

According to the Quebec Cardiovascular Study, the risk of developing CAD in men with MetS is 20 times higher than in the general population. The pathogenesis of cardiovascular complications is based on macro- and microvascular damage. Macrovascular changes are associated with atherosclerosis, which develops under the influence of oxidized LDL, triggering a proinflammatory reaction in the vascular wall involving immune-competent cells and the cytokines they synthesize. In patients with type 2 DM and dyslipidemia, the production of oxygen free radicals increases, which reduces the synthesis of nitric oxide and prostacyclin, promoting vasoconstriction and the progression of atherosclerosis [7].

Furthermore, renal artery involvement in generalized atherosclerosis in patients with type 2 DM is more common over the age of 60. Retrospective data from angiographic studies confirm widespread lesions of the coronary, brachiocephalic, renal, and peripheral arteries [8].

A common pathogenetic link between UL and the aforementioned comorbid pathology is inflammation and free-radical oxidation, leading to renal tubular damage and formation of Randall's plaques, which serve as a foundation for subsequent crystal deposition, initiating nephrolithiasis [9].

Chronic heart failure associated with AH, CAD, and CKD, coupled with the activation of the renin – angiotensin – aldosterone system, is initially compensatory but subsequently leads to cardiac and renal remodeling with the development of fibrosis [10].

The rapid formation of a staghorn calculus, characteristic of patients with MetS-associated diseases, warrants special attention [11]. This is

due to chronic urine acidification and impaired calcium metabolism [12]. Microalbuminuria and a reduced GFR of 25 ml / min indicate significant renal parenchymal damage and confirm the link between UL and CKD [13].

According to current data, the risk of developing UL in patients with MetS increases by 2–3 times, with 50-60% of them experiencing a recurrent course of the disease [14]. Of particular concern is the fact that the combination of these pathologies triples the risk of developing CKD compared to isolated UL [13]. The pathogenesis of this interaction involves several key aspects. Central to this interaction is insulin resistance, leading to reduced citrate excretion and the development of hypercalciuria due to impaired calcium – phosphate homeostasis [12].

Hyperuricemia plays an important role in the pathogenesis, not only promoting urate crystallization but also activating tubulointerstitial inflammation [15]. Elevated uric acid levels are a significant factor in the development and progression of CKD, due to the development of AH against the background of endothelial dysfunction and reduced nitric oxide production. The deposition of uric acid crystals in ischemic areas of renal tissue against the background of elevated blood pressure leads to renal parenchymal damage [16]. Moreover, a high uric acid level itself may indicate reduced renal function due to impaired excretion [17].

Abdominal obesity as a component of MetS independently contributes to reduced renal function through a complex of mechanisms. Under conditions of chronic hemodynamic and metabolic disturbances, increased production of endothelin-1 takes place, which causes pronounced spasm of efferent arterioles and an increase in intrarenal pressure. Simultaneously, possessing growth factor properties, endothelin-1 stimulates the proliferation of cellular elements of renal tissue, leading to oligonephronia, hypertrophy of the remaining nephrons, and ultimately contributing to glomerulosclerosis and the progression of CKD [18].

An additional risk factor is dyslipidemia, which alters the lithogenic properties of urine through the development of hyperoxaluria, hypercalciuria, and hyperphosphaturia against the background of hypocitraturia. These changes significantly increase the risk of stone formation and exacerbate renal tissue damage [19]. These processes explain why in patients with MetS and UL, the rate of GFR decline reaches 5–8 ml / min / year, which is 4–5 times higher than in patients without metabolic disorders [20]. It is also known that in patients with CAD complicated by CHF, nephrolithiasis occurs 1.5 times more frequently, and the rate of GFR decline can be 4 ml / min / year [21]. Patients with MetS form staghorn calculi 3–4 times more often than the general population [12]. Rapid progression of renal dysfunction is noted: the 5-year risk of end-stage CKD is 15–20% compared to 3–5% in patients without MetS [22]. The early appearance of microalbuminuria, which serves as a marker of both diabetic and urate-induced nephropathy, is also characteristic [23]. The GUBBIO study noted that an increase in BMI by 4 kg / m² increased albumin excretion by 1.83 times in men [18].

A particular point of attention in this clinical case is the lack of consistent metaphylaxis over the 30-year history of the disease. The patient had all indications for aggressive metaphylaxis: recurrent nephrolithiasis, metabolic acidosis (pH 5.5), and concomitant metabolic disorders [11, 14]. Despite the recurrent nature of nephrolithiasis (2–3 episodes per year) and identified metabolic disorders, the patient received only episodic therapy (blemaren for 1 month), which is clearly insufficient for secondary prevention. This fact reflects systemic problems in modern urological practice. According to the Urologists' Attitudes on Metabolic Evaluation (NAME, 2021) study, only 38% of urologists regularly prescribe a metabolic evaluation after the first episode of UL [24]. In 72% of cases, they limit themselves to stone composition analysis without assessing urinary lithogenic factors [24]. Metaphylaxis should become a mandatory component of treatment for all patients with UL, especially in the presence of metabolic disorders. Timely initiation of preventive measures is the most effective way to prevent severe complications, including end-stage CKD.

The performed surgical intervention in the volume of PNL in this patient with staghorn nephrolithiasis and MetS-associated diseases demonstrated a number of important clinical aspects. The patient had severe comorbid pathology, which required minimizing intraoperative risks [25, 26]. Limiting the active intervention time to two hours was a justified decision, considering the high surgical risk (ASA III) and reduced renal function (GFR 25 ml / min); the placement of a nephrostomy drain ensured adequate urine drainage. It is important to note that in modern urology, during PNL, it is recommended to adhere to the golden hour rule – achieving maximum stone fragmentation efficiency within the first hour of surgery, which reduces the risk of complications

and improves long-term outcomes [25]. The absence of complications in the postoperative period, rapid restoration of independent urine passage, and stable renal function parameters confirmed the correctness of the chosen strategy, combining sufficient intervention radicality with patient safety [27]. However, the presence of a residual fragment indicates the need for further follow-up and possible retreatment.

CONCLUSION

Diseases associated with MetS are significant risk factors for the recurrent course of UL and the progression of CKD. This clinical case highlights the importance of timely diagnosis of metabolic disorders and their elimination for effective secondary prevention of MetS-associated diseases, including UL, preventing end-stage renal failure, and improving the quality of life.

Medical management of patients with UL against the background of comorbid pathology requires a multidisciplinary approach involving urologists, therapists, nephrologists, endocrinologists, and others. Timely correction of metabolic disorders and effective prevention of stone recurrence can significantly slow the progression of CKD in this category of patients.

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Current Status of Opisthorchiasis Therapy: Praziquantel and Plant-derived compounds

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ABSTRACT

This lecture examines contemporary therapeutic strategies for opisthorchiasis, focusing on infections caused by the liver fluke *Opisthorchis felineus*, endemic in Western Siberia. While praziquantel remains the first-line treatment, its clinical utility is constrained by several factors, including lack of efficacy against juvenile parasite forms and emerging drug resistance. The discussion explores alternative pharmacological approaches, encompassing novel synthetic agents, combination therapies, and compounds targeting parasite-specific metabolic pathways. Special attention is given to plant-derived bioactive substances with experimentally confirmed anti-opisthorchiasis activity, including curcumin, *Thunbergia laurifolia* and *Allium sativum* extracts, and xanthohumol. These phytochemicals demonstrate dual therapeutic potential: direct antiparasitic effects and modulation of infection-associated pathophysiological processes, such as oxidative/nitrosative stress attenuation, inflammatory response suppression, and hepatobiliary fibrosis progression delay. A synergistic treatment paradigm combining praziquantel's anthelmintic properties with the pleiotropic effects of plant-based antioxidants and anti-inflammatory compounds shows particular promise. This strategy may improve parasite clearance rates, reduce treatment-related adverse events, and prevent chronic complications. Further investigation is warranted to refine combination protocols, develop targeted delivery systems, and identify next-generation anthelmintic compounds capable of addressing the limitations of current synthetic therapies.

Keywords: *Opisthorchis felineus*, anthelmintic therapy, praziquantel, combination therapy, antioxidants

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Современное состояние проблемы терапии описторхоза: празиквантел и соединения растительного происхождения

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РЕЗЮМЕ

Представлен обзор современных стратегий терапии описторхоза. Особое внимание уделяется инвазии печеночным сосальщиком *Opisthorchis felineus*, эндемичным на территории Западной Сибири. Несмотря на длительное применение празиквантела в качестве препарата первой линии, его использование сопряжено с рядом существенных ограничений, включая неэффективность против личиночных стадий и потенциальную резистентность у паразитов. Рассматриваются альтернативные подходы к лекарственной терапии с использованием синтетических препаратов, соединений комбинированного состава и агентов, направленных на специфические метаболические системы паразита. Отдельно приводится исчерпывающий обзор биологически активных веществ растительного происхождения, продемонстрировавших в экспериментах противоописторхозную активность (куркумин, экстракты *Thunbergia laurifolia u Allium sativum,* ксантогумол). Ценность этих соединений заключается не только в их потенциальных противопаразитарных свойствах, но и в способности модулировать ключевые патофизиологические процессы на фоне инвазии: подавлять окислительный и нитрозативный стресс, уменьшать выраженность воспалительных реакций и замедлять развитие фиброза гепатобилиарной системы. Наиболее обоснованной стратегией представляется комбинированная терапия, объединяющая противопаразитарное действие празиквантела с разнонаправленными эффектами растительных антиоксидантов и противовоспалительных агентов. Такой подход позволяет повысить эффективность эрадикации паразита, снизить нежелательные побочные эффекты антигельминтной терапии и минимизировать риск развития хронических осложнений инвазии. Подчеркивается необходимость дальнейших исследований для оптимизации комбинированных схем, разработки систем направленной доставки действующих веществ и поиска новых высокоактивных соединений для преодоления текущих ограничений синтетических антигельминтных лекарственных средств.

Ключевые слова: *Opisthorchis felineus*, антигельминтная терапия, празиквантел, комбинированная терапия, антиоксиданты

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

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INTRODUCTION

Liver diseases caused by parasitic trematodes, cestodes, and nematodes continue to pose a substantial global health threat [1]. Among these, trematode infections represent a distinct epidemiological challenge [2], with particular recent emphasis on species from the families *Schistosomatidae*, *Opisthorchiidae*, *Fasciolidae*, and *Paragonimidae* [3–5].

Opisthorchis felineus (*O. felineus*) stands as a principal causative agent of opisthorchiasis, capable of producing both asymptomatic infections and severe hepatobiliary pathology, including cholecystitis, cholangitis, and periductal fibrosis. Chronic *O. felineus* infection is further associated with elevated risk of cholangiocarcinoma development [6–8]. Emerging evidence suggests that chronic *O. felineus* infection may modulate host immune responses, potentially exacerbating comorbid conditions [9]. This parasite remains the dominant etiological agent across extensive territories encompassing Russia, Kazakhstan, and several European nations [8, 10], with infection prevalence reaching peak levels in Western Siberia [8].

The well-documented endemic status of *O. felineus* in Western Siberia, supported by longitudinal surveillance data, highlights the urgent

need to strengthen epidemiological monitoring of transmission patterns, refine diagnostic algorithms, and optimize therapeutic protocols with consideration for regional characteristics and patient comorbidities.

Current research priorities demand systematic consolidation of existing knowledge regarding therapeutic interventions against *O. felineus*-induced opisthorchiasis, with specific focus on: the application of bioactive compounds; their incorporation into combination therapies with praziquantel (PZQ) – the current therapeutic mainstay; and the development of novel antiparasitic agents and treatment strategies to circumvent potential PZQ resistance in adult flukes while enhancing therapeutic efficacy [10, 11].

PRAZIQUANTEL – THE FIRST-LINE PHARMACOTHERAPEUTIC AGENT FOR OPISTHORCHIASIS

Pharmacotherapy remains the principal approach for targeted reduction of opisthorchiasis prevalence, with praziquantel (PZQ) – an isoquinoline derivative – standing as the sole anthelmintic of choice for trematode infections. For opisthorchiasis treatment, WHO recommends either: (1) a dosage regimen of 25 mg / kg PZQ administered three times daily for two to three days, or (2) a single 40 mg / kg dose [10]. However, clinical practice frequently employs an alternative protocol: an initial 50 mg / kg dose followed by 25 mg / kg on the subsequent day [10]. The therapeutic efficacy of PZQ may diminish with prolonged infection duration and higher parasitic load, necessitating repeat treatment courses. Given the compound's hepatotoxicity and other adverse events, such retreatment poses significant clinical concerns [11].

The anti-opisthorchiasis mechanism of PZQ involves selective disruption of Ca^{2+} homeostasis within the parasite. *In vitro* studies demonstrate that introducing PZQ into the culture medium containing adult flukes (maritae) induces intracellular Ca^{2+} accumulation through dissociation of slow calcium channel subunits in the liver fluke's muscular system, resulting in sustained muscle contraction and paralysis [12, 13]. Nevertheless, emerging evidence suggests that calcium channel modulation cannot fully explain PZQ's antiparasitic activity, as pre-incubation of trematodes with cytochalasin D (which lacks calcium transport effects) completely inhibits PZQ efficacy [14]. Paradoxically, juvenile developmental stages exhibit measurable Ca^{2+} elevation upon incubation in PZQ-containing medium despite their well-documented drug resistance [14].

The tegument – a unique syncytial epithelial layer covering parasitic flatworms – may represent another pharmacological target. Current hypotheses propose that PZQ's mechanism includes direct tegumental damage, leading to parasite antigen exposure and subsequent host immune response activation [15]. An additional facet of PZQ's antiparasitic action involves nucleoside transport disruption (specifically adenosine and uridine) via transporter protein inhibition. This induces ATP depletion, reduced adenine/uridine nucleotide pools, and consequent metabolic dysfunction with impaired RNA/DNA synthesis [16].

It should be emphasized that commercial PZQ preparations contain a racemic mixture of isomers, with only the levorotatory isomer demonstrating antiparasitic activity [17].

Despite its high efficacy against trematodes, particularly *O. felineus*, PZQ administration faces several significant limitations. Considerable attention in specialized literature focuses on the pronounced cytotoxicity of PZQ's S-enantiomer [18]. However, the most critical issue – substantiated by multiple clinical observations in endemic regions – involves the potential development of PZQ resistance in trematodes [19]. *In vitro* studies confirm that adult helminths under continuous PZQ exposure develop increased pharmacological tolerance [20]. A particularly controversial yet crucial safety consideration involves the potential association between repeated PZQ courses and cholangiocarcinoma risk [19, 21]. This carcinogenic risk hypothesis stems from well-documented evidence of massive parasite antigen release within 24 hours post-PZQ administration, leading to antigen accumulation, induction of systemic inflammation in patients, and significant intensification of oxidative and nitrosative stress [19]. Conversely, some animal model studies described in separate sources suggest potential reduction of cholangiocarcinoma risk following repeated "infection–PZQ treatment" cycles [22].

Furthermore, PZQ demonstrates neither prophylactic activity nor efficacy against juvenile

parasite stages, representing a critical gap in developing mass chemoprevention strategies for high-incidence regions [23].

The cumulative evidence regarding PZQ's cytotoxicity, resistance development, and potential carcinogenic risk underscores the urgent need for novel therapeutic strategies and agents against opisthorchiasis. Promising candidates include synthetic benzimidazole derivatives (albendazole, mebendazole) and tribendimidine – compounds with fundamentally different molecular targets in the parasite. Particular interest lies in developing hybrid molecules that combine antiparasitic activity with improved safety profiles. As will be discussed below, these approaches offer a potential for overcoming PZQ's limitations while maintaining therapeutic efficacy against *O. felineus* and related trematodes.

ALTERNATIVE PHARMACOLOGICAL STRATEGIES FOR OPISTHORCHIASIS THERAPY USING SYNTHETIC COMPOUNDS

During the 1980s, *in vitro* and *in vivo* experiments, including clinical studies, were conducted to evaluate the specific anti-opisthorchiasis activity of albendazole and mebendazole [24]. Previously, these drugs were extensively used for treating soil-transmitted nematode infections [25].

Albendazole and mebendazole belong to the same drug class (benzimidazole derivatives) and exhibit broad-spectrum antiparasitic activity, including efficacy against nematodes, cestodes, and liver fluke infections [26, 27]. These agents disrupt microtubule function in parasitic cells by inhibiting β-tubulin polymerization into microtubules, subsequently leading to reduced glucose transport, glycogen depletion in *Opisthorchis*, impaired tegumental protein secretion, and neuromuscular transmission deficits [10, 26]. It should be noted that mebendazole acts as a direct active molecule against the parasite, whereas albendazole requires metabolic activation through transformation into a sulfoxide derivative [27]. Crucially, albendazole's pharmacologically active metabolite demonstrates greater affinity for parasitic β-tubulin compared to mebendazole, penetrates helminth tissues more effectively, and may additionally disrupt ATP synthesis. Indeed, the therapeutic regimen involving albendazole (10 mg / kg for 7 days) serves as an alternative to conventional PZQ and is recommended by the US Centers for Disease Control and Prevention for liver fluke infections. Albendazole dosing regimens for *Clonorchis sinensis* infection – 8 mg / kg twice daily for 5 days and 10 mg / kg twice daily for 7 days – demonstrated 100% efficacy, while 5 mg/kg twice daily for 7 days achieved cure in 27 of 32 study participants [10].

However, albendazole administration (400 mg twice daily for 7 days) against *Opisthorchis viverrini* yielded only 33.3% efficacy (complete cure in 9 of 27 patients), despite a 95.0% reduction in egg output [28]. Low-dose albendazole (single 400 mg dose) and mebendazole (400 or 500 mg) showed minimal efficacy against both *C. sinensis* and *O. viverrini*. Another source evaluating albendazole's anti-opisthorchiasis activity reported moderate therapeutic efficacy with twice-daily 400 mg dosing for 3–4 days, despite >92% reduction in fecal egg counts [28]. Conversely, W. Sangkam et al. investigated mechanisms of albendazole, niclosamide, and mebendazole against *O. viverrini*, assessing oxidative stress intensity, worm motility, and tegumental morphology [29]. The authors demonstrated significant potential of these agents as alternative therapeutics for *O. viverrini* infection [29].

Particular interest merits efforts to develop combined pharmacological agents incorporating synthetic drugs alongside plant-derived bioactive compounds. Recently, a novel complex of albendazole with arabinogalactan polysaccharide – derived from *Larix sibirica* and *Larix gmelinii* wood – was synthesized, and its anthelmintic activity against *O. felineus* was evaluated [30]. The synthesized albendazole-arabinogalactan complex demonstrated high efficacy in suppressing liver fluke infection, exhibiting anthelmintic activity at doses tenfold lower than either constituent compound administered separately. Moreover, significant reductions in overall toxicity and selective hepatotropic adverse effects were documented for the complex compared to isolated administration of its components. The authors concluded that the albendazole-arabinogalactan complex represents a safer and more effective anti-opisthorchiasis agent than albendazole alone, thereby establishing a promising pathway for novel anthelmintic development [30].

Beyond strategies for enhancing classical anthelmintic efficacy through hybrid complex formation, an alternative approach involves employing original synthetic agents. The next stage in searching for PZQ alternatives focused on evaluating compounds with innovative chemical structures. Among these stands tribendimidine – a synthetic amidinophenylimidazole derivative.

Tribendimidine was approved by China's National Medical Products Administration for treating soil-transmitted helminthiases and has also demonstrated high efficacy against liver fluke infections [31]. As with PZQ, tribendimidine's precise pharmacological mechanism remains incompletely understood. Current hypotheses suggest that increased lysophospholipid levels induced by this drug activate various helminth protein kinases – including tyrosine kinases, protein kinase C, and mitogen-activated protein kinases – leading to tegumental cell damage and host immune system recognition of parasite-specific antigens [32]. When administered as a single 400 mg dose to adults, tribendimidine achieved complete cure in 50% of *C. sinensis*-infected patients and 91.5% of *O. viverrini* cases [33, 34].

Moreover, the reduction rate of egg output in opisthorchiasis patients exceeded 99% [34]. Additional *in vivo* and *in vitro* experiments have demonstrated tribendimidine's potential efficacy specifically against *O. felineus* [11].

While the search for fundamentally new compounds (including tribendimidine) continues, PZQ remains the gold standard for opisthorchiasis pharmacotherapy. This undisputed status drives intensive research efforts to overcome its limitations through structural derivative development [35]. Studies by Novosibirsk researchers evaluated the efficacy of a supramolecular PZQ complex with disodium glycyrrhizinate against *O. felineus* infection in hamsters. *In vitro* experiments demonstrated that the "PZQ–disodium glycyrrhizinate" complex induced rapid parasite immobilization (1.5 times faster than PZQ alone) and caused significant tegumental damage [36, 37].

In vivo studies revealed the developed PZQ–disodium glycyrrhizinate complex surpassed standard PZQ in efficacy against *O. felineus*, attributable to both enhanced active substance (PZQ) bioavailability and disodium glycyrrhizinate's pronounced anti-inflammatory effects.

However, numerous studies testing PZQ derivatives have failed to demonstrate superior efficacy compared to the parent molecule. The promising *in vitro* activity of potential PZQ derivatives often doesn't correlate with animal study results, as their pharmacokinetics and biotransformation profiles constitute key determinants of *in vivo* efficacy [38].

The aforementioned evidence underscores the necessity for developing novel pharmacological strategies for opisthorchiasis chemotherapy, focusing on identifying molecular targets within the parasite – particularly specific parasitic proteins or metabolic pathway components [39]. Beyond direct anthelmintic effects, targeting critical metabolic processes essential for parasite survival represents a promising direction. In this context, hemozoin – a crystalline heme detoxification product – holds particular interest, as its formation proves critical for blood-feeding trematodes. Hemozoin (β-hematin) formation constitutes a fundamental physiological process in *Opisthorchis*, enabling neutralization of toxic heme [40, 41].

Chloroquine, a classic antimalarial drug, exerts its primary effect by inhibiting heme biocrystallization into hemozoin. This leads to soluble heme accumulation, which generates reactive oxygen species, inducing oxidative stress and parasite death [42]. As early as 1955, chloroquine demonstrated moderate anti-opisthorchiasis activity in *O. viverrini*-infected patients [42]. Recent studies confirmed hemozoin crystal formation in *O. felineus* and *C. sinensis* trematodes, while being absent in the closely related *O. viverrini* [40, 41].

This observation likely accounts for the limited therapeutic efficacy of chloroquine observed in *O. viverrini*-infected patients. Importantly, when targeting the hemozoin-producing *O. felineus*, chloroquine exhibits significant potential for disrupting a vital metabolic pathway essential for parasite survival. The drug's inhibition of hemozoin biogenesis results in the accumulation of cytotoxic free heme, subsequent generation of reactive oxygen species, and ultimately parasite death – a mechanism analogous to its well-characterized action against both *Plasmodium spp*. and *Schistosoma* parasites. These findings highlight the compelling rationale

for investigating chloroquine's therapeutic potential against *O. felineus*-induced opisthorchiasis, particularly in endemic Siberian populations.

In a comprehensive screen of novel benzimidazole derivatives targeting adult *S. mansoni* in murine models, several compounds demonstrated remarkable anthelmintic efficacy (70 P.85%) comparable to PZQ. Notably, these compounds exhibited broad-spectrum activity against both mature and juvenile developmental stages. The study identifies a promising new class of potential antischistosomal agents, with mechanistic studies suggesting their activity stems from selective inhibition of hemozoin formation through specific **β**-hematin binding and subsequent disruption of crystallization processes [43].

The cytochrome P450 system of *O. felineus* represents another promising target for novel trematocidal agents. This enzyme exhibits high activity in opisthorchid tissues and plays a crucial role in parasite physiology [44, 45]. Published evidence indicates that inhibitors of heme-containing enzymes (particularly azole derivatives) suppress parasitic cytochrome P450 activity and significantly reduce *O. felineus* viability [44, 45]. Among azole derivatives demonstrating *in vitro* efficacy against *O. felineus* are the antifungal agents miconazole and clotrimazole [46]. However, the synergistic effects of PZQ-clotrimazole and PZQ-miconazole combinations observed *in vitro* failed to translate to *in vivo* models [47].

Thus, despite PZQ's dominant position in opisthorchiasis treatment, accumulated evidence reveals critical limitations: inefficacy against juvenile parasite stages, emerging resistance risks in endemic areas, and observed correlations with cholangiocarcinogenesis in certain studies. These findings have spurred the exploration of alternative synthetic agents. Research confirms moderate efficacy of benzimidazoles (albendazole, mebendazole), particularly during extended regimens, though their effectiveness varies by trematode species and dosing protocols. A significant breakthrough involves hybrid compound development, exemplified by the albendazole–arabinogalactan complex demonstrating enhanced anthelmintic activity with reduced hepatotoxicity compared to monotherapy. Tribendimidine, another promising candidate, shows high efficacy against *O. viverrini* with favorable safety profiles. Parallel efforts focus on PZQ molecular modifications, including supramolecular complexes with disodium glycyrrhizinate to improve bioavailability and confer complementary anti-inflammatory effects. Investigations of parasitic enzyme inhibitors (particularly *O. felineus* cytochrome P450) reveal azole derivative potential, while chloroquine application (especially combined with PZQ) in *O. felineus*-endemic regions may theoretically provide synergistic action through dual targeting: disrupting calcium homeostasis (PZQ) and heme metabolism (chloroquine). The critical remaining challenge involves translating *in vitro* findings to clinical practice, hindered by complex pharmacokinetics and insufficient metabolic profiling of novel compounds.

These limitations of synthetic approaches – particularly their toxicity during repeated courses and unresolved inefficacy against juvenile forms – underscore the growing importance of investigating plant-derived bioactive compounds, which will be examined in detail in the following section. Their fundamental advantage lies in multicomponent action that combines direct anthelmintic activity with pathophysiologically grounded effects: oxidative stress suppression, inflammation mitigation, and fibrogenesis inhibition.

Moreover, as will be discussed subsequently, combinations of plant antioxidants with PZQ may unveil novel therapeutic prospects. Such formulations could simultaneously neutralize the adverse consequences of intoxication associated with oxidative stress and parasite antigen release, while potentially reducing risks of chronic infection complications.

PLANT-DERIVED BIOACTIVE COMPOUNDS AS PROMISING AGENTS FOR OPISTHORCHIASIS THERAPY

Modern parasitology is increasingly exploring the potential of natural biomolecules not only as sources of novel anthelmintic compounds, but also as modulators of invasion-related pathophysiological consequences. Research into plant-derived bioactive substances for opisthorchiasis treatment focuses on their capacity to complement and potentiate PZQ effects, mitigate its adverse reactions, and crucially – disrupt the cascade of events leading to chronic bile duct inflammation.

Recent years have witnessed the identification of several promising plant-derived bioactive compounds for developing novel anti-opisthorchiasis agents. It should be emphasized that the majority of these substances exhibit pronounced antioxidant activity. Regrettably, in contrast to schistosomiasis research, studies evaluating the anti-opisthorchiasis potential of antioxidant biomolecules remain relatively limited [24]. A notable exception is curcumin – a polyphenolic curcuminoid derived from *Curcuma longa* rhizomes – which has garnered particular research interest as a promising candidate for new anti-opisthorchiasis agents. Experimental evidence demonstrates that curcumin administration in *O. viverrini*-infected hamsters significantly reduces oxidative DNA damage while suppressing the expression of oxidative stress-associated genes (iNOS, NF-κB, and COX2) [48]. Conversely, during opisthorchiasis infection, curcumin upregulates antioxidant defense genes (superoxide dismutase types 2 and 3, and catalase). These combined effects result in substantial improvement of hepatic histopathology through suppression of inflammatory infiltration and periductal fibrosis [49]. The authors propose that curcumin mitigates DNA damage by simultaneously suppressing inflammatory responses and restoring systemic oxidative balance during opisthorchiasis [48]. Recent findings reveal that nanoencapsulated curcumin–PZQ co-administration enhances therapeutic efficacy in hamsters, significantly reducing periductal fibrosis while preserving bile canaliculi morphology and maintaining normal bile acid metabolism gene expression – effects not observed with curcumin monotherapy [50]. Another study investigated both *in vitro* and *in vivo* anti-opisthorchiasis activity of a supramolecular curcumin–disodium glycyrrhizinate complex, demonstrating that the developed formulation induces more pronounced parasite immobilization and tegument damage compared to curcumin alone. *In vivo* evaluation showed the "curcumin–disodium glycyrrhizinate" complex exhibits moderate anthelmintic activity (50–60% parasite burden reduction), while being less efficacious than PZQ, yet significantly attenuates hepatic inflammation and fibrosis progression through its superior antioxidant and anti-inflammatory properties [19].

One of the central mechanisms in opisthorchiasis-associated complications involves disruption of hepatic redox homeostasis, where the glutathione system – the primary antioxidant defense mechanism of hepatocytes – plays a pivotal role. Studies of chronic *O. felineus* infection demonstrate significant depletion of reduced glutathione (GSH) alongside increased oxidized forms (GSSG), promoting excessive reactive oxygen species accumulation, activation of pro-inflammatory signaling cascades, and stimulation of hepatic stellate cells, collectively driving fibrogenesis progression [51]. These findings validate pharmacological modulation of the glutathione system as a promising strategy for preventing opisthorchiasis-induced fibrosis, encompassing direct SH-group donors (N-acetylcysteine), glutathione synthesis stimulators, or complex antioxidants capable of restoring redox balance and interrupting the pathogenetic cascade of long-term invasion complications [51].

Another noteworthy research subject – the aqueous extract of blue trumpet vine (*Thunbergia laurifolia*) leaves – when administered to hamsters with experimental *O. viverrini* infection, suppressed inflammatory cell aggregation in peribiliary tissues while showing no hepatotoxicity. However, the extract itself demonstrated no significant direct anti-opisthorchiasis activity. Remarkably, co-administration of *T. laurifolia* extract with PZQ not only reduced inflammatory cell aggregation but also suppressed opisthorchiasis-associated cholangiocarcinoma development [52]. This effect may be attributed to decreased serum ALT levels, indicating reduced hepatocyte damage. The most significant results emerged when researchers administered the *T. laurifolia* extract not concurrently with PZQ, but rather following completion of standard anthelmintic therapy [53]. This sequential treatment protocol's efficacy likely stems from suppression of either inflammatory reactions or host immune responses to parasite death. Indeed, the extract's pronounced anti-inflammatory activity appears to modulate chronic infection-related immune reactions, improving hepatic histopathology and restoring organ function [53]. *In vitro* studies of *T. laurifolia* extract's specific anti-opisthorchiasis activity against both juvenile and adult *O. viverrini* forms revealed decreased motility and survival rates at both stages. Scanning electron microscopy showed minimal tegumental alterations

compared to positive controls, despite significantly elevated reactive oxygen species levels [54].

Finally, a study investigating the antifibrotic properties of xanthohumol demonstrated that this polyphenolic compound – whether administered alone or in combination with PZQ – significantly reduced DNA damage, intracellular iron content, TfR-1 protein expression, and most importantly, decreased fibrotic areas [55]. The authors' findings suggest potential applications of xanthohumol combined with PZQ for preventing opisthorchiasis-associated cholangiocarcinoma development [55].

Considerable interest also surrounds the work by P. Pechdee et al., which conducted a comprehensive analysis of *A. sativum* (garlic) extract effects on isolated *O. viverrini* adult flukes [56]. The study revealed substantial increases in reactive oxygen species levels and structural damage to parasite bodies in *A. sativum*-treated groups. These findings correlated with overall motility reduction and increased mortality rates among mature flukes [56].

The combination of PZQ anthelmintic therapy with plant-derived bioactive compounds exhibiting anti-inflammatory, antioxidant, and hepatoprotective properties may substantially enhance anti-opisthorchiasis treatment efficacy. This combined approach not only potentiates antiparasitic effects but also mitigates pathological consequences of infection, including bile duct fibrosis, chronic inflammation, and oxidative stress.

Bioactive plant compounds (e.g., curcumin, silymarin, flavonoids, and polyphenols) demonstrate the ability to modulate immune responses, suppress proinflammatory cytokines (TNFα, IL-6), and reduce reactive oxygen species levels, thereby attenuating hepatobiliary system damage progression. Furthermore, their capacity to inhibit cell proliferation and angiogenesis may contribute to reducing cholangiocarcinoma risk – one of the most severe complications of chronic opisthorchiasis.

Indeed, the available scientific literature indicates that the redox status of both parasite and host, along with liver fluke-induced inflammatory responses, may represent primary biological targets for trematode infection treatment strategies.

CONCLUSION

Opisthorchiasis caused by the liver fluke *O. felineus* remains a significant public health challenge, particularly in highly endemic regions, such as Western Siberia. Chronic parasite persistence in the hepatobiliary system associates with severe pathologies, including chronic cholangitis and progressive periductal fibrosis. PZQ, while remaining the therapeutic cornerstone for decades, exhibits fundamental limitations: complete lack of efficacy against larval developmental stages, potential hepatotoxicity during repeated treatment courses, growing threats of drug resistance development in endemic foci, and a scientifically debated yet potentially significant association between mass helminth die-off following PZQ administration and intensification of oxidative/nitrosative stress – established promoters of carcinogenesis.

As this lecture demonstrates, overcoming these limitations requires moving beyond PZQ monotherapy toward developing integrated, multi-target therapeutic strategies. The search for alternative synthetic compounds, such as tribendimidine, hybrid complexes (e.g., albendazole–arabinogalactan), or inhibitors of specific parasitic enzymes (*O. felineus* cytochrome P450) has revealed promising avenues. However, translating these agents into clinical practice remains challenging due to difficulties in converting encouraging *in vitro* results to *in vivo* efficacy, primarily stemming from complex pharmacokinetics and metabolic transformation issues. Within this context, plant-derived bioactive compounds gain particular importance. Their unique value derives not only from demonstrated direct anthelmintic activity (as seen with cynaropicrin), but critically from inherent polypharmacology enabling simultaneous targeting of key opisthorchiasis pathogenesis pathways: effective suppression of oxidative/nitrosative stress; modulation and mitigation of chronic inflammation through downregulation of proinflammatory cytokines (TNFα, IL-6) and mediators (iNOS, COX-2); reduced tissue infiltration thereby slowing fibrosis progression; and pronounced hepatoprotective effects improving hepatic histopathology and normalizing functional parameters.

The most promising and scientifically justified approach currently recognized involves combination therapy integrating PZQ's antiparasitic activity with the multifaceted effects of plant-derived antioxidants and anti-inflammatory agents. This synergistic strategy simultaneously

addresses multiple challenges: it potentiates PZQ's primary anthelmintic effect; neutralizes adverse consequences of massive parasite die-off, such as acute oxidative stress and extensive antigen release triggering systemic inflammation; and importantly, enhances overall treatment safety by enabling PZQ dose reduction and consequent toxicity mitigation.

Future research directions are clearly defined by current challenges and opportunities. Key priorities include: advanced development and optimization of combination regimens incorporating PZQ with specific phytocomplexes; precise determination of optimal dosages; evaluation of administration sequences (concurrent versus staggered); and assessment of long-term outcomes. Significant efforts focus on nanoencapsulation technologies to overcome bioavailability limitations and compound instability. Concurrently, screening of natural compounds continues to identify novel agents demonstrating direct anthelmintic activity against *O. felineus*, particularly those effective against PZQ-resistant juvenile forms.

Thus, effective opisthorchiasis control requires an integrative therapeutic paradigm. This approach harmoniously combines proven efficacy of classical anthelmintics with the multidimensional therapeutic impact of bioactive plant complexes. Addressing current treatment challenges necessitates developing comprehensive protocols capable of both reliably eradicating parasites at all developmental stages and protecting patients from the devastating long-term consequences of chronic infection.

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The role of Professor Mikhail G. Kurlov in the Fight Against Tuberculosis in Tomsk Province From the Late 19th to the Early 20th Century

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ABSTRACT

This article examines the work of the prominent physician and healthcare professional Mikhail G. Kurlov in the context of combating tuberculosis in Siberia at the turn of the 20th century. Using archival materials and periodicals, the study highlights his role in establishing a regional network of tuberculosis treatment facilities, introducing advanced methods of diagnosis and therapy, and developing a strategy for public health education in Tomsk province.

Keywords: Mikhail G. Kurlov, tuberculosis, Siberian healthcare, history of medicine

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Роль профессора Михаила Георгиевича Курлова в борьбе с туберкулезом в Томской губернии конца XIX – первой четверти XX в.

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РЕЗЮМЕ

Статья посвящена деятельности выдающегося врача и организатора здравоохранения М.Г. Курлова в контексте борьбы с туберкулезом в Сибири на рубеже XIX–XX вв. На основе архивных материалов и периодической печати показана его роль в зарождении региональной сети противотуберкулезных учреждений, внедрении передовых методов диагностики и терапии, а также в разработке стратегии санитарного просвещения в Томской губернии.

Ключевые слова: М.Г. Курлов, туберкулез, здравоохранение Сибири, история медицины

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

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Mikhail G. Kurlov, Tomsk, 1893

Tuberculosis (TB) known as the white plague posed a serious threat to humanity for centuries, decimating entire settlements and causing the deaths of a large number of people. The most severe situation developed in remote and underdeveloped areas, where low living standards, a lack of basic hygiene norms, and a shortage of medical institutions facilitated the rapid spread of the infection. In particular, within the Russian Empire's Siberian provinces, the disease exhibited an especially aggressive manifestation owing to harsh weather conditions, vast distances between settlements, and a scarcity of skilled professionals. In such a challenging environment, the work of individual physicians who dedicated themselves to combating TB, including renowned scientists, such as Nikolay Pirogov, Ivan Sechenov, and Sergey Botkin, was of immense importance.

Among those who made a significant contribution to addressing this problem was Mikhail G. Kurlov (1859–1932), an ordinary professor of the Department of Medical Diagnostics and the Therapeutic Faculty Clinic at the Medical Faculty of the Imperial Tomsk University – a talented therapist and active public figure whose professional career was largely connected to Siberia. Having arrived in Tomsk at the end of the 19th century, he immediately recognized the gravity of the TB crisis in the region and focused on research, the development of preventive measures, and the search for effective methods of treating this dangerous disease.

An objective assessment of Kurlov's activities in organizing TB control measures requires a preliminary reconstruction of the epidemiological situation characterizing the spread of TB both globally and within the Tomsk Governorate. Such an analysis is a necessary precondition for understanding the scale of the problem faced by Kurlov and his associates and, consequently, for a balanced evaluation of the significance of their contribution.

A retrospective analysis of medical statistics provides compelling evidence of the dominant role of TB in the profile of infectious mortality during the period under review. For instance, statistical data for the German Empire from 1894 indicate that mortality from TB (123,904 cases) exceeded the aggregate mortality rate from five other common diseases: diphtheria, whooping cough, scarlet fever, measles, and typhus (116,905 cases). This quantitative superiority serves as direct proof of the higher pathogenicity and contagious potential of *Mycobacterium tuberculosis* compared to the pathogens of a number of other epidemiologically significant infections of the late 19th century.

This alarming global trend was directly reflected in the realities of Russia, and particularly in the Tomsk Governorate. The local epidemiological situation regarding TB in the late 19th century was also characterized by an exceptionally high mortality rate. Between 1897 and 1900, mortality from tuberculosis averaged 43% of all deaths, corresponding to approximately 343 fatalities annually. Notably, this rate was comparable to analogous data recorded in the major urban centers: Moscow (42.7%) and Vienna (42.7%). Furthermore, a comparative

analysis reveals that rates in the Tomsk Governorate substantially exceeded the level of TB mortality in Rome (34.2%) and London (17.6%) [1].

The reasons for such a catastrophic state of affairs in the Tomsk Governorate, however, were rooted not in a natural-geographical factor ("the unfavorable conditions of the Siberian climate"), but in a completely different aspect – in local sanitary conditions. Unsanitary conditions had become a widespread phenomenon, as clearly demonstrated by the urban environment. For instance, students of the Imperial Tomsk University who lived in the districts of Soldatskaya Slobodka and Verkhnyaya Yelan were in one of the city's most unfavorable sanitary zones. Despite being relatively far from the center, these streets were characterized by extreme levels of dust and filth combined with high building density. The housing here consisted almost exclusively of wooden houses, plastered with clay or clad with painted clapboard, which did not contribute to the maintenance of hygienic standards [2].

The critical state of the medical infrastructure itself exacerbated the situation. As Mikhail Kurlov noted, even the Public Assistance Board Hospital was an "extremely ununequipped" institution: "It would be truly difficult to find other, more unsuitable premises that so completely fail to meet any hygienic conditions. It is a veritable hotbed of stench, filth, and bedbugs; it is, in short, anything vile one can imagine, but certainly not a medical institution..." [3]. Moreover, as the professor emphasized, it was precisely in these conditions that phthisic patients were housed, since other premises were occupied by patients with acute infectious diseases.

Recognition of the gravity of TBsituation and the imperative need for coordinated state intervention served as a powerful impetus for unifying efforts within the medical community. Consequently, based on a submission from the Board of the All-Russian League for the Fight against Tuberculosis and Ruling No. 64 of June 27, 1911 from the Tomsk Provincial Office for Society Affairs, the Tomsk department of the All-Russian League was established. The leadership core of the new organization was formed by prominent representatives of Tomsk scientific and medical elite. The founding members of the department were professors of the Imperial Tomsk University (N. Bereznegovsky, M. Kurlov, and V. Sapozhnikov), Emperor Nicholas II Tomsk Technological Institute (B. Weinberg), and physician Z. Nesmelova. It was at this first meeting that Mikhail Kurlov was elected chairman, and V. Pirussky, the senior physician of the Administration for the Reconstruction of Mountain Sections of the Siberian Railway, was elected vice-chairman.

Endowed with significant autonomy, the Tomsk department, under the leadership of the formed professional medical community, immediately embarked on an ambitious program that extended far beyond the initially planned activities within the city. Key areas of work included: 1) studying the prevalence of TB; 2) public health education; 3) assisting state and public institutions in anti-TB measures; 4) organizing commissions, research projects, lectures, exhibitions, and congresses; 5) publishing activities; and 6) petitioning the authorities and establishing specialized institutions (therapeutic, charitable, and hygienic) [4].

What necessitated such a comprehensive program? The answer is revealed by the statistics on TB morbidity and mortality in Tomsk. In 1911, 394 people died from tuberculosis there. Given a population of approximately 100,000, this resulted in a mortality rate of 40 per 10,000 people [5]. For comparison, in the capital cities, this rate was almost two times lower (in St. Petersburg – 33 deaths per 10,000 people; in Moscow – 22 deaths per 10,000 people).

Confronted with this catastrophic statistic, Kurlov initiated a series of specific measures aimed at improving the situation. Firstly, he attempted to use the Tomsk City Public Administration to push through the idea of expanding hospital facilities for patients with severe forms of TB. The lack of sufficient beds had created a dire situation where " TB patients spent months knocking on the doors of hospitals before securing a place. Until then, in their own corners and apartments, they freely scattered their sputum, for the disinfection of which no measures were taken anywhere" [6]. Secondly, Kurlov insisted on the necessity of disinfecting the premises where TB patients lived, something that had not been done previously (as the prevailing opinion was that TB was not a contagious disease). Thirdly, monitoring of TB patients arriving in Tomsk (at coaching inns, furnished rooms, and hotels) became an important activity, as these patients were potential sources of an epidemic.

TB Center, 24 Nechayevskaya St., Tomsk, 1920s

Suburban TB sanatorium "Gorodok", Timiryazevskoye Village, 1930s

In addition to organizational and sanitary measures, a serious problem highlighted by medical workers was the issue of diagnosis. They emphasized the gravity of untimely TB detection and its catastrophic consequences for public health. In particular, the difficulty of recognizing the disease in its early stages, when symptoms could be minor or non-specific, was underscored. As noted in a petition from the Tomsk department of the All-Russian League for the Fight against Tuberculosis: "The horror of TB and infection by it lies in the fact that this disease is difficult to recognize at its very beginning. A person has already got TB, the infectious agent has already penetrated the human body, but the person is still strong, still working, does not admit the thought that they could have a terrible disease, interacts with acquaintances, and caresses children and their wife, while the infection is active, and in the end – the family and all those around them are infected with TB" [4]. Thus, the absence of effective methods for early diagnosis and specific treatment not only led to patients seeking medical care late but also uncontrollably exacerbated the problem of the infection's spread, negating containment efforts.

A direct consequence of this alarming situation was the practical initiatives of Kurlov aimed at creating specialized medical infrastructure. Given the prevailing circumstances, in 1913, he proposed allocating one of the newly constructed barracks with 25 beds for the "communicable disease hospital", specifically for accommodating TB patients. However, despite the urgency of the problem and the agreement of the City Council representatives, the question of transferring the premises for TB patients was postponed due to the scarlet fever epidemic that broke out in 1913.

Faced with administrative obstacles in organizing an inpatient facility, the Tomsk department of the League adopted a strategy of seeking compromise solutions. Thus, as early as 1912, an attempt was made to open a special outpatient clinic for the reception and treatment of TB patients and providing assistance to their families. However, this initiative also encountered financial constraints. Due to a lack of funds in the city administration for establishing and maintaining a separate medical facility, a temporary compromise was found. A decision was made to organize the admission of TB patients at the city treatment facility. According to this decision,

doctors "during hours free from duties" and "by mutual agreement with the administration of the treatment facility" could provide care to such patients. As a result, the staff of this unit included a specialist doctor, two paramedics, and an attendant. This team conducted sessions 4 times a week, primarily from 5:00 PM until midnight, intended "exclusively for the citizens of Tomsk" [6].

While addressing practical medical tasks, Mikhail Kurlov and his associates launched an active educational campaign, considering public health education to be a key element in combating TB. As part of this strategy, popular science articles were published in the newspaper "Sibirskaya Zhizn" (Siberian Life), among which the work «The Latest Views on the Spread of Consumption» [7] is of particular interest. It contained Kurlov's analysis of epidemiological processes in the urban environment, methods of infection containment, and the problem of re-infections.The most large-scale project in this area was the publication of a one-day newspaper, "Bely Tsvetok" (White Flower) [8] issued on May 18, 1914 on behalf of the Tomsk department of the All-Russian League for the Fight against Tuberculosis, edited by Professor M. Kurlov. Although the publication was limited to a single issue, its content was comprehensive, combining the functions of a public report and a tool for health education. For instance, to demonstrate concrete results of their work, the newspaper featured financial and organizational reports, including a report on the activities of the Guardianship for the Indigent Tuberculosis Patients

of Tomsk for 1913. According to this document, during that year, the guardianship held 16 meetings and organized three fundraising events: a cinema screening at the "Illusion Globus" electric theater, a public festival, and a theatrical performance. The proceeds were directed to provide material assistance to 40 impoverished patients, primarily in the form of foodstuffs (meat, milk, bread, and eggs), as well as clothing, footwear, and, in critical cases, monetary allowances for child support, burial of the deceased, or repatriation of non-resident patients to their hometowns.

The exceptional scientific quality of these publications was ensured by authors who would go on to become prominent experts in their respective fields, including V. Markuzon, later the author of one of the first monographs on childhood tuberculosis in the USSR and head of the clinic at the Central Institute for the Protection of Children and Adolescents in Moscow; P. Butyagin, a pioneer of experimental and clinical bacteriology in Siberia; N. Bereznegovsky, an outstanding surgeon who founded the East Siberian Prosthetic Institute in Tomsk; and I. Altshuller, the personal physician to Anton Chekhov who later became head of the "Birkenwerder" TB sanatorium near Berlin.

A unique method employed to heighten the journalistic impact on readers involved placing aphoristic statements between articles, rendered in a distinctive font. For example: "The fight against tuberculosis requires a clear mind, a responsive heart, and a generous hand", and "Tuberculosis is a social evil, and the fight against it will be productive only with the active participation of society and the masses, and with corresponding efforts from the government". This rhetorical device allowed for the concise formulation of the movement's key ideas, emphasizing the social nature of the disease and the necessity of collective efforts.

These declarative appeals found direct practical implementation in the organization of large-scale charity events, the most important of which was the "White Flower" campaign. The collection of donations during this event served as the central source of funding for anti-TB measures. Consequently, the League's departments actively developed interregional cooperation to increase the reach of their collections, striving to create a unified charitable space and consolidate resources to combat the disease.

Celebration of the Camomile Day, the second day of the White Flower campaign, Tomsk, May 20, 1911. Students of Tomsk Imperial University sold camomiles in public places and collected donations for anti-TB initiatives

A vivid example of such cooperation was the collaboration between the Tomsk department of the League and the Irkutsk department regarding the organization of a collection drive involving the sale of daisies at stations of the Trans-Siberian Railway [9].

The funds accumulated through this activity and the acute awareness of the problem's severity prompted the Tomsk department of the League to transition from isolated campaigns to the implementation of a large-scale infrastructure project. To counter the spread of early forms of TB in the Tomsk Governorate during 1913–1914, plans were initiated to build a "sanatorium for the treatment of initial forms of tubercular diseases". A land plot of 2,153.56 square fathoms (which is currently equivalent to 9,803.5 m^2) was chosen for this purpose, located on the bank of the Ushaika River near Petrovskaya Street (now Yakovleva Street), where an institution for 15–17 patients was intended to be built. It is important to emphasize that the sanatorium was planned to admit patients with the early stages of TB, while severe cases were to be directed to the hospital, demonstrating an aspiration to create a differentiated care system.

However, significant difficulties arose immediately during the implementation of this project, indicating a clash between progressive medical initiatives and the city's environmental and social realities. A key deterrent was concern over the potential negative impact of the construction on the riverine ecosystems of the Tom and Ushaika Rivers. In particular, serious objections were raised regarding the possible pollution of the water bodies, given their active use by the local population for domestic needs, including drinking water supply. An additional argument used by the project's opponents was the recreational use of the Ushaika River, where children swam and laundry was rinsed: "the impoverished population near the river uses it not only for domestic needs and for drinking, but also, the Ushaika River is used for children's bathing and for rinsing laundry" [10].

Despite the identified environmental risks and subsequent public discussions, the Tomsk City Duma decided to allocate the land plot for the sanatorium's construction. However, the project's implementation was blocked by the outbreak of the First World War (1914–1918) and, subsequently, the Civil War (1918–1922). The worsening epidemiological situation coupled with the impossibility of completing the original plan prompted Kurlov to initiate the development of an alternative solution, which was realized in October 1924 with the establishment of a comprehensive anti-TB infrastructure in Tomsk.

This infrastructure included a city dispensary with a 40-bed inpatient facility, night and day preventative clinics, a laboratory, a department for active forms of TB at the Provincial Hospital, and the "Gorodok" suburban sanatorium in the village of Timiryazevskoye. The scale of the problem is illustrated by data from the dispensary's first year of operation: 14,664 visits were recorded by patients with various forms of TB (pulmonary, bone, glandular, etc.) and non-specific respiratory diseases. The institution's preventive activities included conducting sanitary inspections of 12 city establishments and organizing 8 TB units at key enterprises (Kozhzavod, Vodosvet, Mashinstroy, etc.). These efforts were coordinated by the Council of Social Assistance attached to the dispensary, which developed plans for mass screenings and measures to improve working conditions at industrial sites [11].

As the conducted research demonstrates, professor Mikhail Kurlov played a pivotal role in organizing the fight against TB in the Tomsk Governorate from the late 19th to the first quarter of the 20th century. Under his leadership, a comprehensive healthcare system was established, uniting outpatient services, inpatient treatment, and social support for patients. He actively introduced progressive methods of diagnosis and therapy, while simultaneously developing a systemic strategy for public health education. As part of this activity, he gave popular science lectures, published articles in the newspaper "Sibirskaya Zhizn", and edited the specialized publication "Belyi Tsvetok" (White Flower).

An important focus of the work was the organization of charitable events and interregional cooperation, including the coordination between the Tomsk and Irkutsk departments of the All-Russian League for the Fight against Tuberculosis to conduct collection drives at stations of the Trans-Siberian Railway. A significant achievement was the formation of a regional network of specialized institutions, which included the sanatorium project for treating early forms of TB initiated in 1913–1914, as well as the anti-TB dispensary infrastructure established in 1924–1925, complete with preventative clinics,

a laboratory, and the “Gorodok” sanatorium in the village of Timiryazevskoye in the Tomsk district of the Tomsk province.

The work of Kurlov contributed not only to the creation of specialized anti-TB institutions but also to the formation of a comprehensive model for controlling socially sensitive diseases. The organizational solutions he developed, based on the principles of the emerging phthisiology, laid the institutional foundation for the development of the Siberian healthcare system in the subsequent Soviet period.

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Издательский дом Сибирского государственного медицинского университета представляет серию книг

«Наследие томской медицины»

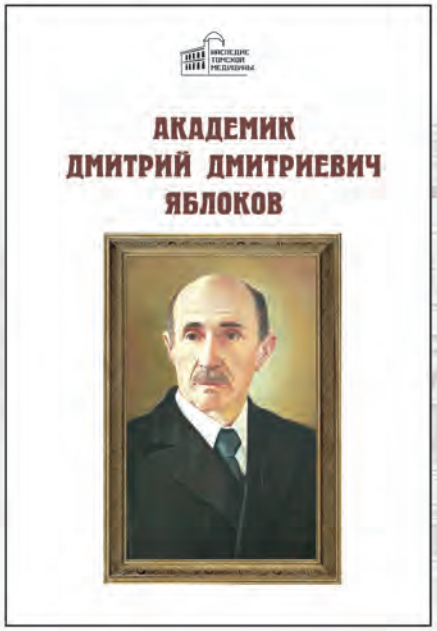
Книга посвящена 130-летнему юбилею кафедры госпитальной хирургии СибГМУ. Приведены биографические данные 79 сотрудников клиники и кафедры госпитальной хирургии в период с 1892 по 2022 г. Им предшествует подробная статья, характеризующая основные научно-практические достижения коллектива на каждом историческом отрезке. В издании упомянуты не только выдающиеся хирурги, звезды мировой величины, но и рядовые профессора, доценты, ассистенты, врачи-ординаторы, многие из которых связали с кафедрой и клиникой всю свою трудовую биографию. При изложении материала наряду с традиционными источниками информации использованы автобиографические документы, данные из семейных архивов, производственные характеристики нередко с сохранением авторского стиля.

Это позволяет полнее ощутить атмосферу в обществе и рабочем коллективе в разные годы существования клиники. Текстовая информация сопровождена богатым иллюстративным материалом, многие фотографии опубликованы впервые.

Издание предназначено для хирургов, студентов старших курсов врачебных факультетов, специалистов по истории медицины.

Трёхтомная иллюстрированная летопись одного из старейших и наиболее авторитетных медицинских вузов России – Сибирского (Томского) государственного медицинского университета является по сути первой серьёзной попыткой осветить более чем 140-летнюю историю этого прославленного университета.Особенностью издания является его богатейший иллюстративный материал, включающий более четырёх тысяч фотографий (в том числе ранее практически неизвестных), и никогда не публиковавшиеся до этого крайне любопытные и интересные факты о жизни университета, его студентов и профессоров, воспоминания и рассказы выпускников и преподавателей вуза.

Для самого широкого круга читателей, интересующихся историей российских университетов, отечественного высшего медицинского образования и науки, развитием клинических и научно-медицинских школ, здравоохранения, историей Томска, Сибири, России…



В книге представлены биография и обзор научной, педагогической и общественной деятельности выдающегося ученого, терапевта, клинициста, академика АМН СССР, Героя Социалистического труда, лауреата Сталинской премии Дмитрия Дмитриевича Яблокова (1896-1993).

Для врачей, студентов, всех интересующихся историей медицины.

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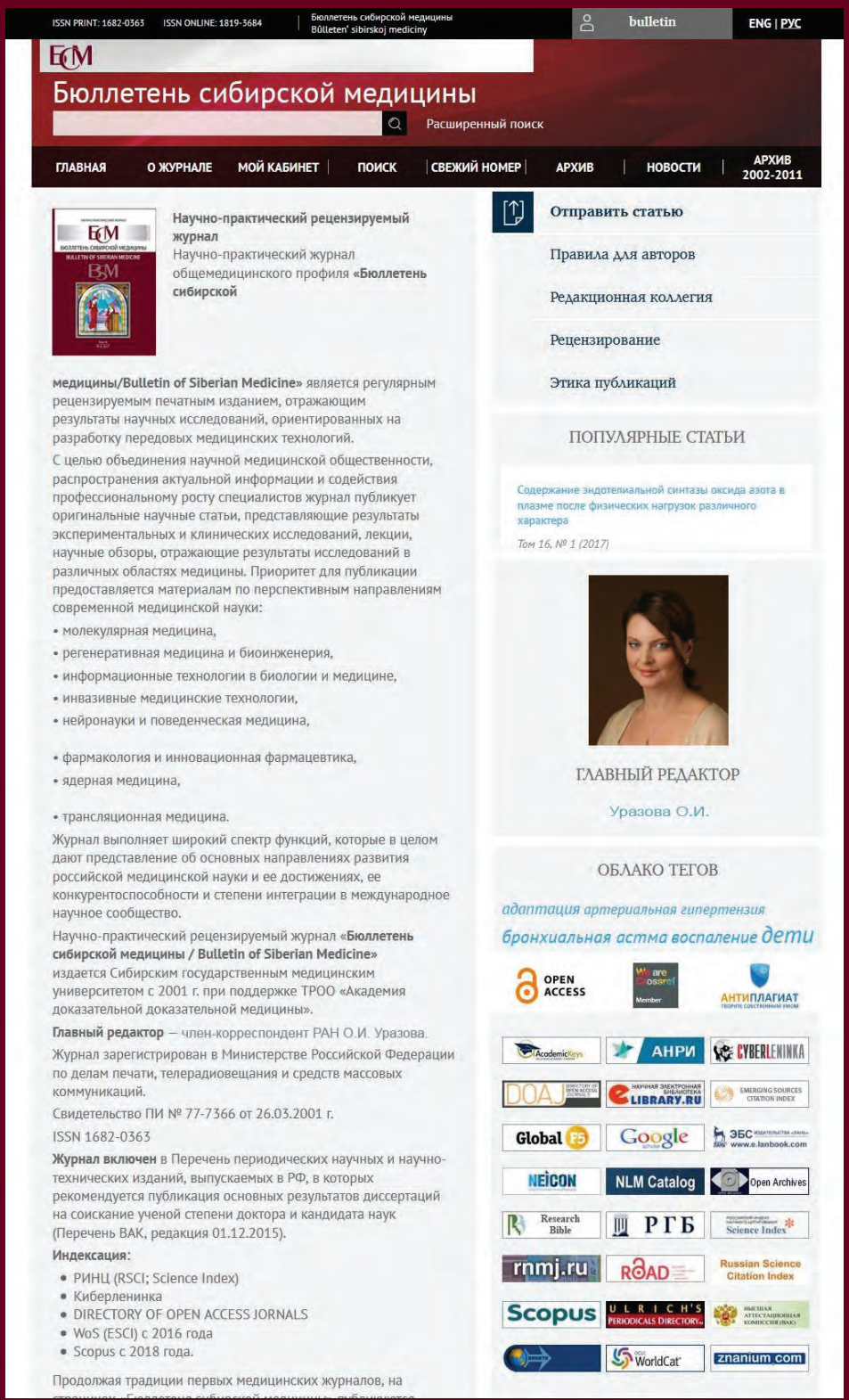

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